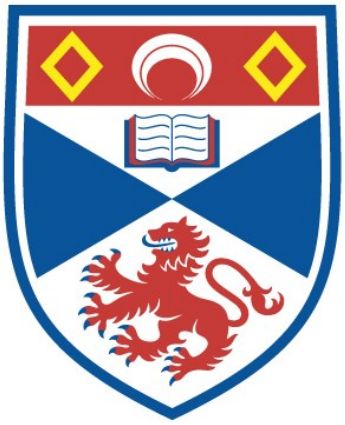
STUDIES ON THE LAMPBRUSH CHROMOSOMES OF THE AMERICAN NEWT NOTOPHTHALMUS (TRITURUS) VIRIDESCENS

Sheila Hartley

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1977

STUDIES ON THE LAMPBRUSH CHROMOSOMES OF THE AMERICAN NEWT *Notophthalmus (Triturus) viridescens*

by

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A thesis submitted for the Degree of Doctor of Philosophy

February, 1977.

DECLARATION

I hereby declare that this thesis is my own composition, and that the experimental work was performed by me alone, except for some of the data included in Chapter V which has been submitted for publication jointly with Professor H.G. Callan in the Journal of Cell Science.

None of the material in this thesis has been submitted for any other degree.

SHEILA E. HARTLEY

11th February, 1977.

CERTIFICATE

I certify that Miss Sheila Edna Hartley has spent 12 terms at research work on the lampbrush chromosomes of the newt, Notophthalmus viridescens, that she has fulfilled conditions of Ordinance No. 12 (St. Andrews) and that she is qualified to submit the accompanying thesis for the degree of Doctor of Philosophy.

H.G. CALLAN.

11th February, 1977.

UNIVERSITY CAREER

I entered the University of St. Andrews in October 1969, and graduated with an upper second class Honours B.Sc. in June 1973.

In October 1973 I started post-graduate research in the Zoology Department of the University of St. Andrews on the lampbrush chromosomes of the newt Notophthalmus viridescens. The results of my work are presented here for the degree of Doctor of Philosophy.

C O N T E N T S

C H A P T E R III.

C H A P T E R IV.

C H A P T E R V.

ABSTRACT

Observations have been carried out on the lampbrush chromosomes of Notophthalmus (Triturus) viridescens, the American newt; especially of chromosome II and XI. Chromosome II regularly bears two and occasionally three pairs of giant loops situated close to the centromere. The giant loops are distinguished from the majority of the normal loops by the greater bulk of their matrix and their length, which may vary from 60 µm to 300 µm. The giant loops are usually observed as single loop pairs arising from a single chromomere but occasionally they occur as multiple loop pairs from a single chromomere. The giant loops also show variation in the distribution of their RNP matrix and more than one polarized matrix unit may be present on a single loop. The size of the loops and the matrix distribution pattern around any one loop pair is constant in oocytes ranging in size in any particular animal.

The effect of increasing and decreasing the metabolic rate of the newt, by using hormone injections and cold treatment respectively, on the length and matrix distribution patterns of the giant loops was investigated. Hormone injections may cause changes in loop length, usually an increase but in one case a decrease, or have no effect. This may be due to the initial loop length or the level of gonadotrophin already present in the animal. After hormone injections the matrix distribution pattern, in multipolarized loops, alters with an increase in the proportion of the loop occupied by the first matrix unit. The constancy of loop characteristics over a range of oocyte sizes and the effect of hormone treatment are discussed in terms of matrix roving round a stationary loop axis.

Cold treatment causes a dramatic shortening of the majority of lateral loops but leaves the giant loops virtually unaltered. Autoradiographic experiments have shown that the rate of RNA synthesis in the giant loops

is half the rate of synthesis on the ordinary loops and that the RNA transcribed by the giant loops contains very little guanine. These results are discussed in terms of differing rates of RNA polymerase movement or attachment, different types of RNA polymerase molecules and the organization of highly repetitive sequences in the genome.

The effect of inversion heterozygosity on chiasma distribution in bivalent XI was also studied. 15 out of 94 females studied were heterozygous for an inversion involving almost the whole of the longer arm of bivalent XI and including the sequentially labelling loops situated close to the end which are transferred, by the inversion, to a position close to the centromere. The chiasma distribution in normal bivalents XI was compared with that of normal bivalents II and inversion heterozygote bivalents XI. Normal bivalents XI have chiasmata restricted to the chromosome ends while normal bivalents II have unrestricted distribution of chiasmata. In inversion heterozygote bivalents XI no chiasmata at all are formed in the longer inverted arm pair and chiasmata become distributed throughout the length of the shorter non-inverted arm pair. Chiasma distribution was found to be similar in both sexes. These results are discussed in terms of the availability of recombination nodules and the time of their association with the synaptonemal complex.

CHAPTER I.

GENERAL INTRODUCTION TO THE LAMPBRUSH CHROMOSOMES OF *Notophthalmus (Triturus) viridescens* with special reference to the giant loops on chromosome II.

Lampbrush chromosomes, which are characteristic of diplotene of 1st meiotic prophase, were first observed by Flemming (1882) in sections of salamander oocytes and by Rückert (1892), who gave them their name, in the oocytes of the shark *Pristiurus*. Until 1937, when Duryee developed a technique for isolating lampbrush chromosomes into an appropriate saline and observing them in an unfixed state, there was little interest in them. Gall (1952, 1954) made important advances in technique by using phase-contrast optics and inverting the train of his phase-contrast microscope, thus making observation of the unfixed chromosomes possible at high magnification.

Following these advances in technique, lampbrush chromosomes have been observed in the oocytes of a wide range of animals including molluscs (Callan, 1957; Ribbert & Kunz, 1969; Bottke, 1973), insects (Kunz 1967a and b: Bier, Kunz & Ribbert, 1969), fish (Rückert, 1892; Dodson, 1948), Amphibia (Flemming, 1882; Gall, 1952, 1954, 1966; Gall & Callan, 1962; Callan, 1963, 1966; Callan & Lloyd, 1960a and b; Müller, 1974) and man (Baker & Franchi, 1967), and in the spermatocytes of certain insects such as *Chironomus* (Keyl, 1975) and *Drosophila* (Meyer, 1963; Hess, 1965; Hennig, Mayer, Hennig & Leoncini, 1973). Recently lampbrush-like chromosomes have been observed in some plants (Grun, 1958; Spring, Scheer, Franke & Trendelenburg, 1975).

The lampbrush chromosomes found in amphibian oocytes have proved to be extremely amenable to study for several reasons. Oocytes are easily obtainable and lampbrush stages are found for several months each year. The chromosomes are large, ranging in size from 60 µm for the smallest *Xenopus* chromosome (Müller, 1974) to over 600 µm for the longest chromosomes

in Triturus viridescens (Gall, 1954) and 1000 µm for the longest chromosome in Salamandra salamandra (Mancino, Barsacchi & Nardi, 1969) and individual chromosomes within species may be recognized on the bases of their relative lengths, centromere positions in cases where the centromeres can be distinguished, and positions of landmark structures such as spheres and loops with special morphology. As lampbrush chromosomes are found during diplotene of 1st meiotic prophase, the homologous chromosomes are paired to form bivalents and are held together at various points along their length which may be chiasmata or places where "gene products" have fused e.g. inter-homologue fusion occurs between the "lumpy" loops around the centromere of chromosome II of Triturus cristatus carnifex and T.c. karelinii (Callan & Lloyd, 1960b).

Amphibian lampbrush chromosomes have been the objects of study of many workers (Gall, 1952, 1954, 1966; Gall & Callan, 1962; Callan & Lloyd, 1960a and b; Miller, 1965; Mancino & Barsacchi, 1965, 1966, 1969). Gall (1952) described lampbrush chromosomes as threads from which thousands of loops project laterally. The central axis of each thread is made up of a single row of chromomeres which bear the loops as lateral projections; the lateral loops occurring in pairs (Callan, 1963). Since Gall's early observations it has been found by a variety of techniques: Feulgen staining, enzymatic digestion and electron microscopy, that the chromomeres, their connecting inter-chromomeric strands and the axes of the lateral loops are made up of deoxyribonucleoprotein (DNP) (Dodson, 1948; Gall, 1954; Callan & Macgregor, 1958; Macgregor & Callan, 1962; Miller, 1965). The DNP axes of the lateral loops are surrounded by a matrix of ribonucleoprotein (RNP) (Gall, 1956; Callan & Macgregor, 1958; Macgregor & Callan, 1962) which shows a gradation in thickness around the loop (Callan, 1955; Callan & Lloyd, 1960b) so that there is a thin insertion and a thick insertion of the loop into each chromomere. Studies of deoxyribonuclease (DNase) action and of electron microscopy

have provided evidence that each loop axis is composed of one DNA double helix and that the inter-chromomeric strands are composed of two such helices (Gall, 1963; Miller, 1965) i.e. each chromatid is a single double helix molecule of DNA. It should however be mentioned that Ullerich (1970) working on the Anura and Fabergé (1970) on the newt Taricha describe segments in which the loop axis appears to be composed of two double helices.

In 1954, Gall characterized the chromosomes of Triturus viridescens (n = 11) in a lampbrush chromosome map on the basis of their lengths such that the chromosome with the longest arm was denoted chromosome 11. The centromeres in T. viridescens resemble normal chromomeres but are larger and although they do not bear lateral loops, they occasionally have spheres attached to them. The left arm of the chromosome was selected as the one which projects furthest from the centromere. In 1960, Callan & Lloyd characterized the lampbrush chromosomes of four subspecies of Triturus cristatus: cristatus, carnifex, karelinii and danubialis (n = 12) and they numbered the chromosomes so that the longest chromosome was denoted chromosome I. Other workers have used this convention in constructing working maps of amphibian lampbrush chromosomes (Barsacchi, Busotti & Mancino, 1970; Callan, 1966; Giorgi & Galleni, 1972; Lacroix, 1968; Mancino & Barsacchi, 1965, 1966, 1969; Mancino et al., 1969; Müller, 1974; Nardi, Raggbianti & Mancino, 1972) and so with Dr. Gall's agreement, the map of Notophthalmus (Triturus) viridescens has been redrawn accordingly (Callan & Lloyd, 1975).

The American red spotted newt, Notophthalmus (Triturus) viridescens differs from the European species of Triturus in the number of chromosomes it possesses; Notophthalmus has 11 pairs whilst Triturus has 12. In the chromosome complement of N. viridescens, chromosomes II, III, VI, VIII and XI are recognized by the landmark structures that they bear (Fig. 1). The

Fig. 1. Working map of the lampbrush chromosomes of Notophthalmus (Triturus) viridescens.

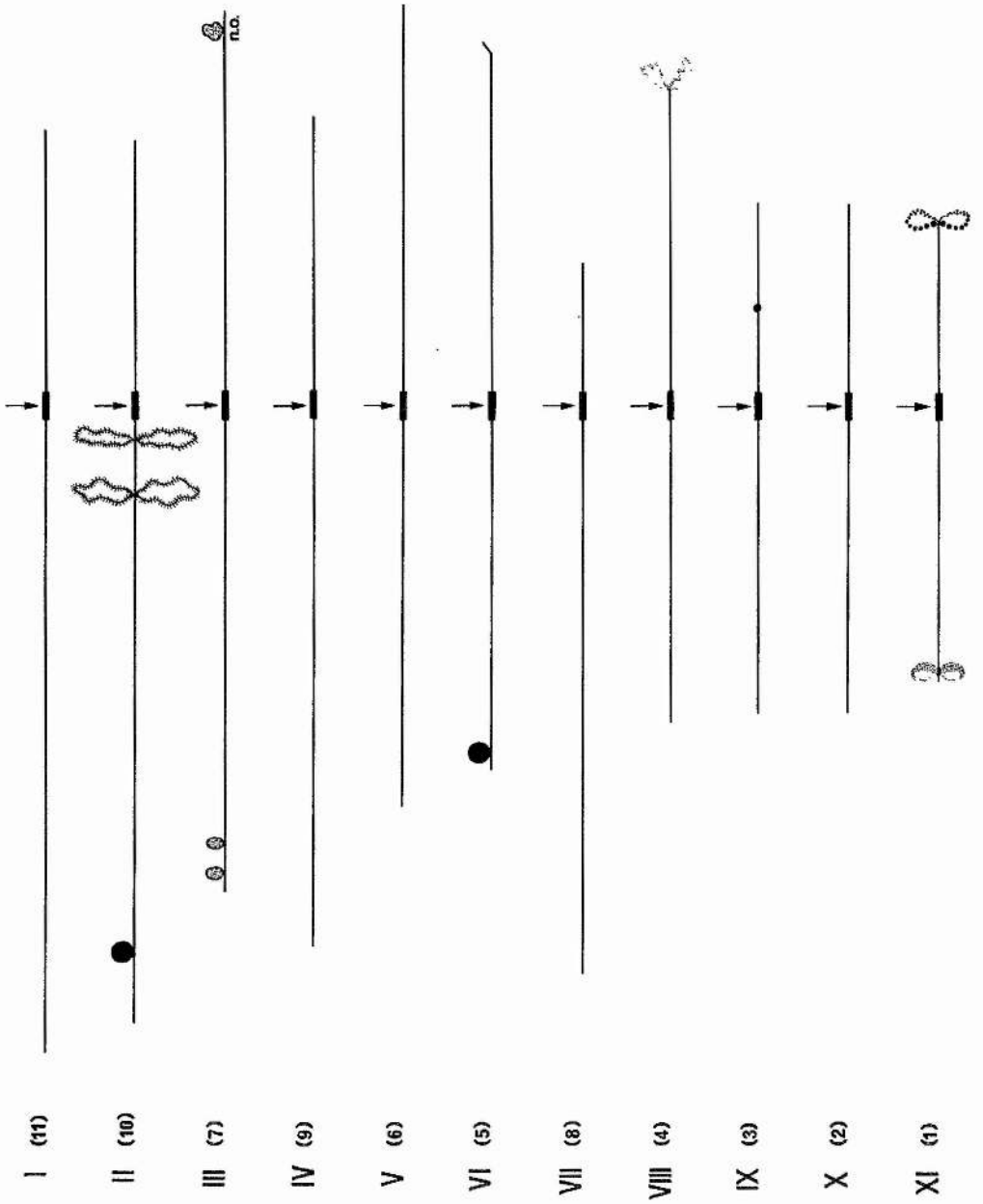
Numbers in brackets are Gall's (1954) original numbers.

Arrows indicate the centromeres.

n.o. = nucleolus organizer.

(From Callan & Lloyd, 1975. Reprinted with the kind permission of Professor H.G. Callan).

Fig. 1



remainder of the chromosomes are distinguished by their relative lengths and centromere positions. In Gall's (1954) original working map the giant loops on chromosome II were drawn at a single locus, 0.55. Loci of landmark structures are denoted in decimal fractions when the chromosome is taken to be of unit length and then divided into hundredths, with the end of the left arm being taken as zero. In Callan & Lloyd's (1975) amended map two pairs of giant loops are drawn at loci 0.60 and 0.66 on chromosome II. Callan (unpublished observation) has noted that the giant loops may exhibit multiple polarity i.e. more than one thin to thick matrix unit round each loop.

A study of the lampbrush chromosomes of N. viridescens and in particular chromosome II and its giant loops was undertaken in an effort to gain further understanding of the fundamental structural and functional organization of lampbrush chromosomes.

MATERIALS AND METHODS

Animals: Adult females of the American red spotted newt, Notophthalmus (Triturus) viridescens were obtained from Lee's Newt Farm, Oak Ridge, Tennessee and kept in the laboratory at 18°C.

Lampbrush Chromosome Preparations: To obtain oocytes a newt was anaesthetized with MS 222 (Sandoz) at a concentration of 1 gm/litre, a small incision was made in the abdominal wall and part of the ovary removed. The piece of ovary was placed in an embryo cup which was sealed and kept on ice.

The techniques used for isolating oocyte nuclei and removing nuclear membranes were as described by Callan & Lloyd (1960a). Oocyte nuclei were isolated in embryo cups in a 3 : 1 unbuffered mixture of 0.1 M potassium and sodium chloride. The nuclei were then transferred to a preparation chamber, a 3" by 1" "bored" slide with a coverslip sealed across the hole with low

melting point paraffin wax, containing a solution of this "3 : 1" saline with calcium chloride at some concentration between 0.5×10^{-4} M and 1.0×10^{-4} M. Calcium ions aid the dispersal of the nuclear sap but at concentrations above 1.0×10^{-4} M cause stiffening of the lateral loops of the lampbrush chromosomes. A coverslip was placed over the preparation and sealed in position with vaseline.

Preparations of lampbrush chromosomes were made from oocytes whose diameter was in the range 0.60 mm to 1.00 mm, for it is at this stage that the lateral loops are maximally extended. The preparations were observed with a Zeiss inverted (plankton) phase-contrast microscope. Photographs were taken with a single exposure camera loaded with Pan F film and drawings were made with either a Zeiss or a Reichert camera lucida attached to the Zeiss microscope.

OBSERVATIONS

Fig. 1 is the working map of the lampbrush chromosomes of N. viridescens showing the relative lengths, centromere positions and landmark structures of the 11 bivalents. Chromosome II, the second longest chromosome in the complement (figs. 2, 4 and 5), regularly bears two pairs of giant loops close to the centromere on the left arm (figs. 1 and 6a). Occasionally a third pair of giant loops may be present on this arm (figs. 3 and 6b). Callan & Lloyd (1975) placed the two pairs that appear on their map (fig. 1) at loci 0.60 and 0.66. The third pair, when present, is located at position 0.63 (fig. 3). The giant loops are distinguished from the rest of the loops by the greater bulk of their matrix and their length. However there is some variation from animal to animal not only in respect of number of pairs of giant loops but also with respect to their length and pattern of matrix distribution.

Fig. 2. Camera lucida drawings of chromosome II.

C = centromere

Ch = chiasma

S = sphere

TF = terminal fusion.

Top: Heterozygous for 3 pairs of giant loops; the pair nearest the centromere is missing on one homologue.

Middle: Heterozygous for 3 pairs of giant loops; the pair furthest from the centromere is missing on one homologue.

Bottom: Homozygous for 2 pairs of giant loops.

Fig. 2

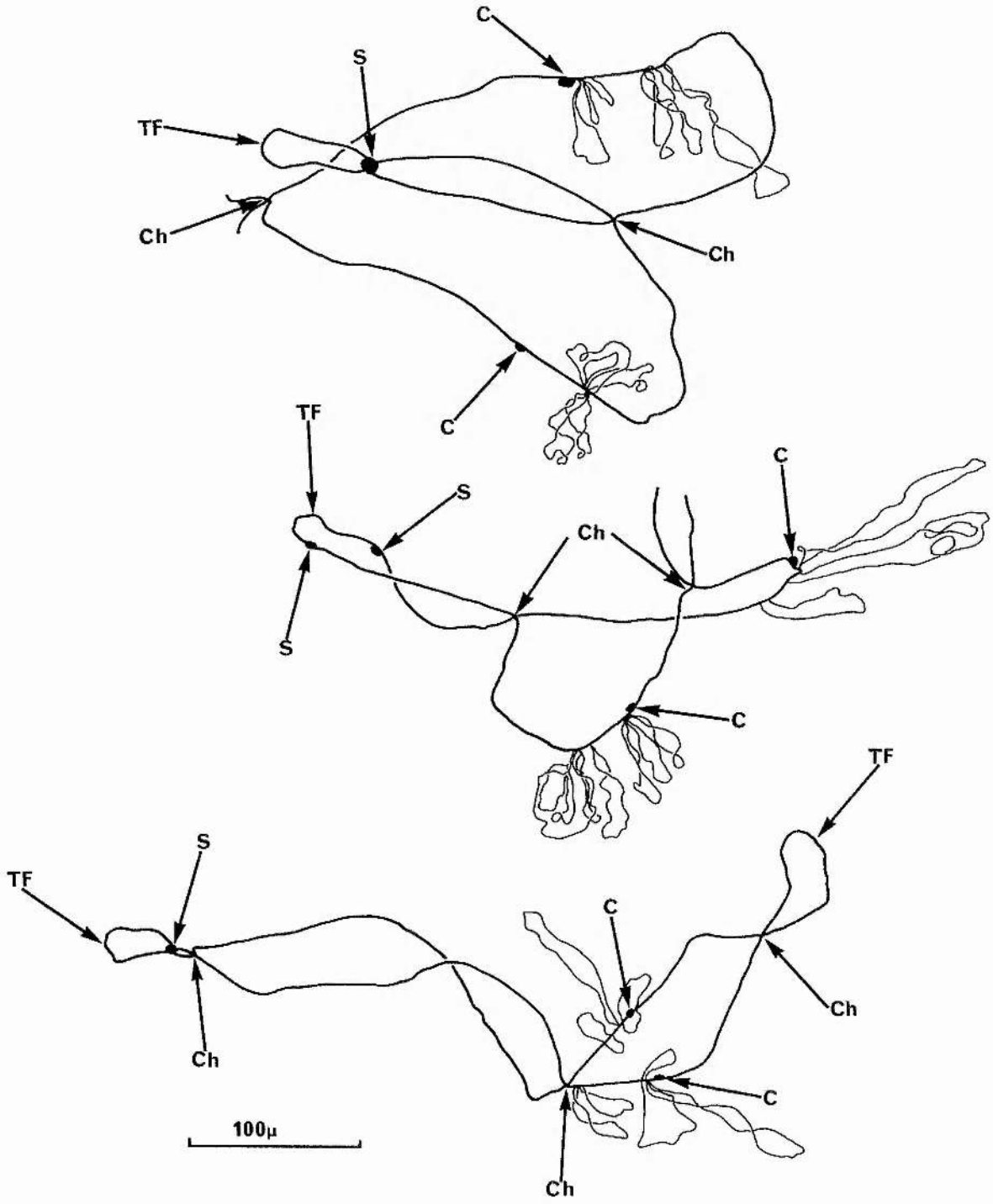
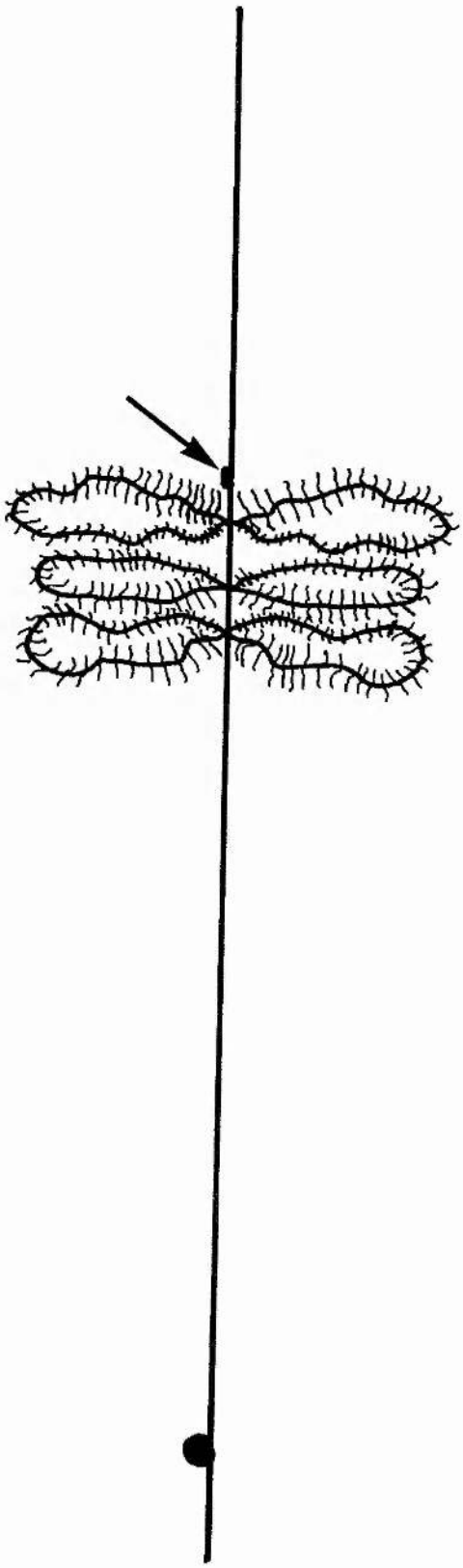


Fig. 3. Diagram of chromosome II to show the 3 possible loci for giant loops at 0.60, 0.63 and 0.67.
The other landmark structure is a sphere at locus 0.08.
The arrow indicates the centromere.

Fig. 3

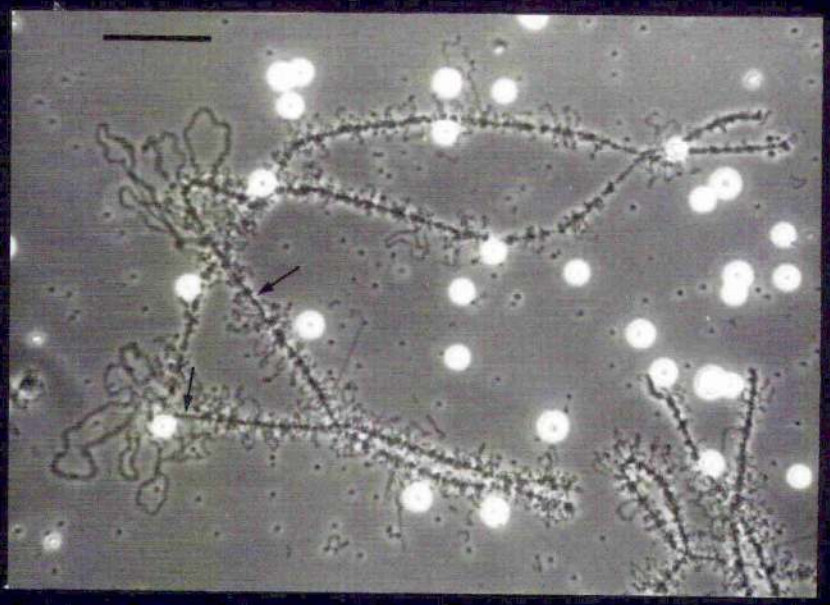


Although at each locus there is usually only a single loop pair (fig. 7a) there may, more rarely, be multiple loops coming from one locus (fig. 7b). The size of the giant loops ranges from approximately 300 µm for very long examples (fig. 8a) to only 60 µm for small examples (fig. 8b) but even these small examples are longer than the ordinary loops whose average length is between 40 µm and 50 µm. The matrix surrounding the loop axis may show interruptions and several different matrix distribution patterns have been observed (fig. 9). There may be a single polarized matrix unit running the length of the loop (fig. 9a), a single polarized matrix unit which stops abruptly, before the axis goes back into the chromomere (fig. 9b), or one or two interruptions in the matrix so that there are two or three polarized matrix units along the length of one loop i.e. a multipolarized loop (figs 8a & 9c).

Oocytes have been examined within the size range 0.60 mm - 1.00 mm diameter and the matrix distribution pattern of any one pair of giant loops is the same throughout the size ranges in an individual animal. Where interruptions occur, they occur at the same level on sister loops and on the homologous pair, if present, on the homologous chromosome. In oocytes above 1.0 mm diameter the lateral loops of the lampbrush chromosomes begin to retract into the chromomeres and the chromosomes start to condense. Because of this, observations made on the giant loops described in the following chapters were made on oocytes of 0.60 mm - 0.80 mm diameter. Some animals have been observed to be heterozygous for one or more pairs of the giant loops (fig. 2).

Fig. 4. Phase contrast photograph of entire chromosome II with 2 pairs of giant loops.
The arrows indicate the centromeres.

Scale = 50 µm



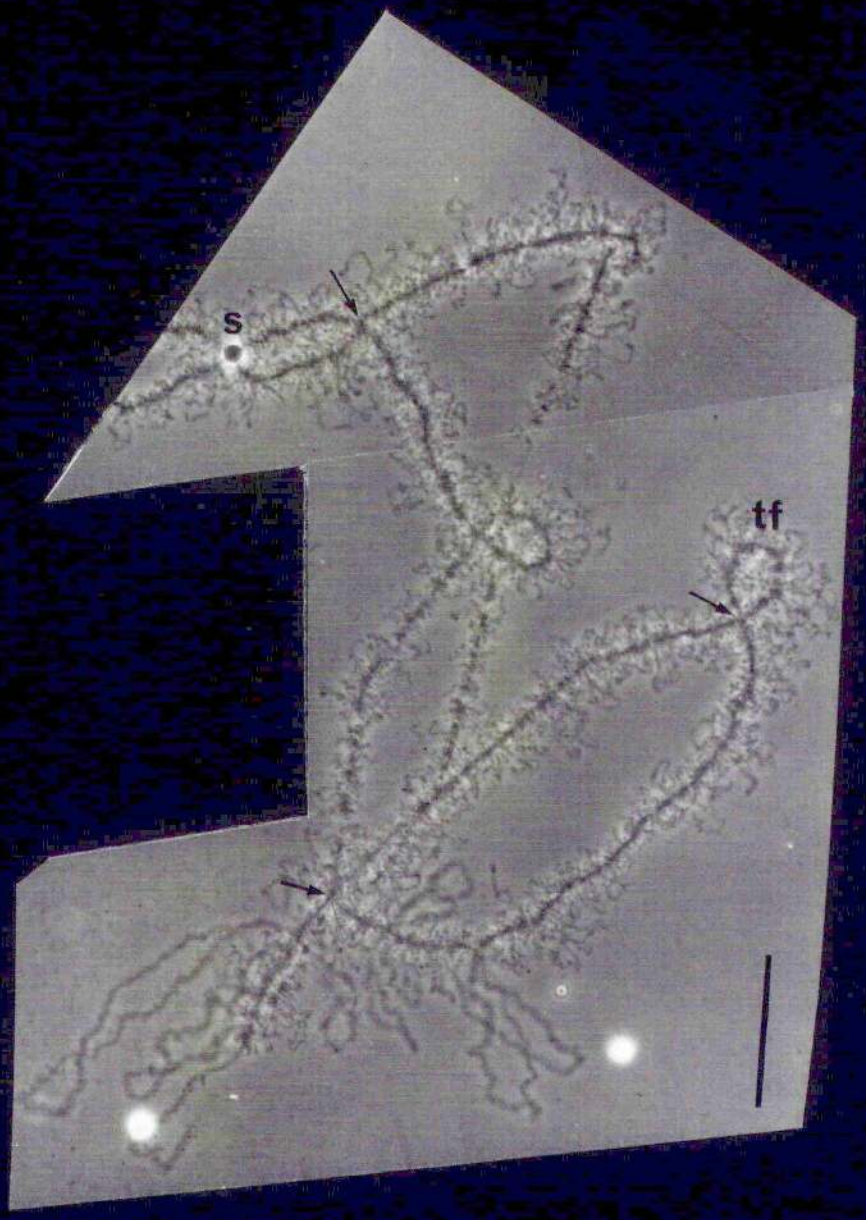

s
tf

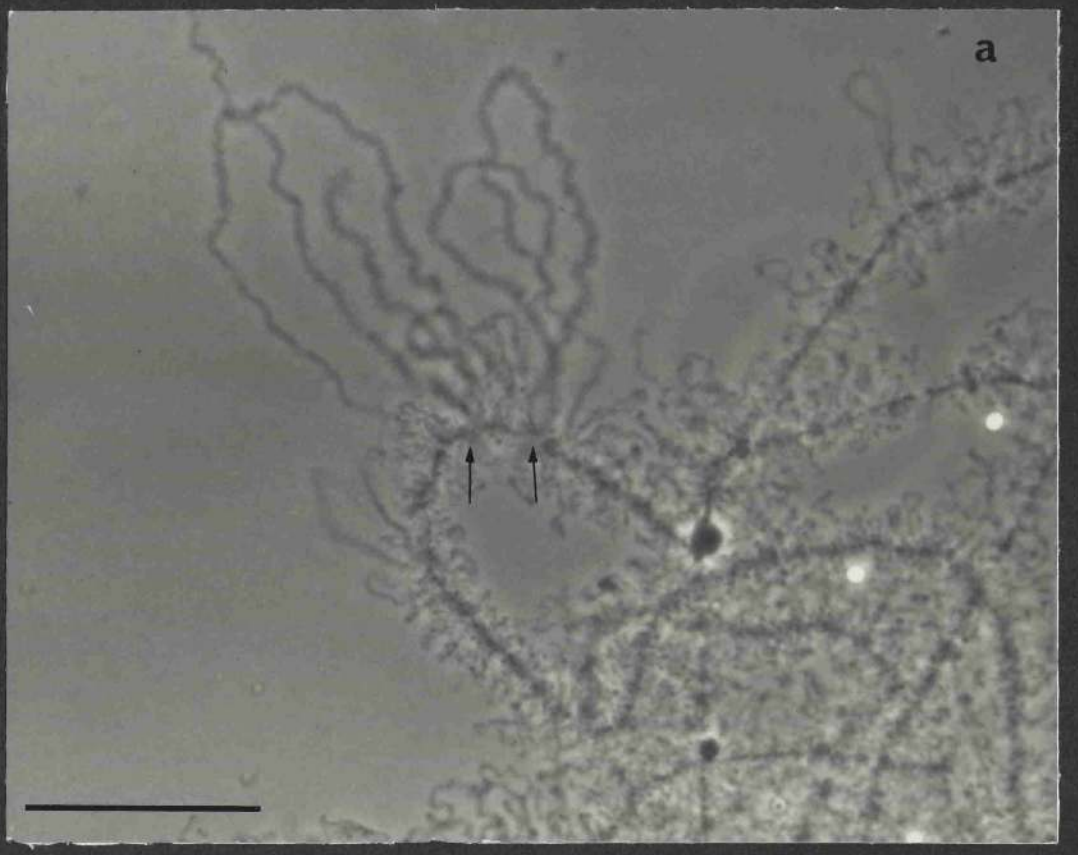
Fig. 6. Phase contrast photographs to show the alternatives for the number of giant loop loci.

a. Two pairs of giant loops whose points of origin are marked by the arrows.

Scale = 50 μm

b. Three pairs of giant loops whose points of origin are marked by the arrows;

Scale = 50 μm.


a

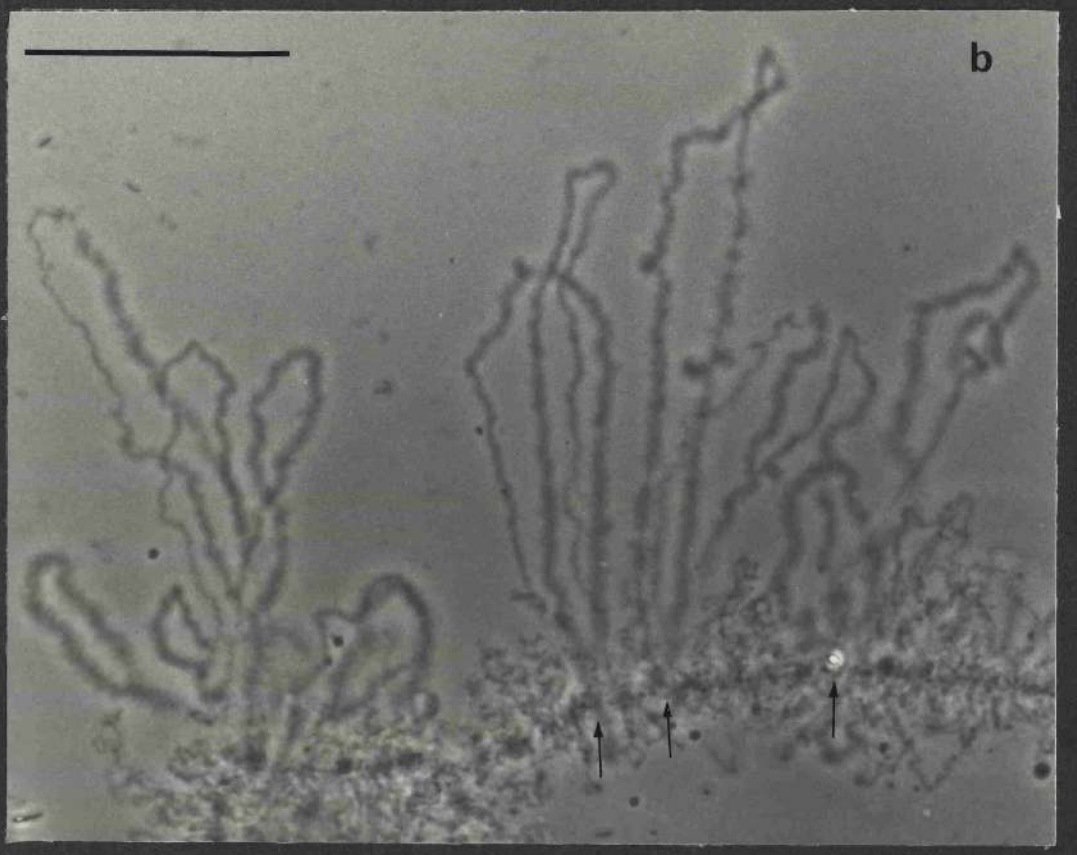
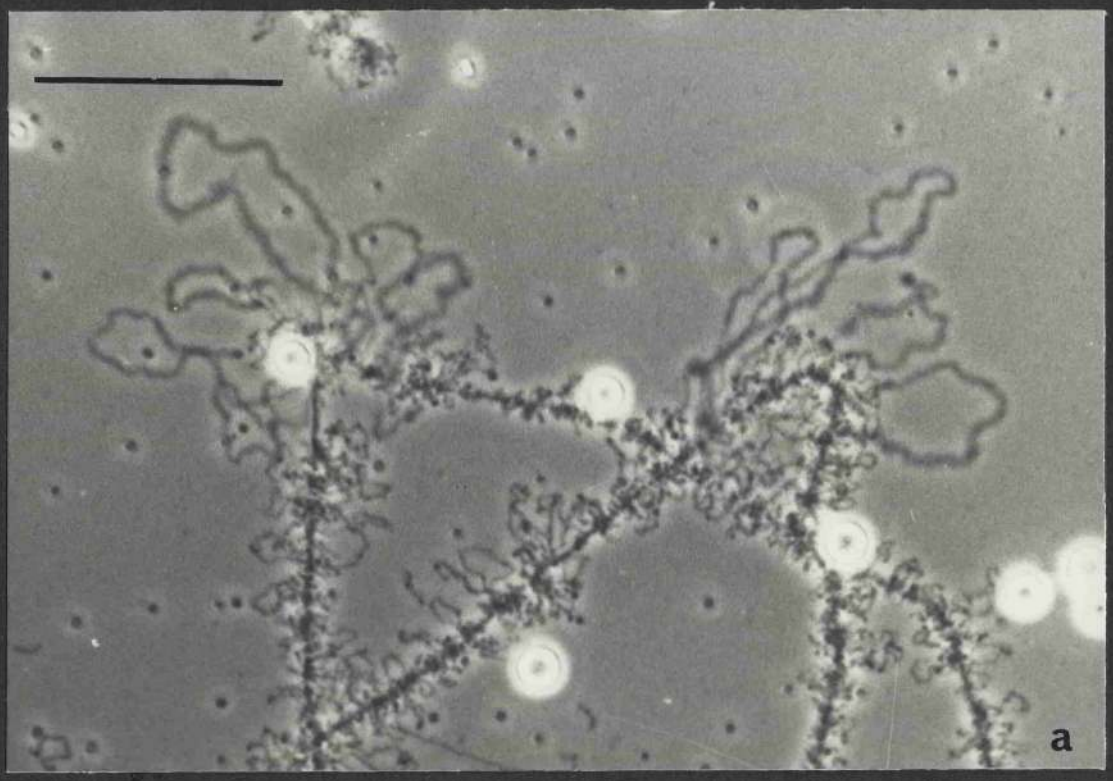

b

Fig. 7. a. Phase contrast photograph of single loop pairs at the giant loop loci.

Scale = 50 μm

b. Phase contrast photograph of multiple loop pairs at the giant loop locus indicated by the arrow.

Scale = 50 μm


a

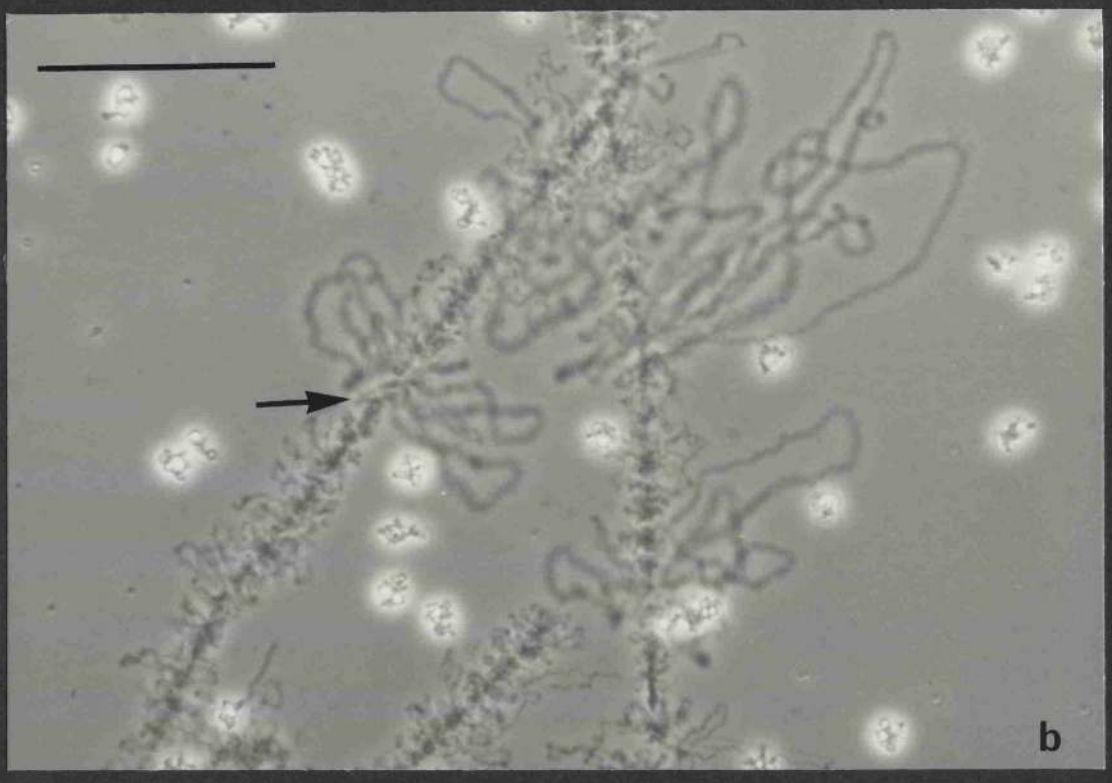

b

Fig. 8. Phase contrast photographs to show the differences in giant loop length.

Scale = 50 μm

a. Long example approximately 300 μm in length. The arrow marks an interruption in the matrix.

b. Short example only 60 - 70 μm in length.

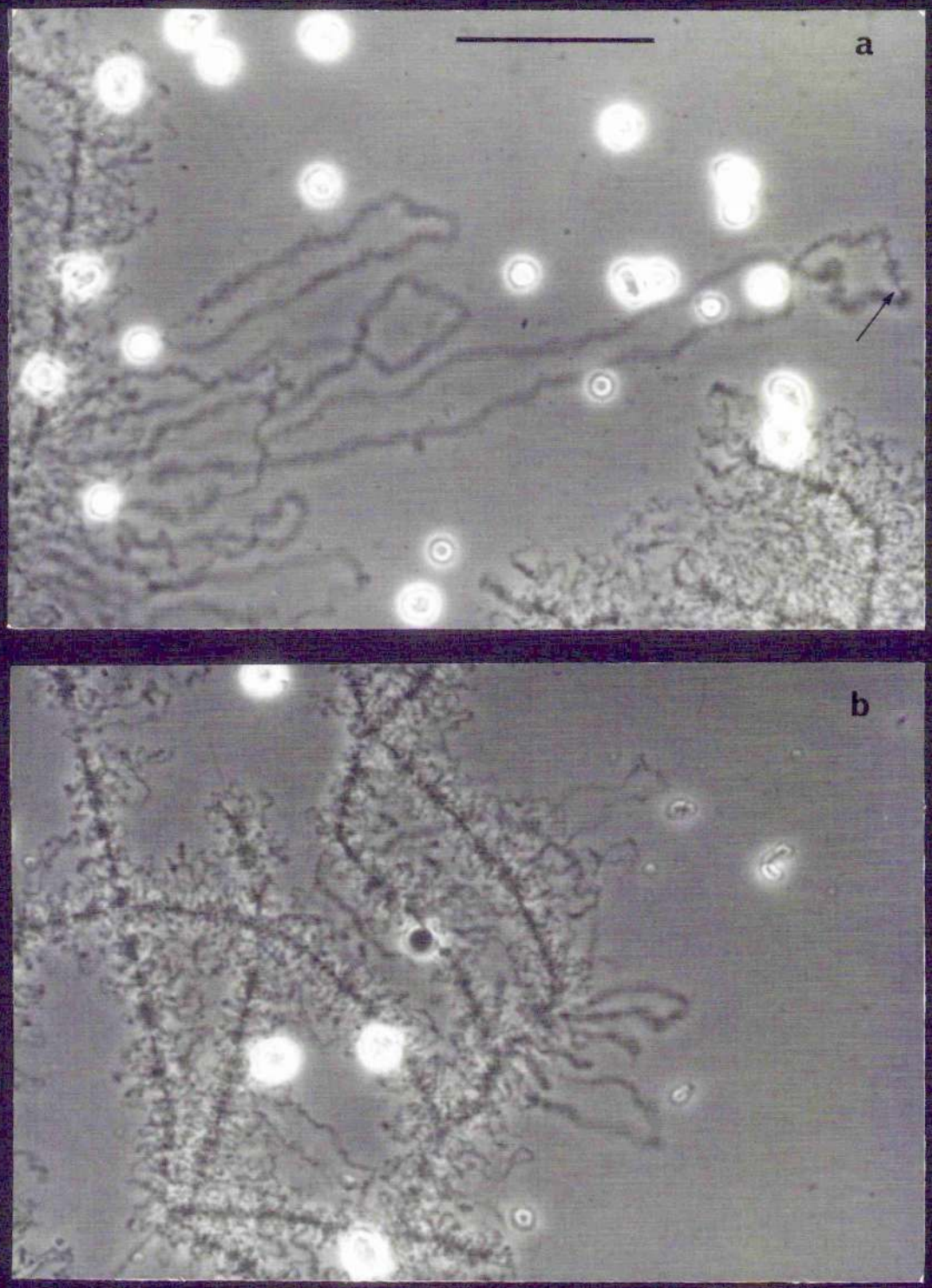

a
b

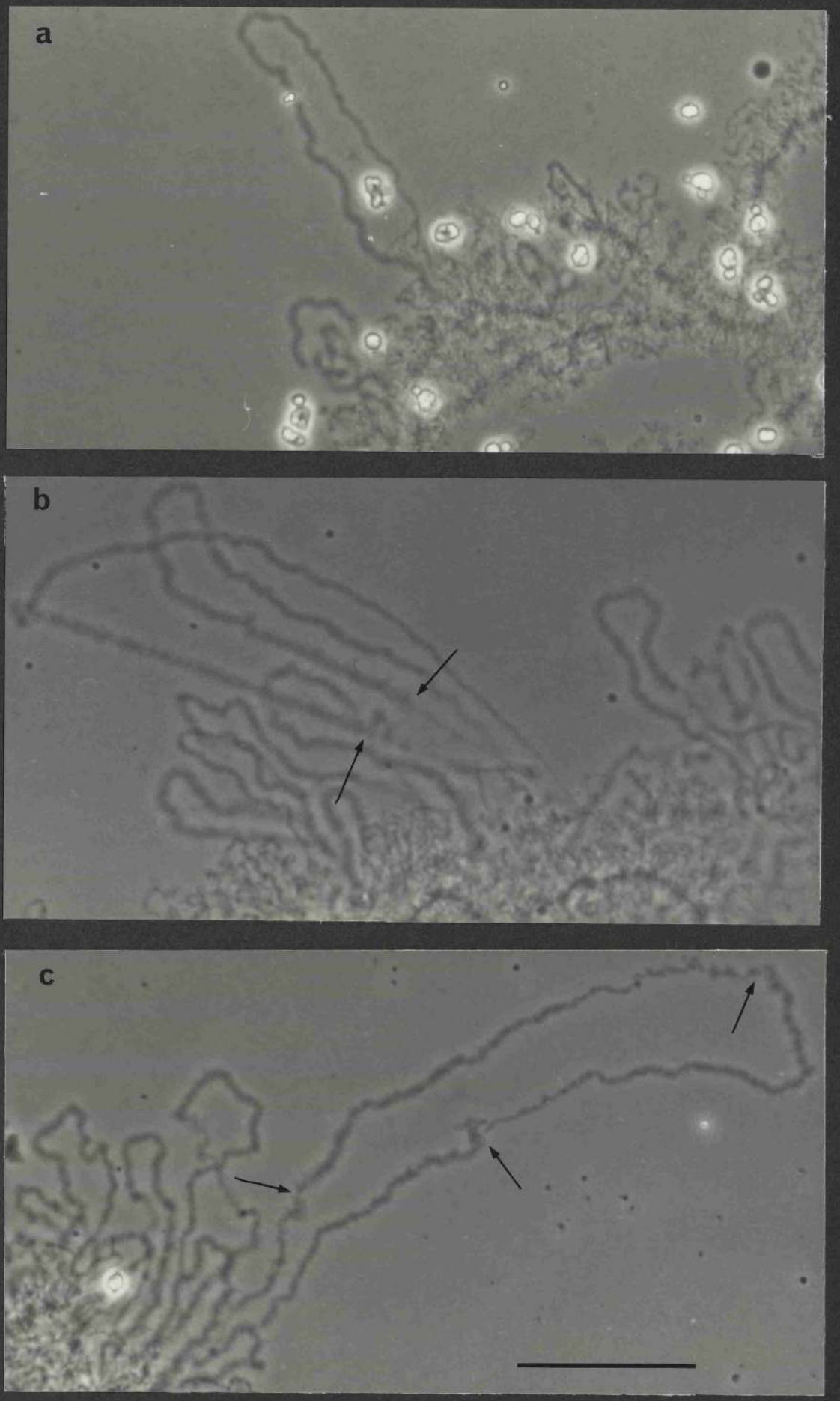
Fig. 9. Phase contrast photographs to show the alternative patterns of matrix distribuion round the giant loops.

Scale = 50 μm

a. Single matrix unit running the entire length of the loop.

b. Single matrix unit which stops before the axis returns to the chromomere (arrowed).

c. Several matrix units in one loop.
Arrows mark the termination points of individual matrix units.


a
b
c

CHAPTER II.

PHYSIOLOGICAL EXPERIMENTS

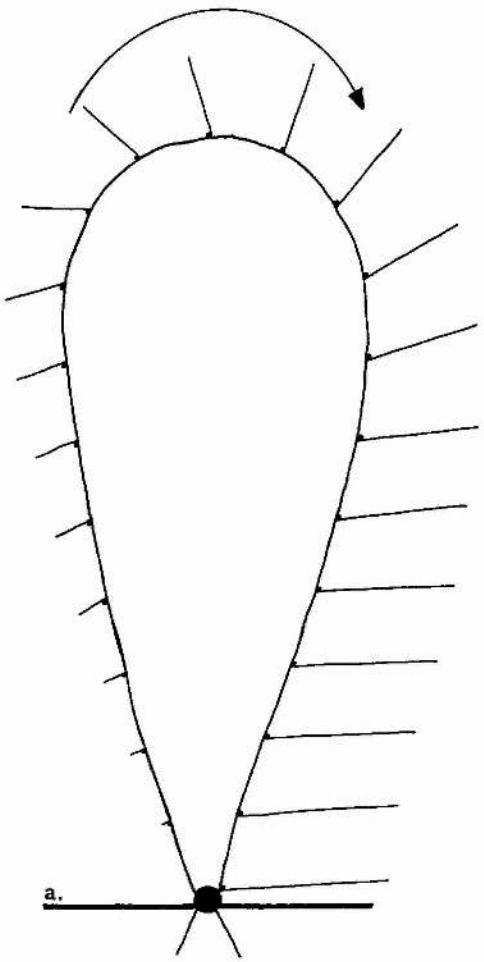
INTRODUCTION

When Callan & Lloyd (1960b) observed the polarized asymmetry of the RNP matrix which is attached along the lengths of the lateral loops of lampbrush chromosomes they proposed a theory to account for this polarization. They proposed (Callan & Lloyd, 1960a) that the thin end of the loop, with little matrix RNP, represented that part of the loop axis DNA which had most recently been extended from the "parent" chromomere, and that the thick end, with much matrix RNP, represented that part which had first extended and which therefore had been engaged longest in RNA synthesis. This model assumes that there are "new" and "old" regions of axis and implies that the amount of matrix at any one point along the axis is directly related to the length of time that that point has been out of the chromomere, synthesizing RNA and accumulating matrix.

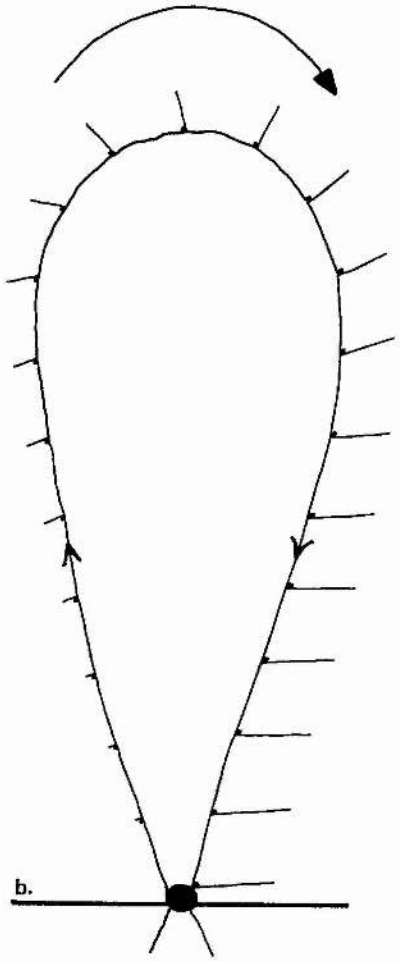
This model took no account of transcriptional movement (the nature of RNA transcription was ill-understood at the time) and is now known to be wrong. However, there are two possible alternative models to account for matrix polarity (fig. 10). One model (fig. 10a) proposes that there is a stationary loop axis and that transcriptional movement alone is responsible for matrix polarity. This model assumes an initiation point for RNA synthesis at the thin end of the loop and then movement of the transcribing RNA molecules together with the protein which makes up the RNP matrix. The alternative model (fig. 10b) proposes that axis and matrix move together. This model assumes that axial and transcriptional movement both contribute to matrix polarity.

Fig. 10. Diagrams to show the two possible alternative explanations for matrix polarity.
The arrows indicate the direction of movement.
A matrix packing factor (Miller & Beatty, 1969) of 10 : 1 has been assumed.

Fig. 10



Transcriptional movement alone responsible for matrix polarity



Transcriptional and axial movement equally responsible for matrix polarity

Snow & Callan (1969) obtained evidence which might be used to support either theory following actinomycin D poisoning of the lateral loops of the lampbrush chromosomes of T.c. cristatus. They found that whilst most of the lateral loops recover their capacity to synthesize RNA in full 2 days after treatment, there are occasional normal loops which do not recover completely and in which the RNA-synthesizing capacity is restricted to a region adjoining the thinner insertion of the loop into the parent chromomere. They also found that during the course of recovery following actinomycin D poisoning the giant granular loop on chromosome XII present at the time of treatment is progressively replaced by a new giant loop lying between the parent chromomere and the original loop's dense tip. The filament that appears between the parent chromomere and the original loop's dense tip may be "new" loop axis, or it may be axis that has been released as a result of matrix moving round a stationary axis. Snow & Callan were reluctant to attribute the polarized asymmetry of loop matrix RNP to transcription alone because of the dimensions of the great majority of lateral loops. Many of the ordinary lateral loops of T.c. cristatus are well over 100 μm long, yet the thickness of their loop matrix at its widest point may be no more than a micron or two. If the length of the transcribed RNA molecules were, to a first approximation, equal to the length of DNA traversed during transcription, Snow & Callan envisaged that a "transcription only" explanation would require there to be much more bulky matrix on ordinary loops than is, in fact, found.

Not all lateral loops possess single polarized matrix units running the length of the loop. T.c. karèlinii bears a pair of identifiable loops on the left arm of chromosome XII that have intercalary portions of their axes bare of matrix (see fig. 24c of Callan & Lloyd, 1960a). In the axolotl, Ambystoma mexicanum, the majority of lateral loops are multipolarized i.e. have more than one thin to thick matrix unit in the loop (Callan, unpublished

observation). There are multipolarized loops close to the left end of chromosome XII in T.c. cristatus (Makarov & Safronov, 1976) and the giant loops on chromosome II of N. viridescens may also show multipolarity (see Chapter I). The interruptions in the matrix are thought to be initiation/termination sites for RNA synthesis corresponding to multiple transcription units in a single loop.

The majority of lateral loops label uniformly throughout their lengths if preparations are made a few hours after administration of tritiated (^{3}H-)uridine but the giant granular loops on chromosome XII of T.c. cristatus show a sequential labelling pattern (Gall & Callan, 1962). Gall & Callan also found that injection of gonadotrophin into the newts increases the rate of travel of labelling in the giant granular loops by a factor of 2. Macgregor (1963) investigated the effects of gonadotrophin injections on the giant fusing loops of chromosomes X, XI and XII of T.c. carnifex. The giant fusing loops are so called because of the tendency for their matrices to fuse together; sister loop fusion is common and interhomologue fusion may occur. Intra-loop fusion of the matrix tends to obscure the underlying ordinary structure of these loops which label uniformly after administration of ^{3}H-uridine. Macgregor found that, depending on their initial size, these loops remained unchanged or decreased in size, the changes being correlated with alteration of the metabolic activity of the oocytes as a result of increasing the hormone level in the animals.

Following on from these observations I conducted experiments to observe the effect of altering the metabolic activity on loop length and on the position of the initiation/termination sites present in multipolarized loops of N. viridescens. Hormone injections were used as a means of increasing, and cold treatment as a means of decreasing metabolic activity. These experiments were used to test whether the variations in the giant loops

described in Chapter I could be attributed to physiological, rather than genetic, causes. Genetic experiments on N. viridescens are impracticable because of the "red eft" stage, a terrestrial phase lasting from $2\frac{1}{2}$ to 4 years, in the life history (Reinke & Chadwick, 1939).

MATERIALS AND METHODS

(a) Hormone Treatment: Lampbrush chromosome preparations were made according to the method described in Chapter I before and after animals received hormone injections. Each animal was given two subcutaneous injections of chorionic gonadotrophin (op. 776; Ciba, Aktiengesellschaft, Basel) two days apart; the first injection being given one or two days after an initial examination of the chromosomes. Concentrations ranged from 10 I.U. in 0.05 ml to 100 I.U. in 0.1 ml of distilled water or newt ringer (0.01 M 3:1 KCl:NaCl, 0.01 M Tris HCl pH 7.8). Samples of oocytes were taken at intervals following the second injection.

Out of 30 experimental animals only 8 survived both injections, some died after the initial operation but more died following the first injection. This was unexpected because T.c. cristatus will survive 2 injections each of 200 I.U. of chorionic gonadotrophin (Gall & Callan, 1962), T. vulgaris will tolerate injections of 100 I.U. (Callan, personal communication) and chorionic gonadotrophin is regularly used to cause ovulation in both these species. Various methods were employed to counter this: some animals were anaesthetized for the injections, others were not; the concentration of hormone was varied as was the solvent used; but with no success. The cause of death is not known.

(b) Cold Treatment: Preparations of lampbrush chromosomes were made according to the method outlined in Chapter I before and after the animals received cold treatment. Animals were given cold treatment by placing them at 5°C in a cold incubator for one week immediately following an initial examination of the chromosomes.

In these experiments again few animals survived the treatment, only 4 out of 13 experimental animals, which may have been due to placing them in the cold before they were completely recovered from the initial operation. However, when the reverse experiment was attempted i.e. placing animals at 5°C for one week and then observing recovery, none of the 4 experimental animals survived the cold shock. This is somewhat surprising as Gall (1966) states that N. viridescens will survive quite well at temperatures between 5° and 10°C, and both he and Dr. O.L. Miller Jr. (personal communication) store their newts at this temperature.

OBSERVATIONS

(a) Hormone Treatment: The effect of hormone injections on loop length is very variable and no constant pattern of response emerges. Table 1 shows the effect of hormone injections on the length of the giant loops in seven animals. Each column represents the loop pair at one locus. One of the animals, H/VI, was heterozygous for the pair of loops whose initial mean length was 172 µm. The loop pairs in newts H/II, H/III, H/IV and H/VI showed little change in length following hormone treatment (fig. 12). The loop pairs examined in H/I, H/V and H/VII show changes in length; steady increases in the cases of those in H/V and H/VII and an initial decrease followed by recovery in H/I (fig. 13). The effect of hormone injections on matrix distribution was more constant. Prior to hormone treatment newts H/I and H/II had interrupted matrix with two polarized matrix units in each

TABLE 1. Effect of hormone injections on loop length

Animal / Time	Mean loop length (μm) of giant loops												
	H/I	H/II	H/III	H/IV			H/V			H/VI		H/VII	
Number of preparations analysed	4	3	5	5	3	4	3	4	2	4	5	3	3
Before injection	241 207–328	190 175–212	112 75–162	64 40–125	75 49–118	86 42–172	101 75–141	118 92–207	113 94–141	172 130–235	193 137–247	122 85–200	61 50–80
2 days after 2nd injection	–	166 131–217	–	–	–	–	173 132–200	192 141–303	211 174–266	130 72–187	219 155–322	172 147–202	183 140–277
4 days after 2nd injection	131 96–191	183 143–222	132 80–245	85 54–115	63 49–87	67 60–75	255 188–365	284 242–313	208 195–221	204 120–285	178 135–220	–	–
7 days after 2nd injection	230 125–312	–	–	–	–	–	–	–	–	–	–	–	–
10 days after 2nd injection	196 147–282	–	–	–	–	–	–	–	–	–	–	–	–
Injection dosage	2 x 100 IU in 0.1 ml distilled water			2 x 100 IU in 0.1 ml distilled water			2 x 100 IU in 0.1 ml distilled water			2 x 10 IU in 0.05 ml newt ringer		2 x 20 IU in 0.1 ml newt ringer	

Each column of figures represents one loop pair. The lower hyphenated figures are the lower and upper limits of the range of size variation shown by the loops measured and the upper figure the mean.

loop of the pair of giant loops examined. The effect of hormone treatment on matrix distribution can be seen in Table 2 and fig. 11. In both animals the length of the first matrix unit occupies a greater proportion of the loop length following hormone injections. In the case of H/I (figs 11a and 13) the first matrix unit occupies 20% more of the loop length than originally, although the actual loop length has been halved (fig. 13b), four days after the second injection. Seven days after the second in ection the loop has recovered to its original length but the first matrix unit occupies 60% of the loop (fig. 13c) as compared to 43% before injection. At ten days after the second injection 55% of the loop is occupied by the first matrix unit (fig. 13d). In newt H/II the proportion of the loop occupied by the first matrix unit alters significantly from 51% before injection to 95% two days after the second injection and 65% four days following injection but there was no accompanying change in overall loop length (fig. 11b).

(b) Cold Treatment: Four animals survived the cold treatment that they were given. Before the treatment the giant loops on chromosome II were long, approximately 200 μm, and the normal loops were approximately 20 μm in length (fig. 14a). After one week in the cold incubator at 5°C there was no significant change in the appearance and length of the giant loops but the ordinary loops appeared much shorter (fig. 14b). The chromosomes, in general, had the appearance of those from mature oocytes in which the loops are beginning to retract into the chromomeres and the chromosome to condense, when in fact, the preparations were made from oocytes in the 0.60 - 0.80 mm diameter range in which the chromosomes and lateral loops are normally fully extended.

TABLE 2. Effect of hormone injection on loop length and matrix distribution

	Mean loop length (µm)		Mean length of 1st matrix segment (µm)		1st segment as % of total loop length	
	H/I	H/II	H/I	H/II	H/I	H/II
Before injection	241	190	104	97	43	51
2 days after injection	-	166	-	158	-	95
4 days after 2nd injection	131	183	84	119	64	65
7 days after 2nd injection	230	-	137	-	60	-
10 days after 2nd injection	196	-	107	-	55	-

In the case of H/I 4 preparations were analysed and for H/II 3 preparations were analysed. The figures for mean loop length in columns 1 and 2 are taken from Table 1.

Fig. 11. Diagrams to show the effect of hormone injections on loop length and matrix distribution.

Arrows indicate the extent of the 1st matrix unit.

a. Newt H/I

(i) The 1st matrix unit occupied 43% of the total loop length prior to injection.

(ii) 4 days after the 2nd injection, the loop length had been halved and the 1st matrix unit occupied 64% of the total loop length.

(iii) 7 days after the 2nd injection, the loop length had returned to its original value but 60% of the loop was occupied by the 1st matrix unit.

(iv) 10 days after the 2nd injection there was little change in loop length and the 1st matrix unit occupied 55% of the loop.

b. Newt H/II

(i) Prior to injection the 1st matrix unit occupied 51% of the total loop length.

(ii) 2 days after the 2nd injection there had been no significant change in loop length but the 1st matrix unit occupied 95% of the loop.

(iii) 4 days after the 2nd injection there had been no change in loop length and the 1st matrix unit occupied 65% of the total loop length.

Fig. 11

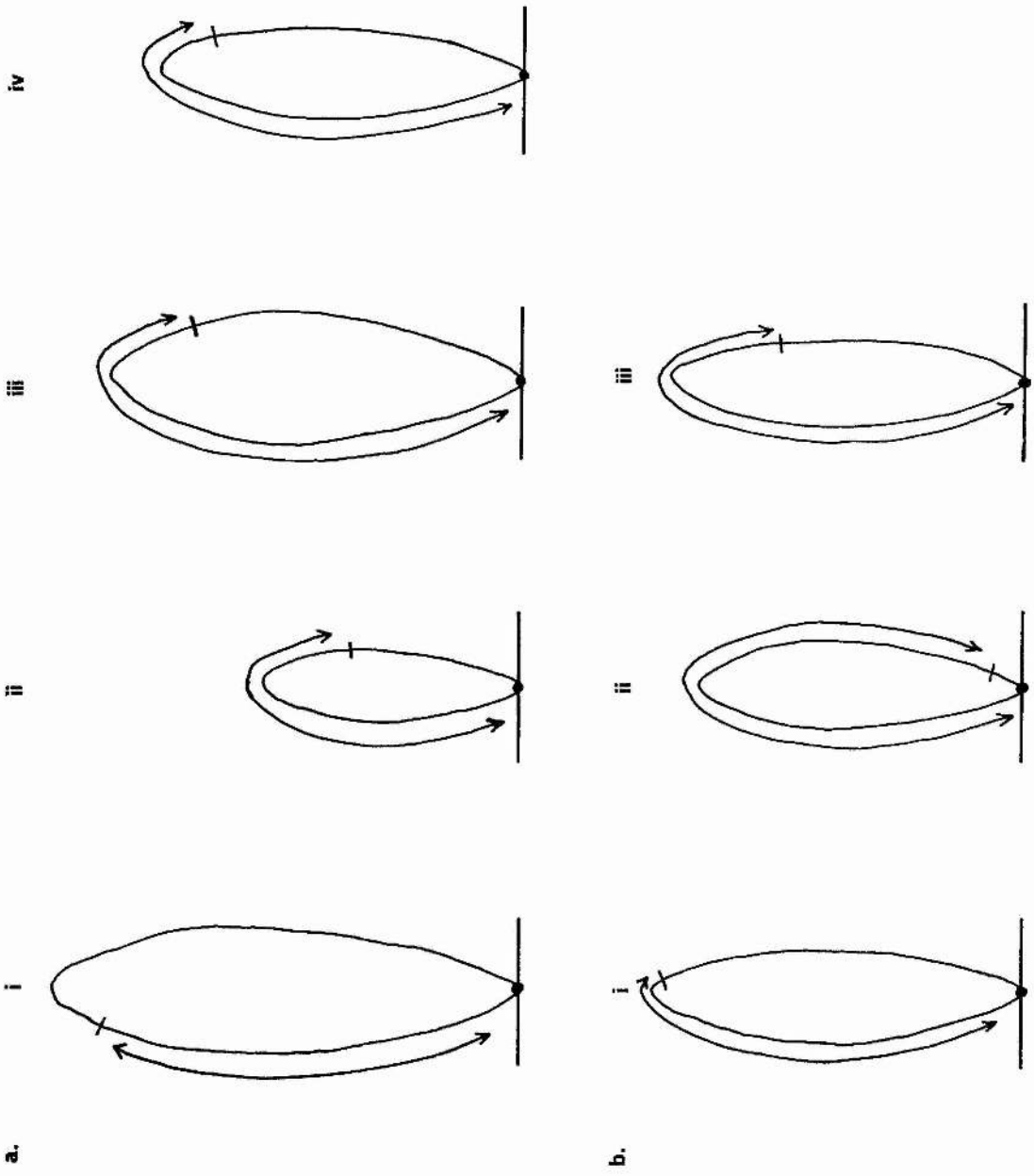


Fig. 13. Phase contrast photographs to show the effect of hormone injections on loop length and matrix distribution in newt H/I.

The same pair of giant loops is shown before injection (a) and at intervals following the 2nd hormone injection (b) 4 days, (c) 7 days, (d) 10 days.

The arrowheads mark the termination point of the 1st matrix unit.

Scale = 50 μm

Fig. 14. Phase contrast photographs to show the effect of cold treatment on loop length.

Scale = 50 μm

a. Giant loops on chromosome II and surrounding ordinary loops before cold treatment.

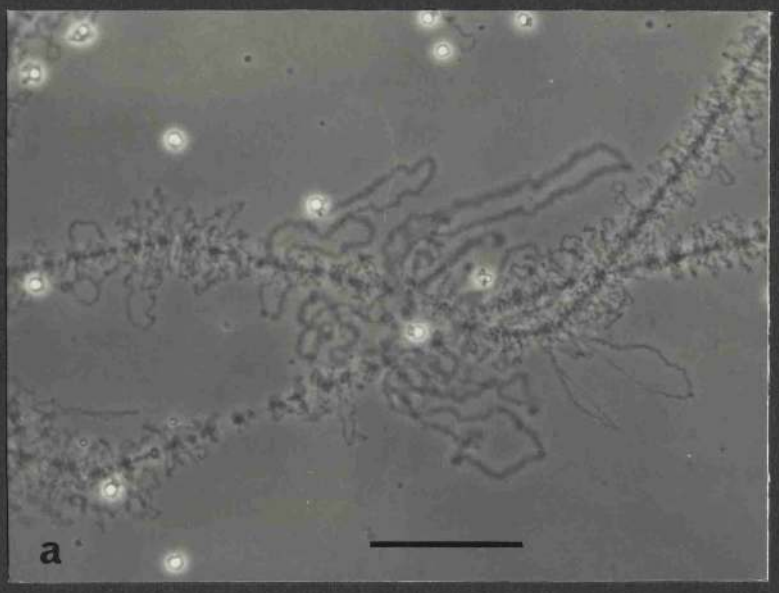
b. Giant loops on chromosome II and surrounding ordinary loops after one week at 5°C. There has been little change in the length and appearance of the giant loops but the ordinary loops have shortened considerably.

Fig. 13. Phase contrast photographs to show the effect of hormone injections on loop length and matrix distribution in newt H/I.

The same pair of giant loops is shown before injection (a) and at intervals following the 2nd hormone injection (b) 4 days, (c) 7 days, (d) 10 days.

The arrowheads mark the termination point of the 1st matrix unit.

Scale = 50 μm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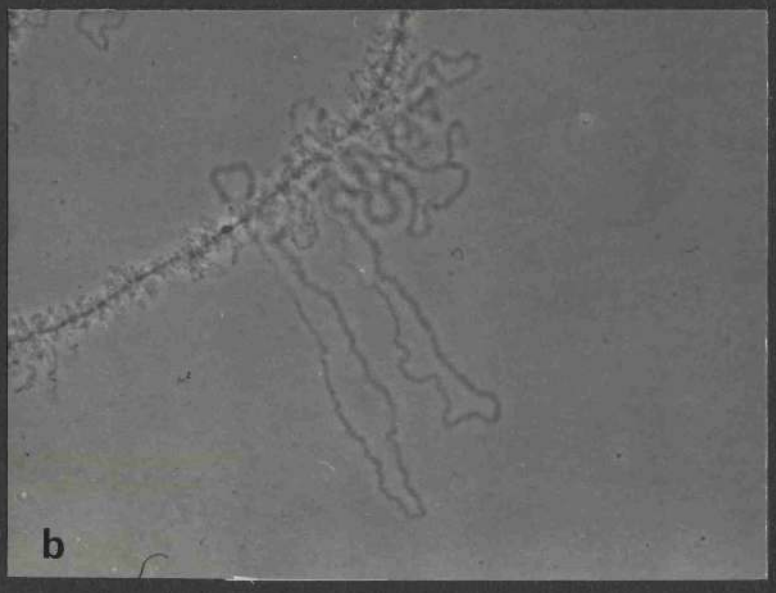
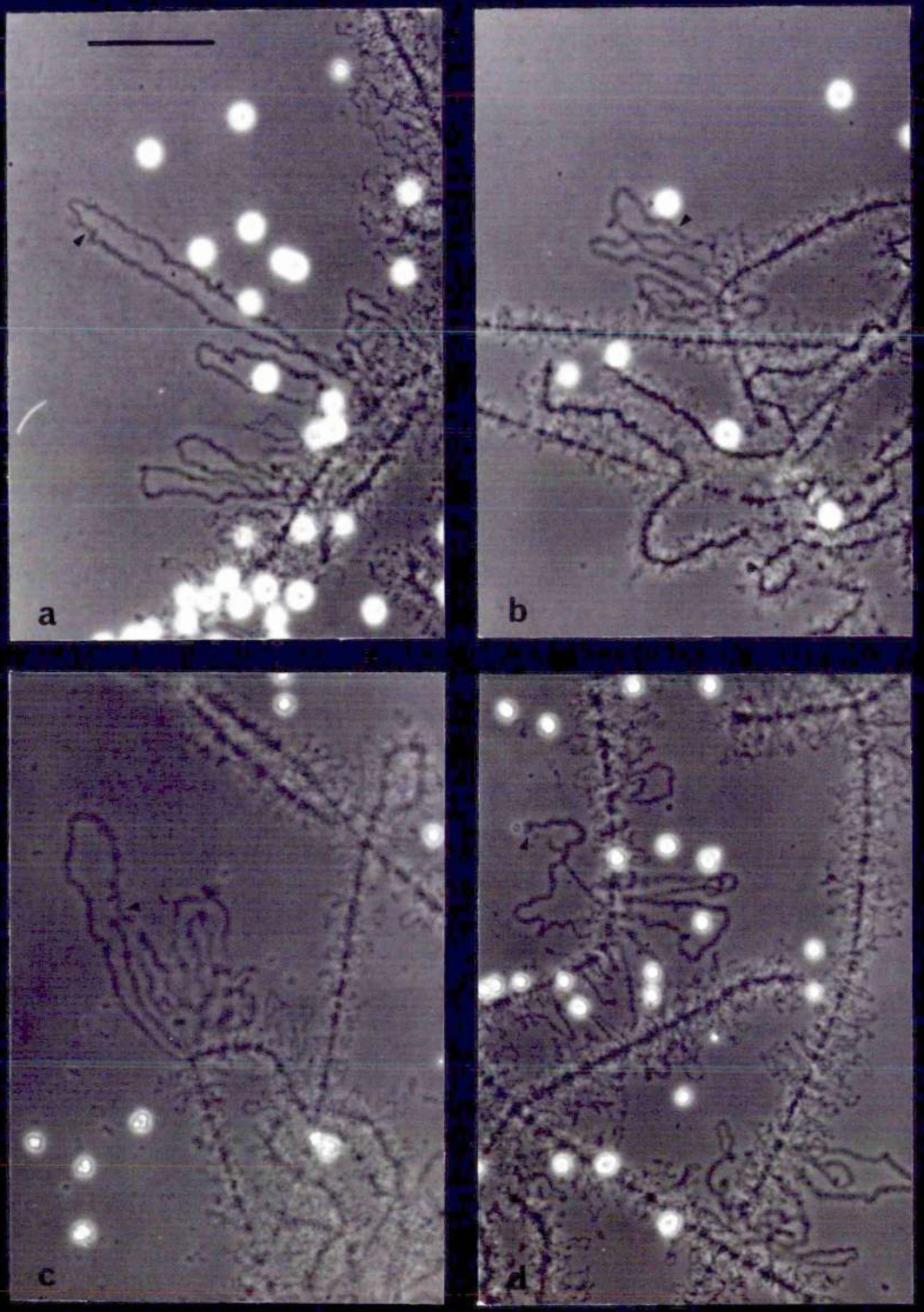

b

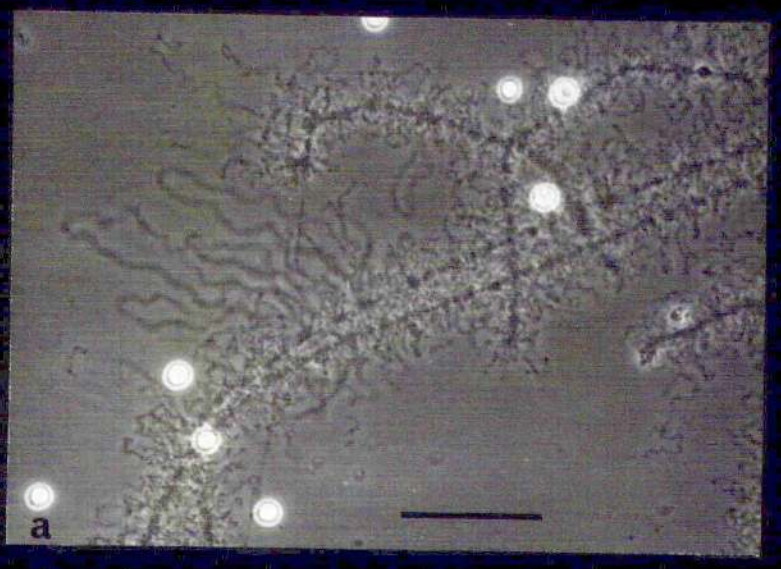
Fig. 14. Phase contrast photographs to show the effect of cold treatment on loop length.

Scale = 50 μm

a. Giant loops on chromosome II and surrounding ordinary loops before cold treatment.

b. Giant loops on chromosome II and surrounding ordinary loops after one week at 5°C. There has been little change in the length and appearance of the giant loops but the ordinary loops have shortened considerably.


a
b
c
d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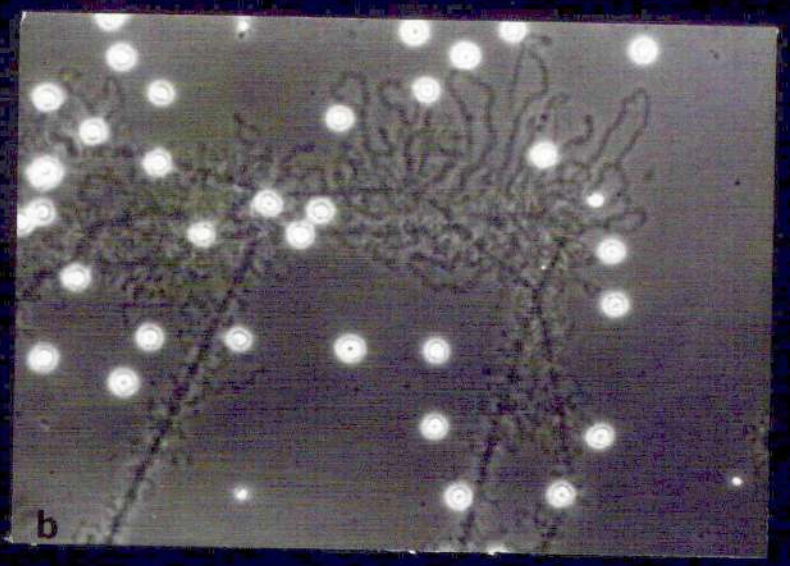

b

C H A P T E R III.

RNA SYNTHESIS

INTRODUCTION

The transcription of RNA by the lateral loops of amphibian lampbrush chromosomes is well established following the early observations of several groups of workers (Gall, 1958, 1963; Gall & Callan, 1962; Izawa, Allfrey & Mirsky, 1963).

Whilst the majority of lampbrush loops are uniformly labelled along their whole length an hour or two after the administration of radioactive isotope in the form of one of the precursors of RNA, there are some lampbrush loops which show different labelling patterns. Gall & Callan (1962) observed that the giant granular loops near the left end of chromosome XII in T.c. cristatus show a sequential labelling pattern following the administration of ^{3}H-uridine by injection. A few hours after the injection of ^{3}H-uridine only a short region near the thin insertion was labelled, after two days the giant granular loops were labelled along 1/5 of their length. Four days after injection ½ the length of the loops was labelled, after 6 days 2/3 of the length was labelled. Fourteen days after injection labelling was complete. Makarov & Safronov (1974) have found the giant granular loops on chromosome XII of T.c. cristatus labelled along 2/3 of their length two days after injection of ^{3}H-uridine and completely labelled 5 days after injection. They found, (Safronov & Makarov, 1975) as did Gall & Callan, that injection of chorionic gonadotrophin increased the rate of movement of labelled RNA by a factor of 2. Evidently the same species of newt can show different rates of movement of RNA in different individuals, which may be due to intrinsic differences in their physiological state. Nardi et al. (1972) found a similar sequential pattern of labelling for one of the pairs of giant fusing loops on chromosome X in Triturus marmoratus. They also

found that the fibrillar loops on chromosome X have a low uptake of ^{3}H-uridine when compared to the rest of the loops. In N. viridescens the sequential loops at the left end of chromosome XI are so called because of their labelling pattern with ^{3}H-uridine; they behave just like the giant granular loop of T.c. cristatus (Gall, 1963b). In addition Gall (unpublished observations) has noticed that the rate of incorporation of ^{3}H-uridine into the giant loops on chromosome II is considerably lower than the rate of incorporation into the generality of the much smaller loops on the chromosomes.

In view of this observation of Gall's and my own observation that the giant loops show a lower response to cold treatment than the ordinary loops (see Chapter II), I undertook experiments to study the incorporation patterns of all four precursors of RNA into the RNA transcribed by the giant loops on chromosome II of N. viridescens.

MATERIALS AND METHODS

Administration of isotope: Oocytes were incubated in vitro with the radioactive precursors of RNA separately. ^{3}H-adenine (No. 408130B, 23 Ci/mM), ^{3}H-cytidine (No. 401571, 27.3 Ci/mM), ^{3}H-guanosine (no. 380302, 19 Ci/mM) and ^{3}H-uridine (No. 345471, 25 Ci/mM) were obtained from the Radiochemical Centre, Amersham. For the experiments 50 µCi of each precursor was put into separate embryo cups. These were allowed to dry down overnight and then half an ovary, with a little coelomic fluid, placed in each so that oocytes, all from one animal, could be compared directly in their capacity to incorporate the separate precursors. The embryo cup was sealed and incubation proceeded for 4 hours at room temperature before the embryo cup was placed on ice. At intervals during incubation the ovary was twirled round in an attempt to ensure uniform precursor accessibility to all the oocytes.

Centrifuged chromosome preparations: Lampbrush chromosomes were isolated, as described in Chapter I, into a 25 mm diameter ring chamber suitable for centrifugation. These chambers were made by cutting and grinding a 3" by 1" bored slide and then sealing a 22 mm circular coverslip across the hole in the centre of the ring with low melting point paraffin wax. After isolation, the chromosome preparation was covered with a 22 mm diameter coverslip. The ring chambers were placed in polypropylene centrifuge tubes containing flat-topped Araldite plugs, and were spun at 3,000 rpm (1055 g) for 5 minutes in a Servall bench centrifuge fitted with a swinging head rotor. After centrifugation the lateral loops of the lampbrush chromosomes are flat and anchored to the floor of the chamber.

Autoradiography: Centrifuged preparations of lampbrush chromosomes were made and fixed in a 4% solution of commercial formalin for 15 minutes (they may be left overnight at this stage if necessary). The coverslip, with attached preparation, was removed from the ring chamber, washed in running filtered tap water to remove the formalin, placed for 5 minutes in freshly prepared ice-cold 5% trichloroacetic acid for extraction of any unincorporated precursors, again washed in running filtered tap water, followed by distilled water. The preparations were then taken through an alcohol series to two changes of xylene to remove the wax from the coverslips, two changes of acetone to remove the xylene and air-dried. The coverslips were attached to 3" by 1" microscope slides with the minimum amount of Canada Balsam necessary, with the chromosome preparation uppermost and exposed.

As controls, several slides were digested with ribonuclease (RNase). The slides were placed in a solution of RNase-A from Bovine pancreas, obtained from the Sigma Chemical Company, in phosphate buffer at pH 6.0, at a concentration of 0.1 mg/ml (phosphate buffer : 4.5 ml $^{M}/15$ Na_2HPO_4,

25.5 ml $^{M}/_{15}$ KH_2PO_4, 170 ml distilled water). They were left for 1 hour at 40°C, then washed in distilled water and air-dried.

All the preparations were filmed in a darkroom with Kodak NTB-2 dipping emulsion diluted with distilled water to half its original strength. After drying in a stream of air, the preparations were placed in light-tight boxes sealed with adhesive tape, and stored in a refrigerator during exposure. The autoradiographs were developed for 2½ minutes in freshly prepared Kodak D-19 at 20°C, washed in distilled water, fixed in Kodak Unifix, washed in filtered tap water followed by distilled water and air-dried. Study of the autoradiographs was made by placing a drop of distilled water and then a coverslip over the preparations and observing them under phase contrast optics using a Zeiss standard W-L microscope.

For photography the preparations were stained in 0.0375% toluidine blue for 45 minutes and photographed through an Ilford 404 green filter and a green Zeiss interference filter using a x 40 apochromatic oil immersion objective and bright field optics. Immersion oil placed directly on the preparation can be removed by petroleum ether followed by acetone and air-drying.

Analysis of Autoradiographs: Oocytes from five animals were allowed to incorporate the individual precursors. Test slides were developed at intervals to ascertain appropriate exposure times, which varied from 7 - 17 days in the cases of adenine, cytidine and uridine; to achieve a comparable level of labelling for guanosine it was necessary to expose the slides for between 12 and 26 weeks.

The developed autoradiographs were analysed in the following way. Camera lucida drawings, using a Reichert camera lucida, were made of the giant loops on chromosome II and a number of unidentified ordinary loops

which happened to lie conveniently such that the total length of the ordinary loops which were drawn exceeded that of the giant loops in each preparation analysed. The number of silver grains was counted over the drawn loops. The average width of the giant loops was found to be 1.65 μm and that of the ordinary loops 0.83 μm. The correction for background was made by multiplying the assumed area of the loops by the background correction factor to calculate the number of grains over the loops which could be attributed to background. This figure was then subtracted from the original grain count to give the corrected grain count. The background correction factor was derived by counting the number of grains in six different areas on each slide, selected at random; each area measured 900 μm^2. Grains were counted within the area and lying on one vertical and one horizontal line bordering each area, the same lines being used for each area counted. The number of grains per μm^2 was calculated for each preparation and this was the background correction factor. The corrected grain count was then divided by the measured loop length to derive the number of grains per μm. A ratio of grains per μm for giant loops to ordinary loops was then calculated.

OBSERVATIONS

Fig. 15 shows the incorporation of the four precursors into the RNA transcribed by the giant loops. All four precursors are incorporated at a much lower level into the giant loops than into the ordinary loops. Tables 3, 4, 5 and 6 give analyses of the data and compare the level of labelling in the giant loops with the level of labelling in the ordinary loops within each preparation. The ratios for giant loops to ordinary loops thus derived confirm the photographic evidence in fig. 15 that the level of labelling is lower in the giant loops than it is in the ordinary loops. In the cases of adenine (fig. 15a, Table 3) and uridine (fig. 15d, Table 6) the ordinary loops incorporate twice as much label as the giant loops; for cytidine

TABLE 3. Incorporation of ^{3}H-adenine into the giant loops on chromosome II and the ordinary loops of *N. viridescens*

Female	Oocyte	Type of loop	Total length drawn (μ)	Total grain count	Assumed loop area (μ^2)
1 (Exposed 17 days)	1	GL	815	335	1353
		Ordinary	769	605	638
	2	GL	804	266	1334
		Ordinary	875	559	726
	3	GL	344	130	572
		Ordinary	1029	562	854
	4	GL	735	227	1219
		Ordinary	1000	607	830
2 (Exposed 14 days)	1	GL	921	289	1529
		Ordinary	835	568	693
	2	GL	414	273	688
		Ordinary	693	853	575
	3	GL	738	556	1225
		Ordinary	993	962	824
	4	GL	316	196	526
		Ordinary	773	659	642
	5	GL	578	390	960
		Ordinary	957	700	794
3 (Exposed 14 days)	1	GL	461	245	766
		Ordinary	1213	730	1006
	2	GL	367	206	609
		Ordinary	1136	946	943
	3	GL	462	222	767
		Ordinary	1314	1201	1090
	4	GL	365	153	607
		Ordinary	614	540	509
4 (Exposed 10 days)	1	GL	822	73	1364
		Ordinary	901	159	748
	2	GL	857	192	1422
		Ordinary	941	444	781
	3	GL	861	103	1429
		Ordinary	762	208	632
	4	GL	851	62	1413
		Ordinary	850	139	705
5 (Exposed 7 days)	1	GL	547	44	909
		Ordinary	906	201	752
	2	GL	803	396	1333
		Ordinary	709	641	588
	3	GL	928	370	1541
		Ordinary	918	505	762
	4	GL	901	132	1496
		Ordinary	747	170	620
	5	GL	592	64	982
		Ordinary	820	314	681
	6	GL	675	221	1120
		Ordinary	845	494	702
	7	GL	368	83	612
		Ordinary	831	315	690
	8	GL	351	43	583
		Ordinary	717	229	595

Background correction factor (grains/μ^2)	Applied background correction	Corrected grain count	Level of labelling (grains/μ)	Relative level of labelling (GLs/Ordinary)
0.02	27	308	0.38	0.49
0.02	13	592	0.77	
0.02	27	239	0.30	0.48
0.02	14	545	0.62	
0.02	11	119	0.34	0.64
0.02	17	545	0.53	
0.02	24	203	0.28	0.47
0.02	17	590	0.59	
0.03	45	244	0.26	0.40
0.03	21	547	0.65	
0.06	41	232	0.56	0.47
0.06	34	819	1.18	
0.09	110	446	0.60	0.67
0.09	74	888	0.89	
0.05	26	170	0.54	0.67
0.05	32	627	0.81	
0.03	29	361	0.62	0.87
0.03	24	676	0.71	
0.02	15	230	0.50	0.85
0.02	20	710	0.59	
0.02	12	194	0.53	0.65
0.02	19	927	0.82	
0.06	46	176	0.38	0.44
0.06	65	1136	0.86	
0.03	18	135	0.37	0.43
0.03	15	525	0.85	
0.01	13	60	0.073	0.43
0.01	7	152	0.17	
0.02	28	164	0.19	0.42
0.02	16	428	0.45	
0.02	29	74	0.086	0.34
0.02	13	195	0.25	
0.01	14	48	0.056	0.37
0.01	7	132	0.15	
0.01	9	35	0.064	0.30
0.01	8	193	0.21	
0.02	27	369	0.46	0.52
0.02	12	629	0.89	
0.02	31	339	0.36	0.68
0.02	15	490	0.53	
0.01	15	117	0.13	0.59
0.01	6	164	0.22	
0.01	10	54	0.091	0.25
0.01	7	307	0.37	
0.02	22	199	0.29	0.51
0.02	14	480	0.57	
0.01	6	77	0.21	0.57
0.01	7	308	0.37	
0.01	6	37	0.10	0.32
0.01	6	223	0.31	

Mean relative level of labelling 0.51 (± 0.10 SEM)

TABLE 4. Incorporation of ^{3}H-cytidine into the giant loops on chromosome II and the ordinary loops of *N. viridescens*

Female	Oocyte	Type of loop	Total length drawn (μ)	Total grain count	Assumed loop area (μ^2)
1 (Exposed 17 days)	1	GL	270	114	449
		Ordinary	883	423	731
	2	GL	631	455	1047
		Ordinary	1241	825	1030
	3	GL	511	375	849
		Ordinary	1184	869	983
	4	GL	838	431	1392
		Ordinary	1065	536	884
	5	GL	922	410	1530
		Ordinary	674	362	559
	6	GL	569	290	945
		Ordinary	1061	665	881
	7	GL	463	206	768
		Ordinary	711	369	590
	8	GL	538	247	893
		Ordinary	893	462	741
2 (Exposed 14 days)	1	GL	593	439	985
		Ordinary	842	731	699
	2	GL	619	506	1028
		Ordinary	751	680	624
	3	GL	891	663	1479
		Ordinary	948	704	787
	4	GL	413	363	686
		Ordinary	833	768	691
	5	GL	652	307	1082
		Ordinary	822	419	682
	6	GL	393	395	652
		Ordinary	689	858	572
3 (Exposed 14 days)	1	GL	407	327	676
		Ordinary	943	909	782
	2	GL	486	364	806
		Ordinary	975	802	809
	3	GL	390	257	647
		Ordinary	786	614	653
	4	GL	514	387	853
		Ordinary	803	713	667
	5	GL	528	367	877
		Ordinary	1175	902	975
	6	GL	403	244	670
		Ordinary	1034	750	859
4 (Exposed 10 days)	1	GL	220	241	365
		Ordinary	555	592	461
	2	GL	496	301	824
		Ordinary	615	409	510
	3	GL	291	333	483
		Ordinary	444	493	369
	4	GL	384	304	605
		Ordinary	660	772	548
5 (Exposed 7 days)	1	GL	579	632	961
		Ordinary	711	1119	590
	2	GL	400	378	664
		Ordinary	667	622	554
	3	GL	615	406	1022
		Ordinary	894	656	742
	4	GL	344	446	571
		Ordinary	760	1073	631
	5	GL	461	237	765
		Ordinary	847	521	703
	6	GL	350	240	581
		Ordinary	742	516	616
	7	GL	376	235	624
		Ordinary	791	527	657

Background correction factor (grains/μ^2)	Applied background correction	Corrected grain count	Level of labelling (grains/μ)	Relative level of labelling (GLs/Ordinary)
0.01	4	110	0.41	
0.01	7	416	0.47	0.87
0.01	10	445	0.71	
0.01	10	815	0.66	1.08
0.02	17	358	0.70	
0.02	20	849	0.72	0.97
0.03	42	389	0.46	
0.03	27	509	0.48	0.96
0.03	46	364	0.39	
0.03	17	345	0.51	0.76
0.01	9	281	0.49	
0.01	9	656	0.62	0.79
0.02	15	191	0.41	
0.02	12	357	0.50	0.82
0.02	18	229	0.43	0.86
0.02	15	447	0.50	
0.03	30	409	0.69	
0.03	21	710	0.84	0.82
0.04	41	467	0.75	
0.04	25	655	0.87	0.86
0.03	44	619	0.69	
0.03	24	680	0.72	0.96
0.03	21	362	0.88	
0.03	21	747	0.90	0.98
0.01	11	296	0.45	
0.01	7	412	0.50	0.90
0.04	26	369	0.94	
0.04	23	835	1.21	0.78
0.02	13	314	0.77	
0.02	16	893	0.95	0.81
0.03	24	340	0.70	
0.03	24	778	0.80	0.88
0.02	13	244	0.63	
0.02	13	601	0.76	0.83
0.03	26	361	0.70	
0.03	20	693	0.86	0.81
0.03	26	341	0.65	
0.03	29	873	0.74	0.88
0.01	7	257	0.59	
0.01	9	729	0.72	0.82
0.05	18	225	1.01	
0.05	23	569	1.02	0.99
0.02	16	285	0.57	
0.02	10	399	0.65	0.88
0.07	34	299	1.03	
0.07	26	467	1.05	0.98
0.02	12	292	0.80	
0.02	11	761	1.15	0.70
0.02	19	615	1.06	
0.02	12	1107	1.56	0.68
0.01	7	371	0.93	
0.01	6	616	0.92	1.01
0.01	10	398	0.65	
0.01	7	649	0.73	0.89
0.01	6	440	1.28	
0.01	6	1067	1.40	0.91
0.01	8	229	0.59	
0.01	7	514	0.61	0.97
0.01	6	234	0.67	
0.01	6	510	0.69	0.97
0.01	6	229	0.61	
0.01	7	520	0.66	0.92
Mean relative level of labelling				0.88 (± 0.08 SEM)

TABLE 5. Incorporation of ^{3}H-guanosine into the giant loops on chromosome II and the ordinary loops of *N. viridescens*

Female	Oocyte	Type of loop	Total length drawn (μ)	Total grain count	Assumed loop area (μ^2)
1 (Exposed 26 wks)	1	GL	793	83	1316
		Ordinary	1079	303	896
	2	GL	307	30	509
		Ordinary	721	221	598
	3	GL	443	36	736
		Ordinary	630	216	533
	4	GL	253	55	420
		Ordinary	706	255	586
	5	GL	537	61	891
		Ordinary	703	246	584
2 (Exposed 26 wks)	1	GL	764	108	1268
		Ordinary	1085	526	901
	2	GL	976	207	1620
		Ordinary	1272	660	1056
3 (Exposed 25 wks)	1	GL	234	23	388
		Ordinary	412	178	342
	2	GL	302	62	502
		Ordinary	682	479	566
	3	GL	289	27	481
		Ordinary	783	283	650
	4	GL	318	33	529
		Ordinary	544	155	452
	5	GL	657	86	1091
		Ordinary	850	494	705
4 (Exposed 12 wks)	1	GL	549	38	912
		Ordinary	599	116	498
	2	GL	626	83	1039
		Ordinary	460	196	382
	3	GL	544	19	903
		Ordinary	596	45	495
	4	GL	697	51	1158
		Ordinary	584	182	485
	5	GL	164	19	273
		Ordinary	391	117	324
5 (Exposed 12 wks)	1	GL	326	15	540
		Ordinary	889	106	738
	2	GL	618	27	1026
		Ordinary	729	68	605
	3	GL	738	33	1225
		Ordinary	744	93	618
	4	GL	442	23	735
		Ordinary	889	141	738
	5	GL	439	16	730
		Ordinary	786	82	652
	6	GL	525	17	871
		Ordinary	847	111	704
	7	GL	326	25	540
		Ordinary	789	100	655
	8	GL	495	20	821
		Ordinary	840	169	697
	9	GL	368	20	611
		Ordinary	771	159	640

Background correction factor (grains/μ^2)	Applied background correction	Corrected grain count	Level of labelling (grains/μ)	Relative level of labelling (GLs/Ordinary)
0.01	13	70	0.09	0.33
0.01	9	294	0.27	
0.01	5	25	0.08	0.27
0.01	6	215	0.30	
0.02	15	21	0.05	0.16
0.02	11	205	0.32	
0.01	4	51	0.20	0.57
0.01	6	249	0.35	
0.01	9	52	0.10	0.29
0.01	6	240	0.34	
0.04	50	58	0.08	0.18
0.04	36	490	0.45	
0.06	97	130	0.13	0.28
0.06	63	597	0.47	
0.02	8	15	0.06	0.14
0.02	7	171	0.42	
0.05	25	37	0.12	0.18
0.05	28	451	0.66	
0.02	10	17	0.06	0.18
0.02	13	270	0.34	
0.02	11	22	0.07	0.26
0.02	9	146	0.27	
0.02	22	64	0.10	0.18
0.02	14	480	0.56	
0.02	18	20	0.036	0.20
0.02	10	106	0.177	
0.04	40	43	0.07	0.18
0.04	15	181	0.39	
0.01	9	10	0.018	0.27
0.01	5	40	0.067	
0.02	23	28	0.040	0.14
0.02	10	172	0.29	
0.02	5	14	0.085	0.30
0.02	6	111	0.28	
0.01	5	10	0.031	0.28
0.01	7	99	0.11	
0.01	10	17	0.027	0.32
0.01	6	62	0.085	
0.01	12	21	0.028	0.23
0.01	6	87	0.12	
0.01	7	16	0.036	0.24
0.01	7	134	0.15	
0.01	7	9	0.021	0.22
0.01	7	75	0.095	
0.01	8	9	0.017	0.14
0.01	7	104	0.12	
0.01	5	20	0.061	0.51
0.01	7	93	0.12	
0.01	8	12	0.024	0.13
0.01	7	162	0.19	
0.01	6	14	0.038	0.19
0.01	6	153	0.20	

Mean relative level of labelling 0.245 (± 0.06 SEM)

TABLE 6. Incorporation of ^{3}H-uridine into the giant loops on chromosome II and the ordinary loops of *N. viridescens*

Female	Oocyte	Type of loop	Total length drawn (μ)	Total grain count	Assumed loop area (μ^2)
1 (Exposed 17 days)	1	GL	600	145	996
		Ordinary	892	412	741
	2	GL	744	162	1235
		Ordinary	792	372	656
	3	GL	596	146	990
		Ordinary	882	398	732
	4	GL	558	104	926
		Ordinary	927	313	769
	5	GL	931	160	1545
		Ordinary	873	243	724
	6	GL	1292	306	2145
		Ordinary	1173	465	973
	7	GL	823	181	1366
		Ordinary	1188	468	986
2 (Exposed 14 days)	1	GL	1269	350	2106
		Ordinary	1327	726	1102
	2	GL	431	227	715
		Ordinary	648	528	538
	3	GL	564	182	937
		Ordinary	1378	883	1144
	4	GL	426	309	707
		Ordinary	526	499	436
	5	GL	520	166	863
		Ordinary	750	438	622
3 (Exposed 14 days)	1	GL	882	239	1464
		Ordinary	1303	795	1082
	2	GL	293	126	487
		Ordinary	696	490	578
	3	GL	317	92	527
		Ordinary	886	376	735
	4	GL	353	65	587
		Ordinary	728	245	604
	5	GL	486	105	806
		Ordinary	928	315	770
	6	GL	425	52	705
		Ordinary	586	172	486
4 (Exposed 10 days)	1	GL	457	116	759
		Ordinary	772	300	640
	2	GL	579	225	961
		Ordinary	510	336	423
	3	GL	917	316	1522
		Ordinary	810	441	672
5 (Exposed 7 days)	1	GL	798	124	1325
		Ordinary	814	346	675
	2	GL	484	112	803
		Ordinary	700	289	581
	3	GL	720	63	1195
		Ordinary	978	240	812
	4	GL	459	85	762
		Ordinary	867	309	719
	5	GL	604	47	1003
		Ordinary	904	217	750
	6	GL	586	69	974
		Ordinary	883	285	733
	7	GL	314	50	521
		Ordinary	694	271	576
	8	GL	639	67	1061
		Ordinary	1036	274	860
	9	GL	357	31	583
		Ordinary	388	70	322

Background correction factor (grains/μ^2)	Applied background correction	Corrected grain count	Level of labelling (grains/μ)	Relative level of labelling (GIs/Ordinary)
0.02	20	125	0.21	0.48
0.02	15	397	0.44	
0.01	12	150	0.20	0.43
0.01	7	365	0.46	
0.01	10	136	0.23	0.52
0.01	7	391	0.44	
0.01	9	95	0.17	0.51
0.01	8	305	0.33	
0.01	15	145	0.16	0.59
0.01	7	236	0.27	
0.02	23	283	0.22	0.58
0.02	19	446	0.38	
0.01	14	167	0.20	0.53
0.01	10	458	0.38	
0.01	21	329	0.26	0.48
0.01	11	715	0.54	
0.01	7	220	0.51	0.63
0.01	5	523	0.81	
0.01	9	173	0.31	0.49
0.01	11	872	0.63	
0.02	14	295	0.69	0.74
0.02	9	490	0.93	
0.01	9	157	0.30	0.52
0.01	6	432	0.58	
0.01	15	224	0.25	0.42
0.01	11	784	0.60	
0.02	10	116	0.40	0.58
0.02	12	478	0.69	
0.01	5	87	0.27	0.64
0.01	7	369	0.42	
0.01	6	59	0.17	0.51
0.01	6	239	0.33	
0.01	8	97	0.20	0.61
0.01	8	307	0.33	
0.01	7	45	0.11	0.39
0.01	5	167	0.28	
0.01	8	108	0.24	0.63
0.01	6	294	0.38	
0.02	19	206	0.36	0.56
0.02	8	328	0.64	
0.02	30	286	0.31	0.58
0.02	13	428	0.53	
0.01	13	111	0.14	0.33
0.01	7	339	0.42	
0.01	8	104	0.21	0.52
0.01	6	283	0.40	
0.01	12	51	0.071	0.30
0.01	8	252	0.24	
0.01	8	77	0.17	0.49
0.01	7	302	0.35	
0.01	10	37	0.061	0.26
0.01	8	209	0.23	
0.01	10	59	0.10	0.32
0.01	7	278	0.31	
0.01	5	45	0.14	0.37
0.01	6	265	0.38	
0.01	11	56	0.088	0.34
0.01	9	265	0.26	
0.01	6	25	0.071	0.42
0.01	3	67	0.17	

Mean relative level of labelling 0.51 (± 0.07 SEM)

(fig. 15b, Table 4) the ordinary loops incorporate 1.1 times as much, for guanosine (fig. 15c, Table 5) the ordinary loops incorporate 4 times as much label as the giant loops. By making the comparisons within single nuclei the precursor pool problem is circumvented as there is a common specific activity of precursor within a single nucleus (although this will vary from nucleus to nucleus).

Edström & Gall (1963) isolated chromosomal RNA from N. viridescens oocytes and analysed its base composition (column 3 of Table 7). Since the ratio for the level of label in the giant loops with respect to the ordinary loops is known, the base ratio of the RNA transcribed by the giant loops can be calculated by multiplying the ratio for the level of label incorporated for each precursor by the amount of that residue as determined by Edström & Gall for the total chromosomal RNA (Table 7). The 5th column of table 7 shows the base ratios of the giant loops' RNA expressed as percentages. There is an extreme imbalance between guanine (9.2%) and cytidine (38.5%), which in turn implies a comparable imbalance between the transcribed and non-transcribed strands of the DNA in the giant loops' axes, with some 4 times more guanine residues in the former than the latter.

It will be recalled that Gall originally noted the relatively low rate of incorporation of ^{3}H-uridine by the N. viridescens giant loops, but this observation by itself did not allow the inference that the rate of RNA transcription on these loops is lower than elsewhere; the giant loops' RNA might merely be relatively deficient in uridine. The results presented here confirm Gall's observation (fig. 16) but go further to show that the rate of RNA transcription on the giant loops is genuinely lower than elsewhere, little more than half the average rate.

TABLE 7. Base ratio of the RNA transcribed on the giant loops (GLs) of Notophthalmus viridescens

Base	Levels of labelling of GLs relative to the generality of ordinary loops	Base ratio of the generality of lampbrush chromosomal RNA of N. viridescens (from Edström & Gall, 1963)	Base ratio of Gls' RNA weighted in accord with the overall composition of chromosomal RNA	Base ratio of GLs' RNA
	%	%		%
Adenine	51	26.6	13.5	24.9
Guanine	24.5	20.5	5.0	9.2
Cytosine	88	23.8	20.9	38.5
Uracil	51	29.3	14.9	27.4
	Rate of RNA transcription on the GLs as compared with that on the generality of ordinary loops:		54.4%	

Fig. 15. Photographs of toluidine blue stained preparations to show the incorporation of (a) adenine (17 days exposure), (b) cytidine (17 days exposure), (c) guanosine (26 weeks exposure), and (d) uridine (17 days exposure) into the RNA transcribed by the giant loops on chromosome II.

Scale = 50 μm

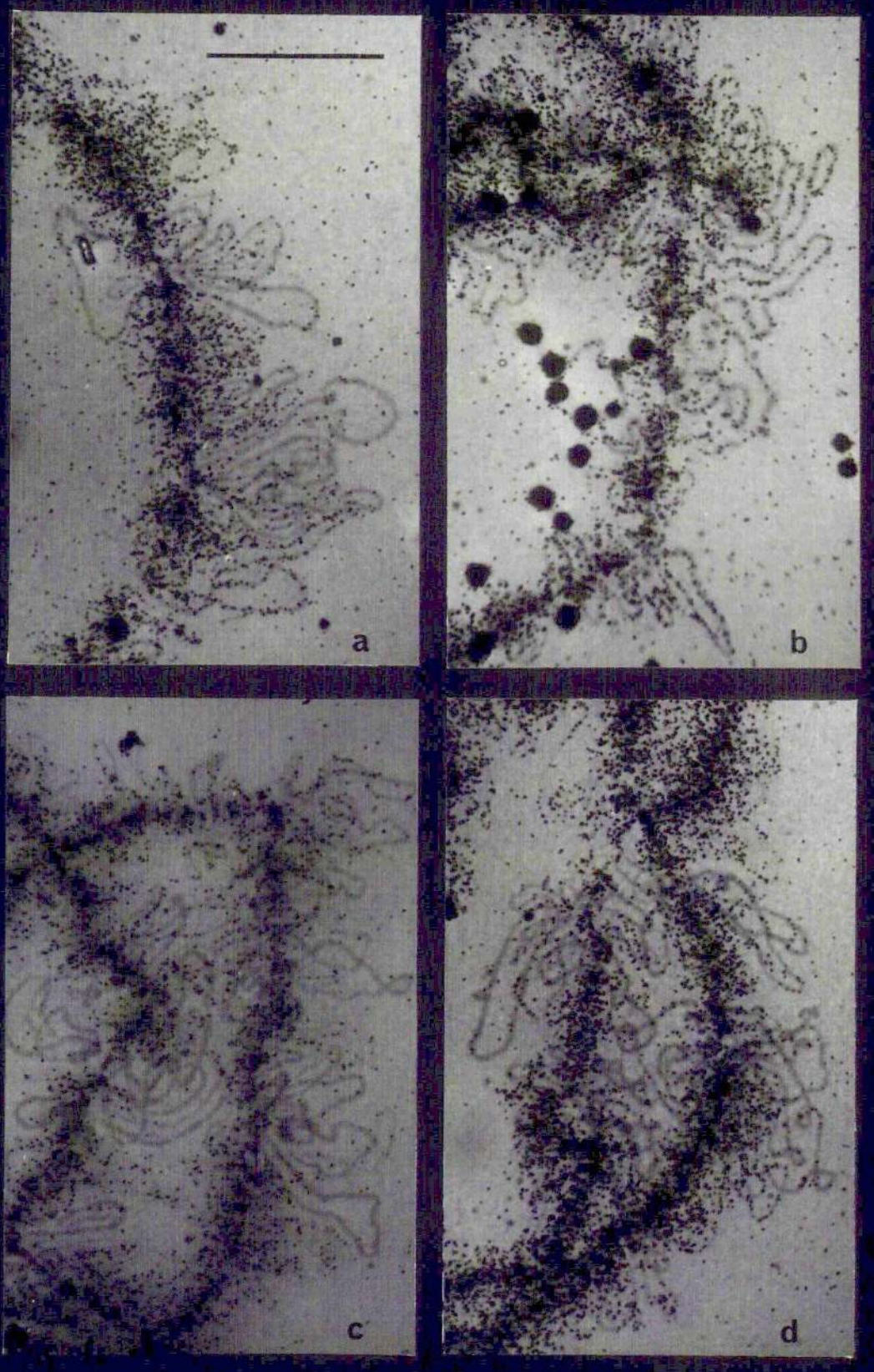

a
b
c
d

Fig. 16. Photographs of toluidine blue stained preparations to show the incorporation of uridine into the RNA transcribed by (a) some very long ordinary loops and (b) the giant loops on chromosome II in the same preparation. (14 days exposure)

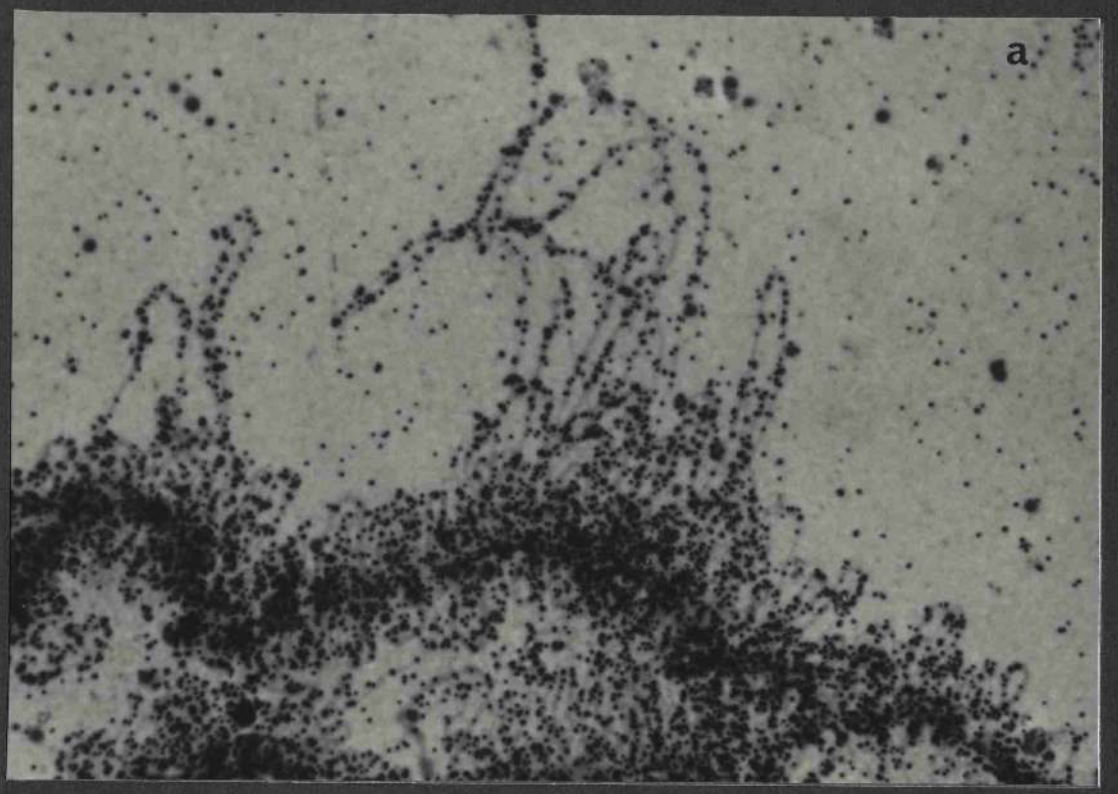
Scale = 50 μm

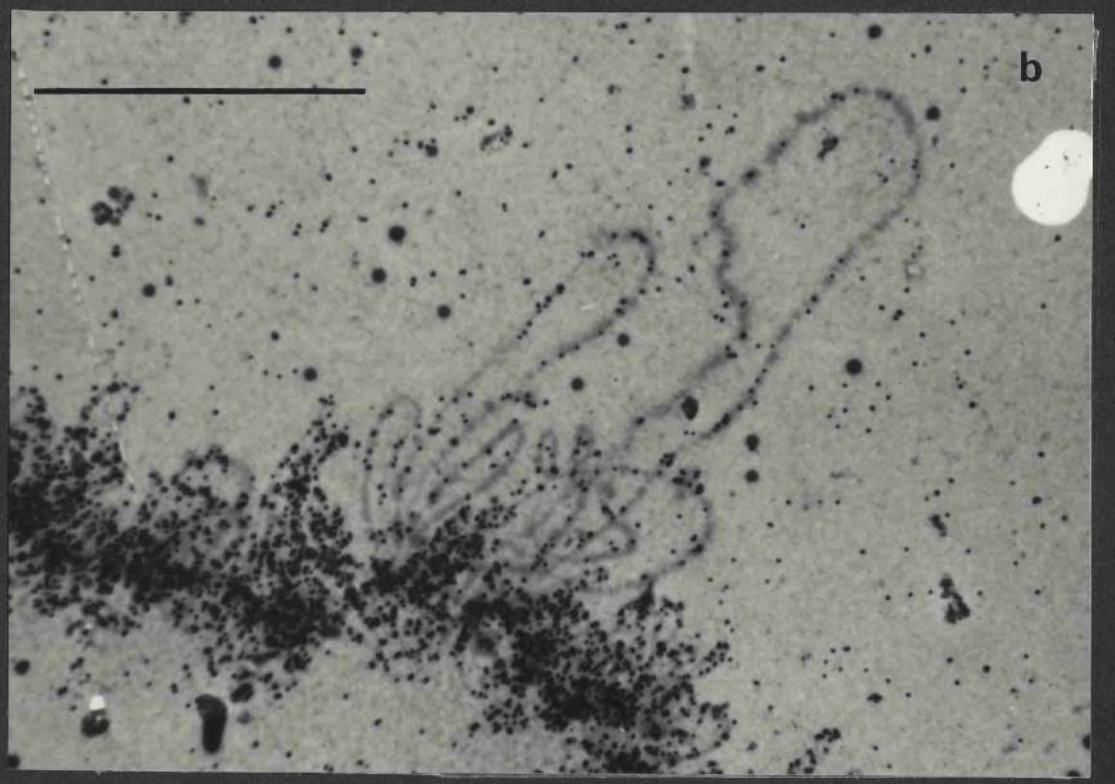
C H A P T E R IV

DISCUSSION

The giant loops on chromosome II of N. viridescens have been termed "normal" (Gall, 1954; Callan, 1963) but they are easily recognised in lampbrush chromosome preparations and show several unusual features. As landmark structures they show more variation in terms of the number of pairs present, their size, and matrix distribution patterns, than other landmark loops such as the "sequential" loops at the left end of chromosome XI in N. viridescens or the giant granular loops near the left end of chromosome XII in T.c. cristatus. Other unusual features are their response to hormone and cold treatment and their low level of labelling following the administration to the oocytes of tritium labelled precursors of RNA.

The presence or absence of loop pairs has been demonstrated to follow simple Mendelian laws (Callan & Lloyd, 1960a & b; Callan, 1963) and heterozygosity for one or more of the giant loop pairs of chromosome II of N. viridescens has been observed (see fig. 2). It is possible that differences in the size and matrix distribution patterns of the giant loops may also be caused by intrinsic genetic differences, although it may equally well be that these variations are related to the physiological condition of the whole animal, rather than of individual oocytes; there is little variation between oocytes from individual animals over the oocyte size range 0.6 mm - 1.0 mm diameter, but this might be ascribable to either cause. Callan & Lloyd (1960a) state that in T. cristatus subspecies the lengths of some loops remain relatively constant in oocytes ranging from 0.8 mm to 1.4 mm diameter. Makarov & Safronov (1976) have found in T.c. cristatus, that age and physiological conditions can alter loop length significantly while their general characteristics remain the


a


b

same. Makarov & Safronov give no details of the size range of the oocytes from which these observations were made. The variations in length that they observe could be caused by the natural regression of the lateral loops' axes into the chromomeres, as maturation to metaphase approaches. It is most likely that changes in loop length from oocytes of a standard size are caused by altered physiological conditions, for both hormone injections and cold treatment affect loop length, as will be discussed in more detail later.

The giant loops on chromosome II of N. viridescens may have interruptions in their matrix and several polarized matrix units within a loop. This is presumably the result of multiple initiation sites for RNA transcription and hence multiple transcription units within single loops. Other instances of multipolarized loops, i.e. loops with more than one thin to thick matrix unit, have been described. Makarov and Safronov have observed multipolarized loops in T.c. cristatus and identified one pair close to the left end of chromosome XII in which there were usually 6 identically orientated matrix units which differed in size from one another, sometimes by as much as a factor of 2. Within this same species they observed more than 10 loops with two matrix units which had opposite polarities to one another. In most instances these had both their thick ends at the insertions into the chromomere, and they could thus be similar to the symmetrical loops described on chromosomes V and VIII of 3 subspecies of T. cristatus (Callan, 1963; Callan & Lloyd, 1975). Makarov & Safronov interpret multipolarized loops as multioperon structures, but do not discount the possibility that they are the result of the decondensation of several chromomeres.

Angelier & Lacroix (1975) observed several examples of more than one matrix unit on the same DNA loop axis when the lampbrush chromosomes of Pleurodeles species were spread for electron microscopy. During this

examination of multiple matrix units they observed that the same axis may may carry units of different length and characteristics and that adjacent units may have opposite polarities. Angelier & Lacroix interpret their observations in terms of multiplicity of transcription units within the organizational unit, the chromomere, and that opposite polarities in adjacent units implies the existence of at least two initiation sites.

Scheer, Franke, Trendelenburg & Spring (1976) have classified lampbrush chromosome loops, from oocyte nuclei of urodele amphibians and primary nuclei of Acetabularia, according to the arrangement of transcriptional complexes when viewed in the electron microscope. They have identified and classified five different arrangements of transcription complexes on a single axis. These range from a single transcription complex per loop to loops in which there are several complexes of different lengths and polarities. They interpret their observations that multipolar loops reflect the transcription of different heterogeneous RNAs and that differences in polarity of complexes on the same axis may reflect transcription from both strands of the DNA axis, using one strand in one region and the other strand in an adjacent region. This would ensure that RNA polymerase complexes would not travel into neighbouring genes, and that RNA polymerases would detach from the termination sites.

Although the giant loops of chromosome II in N. viridescens which are multipolarized are long, not all long giant loops in N. viridescens show multipolarity. Another feature of these giant loops is the occasional occurrence of multiple loop pairs derived from one chromomere. It could be argued that multiple loop pairs at one locus are an alternative to a long multipolarized single loop pair at that same locus so that multiple transcription units within a single chromomere are expressed. However I do not think this can be the case, for single loop pairs at a locus may be

long and interrupted, long and uninterrupted, or short with a single matrix unit. The occurrence of multiple loops at a single locus is well known, especially for the multiple fusing loops on chromosomes XII in T.c. carnifex and T.c. karelinii (Callan & Lloyd, 1960b) and indeed multiple loops per chromomere may be the rule rather than the exception according to the electron microscopic studies of sections of lampbrush chromosomes (Mott & Callan, 1975). Mott & Callan observed lengths of lampbrush chromosomes in which more than one pair of loops arose from most individual chromomeres. Although their observations were carried out on oocytes of a size in which some lateral amalgamation of the chromomeres is to be expected, their interpretation that chromomeres are often complex, bearing more than one pair of loops, is borne out in favourable preparations observed in the light microscope where multiple loop pairs can be resolved. Two other instances of the multiple - single loop pair alternative have been observed: the loops in the left telomere region of chromosome XI of T. vulgaris meridionalis (Barsacchi et al., 1970) and the landmark loop in the left arm of chromosome III of T. helveticus helveticus (Mancino & Barsacchi, 1966). If multiple loop pairs from a single locus are the rule, then structures such as the giant granular loop on chromosome XII in T.c. cristatus, which is always a single loop pair arising from a single chromomere, and similarly the giant fusing loops on chromosome XI in T.c. carnifex are the exception rather than the rule.

The fact that an interruption in the matrix surrounding a lateral loop occurs at the same level in all examples of that particular loop in an individual newt must argue in favour of matrix moving round a stationary loop axis rather than axis and matrix moving together. If axis and matrix moved together, interruptions in the matrix would be expected to occur at different levels on the same loop in oocytes of different sizes, assuming that interruptions in the matrix are termination and initiation sites for

RNA synthesis. Snow & Callan (1969) were reluctant to attribute the polarized asymmetry of loop matrix RNP to transcription alone because of the dimensions of the majority of the lateral loops (see Chapter II). Malcolm & Sommerville (1974) found that the matrix of lateral loops is made up of aggregates of 10 - 20 nm subparticles which reach diameters of 200 - 500 nm. They proposed a model in which the newly formed RNA fibril associates with preformed protein at many sites, to form the 10 - 20 nm subparticles, by interaction between the RNA and the protein, and the 10 - 20 nm subparticles aggregate to form the mature RNP particles that make up the loop matrix. However Malcolm & Sommerville (1977) have subsequently found that the beaded structure of the RNP is broken down by salt and that the various sized particles so released are unstable after RNase treatment. They conclude that the particles do not exist independently of RNA but are formed by periodic condensates of linear RNP as a result of RNA secondary structure and/or protein-protein interaction on the RNA molecule. They propose that the foreshortening of the RNP fibril relative to the length of DNA involved in individual transcriptional matrices is caused by condensation of the RNP fibril at points where hairpin loops occur in the RNA, followed by stabilization of the resulting beaded structure by means of protein-protein interaction. Mott & Callan (1975) studying sections of lampbrush chromosomes prepared for electron microscopy observed particles 30 nm in diameter. They proposed that winding of the nascent RNP fibrils creates the particles and they calculated that by tight packaging 700 nm of fibril 5 nm in cross section could be contained in a 30 nm particle. Whether the subparticles are formed by winding of nascent RNP or condensation of RNP about hairpin loops, close packing of the subparticles could achieve the observed dimensions of the lateral loops on a transcription only model of loop polarity, even for the longest loops, assuming that all the loop length is transcribed. Another possibility is that RNP particles may be

shed not only where the loop axis returns to the "parent" chromomere, but also elsewhere along the loop axis. However this is unlikely considering the frequently observed uniform increase in loop matrix thickness along individual loops.

The results from the experiments involving hormone treatment can be interpreted in terms of matrix moving round a stationary axis. In these experiments two different responses are probably involved: one is the effect of hormone on loop length and the other is the effect of hormone on matrix distribution. These two effects may be mediated by two different components of the hormone.

The effect of hormone injections on loop length was found to be variable and could be the result of either the initial length of the loops involved or the level of gonadotrophin already present in the animal. Macgregor (1963), when studying the effect of gonadotrophin treatment on the giant loops of chromosomes X, XI and XII in T.c. carnifex found that these loops decreased in size or remained unchanged according to whether they were large or small before treatment, large loops decreased in size, small ones remained unchanged. Macgregor proposed that decrease in length of the loops was caused by stimulating oocyte metabolism and increasing demand for the primary products of gene-controlled synthesis and that by loss of matrix the loops become smaller. The increase in length of the giant loops on chromosome II of N. viridescens might be caused by stimulating cellular metabolism and increasing the rate of RNA synthesis by these loops by increasing the density RNA polymerase molecules attached to the DNA axis of these loops, which in normal circumstances are known to have a low uptake of the precursors of RNA (see Chapter III).

The effect of hormone on matrix distribution on the giant loops is rather more constant, though the data is scanty. In loops which have two matrix units there is an initial increase in the length of loop occupied by the first matrix unit, followed by a degree of recovery towards the original level. This may be brought about by masking of the initiation point for RNA synthesis at the level of the interruption and alteration of this site by the hormone binding to the loop axis. Other hormones, steroids, are known to bind to chromatin in the nuclei of their target tissues after associating with a receptor protein in the cytoplasm and moving into the nucleus (reviewed by Lewin, 1974, p. 363-366). From studies on mammalian tissues, gonadotrophins are also known to bind to receptor sites, which consist of protein with some lipid attached, in the cells of their target tissues (Dufau, Charreau & Catt, 1973; Dufau, Charreau, Ryan & Catt, 1974; Danzo, Midgley & Kleinsmith, 1973), and these may act in the same way as the steroid-receptor complexes.

However, whatever may be the mechanism by which the hormone acts, the fact that in one case, that of newt H/II , the distribution of matrix alters without significant change in the length of the loop can be interpreted as matrix moving round a stationary loop axis. This conclusion has also been reached by Makarov & Safronov (1976) following autordaiographic studies on the lampbrush chromosomes of T.c. cristatus. They, as others before them, found that uniformly labelled loops, although the labelling was weak, appeared after as little as 30 minutes incubation of the oocytes with ^{3}H-uridine, and that well labelled loops appeared after only 40 minutes incubation.

A model proposed by Pelling (1972), in which the density of RNA polymerase molecules along a DNA fibre is responsible for the size of puffs in polytene chromosomes, could be applied to lampbrush chromosomes

although the polymerase density apparently differs between the lampbrush chromosome loop and the polytene chromosome puff by at least one, and frequently more than two, order of magnitude. Miller & Hamkalo (1972) estimate, from their electron micrographs, that on a loop of average length 50 μm there would be approximately 1400 RNA polymerase molecules; Pelling (1972) estimates, from RNA to DNA ratios, that a polytene chromosome puff of average size would operate with only 2 polymerases per DNA strand and a Balbiani ring would only contain about 120 RNA polymerase molecules per strand. Uncoiling of the DNA in the lateral loop could be caused by decreasing the distance between polymerase molecules, i.e. increasing their density, which would impose rigidity on the DNA and prevent tight coiling. Factors which affect the rate of attachment of polymerase molecules, such as gonadotrophin treatment and cold treatment, would then affect the length of the lateral loops. Subjecting animals to cold treatment affects the length of the lateral loops of N. viridescens' lampbrush chromosomes. The giant loops on chromosome II are affected to a lesser extent than the ordinary loops which show considerable shortening.

The simplest explanation for this might be reduction in the rate of attachment of RNA polymerase molecules to the loop axis. This would increase the distance between polymerase molecules and allow the DNA axis to fold up on itself and thus shorten the loop. The giant loops on chromosome II are affected to a lesser extent which might be the result of them already having a lower rate of polymerase attachment. Drawing on the analogy between lampbrush and polytene chromosomes which has been drawn many times (e.g. Callan, 1955; Gall, 1958, 1963b; Beermann, 1967), Daneholt (personal communication to H.G. Callan) has found that the rate at which RNA polymerase molecules become attached to the DNA of a Balbiani ring in the polytene chromosomes of Chironomus tentans is reduced as a result

of cold treatment and that such Balbiani rings are smaller than normal because the DNA between polymerases folds up on itself. The giant loops on chromosome II have a 50% lower rate of RNA synthesis than the ordinary loops as shown by autoradiography (Chapter III) which might be caused either by a low rate of polymerase movement or, if polymerase movement within any one nucleus is constant, a low rate of polymerase attachment. In which case cold treatment might be expected to affect the rate to a lesser extent and so the length of the giant loops would not change as dramatically as the length of the ordinary loops. A further possibility is that a different form of RNA polymerase, from the one transcribing the ordinary lampbrush loops, is operating on the giant loops on chromosome II. Multiple forms of RNA polymerase have been known since Roeder & Rutter (1969) described three distinct polymerase activities in developing sea urchin embryos and two in rat liver nuclei. Wassarman, Hollinger & Smith (1972) described three RNA polymerases found in the germinal vesicle contents of Rana pipiens; type I localized in the nucleolus; type II localized in the nucleoplasm; and type III probably of mitochondrial origin. The type II enzyme was found to be present in large amounts and inhibited by α-amanitin. Roeder (1974a) has distinguished five forms of RNA polymerase from whole cell homogenates of cultured kidney cells and embryonic tissues of Xenopus laevis. These have been designated IA, IB, IIA, IIB and III of which IIA may be of cytoplasmic origin. Types IIA and IIB were found to be equally sensitive to α-amanitin. Roeder (1974b) found that Xenopus laevis oocytes contained types I, IIA, IIB and III RNA polymerase with unusually high levels of types IIA and III. When nuclei were manually isolated from the oocytes it was found that they were lacking in RNA polymerase IIA and that the residual cytoplasm was enriched in this enzyme. Roeder also found that polymerase III accounted for a much higher fraction of activity in oocyte nuclei or whole oocytes

than in somatic cell nuclei or intact cells. Although most of the DNA-like RNA is synthesized by the α-amanitin sensitive type II enzymes (reviewed by Lewin, 1974, p. 320-327) it is possible that the type III enzyme may have some role in the transcription of non-ribosomal RNA, ribosomal RNA being transcribed by the α-amanitin resistant type I enzymes. Indeed if another type of RNA polymerase is operating on the giant loops on chromosome II in N. viridescens it may either have a lower rate of activity or different requirements for its activity from the enzyme transcribing the majority of the lampbrush loops.

Autoradiographic studies on the lampbrush chromosomes of N. viridescens have revealed two unusual features of the giant loops on chromosome II; the low rate of RNA synthesis and the base imbalance in the RNA transcribed by these loops. As far as I am aware the only other base ratio determinations for RNAs as transcribed (i.e. prior to any processing) from restricted chromosome regions are those of Edström & Beermann (1962) for Balbiani ring RNAs on the 4th chromosome of Chironomus tentans. They found that the RNA transcribed by the middle region of chromosome IV in salivary gland cells, which contains Balbiani ring 2, was low in uracil and high in adenine content, the base ratio of RNA being adenine = 38%, guanine = 20.5%, cytosine = 24.5% and uracil = 17.1%. This base imbalance is not nearly so great as that found for the giant loops in N. viridescens but is still significant; there must be twice as much thymine as adenine in the transcribed strand of the DNA in the Balbiani ring. Hybridization experiments have suggested that the DNA in Balbiani ring 2 is internally repeated and that there are, on average, 200 copies of the sequence (Lambert, 1972).

Before I had made a detailed investigation of grain counts over the giant loops of N. viridescens it appeared as though they might be transcribing an RNA entirely lacking in guanine. If this had been substantiated it

would at once have explained the results obtained by Gould, Callan & Thomas (1976) following their observations on the effects of restriction endonucleases on lampbrush chromosomes. Gould *et al.* found that while the giant loops on chromosome II of *N. viridescens* are cut by pancreatic DNase; Endo I, EcoB and EcoK from *E. coli*; and Hind from *Haemophilus influenzae*, the giant loops, and these alone, are resistant to digestion by Hae from *H. aegyptius* which recognises the specific sequence 3' $\frac{CC\ GG}{GG\ CC}$. Although the relative abundance of guanine residues on the transcribed as opposed to the non-transcribed strand significantly diminishes the chance of encountering the sequence 3' $\frac{CC\ GG}{GG\ CC}$ in a random base order (from once in every 256 base pairs to once in every 800 base pairs), this is clearly insufficient to account for the indigestibility of the DNA-containing axes of the two to three hundred micron long giant loops. Gould *et al.* conclude that the DNA axes of the giant loops consist of a number of repeats of a unit sequence. Indeed if the repeat length is of the order of 800 base pairs, given that the giant loops are between 200 μm and 300 μm long, there would be approximately 1000 copies of the sequence per loop.

With this degree of repetitiveness and this base bias, the giant loops could be regarded as satellite DNA although the G + C content of the loops, 47.7% is not significantly different from that of the main band, 44.8%, as calculated from buoyant density gradients (Barsacchi & Gall, 1972). *In situ* hybridization studies have shown that satellite sequences in mammals and amphibians are located at or very close to the centromeres (Jones, 1970; Pardue & Gall, 1970; Barsacchi & Gall, 1972; Macgregor, Horner, Owen & Parker, 1973) but Flamm, Walker and McCallum (1969) found that some satellite sequences in mouse are integrated in the main band DNA although this was not detected cytologically (Jones, 1970).

The fraction of satellite sequences hidden within main band DNA was only 2.3% of the total satellite sequences in mouse, and consequently a very small proportion of the total genome. It is possible that small fractions of satellite sequences in other organisms may be located at positions other than the centromeres, for instance in the giant loops on chromosome II in N. viridescens. Classically, satellite sequences are not transcribed into RNA in vivo (Walker, Flamm & McLaren, 1969). However, a small amount of satellite integrated in the genome might be transcribed along with the non-satellite DNA. Alternatively in the oocyte satellite sequences may require a different RNA polymerase for their transcription in vivo from the one transcribing the bulk of the DNA. There is evidence that a nucleoplasmic RNA polymerase other than the type II enzyme transcribes small molecules such as 5S and 4S RNA (Price & Penman, 1972; Weinmann & Roeder, 1974). If the sequences in the giant loops on chromosome II are satellite sequences it is possible that they are only transcribed in the oocyte when transcriptional activity is high. In this case, and if a second RNA polymerase is required for their transcription, increase in the level of polymerase activity in the oocytes, (X. laevis oocytes contain 4 to 5 orders of magnitude more RNA polymerase activity than somatic cells (Roeder, 1974b)), may increase the amount of the second RNA polymerase sufficiently to allow transcription of the sequences to take place.

There is, however, very little evidence for the transcription of satellite sequences and what there is (Harel, Hanania, Tapiero & Harel, 1968) has been refuted (Bostock, 1971; Walker, 1971) as satellites tend to contain coding sequences which would give rise to simple polypeptides with no obvious function if transcribed and translated. Southern (1970) concluded from sequencing studies that satellite sequences, which are highly repetitive, are shorter than indicated by reassociation studies

and that they emerged through tandem duplication of a short sequence with simultaneous base substitution. Tandem duplication as a basis for the organization of genes is not uncommon and holds for such examples as the ribosomal and 5S RNA genes, which consist of coding sequences separated from each other by non-transcribed spacer regions (Brown & Sugimoto, 1973). Keyl (1965) found that certain bands in the polytene chromosomes of Chironomus thummi thummi contain 2, 4, 8 or 16 times as much DNA as the homologoue bands in the closely related species Ch. th. piger. From cytological observations Keyl concluded that Ch. th. piger was phylogenetically older than Ch. th. thummi and hence the increases in DNA content had arisen from localized tandem duplication.

Studying salmanders of the genus Plethodon, Vlad & Macgregor (1975) found that while chromosome number and the relative dimensions of the chromosomes remain the same within species, the absolute length of the chromosomes and the number of chromomeres increases as the C-value increases between species of this genus. However the increase in chromomere number is not sufficient to account for the increase in DNA amount. If this increase is the result of tandem duplications followed by deviation of the sequences, this may explain why multipolarized loops are not common. 95% of the DNA in the genome is retained in the chromomeres and only 5% is transcriptionally active in the lampbrush loops (Callan, 1963). If tandemly duplicated sequences have deviated to such an extent that the information they contain is no longer sensible (Mizuno & Macgregor, 1974), it might be expected that they would be maintained in a condensed state in the chromomere and be transcriptionally inactive. On the other hand "start" and "stop" sequences may have deviated at the same time so that both informational and deviated sequences are transcribed as a single unit. This type of sequence organization in the loop was proposed by Sommerville & Malcolm (1976) from

reassociation kinetic experiments using primary loop transcript RNA and DNA complementary to oocyte messenger RNA. If this is the case it offers an explanation for the difference in size between the transcriptional unit and the functional messenger molecule.

The lateral loops of lampbrush chromosomes have been shown to follow inheritance patterns dictated by the simple Mendelian genetic laws (Callan & Lloyd, 1960a, b; Callan, 1963). Thus the elucidation of the organization of the transcriptional unit as observed in the lateral loop becomes an elucidation of the organization of the active gene.

C H A P T E R V.

INVERSION HETEROZYGOSITY AND CHIASMA DISTRIBUTION

INTRODUCTION

The lampbrush chromosomes of amphibian oocytes offer a unique opportunity for viewing the effect of structural rearrangements within chromosomes, such as inversions, on chiasma distribution. Inversion heterozygosity is not so easily recognized in lampbrush chromosomes as in the polytene chromosomes of Drosophila or the pachytene chromosomes of Zea mays because the homologous chromosomes, being at diplotene of meiosis, do not lie in register with one another. The identification of inversions in lampbrush chromosomes depends on the displacement of landmark structures from their normal positions and some inversions may not include such structures and therefore are likely to pass unnoticed. However, when landmark structures are included in an inversion it is possible to pinpoint the limits of the inversion as precisely as in polytene chromosomes. Another feature of lampbrush chromosomes is that the positions of chiasmata can be determined accurately, and if inversion heterozygosity affects the chiasma distribution this will be immediately noticable.

The occurrence of inversion heterozygosity in Drosophila polytene chromosomes and in the pachytene chromosomes of maize can be seen by the formation of inversion loops, although inversion loops need not characterize all inversions (McClintock, 1931, 1933). If chiasmata are formed within the limits of an inversion, these can be scored at diplotene and/or on the basis of bridge and fragment frequency at first meiotic anaphase (see Whitehouse, 1973, pp. 125-128). The effect of inversions on crossing over has been extensively studied in Drosophila and maize by genetic analysis but the outcome is necessarily and often grossly distorted by the differential

viabilities of the segregants following meiosis when recombination produces deficient genomes.

Such studies have led to some conflicting results. Sturtevant & Beadle (1936) studying the effects of X-ray induced inversion heterozygosity in the X-chromosome of D. melanogaster found that crossing over is reduced in regions adjacent to the inversion limits, and that long inversions appear to interfere more seriously with pairing in uninverted regions of the X-chromosome than short inversions. On the other hand, Novitski & Braver (1954) when studying the effect of a heterozygous inversion in one arm of a specially constructued X-chromosome, a tandem metacentric, in the same species, found that while crossing over was reduced within the inversion there was little difference in crossing over outside the limits of the inversion. The system in maize is somewhat different from that in Drosophila as Rhoades & Dempsey (1953) have found. They observed the effect of a paracentric inversion in chromosome 3 of maize and found that while recombination values within the inverted segment were greatly reduced there was an increase in the incidence of crossing over for regions adjacent to the inversion.

More recently instances of inversion heterozygosity in the lampbrush chromosomes of Amphibia have been reported. Mancino, Nardi & Barsacchi (1970) found one individual of Triturus vulgaris meridionalis which was heterozygous for a paracentric inversion on chromosome XI. The inversion involved a portion of the chromosome in which chiasmata do not normally form and no influence on chiasma formation was observed. Lacroix & Loones (1974) have observed heterozygous inversions in three chromosomes, V, VI and VIII, of Pleurodeles poireti in the female offspring of crosses involving X-irradiated males and normal females. I will return to consider these later. In a population of Notophthalmus viridescens a spontaneous

heterozygous inversion has been found on bivalent XI which alters the normal chiasma distribution in that bivalent.

MATERIALS AND METHODS

Lampbrush chromosome preparations from N. viridescens were made according to the method outlined in Chapter I. The positions of chiasmata were determined by measuring camera lucida drawings with a map measurer so that fractions of length could be calculated.

94 female newts were examined between 1973 and 1976, of which 15 were found to be heterozygous for an inversion involving almost the whole of the long arm of the shortest chromosome, chromosome XI. Of these 15 heterozygotes, 7 have been studied in detail.

Squash preparations: To compare male and female meiosis, squash preparations were made from testes that had been fixed several years previously in a solution of 3 parts absolute ethanol to 1 part glacial acetic acid. Tissue fixed in this way and stored in fixative in a refrigerator becomes harder with time, and the cells more difficult to dissociate, so the following procedure was used. Pieces of tissue were stained in 1% acetocarmine in 45% glacial acetic acid for approximately 45 minutes, the stain was washed off with 45% glacial acetic acid, and the tissue then tapped out and squashed using a little heat from a spirit lamp to soften the cells. Photographs of male meiosis were taken through an Ilford 807 green filter with a x 100 planapochromatic oil immersion objective and bright field optics.

OBSERVATIONS

The heterozygous inversion in chromosome XI was recognized because it includes the sequentially labelling loops (Gall 1963b, Chapter III) situated close to the left end of the chromosome. The sequential loops bear a mass of "fluffy" matrix and in the normal chromosome XI, when they form a double bridge, they demonstrate their polarity with the thin insertions directed towards the telomere (figs. 23 & 24). The inversion reverses the polarity of these loops so that when they now form a double bridge the thin insertion is instead directed towards the centromere (fig. 25). The sequential loops normally occur at locus 0.02 (figs. 17 and 26) and the inversion transfers these loops to locus 0.55 (figs. 18, 23 & 24), close to the centromere which lies at locus 0.60 (Gall, 1954). Thus the inversion involves virtually the whole of the left arm of chromosome XI, for one break point must be assumed between the sequential loops and the telomere and the other break point immediately to the left of the centromere. I have only observed the inversion in the heterozygous state; the expected frequency of inversion homozygotes, using the Hardy-Weinberg equation would be 1 in 157.

Fig. 19a is a histogram showing the chiasma distribution in 8 animals with normal bivalents XI (53 examples). It will be seen that virtually all the chiasmata are localized close to both ends of the chromosome, 41 chiasmata were scored in the left arm pairs, 50 chiasmata in the right arm pairs. The few interstitial chiasmata were all contributed by exceptional oocytes in 2 animals. The chiasma distribution in bivalent XI, with the chiasmata proterminally localized, is different from that of the longer bivalent II. Fig. 20 is a histogram showing the chiasma distribution in 38 examples of bivalent II. The 107 chiasmata scored, 63 in the left arm pairs and 44 in the right arm pairs are not restricted in their distribution.

Fig. 17. Camera lucida drawings of two normal bivalents XI.

C = centromere

Ch = chiasma

sl = sequential loops

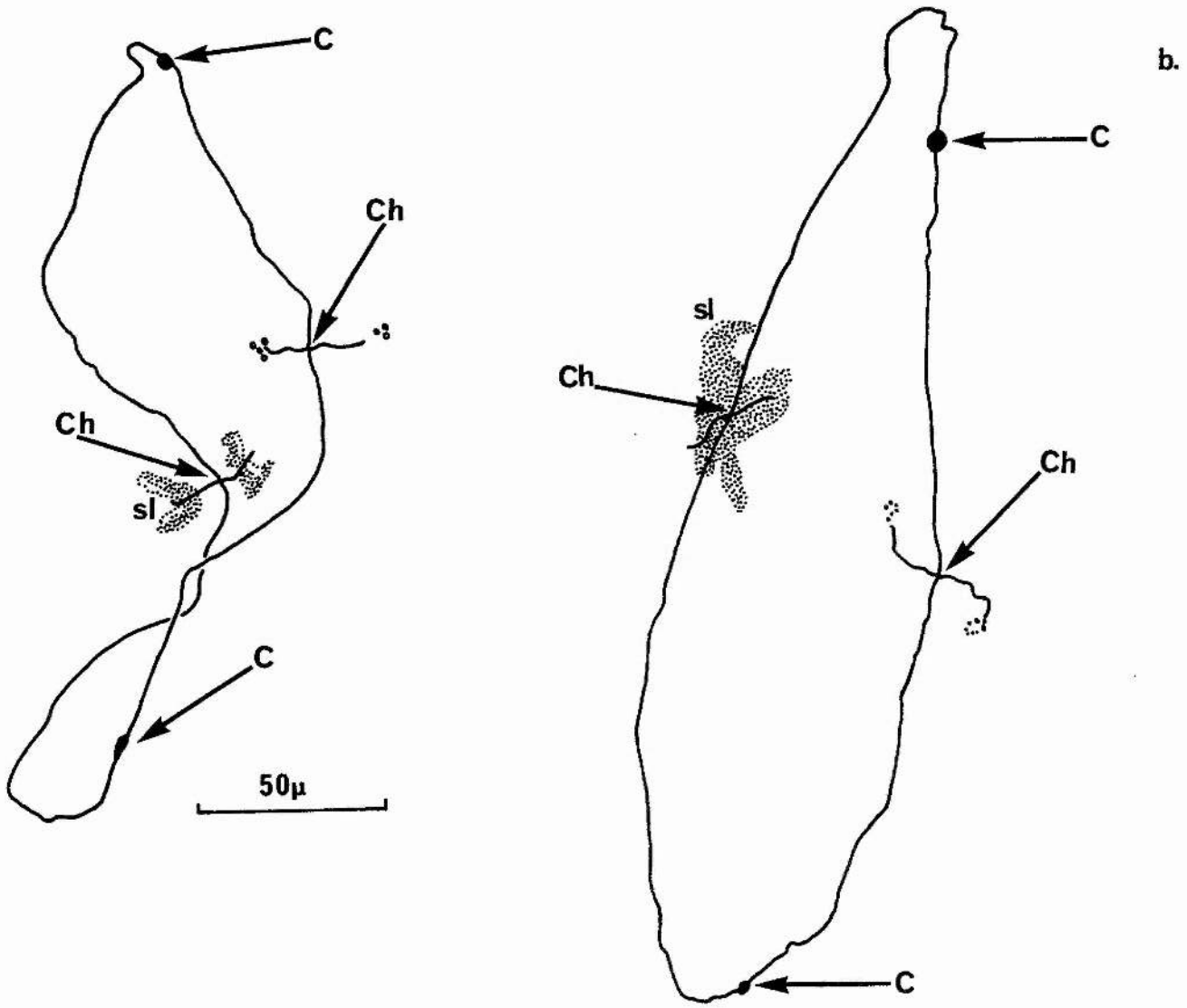


Fig. 17

Fig. 18. Camera lucida drawings of two bivalents XI heterozygous for the inversion in the long arm.

C = centromere

Ch = chiasma

isl = inverted sequential loops

nsl = normal sequential loops

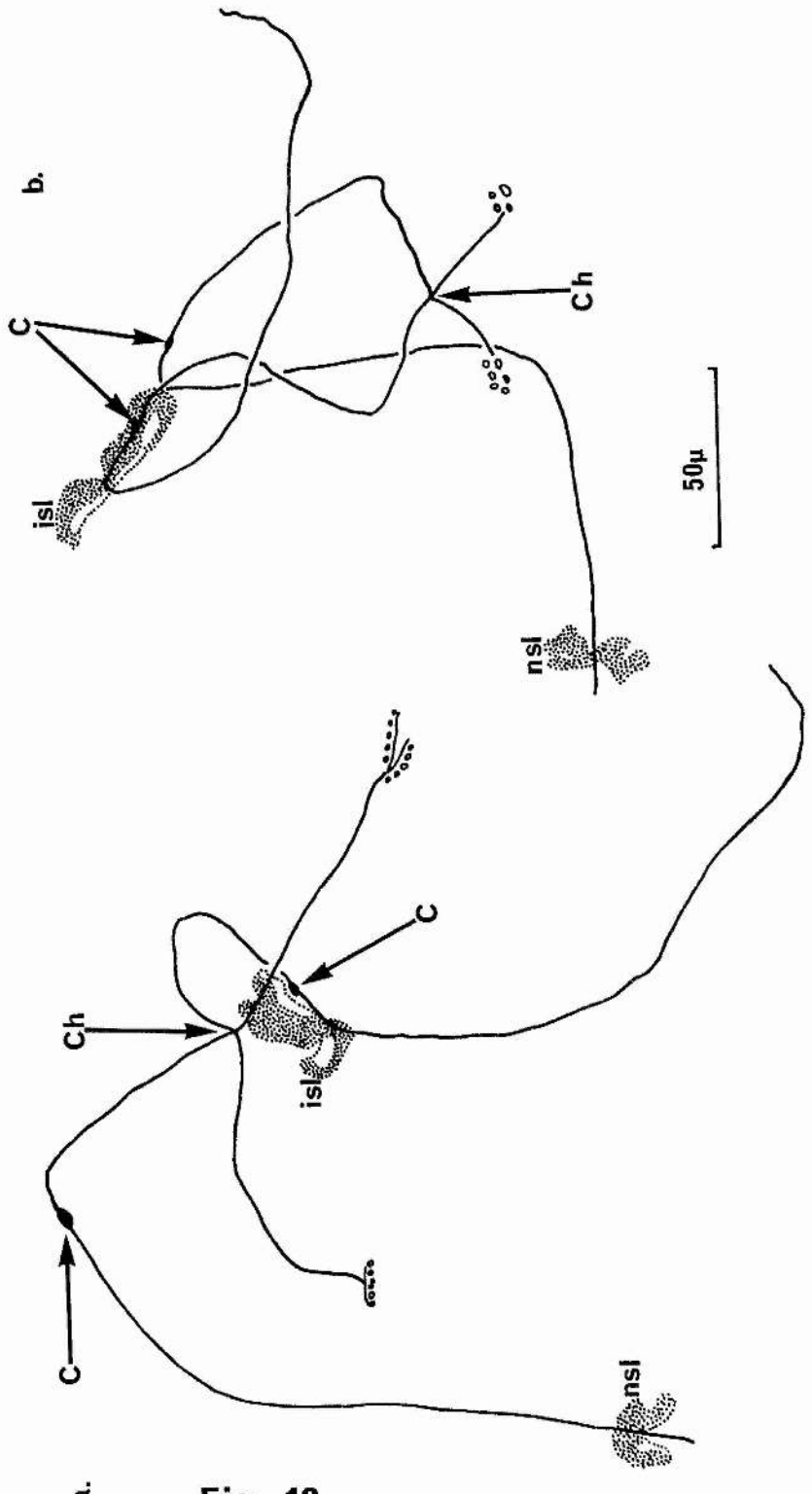


Fig. 18

Fig. 19a. Histogram to show chiasma distribution in 53 examples of normal bivalents XI. The arrowhead marks the position of the centromere. Outlined white areas represent terminal fusions, black areas chiasmata.

Fig. 19b. Histogram to show chiasma distribution in 42 examples of bivalents XI heterozygous for the inversion in the long arm. The arrowhead marks the position of the centromere, and the arrows pointing down mark the limits of the inversion.

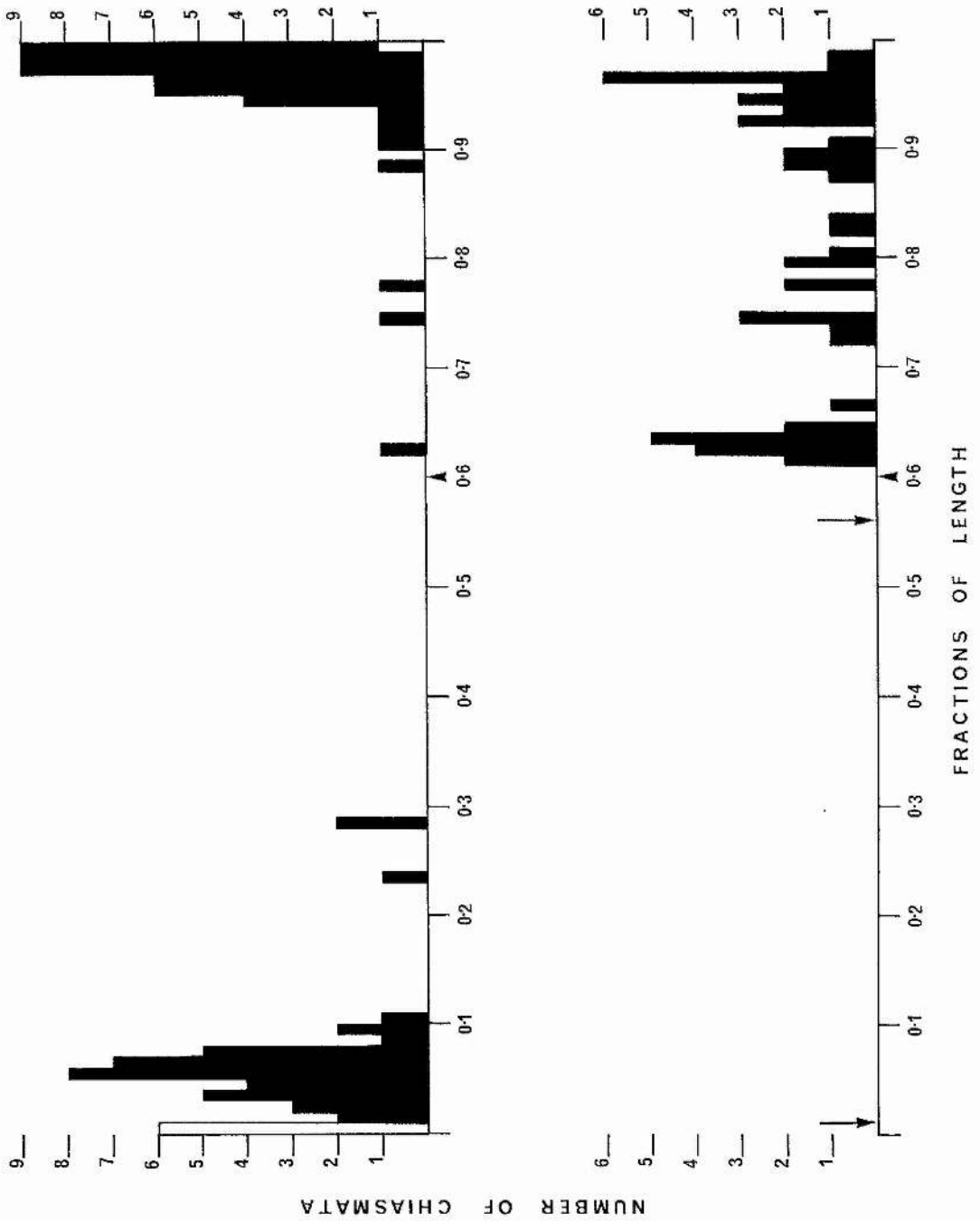

NUMBER OF CHIASMATA
FRACTIONS OF LENGTH
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9

Fig. 19

Fig. 20. Histogram to show chiasma distribution in 38 examples of normal bivalents II. The arrowhead marks the position of the centromere. The outlined white areas represent terminal fusions, black areas chiasmata.

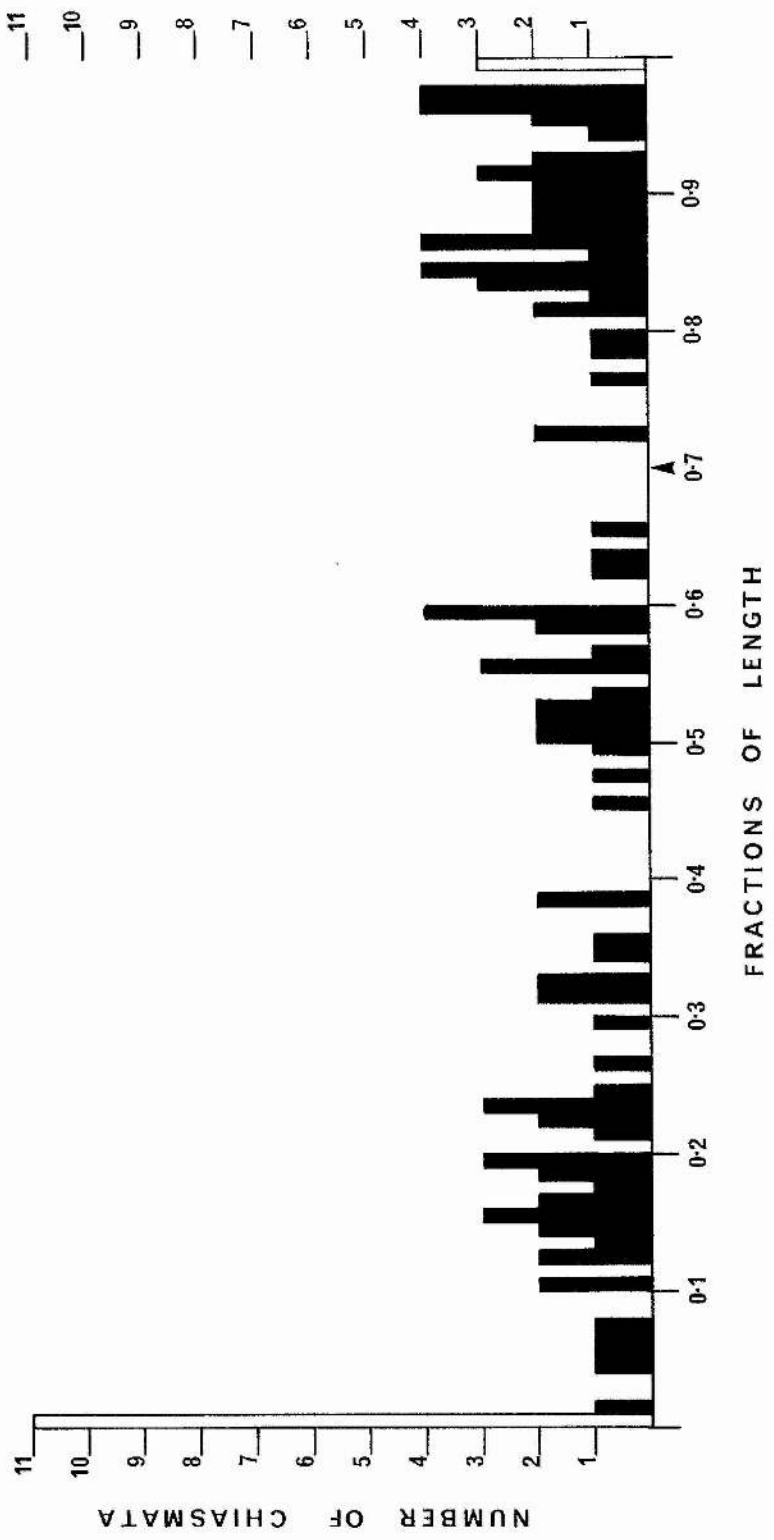

NUMBER OF CHIASMATA
11
10
9
8
7
6
5
4
3
2
1
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
FRACTIONS OF LENGTH

Fig. 20

Chromosome II is approximately twice the length of chromosome XI and if one were to superimpose the position of the centromere of chromosome XI on to that of chromosome II in fig. 20, the end of the left arm of XI would then fall at locus 0.4 and the end of the right arm at locus 0.9. Virtually all the chiasmata formed by XI would then lie between 0.4 and 0.45, and 0.85 and 0.9 on chromosome II, a very different state of affairs from the actual chiasma distribution in chromosome II. The mean chiasma frequency for bivalent II is 2.8, and for bivalent XI is 1.7.

Squash preparations of male meiosis show that at diplotene (fig. 27a and b) and metaphase (fig. 27c and d), in this sex also, chiasmata tend to be proterminally localized in the shorter bivalents, but not in the longer. Chiasma distribution is similar in the two sexes.

Fig. 19b is a histogram showing the chiasma distribution in 42 bivalents XI collected from 7 animals heterozygous for the inversion. There are no chiasmata formed in the longer inverted arm pair. The distribution of 50 chiasmata in the shorter arm pair, i.e. the uninverted arm, is remarkable in two respects. First, chiasmata are now spread throughout the length of the arm. Secondly, more than one chiasma may be formed in that arm (fig. 21b), an event which has never been encountered in normal bivalents XI. Fig. 21a is a histogram showing the distribution of chiasmata in 34 inversion heterozygote bivalents where only a single chiasma was formed in the shorter arm pair and fig. 21b shows the distribution in 8 bivalents where two chiasmata were formed in the shorter arm pair. The solid lines in this figure couple the chiasmata together in pairs showing that when two chiasmata form in the short arm pair the distance between them varies. In fig. 21b it can be seen that there are few chiasmata located close to the telomere.

Fig. 21. Histograms showing chiasma distribution in bivalents XI heterozygous for the inversion in the long arm. The arrowheads mark the position of the centromere and the arrows pointing down mark the limits of the inversion.

a. In 34 bivalents XI with only one chiasma in the short arm.

b. In 8 bivalents XI with two chiasmata in the short arm. The solid lines couple the chiasmata together in pairs showing that when two chiasmata form in the short arm the distance between them varies.

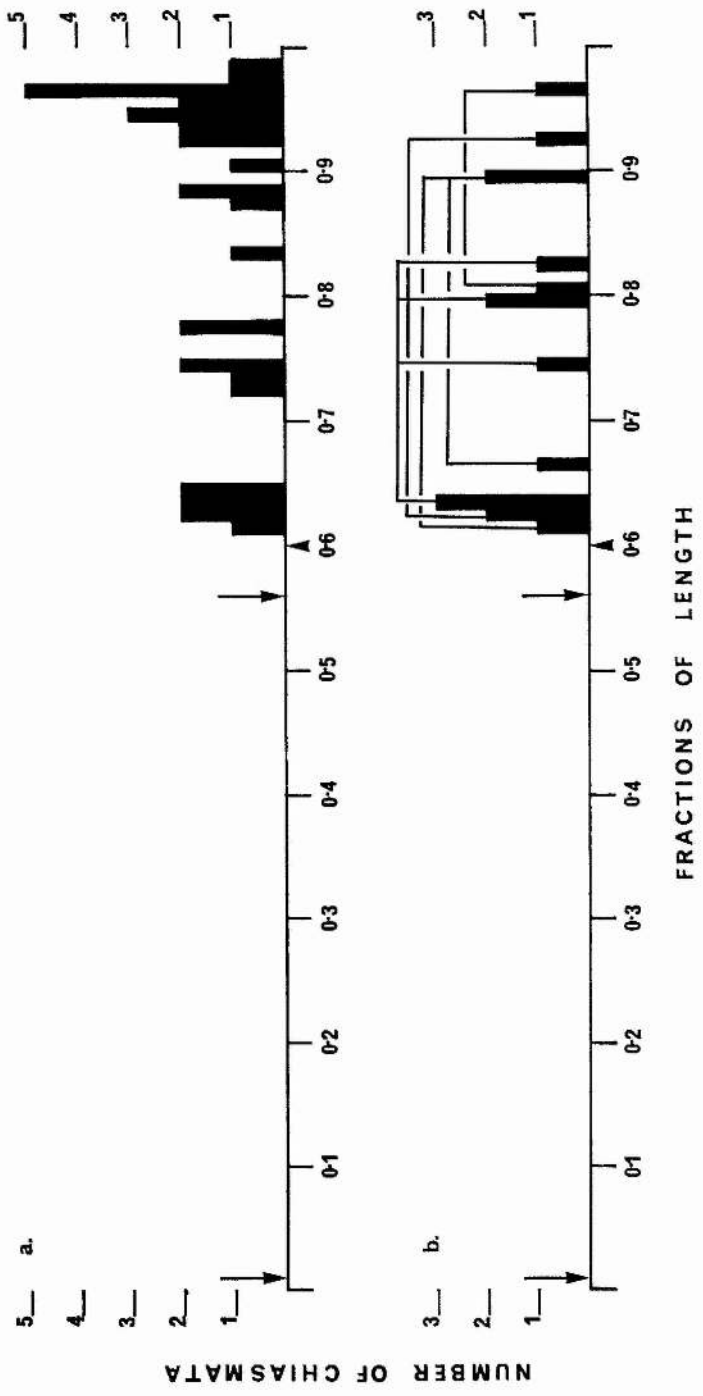

a.
b.
5
4
3
2
1
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
NUMBER OF CHIASMATA
FRACTIONS OF LENGTH

Fig. 21

Of the 7 animals carrying the inversion that were studied in detail, 5 animals showed unrestricted distribution of chiasmata in the shorter arm pair similar to that shown in fig. 22a, while the other 2 animals had chiasmata localized close to the telomere (fig. 22b). There is thus some variation between individuals possessing the inversion in the heterozygous condition.

There is no information available regarding male meiosis in inversion heterozygotes.

DISCUSSION

In N. viridescens it would appear that a similar pattern of chiasma distribution exists, at least in two of the chromosomes, chromosomes II and XI, in both sexes. In other species of urodeles, and also in other animals, where chiasmata are restricted in distribution in one sex, in the other sex chiasmata are not so restricted (Watson & Callan, 1963; Perry & Jones, 1974; Callan & Perry, 1976). For the smallest chromosome of N. viridescens, in structurally homozygous individuals genetic recombination in regions close to the centromere must be a relatively rare event. The difference in chiasma distribution between the long chromosome II and the short chromosome XI in female meiosis of N. viridescens is striking. A similar situation, although not so striking, has been described in the spermatocytes of the grasshopper Stethophyma grossum (Perry & Jones, 1974). Chiasmata are usually procentrically localized in male meiosis of this animal but 4 of the 11 bivalents have interstitial or proterminal chiasmata.

The lack of chiasmata in the longer arm pairs of bivalents XI in inversion heterozygotes is probably due to asynapsis of the whole arm. In amphibians, wherever zygotene has been studied, chromosome pairing begins at the telomeres, which lie close together on the nuclear membrane, and

Fig. 22. Histograms showing chiasma distribution in bivalents XI heterozygous for the inversion in the long arm. The arrowheads mark the position of the centromere and the arrows pointing down mark the limits of the inversion.

a. In 21 bivalents XI from a single animal in which the chiasmata formed in the short arm were not restricted in their distribution.

b. In 12 bivalents XI from 2 animals in which chiasmata in the short arm were confined to the terminal region.

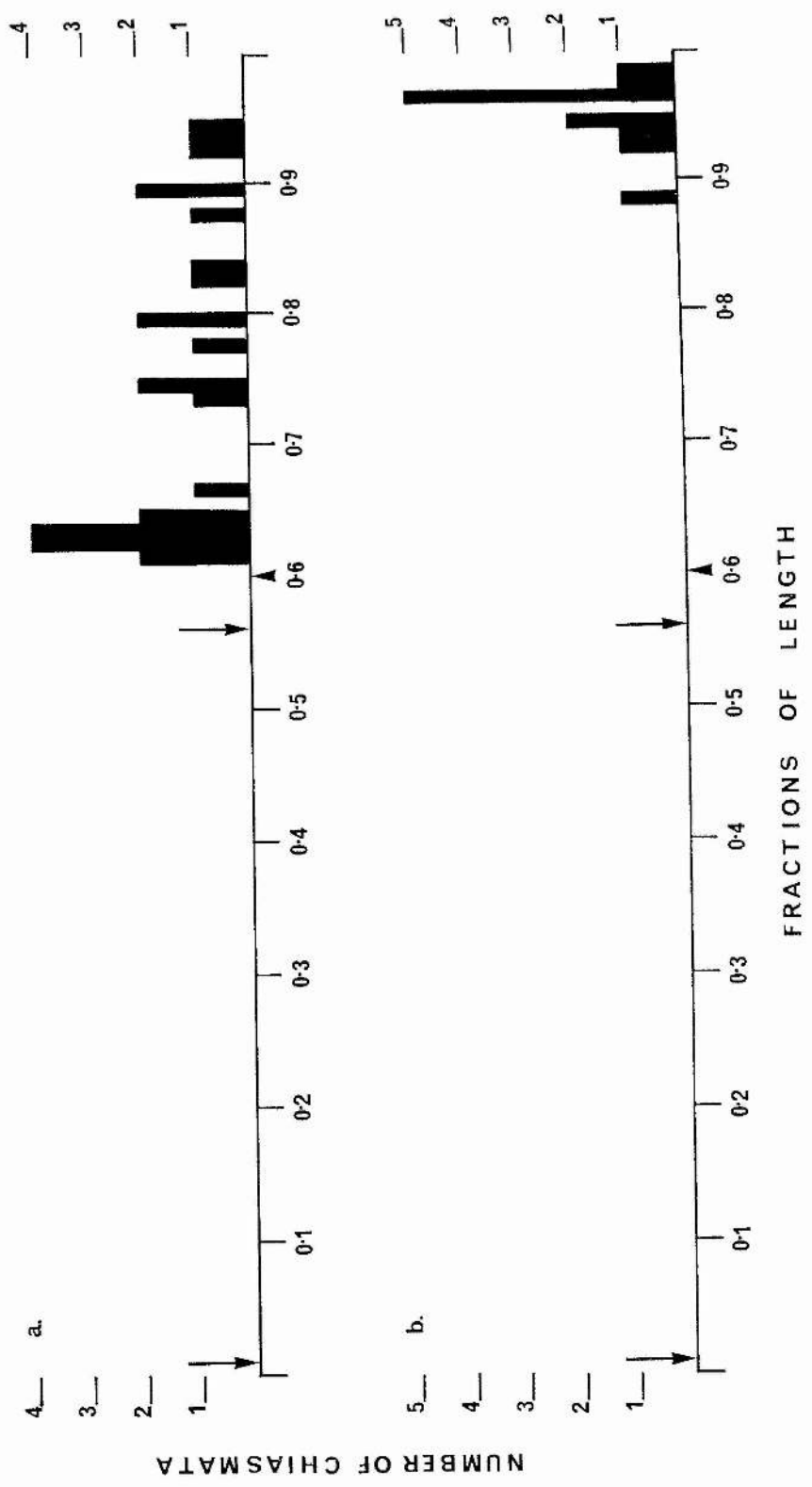

a.
b.
NUMBER OF CHIASMATA
FRACTIONS OF LENGTH
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1
2
3
4
5

Fig. 22

proceeds towards the centromeres generating the classical "bouquet" configuration (Beçak, Beçak & Rabello, 1967; Callan & Taylor, 1968, Kezer, 1970). In inversion heterozygotes the extent of the inversion, involving almost the whole of the longer arm, must impose restrictions on the degree of pairing possible as homologous regions will be at opposite poles of the nucleus.

The inversion described by Mancino et al. (1970) in T.v. meridionalis had its limits outwith the area in which chiasmata are normally formed; chiasmata are procentrically localized in female meiosis in this species, and no alteration from normality of chiasma frequency or distribution was observed in the inversion heterozygotes. Lacroix & Loones (1974) studied the distribution of chiasmata in several structurally heterozygous females of P. poireti. They found a long pericentric inversion on bivalent VI which involved almost the whole of the chromosome (break points at 0.05 and 0.97). Out of 24 bivalents studied 15 did not have chiasmata within the inverted segment, but the remaining 9 had formed several. In this case, because the inversion involves most of the lengths of both chromosome arms, synapsis in the inverted region can take place provided homologous regions happen to lie within neighbouring nuclear domains. Two other pericentric inversions were observed by Lacroix & Loones; one in chromosome V and the other in chromosome VIII and in neither case were chiasmata formed in the inverted region. The inversion in chromosome VIII just included the centromere (the centromere in this chromosome lies at locus 0.55 and the break points occurred at 0.53 and 0.76) but apart from this the situation facing synapsing heterozygous chromosomes VIII must be much like that facing synapsing heterozygous chromosomes XI of N. viridescens and the outcome is the same; total failure to form chiasmata within the inverted region. However there is evidence (Callan, unpublished observations) that

synapsis can take place between mutually inverted chromosome regions during the male meiosis of Triturus vulgaris, for occasional dicentric bridges and short acentric fragments have been observed at first meiotic anaphase in several animals. All such fragments are very short, indicating that the inversions themselves are very short and situated near the chromosome ends.

The most striking feature of the chiasma distribution observed in the inversion heterozygotes of N. viridescens is the shift from proterminal localization to unrestricted distribution within the short uninverted arm of the bivalent. The factor or factors responsible for restricting chiasmata to certain regions of chromosomes still remain to be determined. Darlington (1936, 1940) and more recently Henderson (1969) have proposed that incomplete pairing is responsible for chiasma localization and experimental disruption of pachytene pairing (e.g. heat shocks) causes chiasmata to become more localized (Barber, 1941). However in T. helveticus spermatocytes, whose chiasmata are proterminally localized, aand where pachytene is relatively easy to study, synapsis is complete (Watson & Callan, 1963) and it is now generally accepted that synapsis is complete even in those meioses (e.g. certain grasshoppers) which show strict chiasma localization (reviewed by John, 1976).

Carpenter (1975) has described certain transient structures, recombination nodules, associated with the synaptonemal complex during female meiosis in Drosophila melanogaster. The distribution of recombination nodules, in terms of number per cell and chromosomal localization, show good agreement with the frequency and distribution of recombination events and therefore the availability of these organelles may determine the frequency and distribution of the chiasmata. For this to be the case it

must be assumed that at least one recombination nodule normally becomes assigned to each pair of synapsed chromosomes, otherwise the occurrence of univalents in meioses where the mean chiasma frequency per bivalent is little more than one would be common. Taking the argument further, in meioses where strict proterminal chiasma localization occurs, e.g. T. helveticus spermatocytes (Watson & Callan, 1963), one recombination nodule would be assigned to each bivalent end and there induce a chiasma. This relationship cannot be absolute, however, as chiasma failure in one arm pair of T. helveticus occurs at a frequency of 10 - 20%. If a recombination nodule fails to associate with one bivalent arm end it may become available for association with the synaptonemal complex elsewhere. This could account for the occurrence of two chiasmata in the right arm pair of bivalents XI of N. viridescens which are heterozygous for the long inversion in the left arm.

The observation of compensatory changes in chiasma frequency is by no means novel. Schultz & Redfield (1951) found that when crossing over is suppressed by inversions in two of the major chromosomes of D. melanogaster, the incidence of crossing over in the third chromosome is significantly increased. Darlington (1933) demonstrated a negative correlation, within nuclei, between the number of chiasmata formed by a recognizable extra bivalent in rye and the number of chiasmata formed by all the other 7 bivalents, which implies that there is some kind of unitary control over the number of chiasmata formed in any one nucleus. Mather & Lamm (1935) and Mather (1936a, 1969) made an extensive statistical analysis of cytological data and showed that a similar "competition" for chiasmata operates in Vicia and maize.

The disruption of chiasma localization in the uninverted arm pairs of bivalents XI of N. viridescens which are inversion heterozygotes is not easily explained. Chiasma interference can scarcely explain the distribution of chiasmata in normal bivalents XI, particularly in the longer arm pairs which account for 60% of the chromosome, as the majority of chiasmata formed in this arm pair lie within the 10% of the arm closest to the telomere. It is possible, however, that in the reduced synaptic length of inversion heterozygote bivalents XI chiasma interference does come into play. The histogram fig. 21a shows the frequency and distribution of single chiasmata in the uninverted arm pairs and suggests some clustering of chiasmata close to the centromeres and close to the telomeres i.e. at either end of the synapsed length but not at both ends coincidentally. In other words if a chiasma forms close to the centromere the formation of another, close to the telomere, is suppressed, and vice versa.

Mather (1936b) suggested that the distribution of chiasmata within the chromosomes of D. melanogaster could be comprehended if chiasmata are formed in a regular time sequence starting at the centromere: the first chiasma forming at a certain distance from the centromere and other chiasmata forming subsequently under the restraint of chiasma interference.

If chiasma interference does influence chiasma distribution in the uninverted arm pairs of inversion heterozygote bivalents XI, this control cannot be absolute for 8 bivalents out of a total of 42 formed two chiasmata in the uninverted arm pairs. If recombination nodules are necessary for chiasma formation it may be that the timing of their attachment to the synaptonemal complex is important. If one nodule attaches first and generates a chiasma it may in some way preclude the attachment of another in the same arm pair i.e. cause chiasma interference; on the contrary, if two

nodules happen to attach themselves simultaneously, this might generate two chiasmata much closer together than chiasma interference normally permits. Chiasma interference remains an unexplained though widespread phenomenon, and a challenge to cytologists.

Fig. 23. Phase contrast photograph of bivalent XI heterozygous for an inversion in the long arm of one of the homologues.

C = centromere in the inversion chromosome

ch = chiasma

isl = sequential loops in the inversion chromosome

nsl = sequential loops in the normal chromosome.

Scale = 50 µm

Fig. 24. Phase contrast photograph of bivalent XI heterozygous for an inversion in the long arm of one of the homologues.

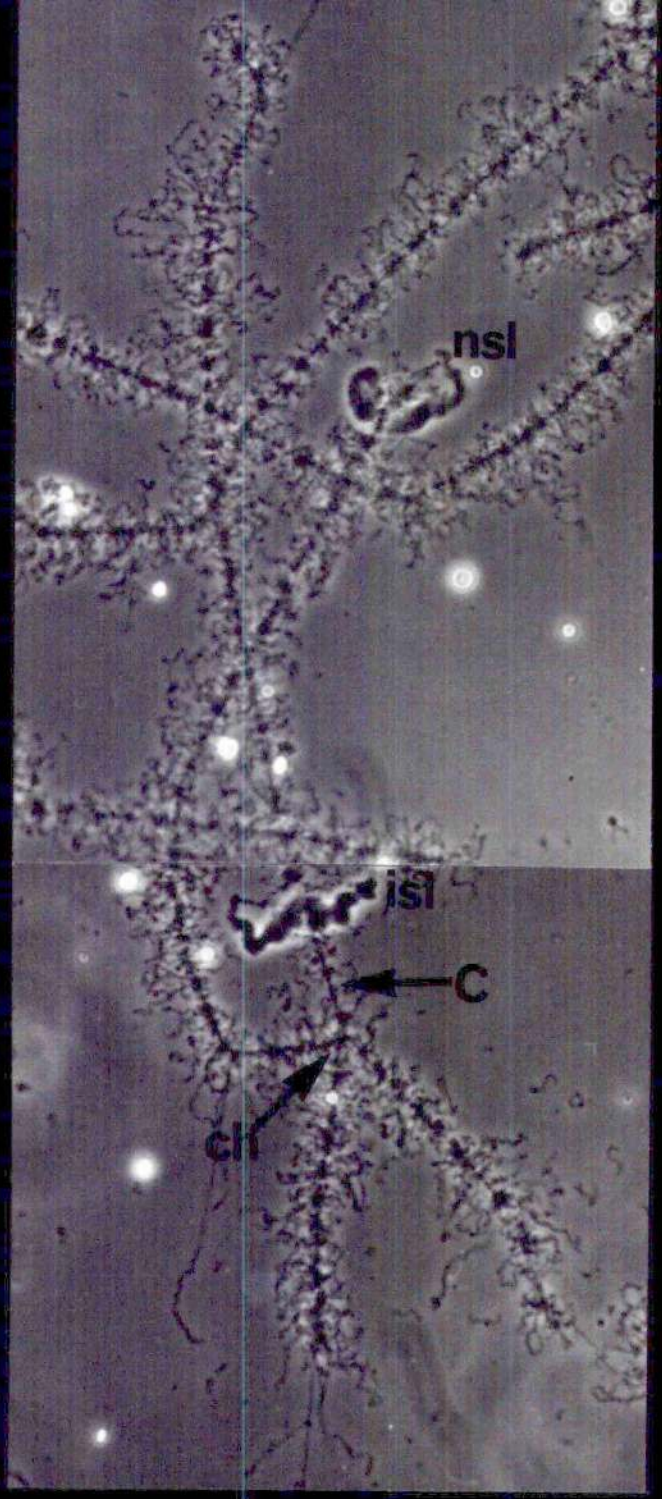
C = centromere in the inversion chromosome

ch = chiasma

isl = sequential loops in the inversion chromosome.

nsl = sequential loops in the normal chromosome.

Scale = 50 µm


nsl
isl
C
cl

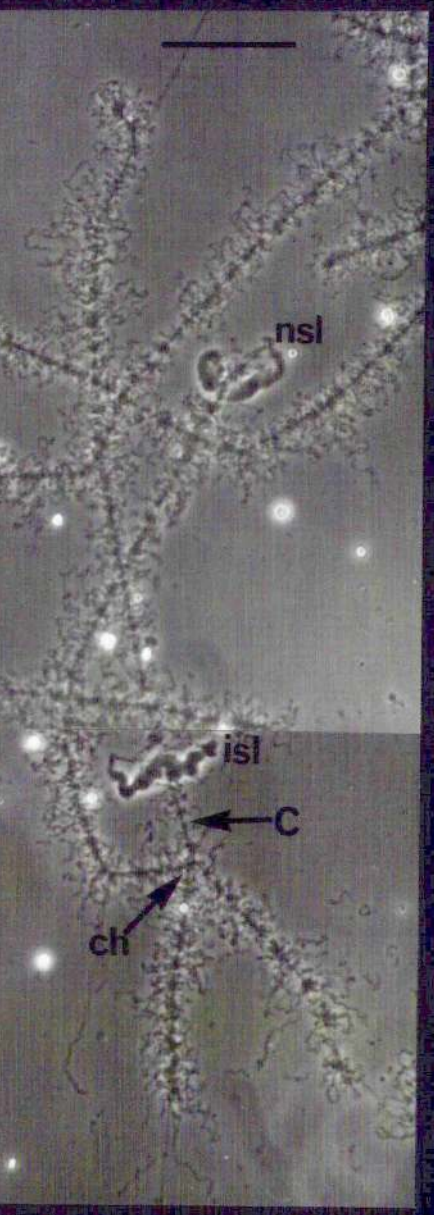

nsl
isl
C
ch

Fig. 24. Phase contrast photograph of bivalent XI heterozygous for an inversion in the long arm of one of the homologues.

C = centromere in the inversion chromosome

ch = chiasma

isl = sequential loops in the inversion chromosome.

nsl = sequential loops in the normal chromosome.

Scale = 50 µm

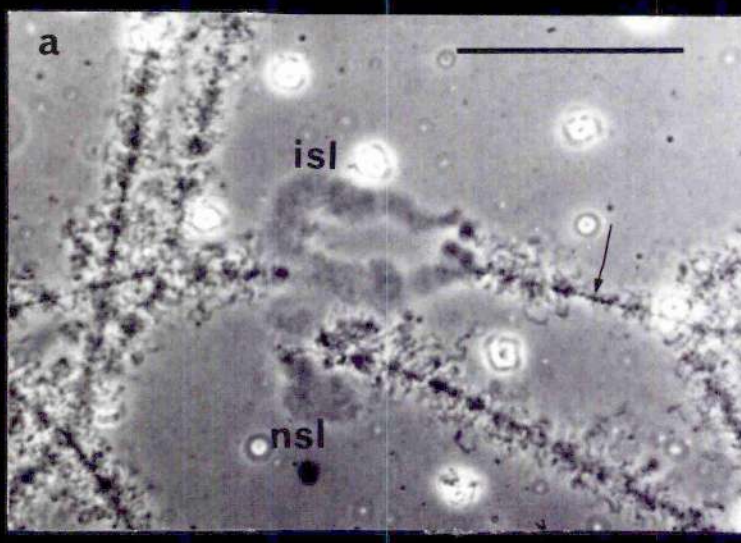
Fig. 26. Phase contrast photograph of a normal bivalent XI.

C = centromere

ch = chiasma

sl = sequential loops which are fused together and masking one of the chiasmata.

Scale = 50 µm


a
isl
nsl

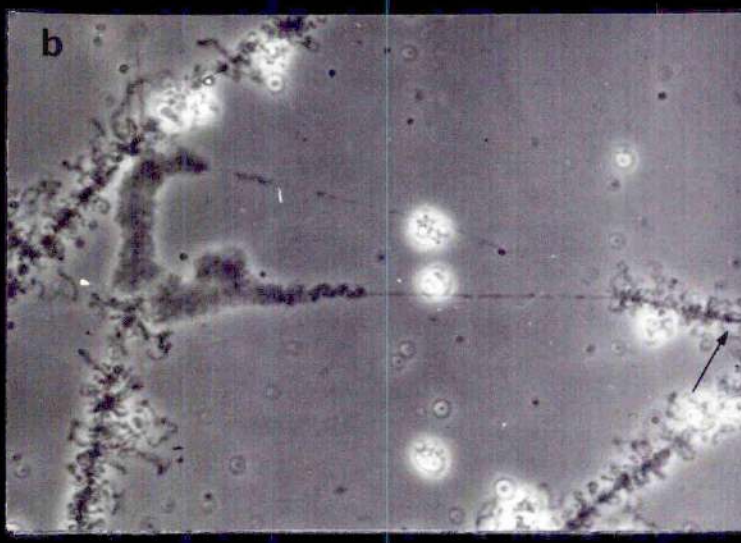
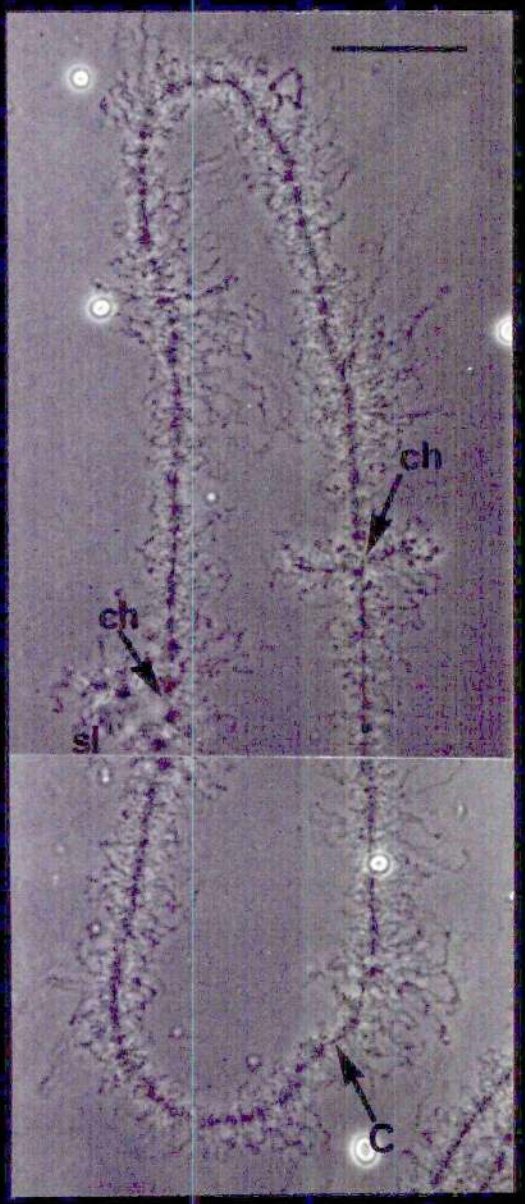

b

Fig. 27. Photographs of carmine-stained squash preparations of male meiosis.

a & b. Diplotene of 1st meiotic prophase. The large arrowheads mark the two longest chromosomes, one of which is probably chromosome II and the small arrowheads mark the two shortest chromosomes, one of which is probably chromosome XI.

c & d. 1st meiotic metaphase.

Scale = 20 μm


ch
ch
sl
C

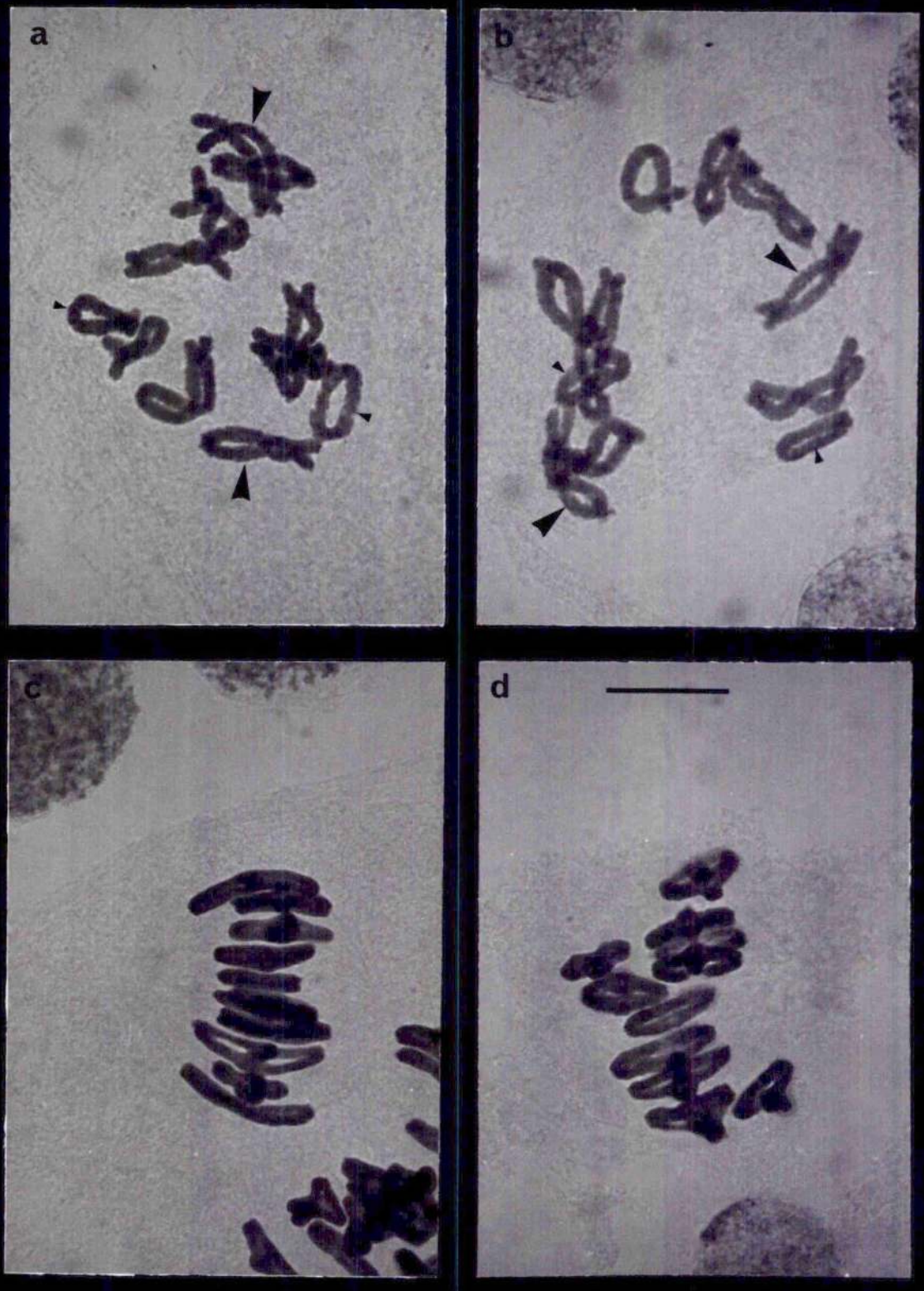
SUMMARY

The morphological variability and transcriptional activity of the giant loops on chromosome II of Notophthalmus (Triturus) viridescens has been studied. The giant loops are distinguished from the ordinary loops by their length and the greater bulk of their matrix, which may consist of more than one transcriptional unit. The loops were studied in respect of their ability to incorporate the individual precursors of RNA, and their response to the physiological stimuli of hormone injections and cold treatment.

It was found that the size of the loops and the matrix distribution pattern of any one loop pair remains constant in oocytes ranging in size in any one animal. Hormone injections were observed to cause alteration in the distribution of matrix in loops having two polarized matrix units. Hormone injections may also cause changes in length of the giant loops. Cold treatment does not significantly affect the lengths of the giant loops but causes marked shortening of the ordinary lateral loops. Autoradiographic experiments have shown that the giant loops synthesize RNA at approximately half the rate of the ordinary loops and that this RNA is deficient in guanine.

These results are discussed in terms of the organization of lateral loops and their attendant matrix, differing rates of attachment and movement of RNA polymerase molecules, different types of RNA polymerase molecules and the organization of highly repetitive sequences in the genome.

Observations on the effect of inversion heterozygosity on chiasma distribution are described for chromosome XI and discussed in terms of the availability of recombination nodules.


a
b
c
d

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