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Some Trends in the Evolution of Very Large Chromosomes

H. C. MacGregor

Phil. Trans. R. Soc. Lond. B 1978 **283**, 309-318
doi: 10.1098/rstb.1978.0030

Phil. Trans. R. Soc. Lond. B. **283**, 309–318 (1978) [309]
Printed in Great Britain

Some trends in the evolution of very large chromosomes

By H. C. Macgregor
Department of Zoology, School of Biological Sciences, University of Leicester, Leicester LE1 7RH, U.K.

[Plates 1–4]

The general features of the arrangement and cytological distribution of repeated sequences in animal chromosomes are reviewed. These features include internal repetitiveness, conservation of clearly functional sequences, rapid divergence of certain classes of repeated sequence with subsequent fixation of families of diverged sequences, and well defined cytological localization of large blocks of sequences in specific parts of the chromosome set. Moderately or 'middle' repetitive (m.r.) sequences constitute a large part of the genomes of higher organisms, they seem to accumulate in a balanced manner within chromosome sets, they are mainly responsible for genome growth, and they are interspersed with sequences of other kinds. Little is known about their cytological distribution.

Four experiments are described, each of which aimed to locate middle repetitive sequences in the chromosomes of a salamander and a newt. Tritiated m.r. DNA from *Plethodon cinereus* binds in a non-random fashion to the meiotic diplotene and mitotic chromosomes of the same species, suggesting a non-random distribution of m.r. sequences on these chromosomes. The same DNA, hybridized *in situ* to the RNA transcripts on the loops of lampbrush chromosomes, produces light and widespread labelling of many loops, but intense labelling of six pairs of loops, each of which lies near to a centromere. Similar experiments in which newt m.r. DNA was hybridized *in situ* to newt lampbrush chromosomes showed heavy labelling of about 30 loop pairs on each of the long heteromorphic arms of chromosome I, but very little labelling elsewhere. Autoradiographs of newt mitotic chromosomes hybridized with newt m.r. DNA showed rather even labelling of all chromosomes including chromosome I. The significance of the heavy labelling of lampbrush loops near centromeres and on the heteromorphic arms of the newt chromosome I is discussed in relation to possible cytological and molecular mechanisms for generating and preserving families of tandemly linked and cytologically localized m.r. sequences.

The range of genome size among organisms is particularly well illustrated by the fact that a single chromomere in a meiotic chromosome from a crested newt has about as much DNA as the entire fourth chromosome of *Drosophila melanogaster*, which in turn has about as much DNA as the entire genome of the bacterium *Escherichia coli*. To a cytologist, this sort of difference is simple to appreciate. One has only to look at chromosomes and take photographs at comparable magnifications. Figures 1–3 show three well known situations. The first is a diploid chromosome set from *Triturus cristatus* ($C = 29$ pg), the second is a set of metaphase chromosomes from a human male ($C = 3$ pg), and the third is a set of mitotic chromosomes from a neuroblast of *D. melanogaster* ($C = 0.14$ pg; see Laird 1971). All three are at the same magnification.

Two general points can be drawn from this demonstration. First, since big chromosomes have more DNA than small ones, the genome of a newt might be expected to contain much more genetic information than that of a man, whose genome will in turn have more information than that of a fruit fly. Secondly, newts, like most tailed amphibians, have very large genomes

indeed. In this paper I shall concentrate upon some of the questions that arise in connection with the origin and evolution of the relatively large genomes of eukaryotes, and, since my own interests are centred around the very large genomes of amphibians, I shall pay special regard to some of the remarkable situations that we find among this particular group of animals.

It is, of course, convenient and sensible to think of genomes as consisting of sequences of nucleotides linked together in a tandem fashion to form the enormously long DNA molecules that we suppose to be the main components of eukaryotic chromosomes. It is therefore appropriate that we should try to determine the arrangement of DNA sequences in chromosomes, and that we should think about how such an arrangement happened to evolve.

TABLE 1

	sequence size range (nucleotide pairs)	copies per genome	function	chromosomal distribution	selection for conservation
satellites	2 (crab) to 1400 (calf)	up to 40 000 000	?	heterochromatin	*very* low
'functional repetitive'	several thousand (rDNA 12 000 to 14 000)	100 to 24 000	ribosomal RNA 5*S* RNA tRNA histone mRNA	localized (e.g. nucleolus organizer)	*very* high
'middle repetitive'	100 low 300 average 4000 high	up to 10 000	?	general (?)	low
single copy	600 to several thousand	1	mRNA transcription	general (?)	high

Table 1 shows a rough classification of the different types of DNA sequence that can be identified in a eukaryotic genome, and some of the characteristics of these types. I do not propose to say anything about single copy or 'unique' sequences, apart from observing that there is a considerable body of evidence to suggest that at least some single copy sequences are structural genes that are transcribed into mRNAs that are subsequently translated into functional polypeptides.

Several trends emerge when we consider the whole spectrum of repetitive DNA sequences. First we have the very fact of repetitiveness. At one end of the scale we have highly repetitive 'satellite' sequences that may be represented by a million or more copies in the genome, as for example, in the cow and the mouse (see Botchan 1974; Southern 1975). At the other end of the scale we have sequences that are classified as 'middle repetitive' (m.r.) that may be represented by a few tens or hundreds of copies. Within this range we have clearly functional sequences such as those for 18*S* and 28*S* ribosomal RNA, 5*S* RNA, transfer RNAs and histones. Studies of several different satellite DNAs, and of the ribosomal and 5*S* DNAs in particular, have revealed a remarkable common feature that has given strong clues as to the mechanism of evolution of repeated sequences. The main clue is internal repetitiveness. The bovine satellite, for example, has an EcoRI cleavage site that exists at 1400 base-pair intervals, but the smallest identifiable repeat is just 300 base pairs long (Botchan 1974). The mouse satellite probably has 3 or 4 levels of repetitiveness, with the largest repeat composed of about 240 base pairs, and the smallest between 9 and 18 base pairs (Southern 1975; Biro, Carr-Brown, Southern & Walker 1975). The 5*S* DNA of *Xenopus* is about 700 base pairs long and is repeated more than

20000 times. Each repeat consists of three clearly identifiable regions, of which one, the so-called A–T-rich spacer, is variable in length and is made up of 15 base-pair sub-repeats (Brown & Sugimoto 1973; Carroll & Brown 1976*a*, *b*). The ribosomal DNA of *Xenopus* exists as a series of several hundred repeats of a sequence of molecular mass $7.2–8.7 \times 10^6$ (about 12000 base pairs in length). About one third of this is known as the non-transcribed spacer region, and it is quite variable in length (Wellauer *et al.* 1976*a*, *b*). The variability is known to reflect differences in the numbers of small sub-repeats, each about 50 base pairs long, in the 'D' region of the spacer. Similar spacer variability has been demonstrated in other organisms (Spring *et al.* 1976; Trendelenburg, Scheer, Zentgraf & Franke 1976) and it doubtless reflects the same basic phenomenon: that of variable numbers of sub-repeats within larger repeats.

The numerical variation of sub-repeats within spacers is copied on a larger scale in the wide variability that one finds in the numbers of repeats of the whole ribosomal sequence that are found at supposedly homologous nucleolus organizers in the same or different individuals. For example, we have recently shown that in one small interbreeding population of *P. cinereus*, nucleolus organizers vary in size from one salamander to another over a range of as much as 15-fold. This means a range of repetition frequency for the main ribosomal sequence of between 218 and 3272 copies, with no two animals having nucleolus organizers of the same size (Macgregor, Vlad & Barnett 1977).

The next remarkable characteristic of repetitive sequences such as satellites and the ribosomal and 5*S* spacers is that they diverge more quickly than the species evolve, and the diverged forms tend to become fixed and homogenized with astonishing rapidity. The non-transcribed rDNA spacer of *X. laevis* is quite different from that of *X. mulleri*, and both are repeated many times over in the nucleolus organizers of the respective animals (Brown, Wensink & Jordan 1972; Brown & Sugimoto 1973). Closely related organisms can have quite different, but nonetheless strikingly homogeneous, satellites. Our recent studies of centromerically localized satellites in eight different populations of *P. cinereus* have shown that these different G+C-rich satellites are distributed alone or in pairs among each of the eight populations. On the basis of their buoyant densities and their inabilities to cross-hybridize with one another, we estimate that these satellites must not only be distinctly different from one another with respect to sequence, but each must be quite homogeneous in the population in which it occurs.

The mechanism of evolution of satellite DNAs will not be easy to identify, for there are many gross variables and subtle sophistications that have to be accounted for. The mouse satellite has a large repeat whose internal repetitiveness provides clues as to possible evolutionary pathways. The satellite sequences of *D. virilis* have remained short and homogeneous, numerous and tandemly linked, betraying their evolutionary history only in so far as they have a few base substitutions that suggest a common ancestral sequence (Gall, Cohen & Atherton 1973; Gall & Atherton 1974). The satellites of certain crustaceans seem to include short sequences that are also represented in satellites from guinea pigs and kangaroo rats (Skinner *et al.* 1974). But let us not delve too deeply into these idiosyncrasies. The existence of repeats and sub-repeats, variation in numbers of tandemly linked repeats, and rapid evolution, fixation, and homogenization, are the features that are most significant in the present context.

To a cytologist, quite the most striking feature of certain repetitive sequences is their localization in certain regions of the chromosome set, and here I turn to evidence that comes from the technique of nucleic acid hybridization *in situ*. Highly repetitive DNA is generally

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concentrated in clearly defined parts of the karyotype. In the simplest situation, a whole family of repetitive sequences is exclusively localized at one site on one chromosome in the set. Some nucleolus organizers and 5*S* loci are like this. *X. laevis* has just one nucleolus organizer. *Zea mays*, the newt *Taricha granulosa*, and man, each have just one 5*S* locus (Wimber, Duffey, Steffenson & Prensky 1974; P. E. Leon, personal communication; Steffenson, Duffey & Prensky 1974; Johnson, Henderson & Atwood 1974). However, man has five nucleolus organizers (Evans, Buckland & Pardue 1974; Ferguson-Smith & Handmaker 1963), all on the short arms of D and G group chromosomes. The chironomid *Glyptotendipes barbipes* has six 5*S* loci (Wen, Leon & Hague 1974). *Notophthalmus viridescens*, an American newt, has 5*S* loci near the centromeres on four or five chromosomes (Hutchison & Pardue 1975; Pukkila 1975), and, most extreme of all, *X. laevis* has 5*S* loci at the ends of the long arms of all its 18 chromosomes (Pardue, Brown & Birnstiel 1973).

When we consider satellite DNA we see the same general kinds of phenomena, except that there are few satellites that are known to be restricted to just one chromosome. In *P. cinereus*, the heavy satellite is found near the centromeres on all 14 chromosomes, and larger chromosomes have more satellite than smaller ones (Macgregor & Kezer 1971; Macgregor, Horner, Owen & Parker 1973). In mouse, the light satellite is found near the centromeres of all the 20 acrocentric chromosomes (Pardue & Gall 1970; Jones 1970). In man, on the other hand, there are four different satellites that are widely scattered over the chromosome set; but again, it must be significant that all these show a preferential localization near to the centromeres of the chromosomes in which they reside (Gosden *et al.* 1975).

In general, then, wherever we have been able to identify, isolate, and probe cytologically for highly repetitive sequences such as ribosomal, 5*S*, or satellite DNA, they have proved to be concentrated into certain regions in the chromosome set. If there are several of these regions, then they are usually on different chromosomes, and they are often found near the centromeres or the telomeres.

So much for ribosomal genes and satellites; now let us turn our attention to middle repetitive DNA. M.r. DNA is particularly important when we are considering the larger eukaryotic genomes, since it constitutes a very substantial component of the genome. Here I will centre my remarks around plethodontid salamanders, since I have worked with these animals and I have some first-hand knowledge of the arrangement of their DNA sequences.

The genome of *P. cinereus* consists of DNA of molecular mass 1.2×10^{13} (20 pg) organized into 14 metacentric and submetacentric chromosomes (Mizuno & Macgregor 1974). About 2% of the genome consists of high density satellite DNA, less than 0.5% consists of ribosomal DNA, 40% consists of single copy sequences, and most of the remainder consists of m.r. sequences. The latter are 200–500 base pairs long, and they have an average repetition frequency of about 7000 times. Such figures are fairly typical for m.r. DNA in a wide variety of organisms: essentially, short sequences repeated several hundreds or thousands of times.

Plethodon has proved to be a particularly useful genus in which to study several aspects of the evolution of m.r. DNA. It has several species whose taxonomic relations are well understood and extensively documented (Highton 1962; Wake 1966), different groups of species have widely different genome sizes, and there are excellent opportunities for cytological studies with regard to mitotic, meiotic and lampbrush chromosomes.

I wish to make three points concerning the m.r. sequences with some special references to *Plethodon*. First, the genome of *P. cinereus* has 20 pg of DNA. That of its relative, *P. dunni*, has

39 pg. Both have 14 chromosomes, and their karyotypes are indistinguishable on any basis other than the gross sizes of the chromosomes. Since the most primitive plethodontids have genomes of around 20 pg, it is reasonable to assume that the large *dunni* genome evolved from a smaller *cinereus* type genome by addition of DNA sequences. Half of the *cinereus* genome consists of m.r. sequences as compared with at least 80 % in the *dunni* genome (Mizuno & Macgregor 1974). So we may suppose that growth of the genome was largely a consequence of accumulation of additional m.r. sequences in such a manner that each chromosome arm acquired new sequences in proportion to its existing load. Immediately we can ask, but not answer, the question: how is such a balanced accumulation of m.r. sequences accomplished?

Secondly, if we compare the m.r. sequences of *P. cinereus* with those of *P. dunni*, two species that have a common ancestor but have been separated from one another for 50–60 Ma, then we find that they have only about 10 % of their m.r. sequences in common (Mizuno, Andrews & Macgregor 1976). Indeed, right across the genus, the general populations of m.r. sequences are markedly different from one group of species to another. We take this to indicate that there is little selective pressure for the maintenance of these sequences, and we think that they cannot therefore have any function in the transcriptive/translative sense. However, about 2.5 % of the m.r. sequences of *Plethodon*, representing about 1 % of the genome, are common to all species of *Plethodon*, and are also found in the genomes of certain other genera of amphibians (Mizuno *et al.* 1976). These common m.r. sequences appear to be the products of a positive selection of certain specific populations of ancestral m.r. sequences that have been extraordinarily well conserved, both with respect to sequence and repetition frequency, over a long period of evolutionary time.

Thirdly, I must mention what must surely be the best known, and the most puzzling, feature of m.r. sequences: their arrangement with respect to one another and with respect to single copy sequences. According to Davidson and his colleagues (Davidson *et al.* 1975*a*, *b*), these short repetitive elements are extensively interspersed among non-repetitive sequences of two or three thousand base pairs in length, and in organisms like *Xenopus* and sea urchins, where the situation has been extensively investigated, a major proportion of the chromosomal DNA is organized in this manner. A different pattern of m.r. arrangement seems to prevail in *Drosophila* and *Apis* (Crain, Davidson & Britten 1976), but we will not concern ourselves with that here. Undoubtedly, interspersion is a fact of genome organization that must be accepted. Nevertheless, in my opinion, the experiments that demonstrate interspersion of m.r. and single copy DNA are unsatisfactory in two ways. First, they look mainly at sequences that form stable duplexes with less than 10 % mismatching, and although they detect less stable duplexes under less stringent conditions, they nevertheless miss, or only dimly resolve, the possibility of large families in which the sequences are related but different in the same sense that the satellites of *D. virilis* are related but different (Gall & Atherton 1974). Secondly, interspersion experiments do not tell us whether well matched members of one family of m.r. sequences are clustered in one part of the genome, in spite of being interspersed with other kinds of sequence. After all, interspersion experiments always begin with DNA that has been more or less sheared, so how can we tell where a sequence resided on a chromosome in relation to other sequences that were more distant than the maximum lengths of the sheared fragments? Essentially, the question remains: are members of a well matched group of repetitive sequences widely scattered throughout the entire chromosome set of an organism, or are they generally – though not necessarily contiguously – clustered in one region? Still less do interspersion

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experiments tell us whether related but different m.r. sequences are concentrated in specific regions of a chromosome set, yet from what is known of the satellites of *D. virilis*, we may be right to expect this kind of concentration.

What I have said so far must be enough to indicate that to reach the arrangements that exist today among DNA sequences in large chromosomes, three basic things had to be accomplished. First, those genes that are transcribed and translated into biochemically functional proteins, or proteins whose sequences are otherwise important, have had to be protected from change. It is surely not unreasonable to ascribe this accomplishment to natural selection. Essentially, base substitutions in sensitive areas will constitute lethal mutations. Structural genes may undergo duplications by one mechanism or another, but this may be irrelevant: as long as there is one good copy of the gene, all will be well. Clearly, there may be selective advantages in some genes being repetitive beyond certain levels. If the repetitiveness falls below those levels, then once again, we have a lethal mutation.

The second thing that had to be accomplished was repetitiveness. In 1973, Smith introduced a remarkable and inspired notion that repetitiveness and the establishment of repetitive families could be brought about by the simple process of unequal crossing over or sister chromatid exchange following or accompanying chromosome replication. In fact, unequal crossing over may be fully capable of producing all the situations that are known to exist among the sequences of eukaryotic genomes. Repeats are a simple matter, so are adjacent reverse repeats, so are palindromes (snap-back sequences). Indeed, it is tempting to say that we need look no further. The cytological opportunities for unequal crossing over and exchange are abundant. It has been estimated from studies of cells of *Triturus cristatus* grown in tissue culture that there may be as many as 15 sister chromatid exchanges (s.c.es) per chromosome set per division (Rudak 1976). We have no reason to suppose that s.c.es do not happen just as frequently in the germ line of that animal. We know that regions of chromosomes that have highly repetitive sequences are often regions that associate in cells, thereby increasing the opportunities for molecular interactions and exchanges between DNA molecules. Specific examples could include chromocentres, which commonly contain highly repetitive satellite DNA, and telomeres, which often come together in meiosis during the formation of the presynaptic bouquet (Pardue *et al.* 1973).

One feature of unequal chromatid exchange in regions where there are interspersed repetitive and single copy sequences is particularly noteworthy. Inevitably, each exchange will give rise to a shorter and a longer product. If one continually selects for the longer product then nothing is lost, repeats are generated, and the 'genome' becomes larger. However, selection for the shorter product may lead to losses. A consideration of this kind has led me to suppose that if unequal exchanges are indeed a major factor in chromosome evolution, then genomes should tend to grow or remain the same. Shrinkage might be dangerous, in the sense that it would carry the risk of inadvertent loss of vital genes.

The third matter that has somehow to be accomplished is interspersion, and this is something that I cannot speculate upon at present, because we do not know enough about interspersion on a scale that goes beyond the lengths of experimental DNA fragments. However, if we are to take interspersion to mean extensive and widespread scattering of members of individual families of repetitive sequences over a chromosome or chromosome set, then I think the problem is considerable, and I cannot see a solution to it at present.

The question that remains unanswered at this stage, and which is related directly to the problem of interspersion, is: how are the m.r. sequences dispersed or interspersed in the

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chromosomes that make up a large genome? Essentially, where are they, and what do they do? I wish to conclude this paper by describing the results of some recent experiments from my laboratory, and speculating just a little on their significance. There are four of these experiments. All of them were aimed at localizing m.r. sequences in chromosomes of *Plethodon* and *Triturus* by the technique of nucleic acid hybridization *in situ*. The experimental conditions are described in detail elsewhere (Macgregor & Andrews 1977). Chromosomes from spermatocytes and gut epithelia were used to determine the gross pattern of distribution of m.r. DNA by DNA/DNA hybridization. Lampbrush chromosomes were used for experiments in which labelled m.r. DNA was hybridized to nascent RNA transcripts on the lateral loops. The latter approach takes advantage of the 'amplifying' effect of the loop RNA in providing many copies *in situ* of the DNA from which it was transcribed. These copies are immediately available for binding with denatured labelled DNA (Pukkila 1975). It is most important at this point to understand clearly the kind of information that is provided by the two approaches. DNA/DNA hybridization locates actual gene sequences, and autoradiographs of *in situ* hybrids will reflect the relative abundance of particular sequences at particular sites. DNA/RNA-transcript hybridization locates sequences by the presence of their transcripts, and autoradiographs can give only the most subjective notion of the abundance of the transcribed sequence.

Let us consider experiments on *Plethodon* first. These were carried out by G. T. Morgan and they will be published in full elsewhere under his name. M.r. DNA from *Plethodon cinereus* (C_0t 0.2–50) was hybridized to spermatocyte and mitotic metaphase chromosomes from the same species. Autoradiographs showed non-random labelling of the chromosomes, with some regions of each chromosome labelling more heavily than others (figures 4 and 5). We do not yet know whether this reflects an overall differential distribution of m.r. sequences or a differential distribution according to repetitive frequency. I am more inclined to the latter explanation. We are currently investigating the possibility that the distribution of m.r. sequences is related to some cytological feature such as the distribution of s.c.es, meiotic crossing over, or synaptic patterns. It is when we consider the lampbrush *in situ* hybrids that particularly interesting situations begin to emerge. M.r. DNA hybridizes weakly to many loops on the lampbrush chromosomes of *P. cinereus*. This tells us that a small proportion of the m.r. sequences is being transcribed on lateral loops at any one time during the lampbrush phase of oogenesis. In sharp contrast, there is a series of six pairs of large loops that label very heavily indeed, even after short autoradiographic exposures. Each of these loop pairs is located immediately next to a centromere bar (figure 6). The centromere bars are pericentric regions of condensed chromatin that are rich in high density satellite DNA. In this connection, it should be remembered that our m.r. DNA does not include satellite sequences. We think that these fast-labelling loops may mark the locations of the 5*S* genes, as described by Pukkila (1975) in *Notophthalmus viridescens*. We suggest that they label fast and heavily because they are made of just one tandemly repetitive sequence, such that all of their transcript is complementary to a component of our labelled probe. Might the sequences that constitute these loops owe their tandem repetitiveness to the same general mechanism and cytological phenomenon that generated the highly repetitive satellite sequences that surround the centromeres?

The situation becomes even more extraordinary when we consider *Triturus cristatus*. Lampbrush chromosomes can, of course, be prepared from this animal with the greatest of ease, and we know a great deal about them, thanks to the thorough and far-sighted studies of H. G. Callan and his colleagues (Callan & Lloyd 1960). We have incubated lampbrush

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chromosomes from this species in a solution of tritiated m.r. DNA (C_0t 0.5–50) and we have made autoradiographs. After exposure of less than 1 week – which is considered to be short by persons accustomed to the technique of hybridization *in situ* – only about 30 pairs of loops are distinctly, and in some cases heavily, labelled. All of the remaining 4000–5000 loops are unlabelled. The most astonishing fact, however, is that all but a few of these fast-labelling loops are located in a region described by Callan & Lloyd (1960) as the heteromorphic arm (ht.a.) of chromosome I (the longest chromosome) (figures 7–9). This arm is of different lengths in the two halves of lampbrush bivalent I, the ht.a. of each half-bivalent carries a different pattern of loops that are morphologically distinctive, and perhaps most importantly, the ht.as never form chiasmata.

Can it be that all of those fast-labelling loops, of which at least three are likely to include the histone genes (Callan, personal communication), are repetitive sites where members of families of m.r. sequences are strictly tandemly linked without interspersion, whereas elsewhere in the genome, families are scattered more widely? Or can it be that the fast labelling loops reflect an oocyte-specific pattern of transcription, and that they are sites of the only m.r. sequences to be spun out into lampbrush loops and purposefully transcribed?

Our fourth and final experiment goes some way towards resolving this matter. It involved hybridizing nick-translated m.r. DNA to mitotic chromosomes from the gut epithelium of *Triturus cristatus*. The outcome was simple. The distribution of label over the chromosomes, after relatively short autoradiographic exposures was rather even, with only slight suggestions of 'hot spots' here and there (figure 10). In no circumstances was there anything special about the labelling of chromosome I. I therefore conclude that the fast labelling of loops on the

Description of plate 1

Figure 1. A set of 24 mitotic metaphase chromosomes from a tissue culture cell of *Triturus cristatus carnifex*. The chromosomes were fixed in methanol acetic acid and stained with Giemsa.

Figure 2. A set of human chromosomes fixed in methanol acetic acid and stained with Giemsa.

Figure 3. A set of mitotic chromosomes in a squash preparation of a neuroblast from *Drosophila melanogaster*. These chromosomes were fixed with ethanol acetic acid and stained with Feulgen.

Figures 1–3 are all shown at the same magnification.

Figure 4. Part of a set of meiotic diplotene chromosomes from the salamander *Plethodon cinereus* after hybridization *in situ* with tritiated m.r. DNA from the same species. These chromosomes show what is considered to be a non-random distribution of the autoradiographic label. This is particularly evident on the long bivalent to the right of the picture.

Figure 5. A set of mitotic chromosomes from the intestinal epithelium of *Plethodon cinereus* after hybridization *in situ* with tritiated m.r. DNA from the same species. Some of these chromosomes show a distinctly uneven or non-random labelling. This is particularly well shown in the two chromosomes indicated by arrows.

Description of plate 2

Figure 6. Autoradiograph of the centromere region on a lampbrush chromosome from *Plethodon cinereus* after hybridization with tritiated m.r. DNA from the same species. The centromere bars are fused to form a cross (C). The majority of loops are unlabelled and too faintly stained to be clearly visible. A pair of large heavily labelled loops arises close to one end of each centromere bar (L). The arrangement of the main chromosome axes have been drawn in to assist with interpretation of the picture. Exposure time 5 days. Figures 4–6 reproduced with the permission of G. T. Morgan.

Figure 7. A dark field micrograph of an autoradiograph of a set of lampbrush chromosomes from *Triturus cristatus carnifex* after hybridization *in situ* with tritiated m.r. DNA from the same species. In this case the labelled probe has bound to the nascent RNA that is in the matrix of the lateral loops on the chromosomes. The two heteromorphic long arms of chromosome I each carry 20–40 very heavily labelled loops which show up with exceptional brilliance in a micrograph of this kind (arrows).

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FIGURES 1–5. For description see opposite.

(*Facing p.* 316)

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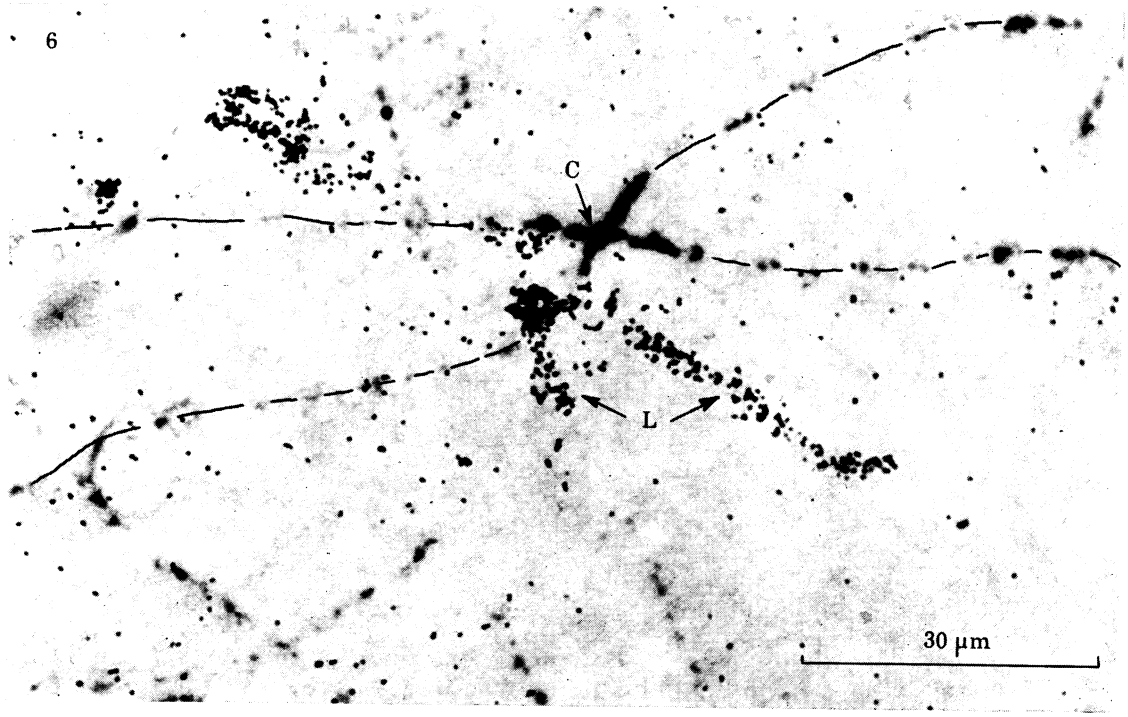

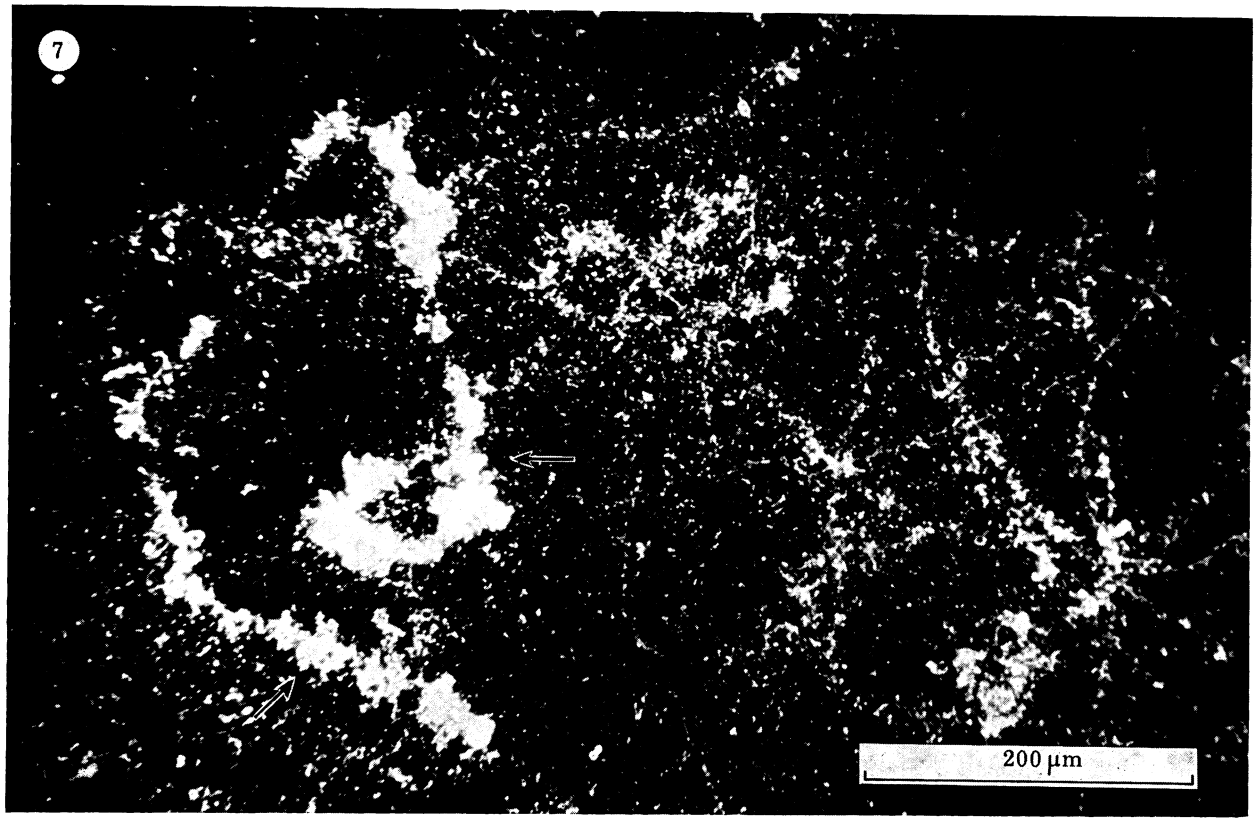

FIGURES 6 AND 7. For description see page 316.

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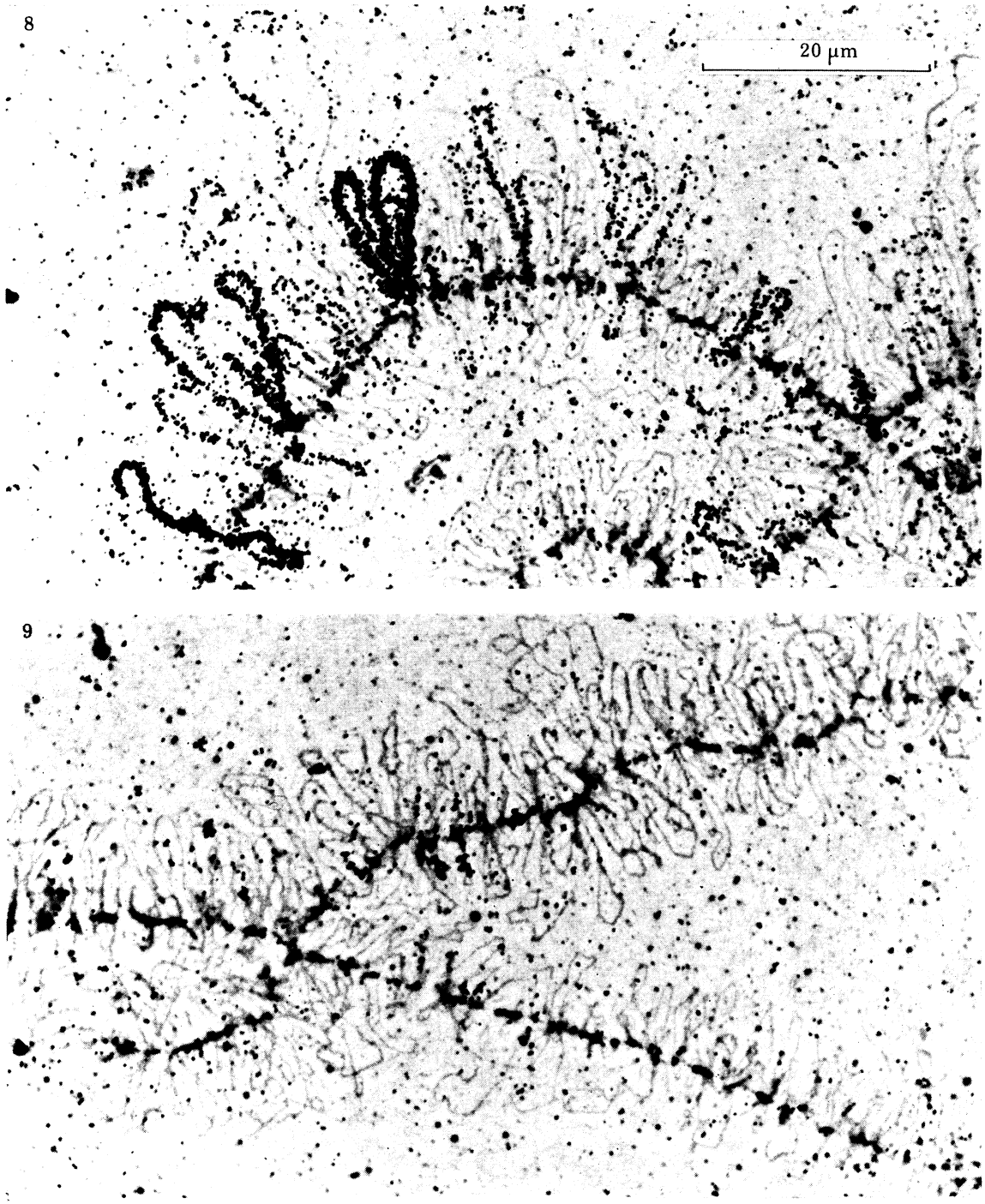

FIGURE 8. An autoradiograph of part of one of the long heteromorphic arms of lampbrush bivalent I from *T.c. carnifex* after hybridization *in situ* with tritiated m.r. DNA from the same species. Many loops of all sizes show exceptionally heavy labelling. The exposure time for this autoradiograph was 7 days.

FIGURE 9. An autoradiograph of another part of the same preparation as shown in figure 8. The chromosome regions shown here are parts of chromosomes II and VII. Note the lack of labelling as compared with the ht.as of chromosome I.

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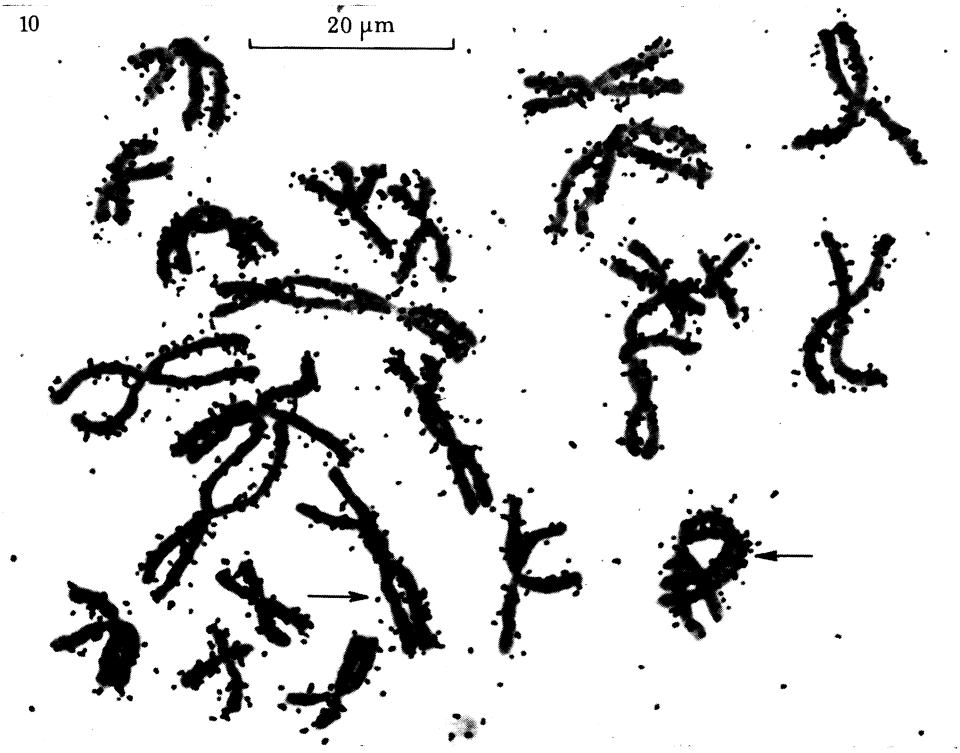

FIGURE 10. An autoradiograph of a set of mitotic chromosomes from the intestinal epithelium of *T.c. carnifex* after hybridization *in situ* with tritiated m.r. DNA from the same species. All of the chromosomes are about equally labelled. The two chromosomes that are thought to be chromosomes I are indicated by arrows. Note that they are not exceptionally heavily labelled as is the case in lampbrush preparations. The exposure time for this autoradiograph was 7 days.

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ht.a. of chromosome I in lampbrush preparations does not indicate that the ht.a. of I is disproportionately rich in m.r. sequences. On the basis of all evidence presently available, I suggest that the fast labelling loops on chromosome I are sites where there is tandem linkage of members of individual families of m.r. sequences, and that there may be many similar sites elsewhere on the chromosome set. However, most of the representatives of this particular class of tandemly linked m.r. sequence that are expressed in the lampbrush stage are situated on the ht.a. of I.

The wider significance of these observations is that they add weight to the notion that members of many families of m.r. sequences may indeed be tandemly linked, without interspersion, that there may be some selectiveness and specificity with respect to the sequences that are transcribed during the lampbrush phase of oogenesis, and that there may be a connection between meiotic crossing over and the arrangement of DNA sequences in a chromosome.

In conclusion then, we may say that the trends that seem to have prevailed in the evolution of large genomes and chromosomes are (1) the generation of repetitiveness through unequal chromatid exchanges, with an inevitable legacy of quantitative heterozygosity, (2) balanced growth of chromosomes, such that each chromosome acquires new repeats in proportion to those already present, (3) conservation of translationally important sequences and rather free divergence of untranslatable sequences, and (4) some kind of interdependence between the arrangement of repetitive sequences and meiotic crossing-over, which we do not yet understand and therefore cannot explain.

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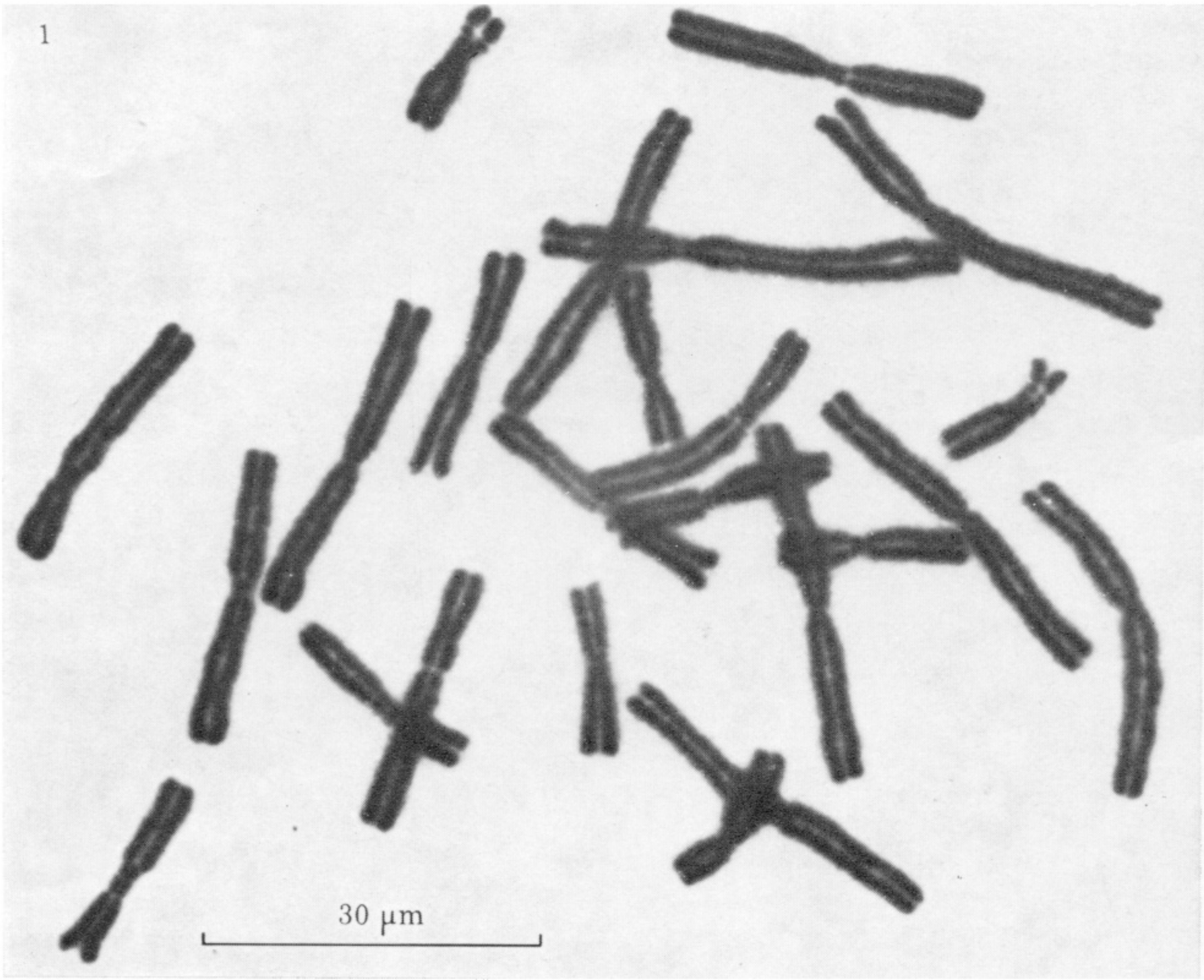
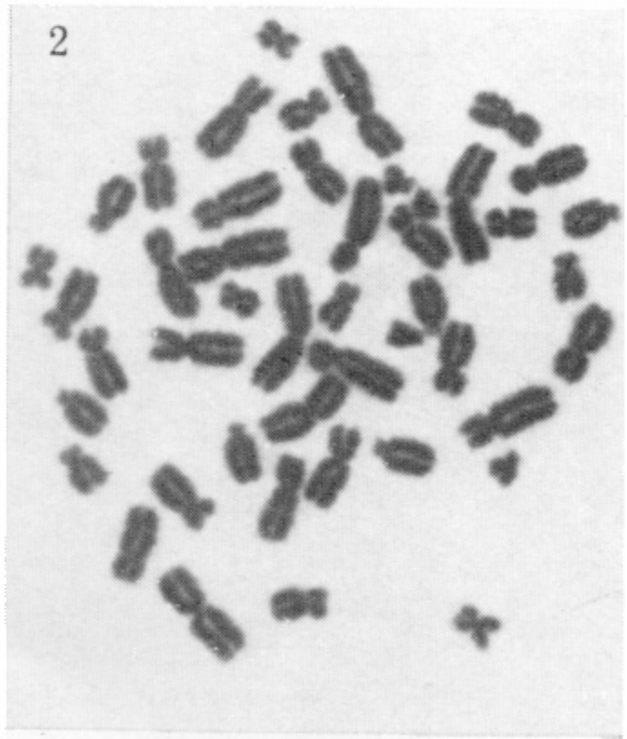
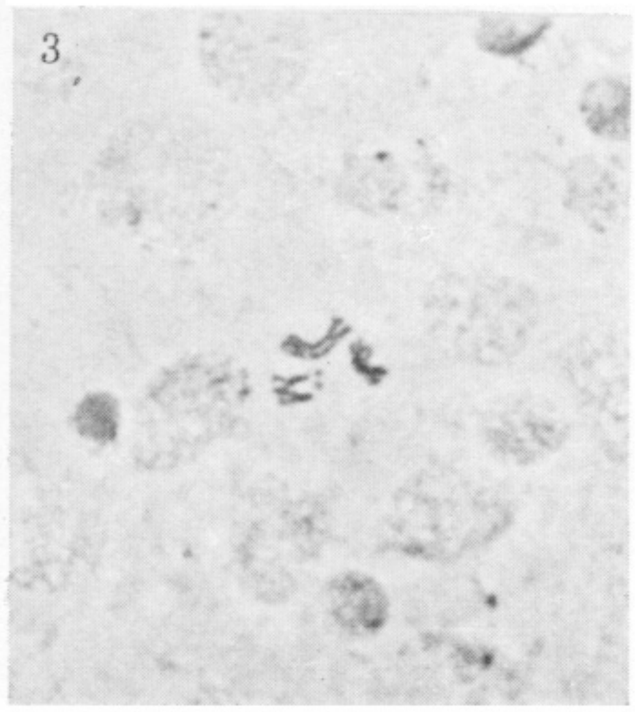
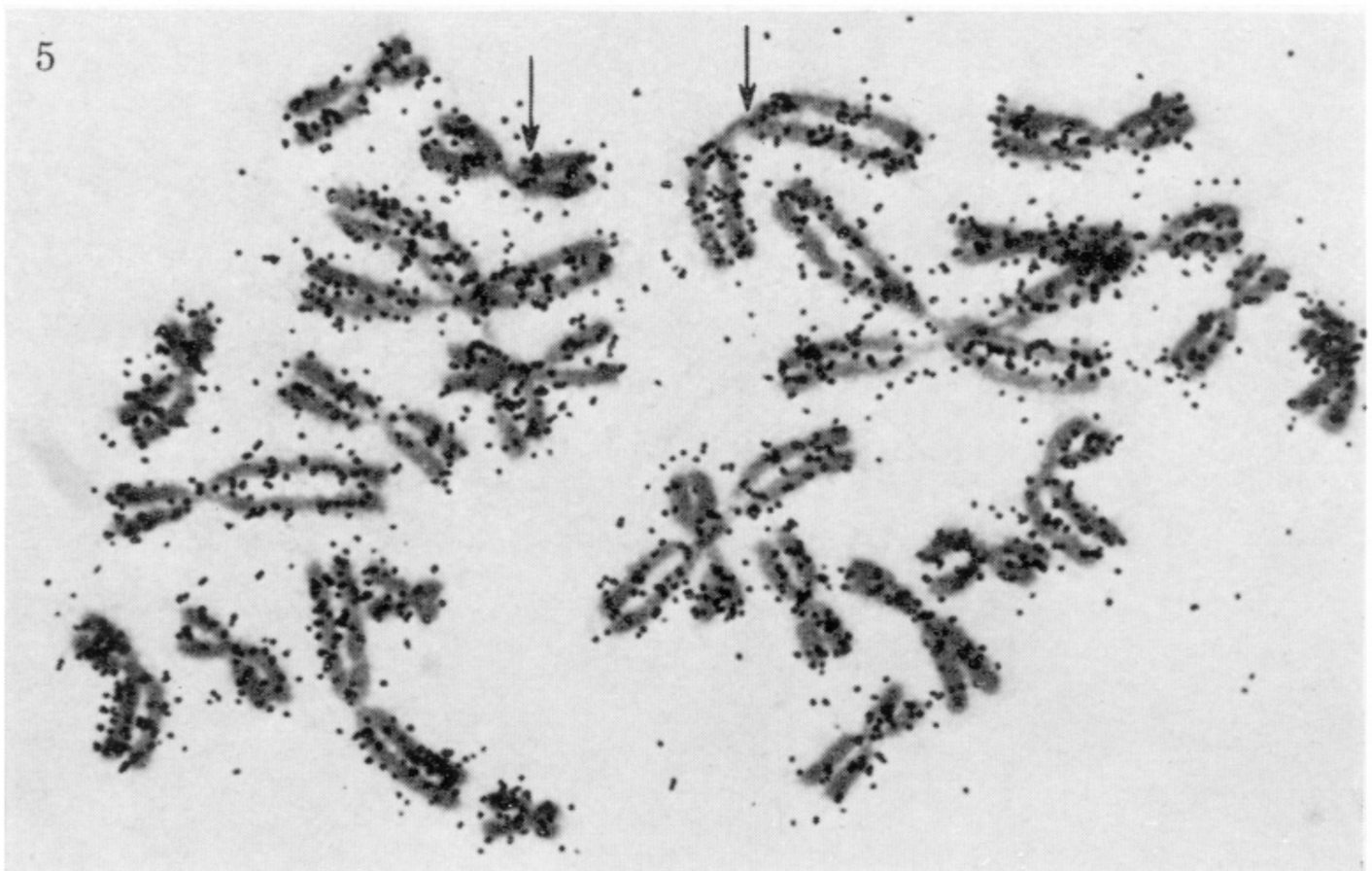
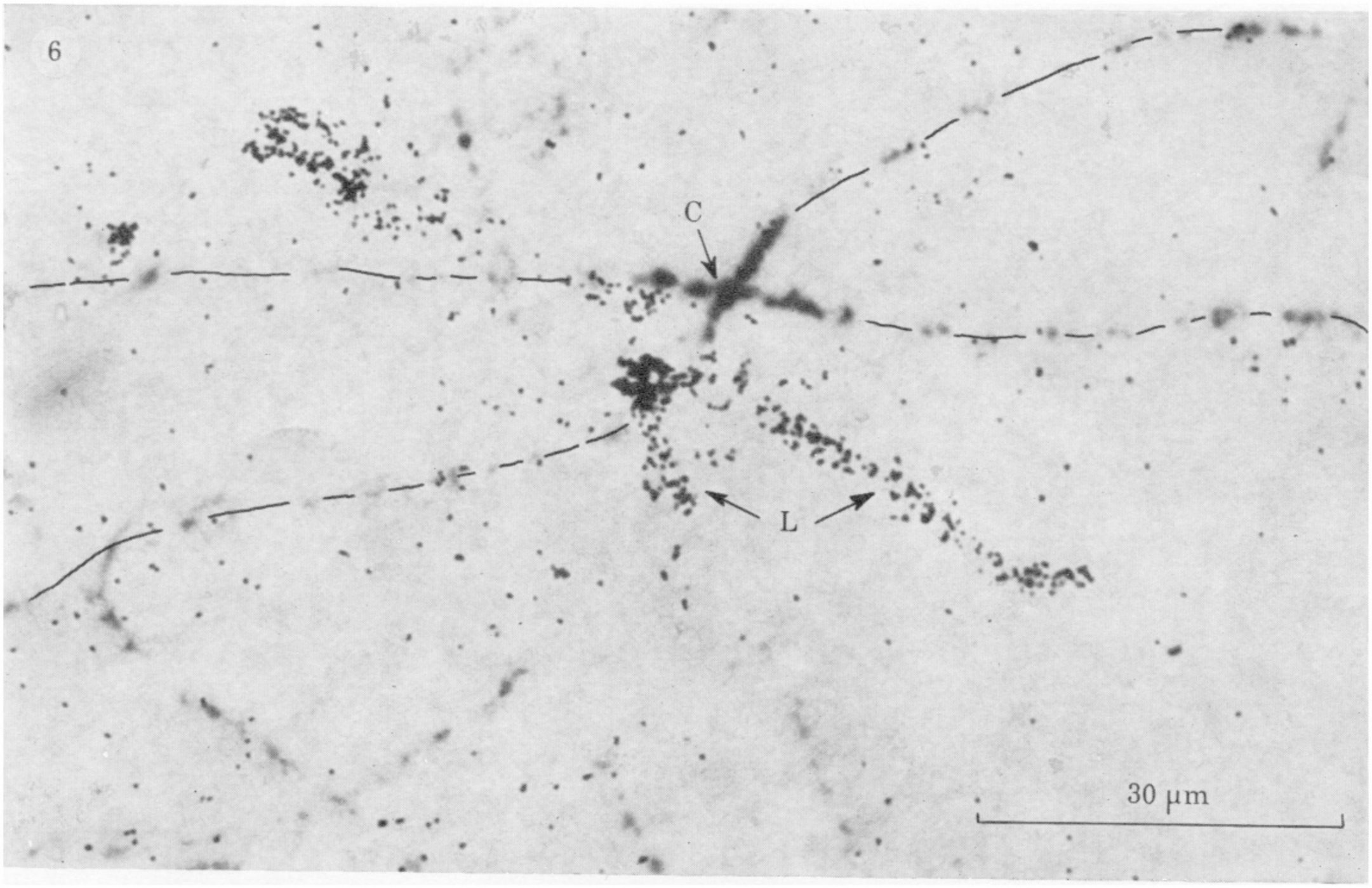


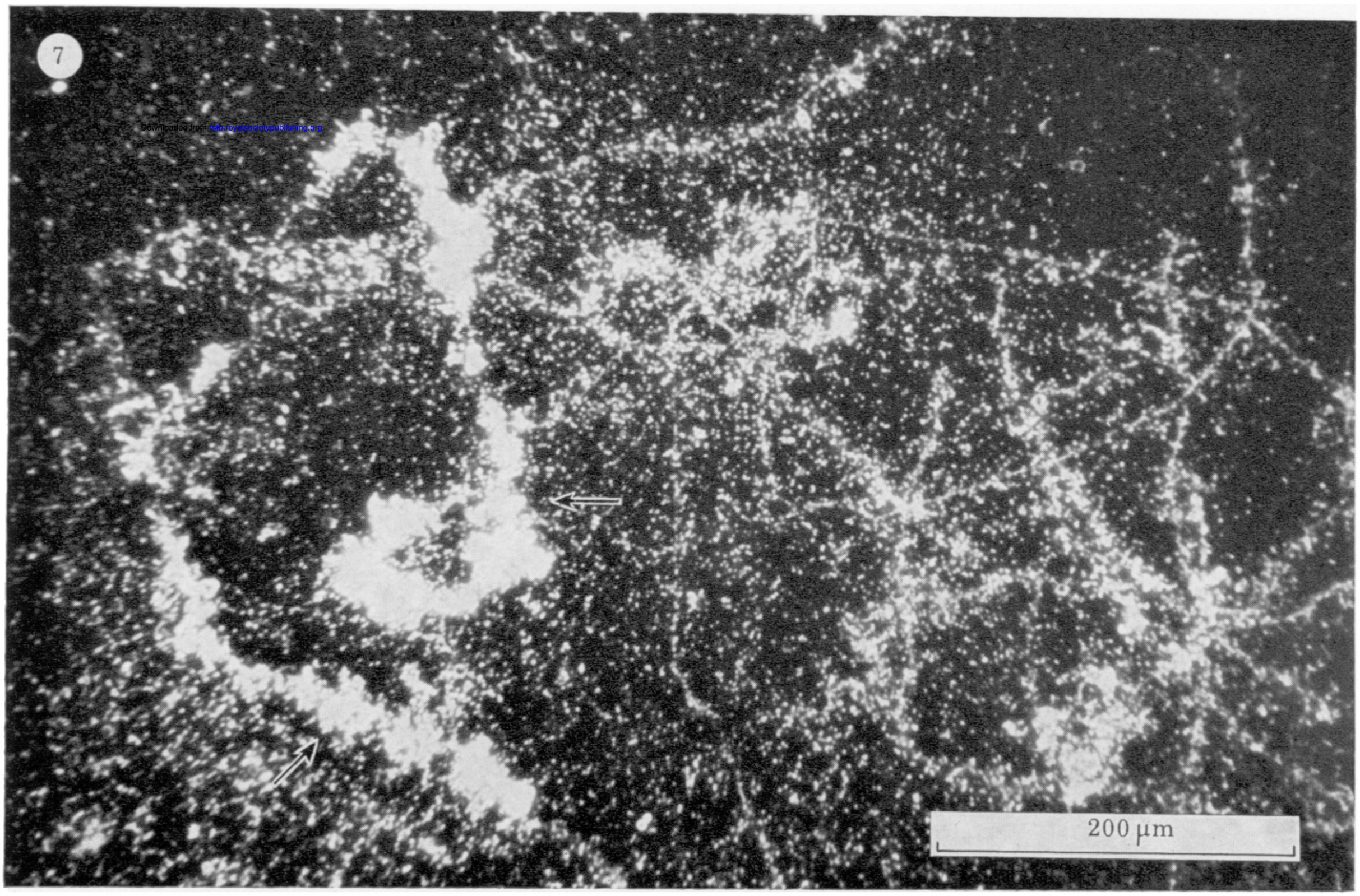




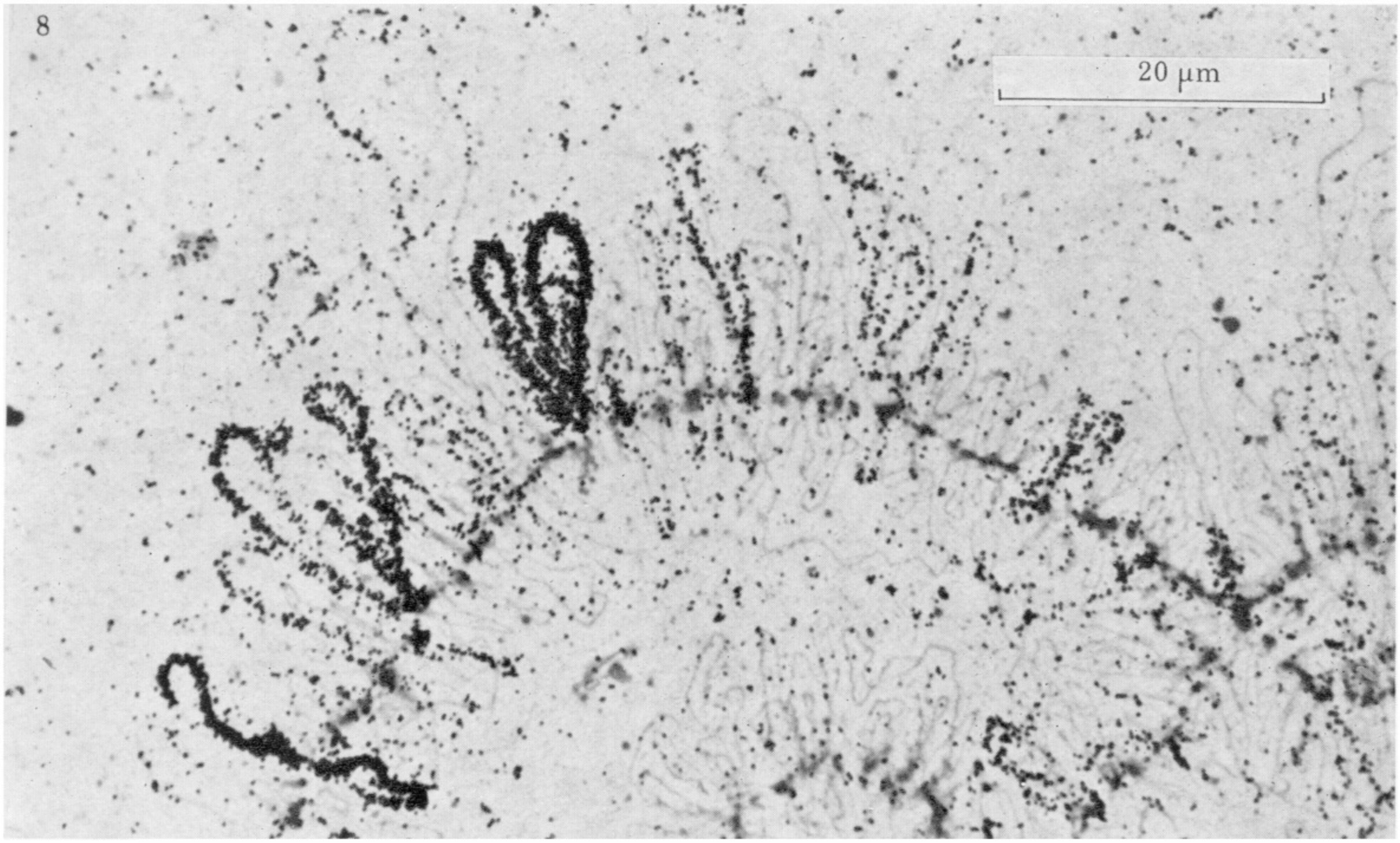


FIGURES 1–5. For description see opposite.





FIGURES 6 AND 7. For description see page 316.



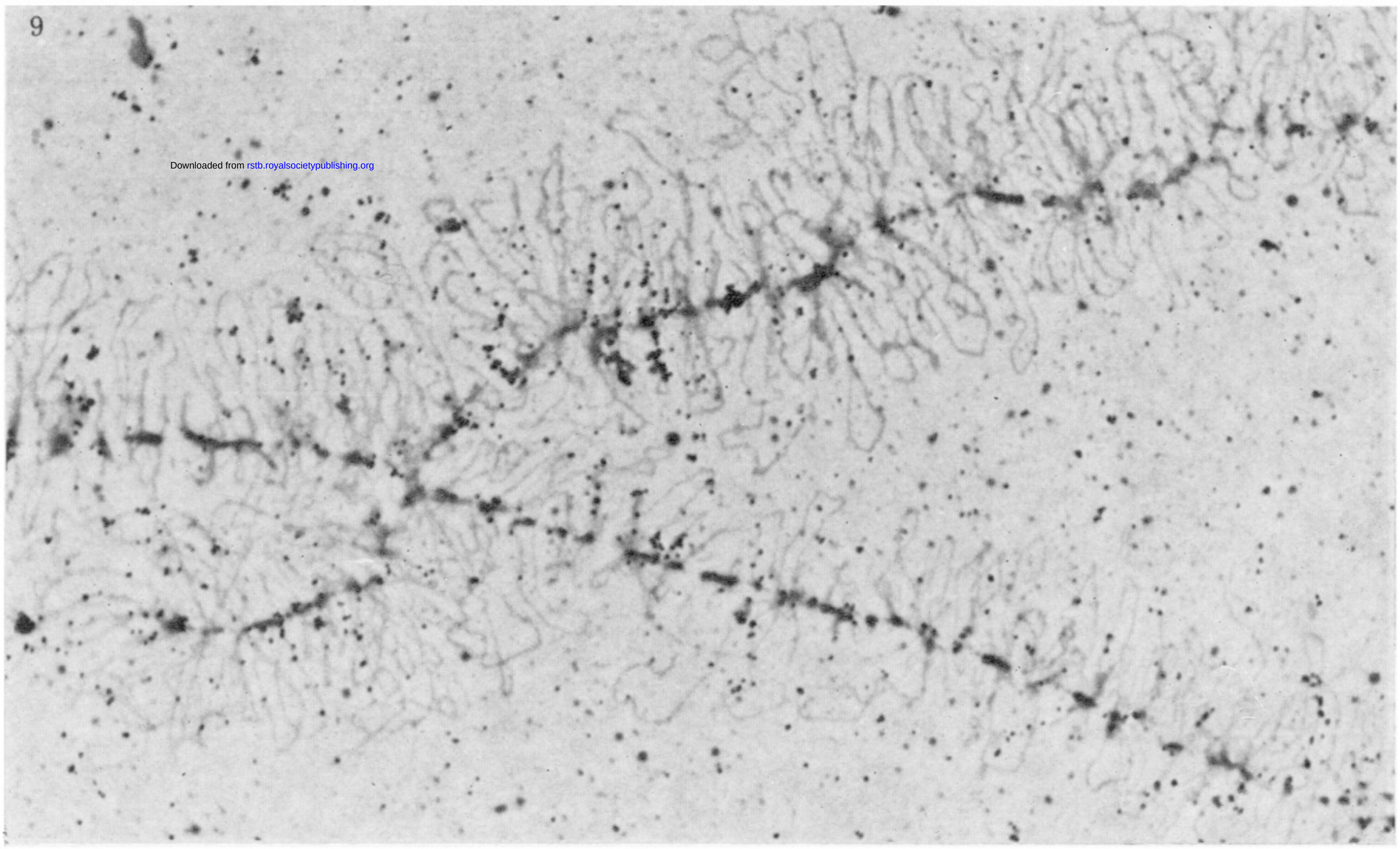


FIGURE 8. An autoradiograph of part of one of the long heteromorphic arms of lampbrush bivalent I from *T.c. carnifex* after hybridization *in situ* with tritiated m.r. DNA from the same species. Many loops of all sizes show exceptionally heavy labelling. The exposure time for this autoradiograph was 7 days.

FIGURE 9. An autoradiograph of another part of the same preparation as shown in figure 8. The chromosome regions shown here are parts of chromosomes II and VII. Note the lack of labelling as compared with the ht.as of chromosome I.

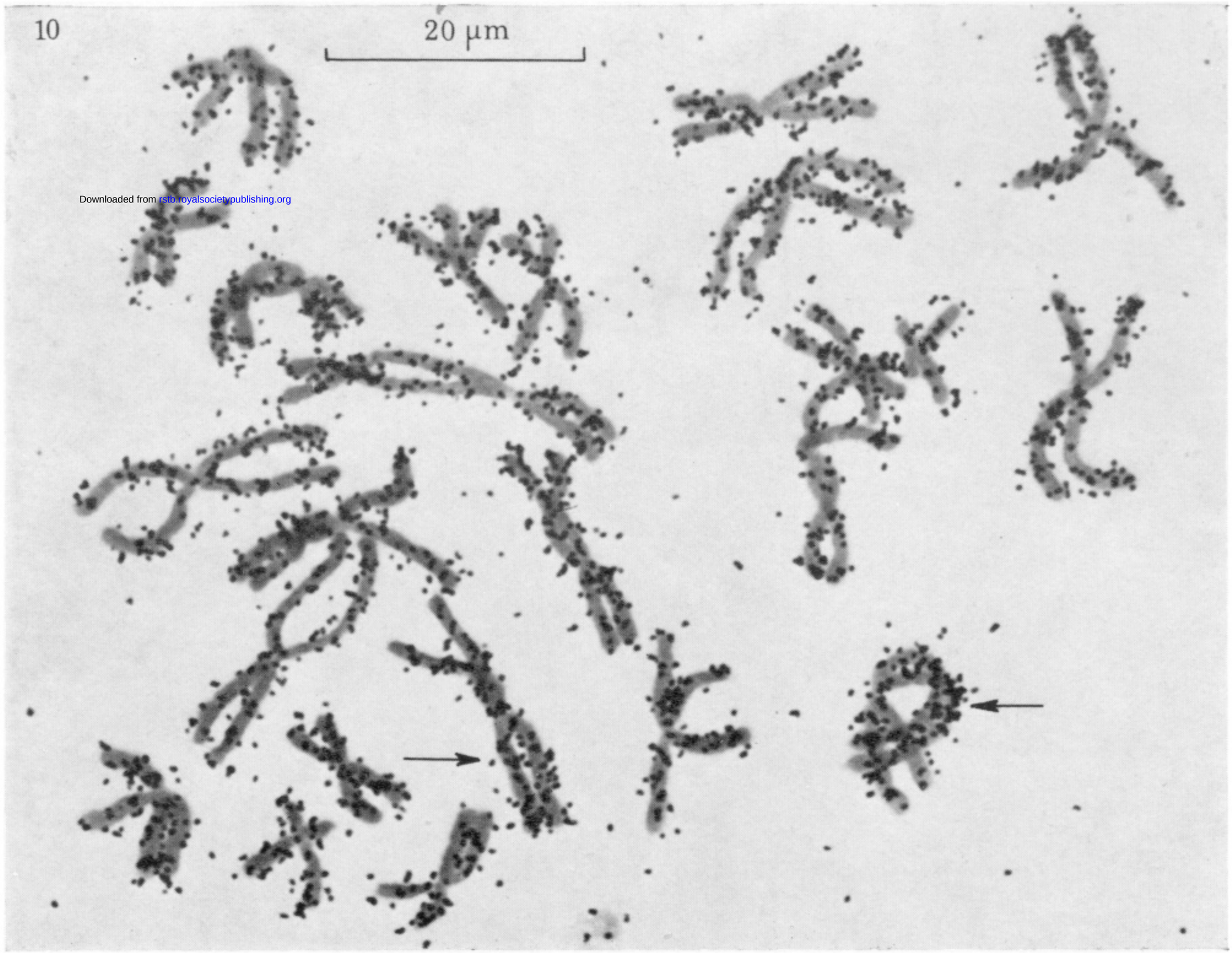


FIGURE 10. An autoradiograph of a set of mitotic chromosomes from the intestinal epithelium of *T.c. carnifex* after hybridization *in situ* with tritiated m.r. DNA from the same species. All of the chromosomes are about equally labelled. The two chromosomes that are thought to be chromosomes I are indicated by arrows. Note that they are not exceptionally heavily labelled as is the case in lampbrush preparations. The exposure time for this autoradiograph was 7 days.