Biochimia et Biophysica Acta, 607 (1980) 397—410

BBA 99650

THE KINETOPLAST DNA OF *TRYPANOSOMA EQUIPERDUM*

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(Received October 19th, 1979)

Key words: Kinetoplast DNA; Maxi-circle; Mini-circle; (Trypanosoma equiperdum)

Summary

We have analyzed the kinetoplast DNA from *Trypanosoma equiperdum* (American Type Culture Collection 30019) and two dyskinetoplastic strains derived from it. The DNA networks from the kinetoplastic strain are made up of catenated mini-circles and maxi-circles, like the networks from the closely-related *Trypanosoma brucei*.

The mini-circles of *T. equiperdum* lack the pronounced sequence heterogeneity of *T. brucei* mini-circles, as shown by the fragment distribution of restriction digests and by the predominance of well-matched duplexes in electron micrographs of renatured DNA.

The electrophoretic analysis of kinetoplast DNA digested with various restriction endonucleases shows the maxi-circle of *T. equiperdum* to consist of circular DNA molecules of $8.4 \cdot 10^6$ daltons, without size or sequence heterogeneity or repetitious segments. A comparison of the sequence of this maxi-circle with that of *T. brucei* ($13.4 \cdot 10^6$ daltons) by restriction endonuclease fragmentation and hybridization shows extensive sequence homology. The size difference between both maxi-circles is due to the deletion of one continuous segment of $5 \cdot 10^6$ daltons.

In the two dyskinetoplastic strains, we cannot detect DNA sequences that hybridize with kinetoplast DNA from *T. brucei* or from the kinetoplastic strain of *T. equiperdum*. In one of these strains, a 'low-density' DNA fraction contained a simple sequence DNA, cleaved by restriction endonuclease HindIII into fragments of 180 base-pairs and multimers of this. The relation of this DNA to kinetoplast DNA, if any, is unknown.

Introduction

The kinetoplast DNA of most members of the order Kinetoplastida consists of catenated networks of mini-circles and maxi-circles. The maxi-circles represent less than 20% of the network mass and they are probably the equivalent of the mtDNA in other organisms. The function of the mini-circles is unknown (see Ref. 1 for review of the literature).

Among the African trypanosomes of the *Trypanosoma brucei* group there are naturally-occurring variants that have apparently lost the ability to make functional mitochondria (see Refs. 1 and 2). Such strains cannot multiply in tsetse flies and they rely on passive transmission by flies or by blood-blood contact. Some of these variants have lost organized kinetoplast DNA networks and are called 'dyskinetoplastic'. Others still contain kinetoplast DNA networks, but in three *Trypanosoma evansi* strains analyzed, these networks did not contain any maxi-circles [3—5].

Hajduk and Cosgrove [6] have previously isolated kinetoplast DNA from another variant trypanosome that cannot make function mitochondria, namely, *Trypanosoma equiperdum*. This variant was found to contain kinetoplast DNA networks composed of catenated mini-circles and the long edge loops considered typical of maxi-circles [4]. These networks closely resemble intact kinetoplast DNA networks from normal *T. brucei*. The recent construction of a restriction map of the maxi-circle of *T. brucei* [7] and the cloning of segments of this DNA in a bacterial host-vector system [8] have made it possible to analyze the possible relation between the maxi-circle of our *T. equiperdum* strain and *T. brucei* in more detail.

In this paper, we show that the maxi-circle of our *T. equiperdum* strain has extensive sequence homology with the maxi-circle of *T. brucei*, but that it lacks a segment of about one third. In addition, we show that the mini-circles of this *T. equiperdum* strain are much less heterogeneous in sequence than those of *T. brucei* (cf. Refs. 4, 5, 9 and 10) and that no kinetoplast DNA sequence is detectable in two dyskinetoplastic *T. equiperdum* strains derived from the kinetoplastic strain. A brief summary of our results has been included in a recent review [5].

Methods

Growth and isolation of trypanosomes. The kinetoplastic (ATCC 30019), spontaneously dyskinetoplastic (ATCC 30023) and acriflavin-induced strains of *T. equiperdum* [6] and the *T. brucei* 427 strain [4] were grown and isolated as described. *T. brucei* 427 is the strain isolated by Cunningham and Vickerman [11] and has been erroneously referred to as *T. brucei* EATRO 427 in previous publications (cf. Refs. 4, 5 and 7) from the Amsterdam laboratory.

Isolation of DNA. The procedure of Fairlamb et al. [4] was followed for isolation of kinetoplast DNA with minor changes as described previously [7]. For the isolation of nuclear DNA, the same lysis and deproteinization procedure was followed, but the DNA was collected by ethanol precipitation rather than by high-speed centrifugation. After ribonuclease treatment and another ethanol precipitation, the DNA was purified by NaI equilibrium density

centrifugation. A 'low-density' fraction of the DNA from the spontaneously dyskinetoplastic strain was isolated by separately collecting the upper part of the broad DNA band: this was re-run in NaI twice, the upper part of the DNA band being collected each time.

Endonuclease digestion and gel electrophoresis of kinetoplast DNA. The restriction endonuclease digestion and agarose gel electrophoresis conditions and the marker DNA used for molecular weight calculations were the same as described previously [7].

Hybridization experiments. Kinetoplast DNA fragments were separated by agarose gel electrophoresis, denatured in situ and blotted onto a nitrocellulose filter by the method of Southern [12]. The filters were hybridized [8] with nick-translated [13] DNA probes or poly(A)$^+$ RNA from *T. brucei*, labelled with polynucleotide kinase and [γ-^{32}P]ATP in vitro [14]. The DNA probes used were the Eco2 (RR2) and Eco3 (RR3) fragments (for nomenclature of the restriction endonuclease-digested fragments, see Ref. 7) from *T. brucei* (427) maxi-circles cloned in *Escherichia coli* using lambda-gt-WES · lambda-B as vector [15] or mini-circles from *T. brucei* cloned in *E. coli*, using plasmid pBR322 as vector [15].

Electron microscopy. A formamide version of the protein monolayer technique was used for the heteroduplex analysis of *T. brucei* and *T. equiperdum* maxi-circle segments and for the analysis of denatured and renatured mini-circles of *T. equiperdum* (for references see Ref. 8).

Analytical CsCl equilibrium density gradient centrifugation. Analytical CsCl equilibrium density gradient centrifugation was carried out in an old Beckman-Spinco Model E analytical ultracentrifuge using Kel-F cells with a path-length of 10 mm. Samples containing 0.5—2 μg DNA were centrifuged at 44 770 rev./min and 20°C in CsCl (ρ = 1.70 g/cm^3), buffered with 20 mM sodium phosphate (pH 7.0) with 1 μg *Micrococcus luteus* DNA (ρ = 1.731 g/cm^3, according to Schildkraut et al. [16]) added as marker. After 16—20 h, ultraviolet-absorption photographs were taken on Kodak Professional Film at several exposure times. The equilibrium density of the sample DNA was calculated from its position in the cell relative to the *M. luteus* DNA using the formula of Sueoka [17]. The tracings of the photograph negatives shown in Fig. 8 were made on a Gilford 2400 spectrophotometer equipped with a linear transport device.

Results

Purified kinetoplast DNA from the kinetoplastic *T. equiperdum* strain was digested with various restriction endonucleases and the digests were electrophoresed through an agarose gel. The ethidium-stained gel in Fig. 1 shows the characteristic picture, already observed for several kinetoplast DNAs: a prominent mini-circle band at $0.6 \cdot 10^6$ daltons, large mini-circle associations retained in the slot and, in between, faint 'extra' bands that are characteristic of the enzyme used. The extra bands in each case are present in stoicheiometric amounts and add up to approx. $8.5 \cdot 10^6$ daltons (Table I). A linear DNA of this size is also released from the network with S_1 nuclease. From these results, we conclude that *T. equiperdum* kinetoplast DNA contains a single type of maxi-circle, which is about two thirds of the size of its counterpart in *T. brucei*.

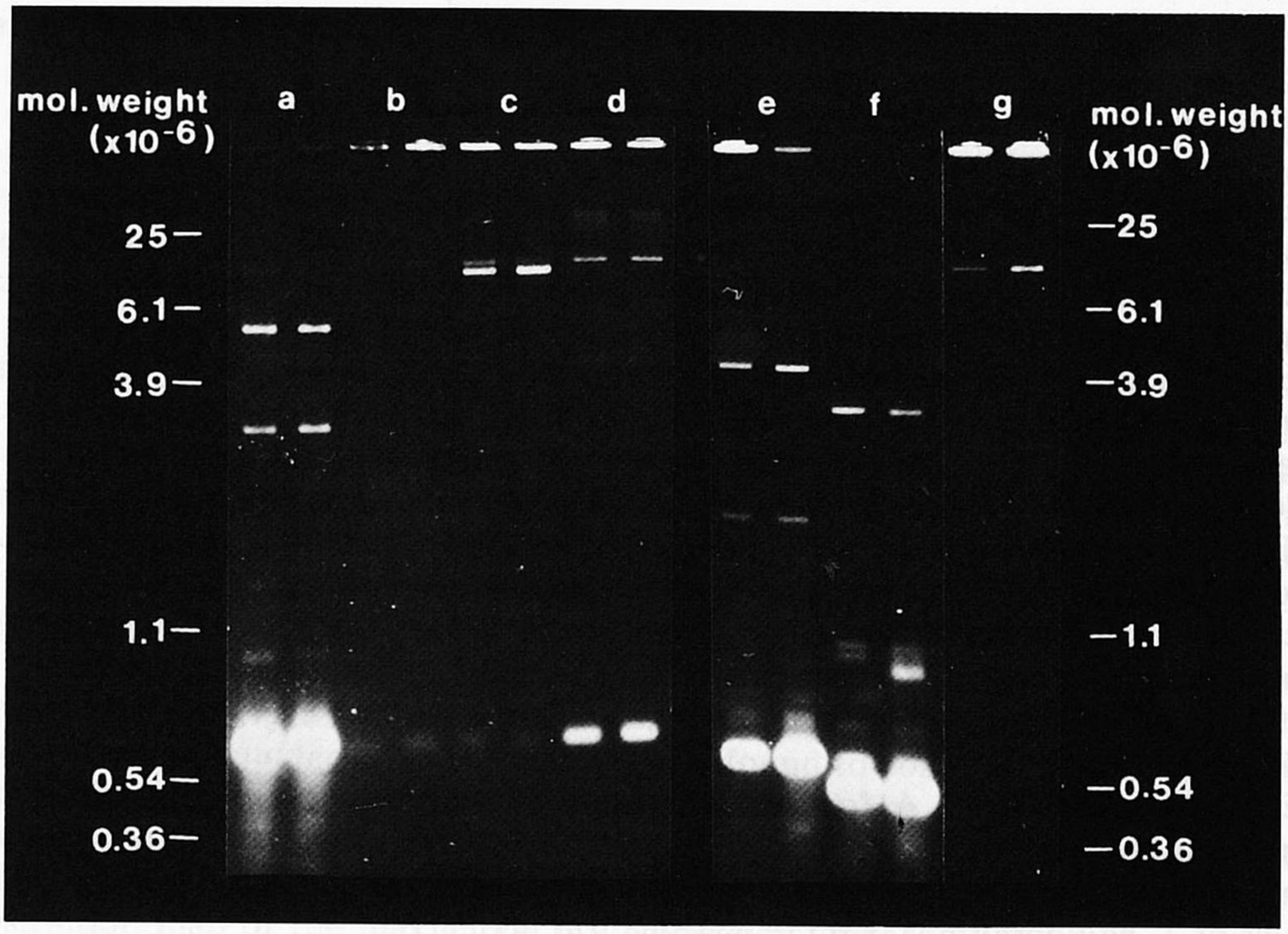

Fig. 1. Electrophoretic analysis of fragments produced by cleavage of kinetoplast DNA from *T. equiperdum* with various restriction endonucleases. The figure is a composite of parts of two separate 0.6% agarose gels, stained with ethidium bromide. Each lane contains 0.5 μg DNA and enzymes used were (a) HhaI, (b) PstI, (c) HindIII, (d) S_1 nuclease, (e) TaqI, (f) MboI and (g) HaeIII. Each enzyme was used at two concentrations, the standard concentration sufficient to obtain complete digestion of *T. brucei* kinetoplast DNA (left-hand lane) and a 4-fold higher concentration (right-hand lane).

This maxi-circle shows no detectable sequence or size heterogeneity, nor signs of repetitious segments.

To get more information on the extent of homology between the maxi-circles of *T. equiperdum* and *T. brucei*, the kinetoplast DNA fragments were blotted onto nitrocellulose filter strips and hybridized with three types of labelled probes.

(a) EcoRI fragments of the maxi-circle of *T. brucei* cloned in a lambda vector in *E. coli*. Of the three fragments, only the smaller two (Eco2 = RR2 and Eco3 = RR3) have been cloned and these span half the maxi-circle [15].

(b) Poly(A)$^+$ RNA from *T. brucei*. We have previously shown [5] that this RNA hybridizes to nuclear DNA and all EcoRI fragments of the maxi-circle but not to mini-circles of *T. brucei*.

(c) An EcoRI digest of kinetoplast DNA from *T. equiperdum*, from which the large network associations not cleaved by EcoRI had been removed by centrifugation (cf. Ref. 7).

The results of these hybridization experiments, presented in Fig. 2, show that the small EcoRI fragment of *T. equiperdum* hybridizes with the Eco3 (RR3) probe (lane c) and only very weakly with the Eco2 (RR2) probe (not visible in lane b), whereas the large EcoRI fragment does not hybridize at all. This fragment does contain sequences homologous to *T. brucei*, however,

TABLE I

MOLECULAR WEIGHTS OF MAXI-CIRCLE FRAGMENTS RELEASED FROM *T. BRUCEI* AND *T. EQUIPERDUM* KINETOPLAST DNA WITH VARIOUS ENDONUCLEASES

Molecular weights were calculated from the electrophoretic mobility of each fragment in a 0.6% agarose gel relative to markers in Methods. The single digest values for *T. equiperdum* fragments are based on at least five experiments; in at least two of these, digests of *T. brucei* and *T. equiperdum* were run by side (cf. Fig. 2). The results for digestion with two enzymes are based on a single experiment (Fig. 3) in the case of *T. equiperdum* kinetoplast DNA, whereas the data for *T. brucei* kinetoplast DNA are taken from Ref. 7.

Mol. wt. ($\times 10^{-6}$) of fragments released by:	Fragment number							Total
	1	2	3	4	5	6	7	
T. brucei maxi-circle								
HapII	7.6	5.7	0.6	—	—	—	—	13.9
HapII × EcoRI *	6.2	1.6	—	—	—	—	—	—
EcoRI	7.0	4.1	2.8	—	—	—	—	14.8
HindIII × EcoRI	4.6	2.3	—	—	—	—	—	—
HindIII	5.5	4.1	3.6	—	—	—	—	13.2
HaeIII	10.0	2.3	1.1	—	—	—	—	13.4
TaqI	4.6	1.65	1.6	1.1	0.64 **	0.4 **	0.25 **	10.3
PstI	12.5	—	—	—	—	—	—	12.5
S_1 nuclease	13.0	—	—	—	—	—	—	13.0
T. equiperdum maxi-circle								
HapII	7.3	0.7—1.0 ***		—	—	—	—	8.0—8.3
HapII × EcoRI	4.5	1.8	1.5	0.7—1.0 ***	—	—	—	8.5—8.8
EcoRI	6.8	1.5	—	—	—	—	—	8.3
HindIII × EcoRI	3.8	2.2	1.5	0.6—1.0 ***	—	—	—	8.1—8.5
HindIII	7.5	0.6 — 1.0 ***		—	—	—	—	8.1—8.5
HaeIII	8.4	—	—	—	—	—	—	8.4
TaqI	3.9	1.65	1.0	0.64	0.4 **	0.25 **	—	7.8
S_1 nuclease	8.4	—	—	—	—	—	—	8.4

* Only fragments derived from the largest EcoRI fragment listed (data from Ref. 7).

** Fragments only detected by hybridization with *T. brucei* maxi-circle DNA.

*** Fragments only detected as part of partial digestion products observed in single digests, e.g., lane c of Fig. 1.

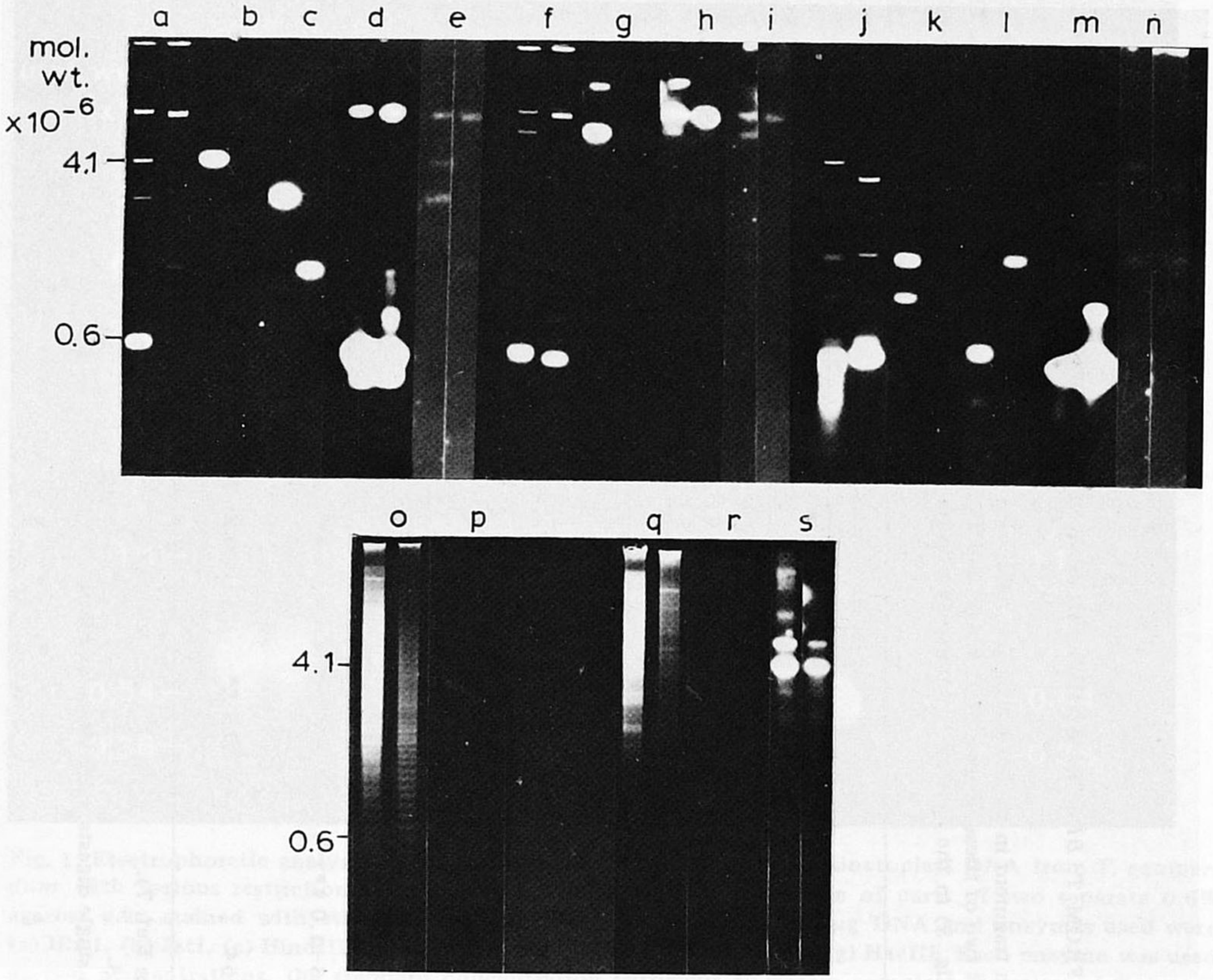

Fig. 2. Characterization of the sequence homology between the maxi-circles of *T. brucei* and *T. equiperdum*. Lanes a, f and j show photographs of ethidium-stained gels containing 0.5 μg kinetoplast DNA digested with (a) EcoRI, (f) HapII or (j) TaqI. The left-hand lane under each letter is the *T. brucei* kinetoplast DNA, the right-hand lane the *T. equiperdum* kinetoplast DNA. Lanes o show a HindIII digest of DNAs from dyskinetoplastic *T. equiperdum* strains: on the left the total cell DNA from the acriflavin-induced dyskinetoplastic strain, on the right a low-density fraction of the DNA from the spontaneously dyskinetoplastic strain. Lane q gives the EcoRI digest of these DNAs. All DNAs were transferred to nitrocellulose filters by a method according to Southern [12] and hybridized with various radioactive probes. Lanes b, g and k are autoradiograms of the filter hybridized with the cloned Eco2 maxi-circle fragment of *T. brucei*: lanes, c, h and l are the same but using the cloned Eco3 fragment; lanes d, m, p and r are the same but using *T. equiperdum* kinetoplast DNA; lanes e, i, n and s are the same but using poly(A)$^+$ RNA. Lanes b—e show autoradiograms corresponding to gel a: lanes g—i show autoradiograms corresponding to gel f, etc. In these experiments the nitrocellulose filter was re-used several times after dehybridization of the labelled probe bound in the previous round of hybridization. Dehybridization was not always complete and this explains the faint hybridization of the *T. brucei* Eco2 fragment in lane d, because this filter was used for hybridization with the Eco2 probe (lane b) in the previous round of hybridization. See Methods for further details.

because it hybridizes both to the kinetoplast DNA of *T. equiperdum* (lane d) and to the poly(A)$^+$ RNA from *T. brucei* (lane e). The homology must be extensive, because the extents of hybridization of the *T. brucei* and *T. equiperdum* maxi-circle fragments in Fig. 2 are about the same, if one takes into account that the maxi-circle content of this *T. equiperdum* kinetoplast DNA preparation is higher than that of the *T. brucei* kinetoplast DNA preparation. The results with the EcoRI digests are confirmed and extended by the results with HapII digests (lanes f—i) and TaqI digests (lanes j—n).

The results suggest that the large EcoRI fragment of *T. equiperdum* contains most of the sequence present in the large EcoRI fragment of *T. brucei*. This was verified by two types of experiment. Firstly, the position of the HapII and HindIII cutting sites in the large EcoRI fragment was determined in double digests, as shown in Fig. 3. The fragments obtained are similar to those obtained in the corresponding double digests of *T. brucei* maxi-circles (see Table I). Secondly, attempts were made to construct heteroduplexes of the linearized maxi-circles from *T. equiperdum* and *T. brucei* to map the regions of homology in electron micrographs. These attempts were unsuccessful because the maxi-circle DNAs as isolated already contained too many nicks, precluding the formation of full-length hybrids that could be oriented relative to the restriction map. However, many shorter molecules were present, showing an extensive mismatch. An example is given in Fig. 4. Such molecules were absent in DNA spread without denaturation-renaturation or in controls containing only *T. brucei* or only *T. equiperdum* kinetoplast DNA.

The map of the T. equiperdum maxi-circle

A tentative interpretation of our results is given in the linearized maps presented in Fig. 5. We assume that most of the sequence of the largest EcoRI fragment of *T. brucei* is conserved in the large EcoRI fragment of *T. equiperdum*. This follows from the apparent conservation of the single HindIII and

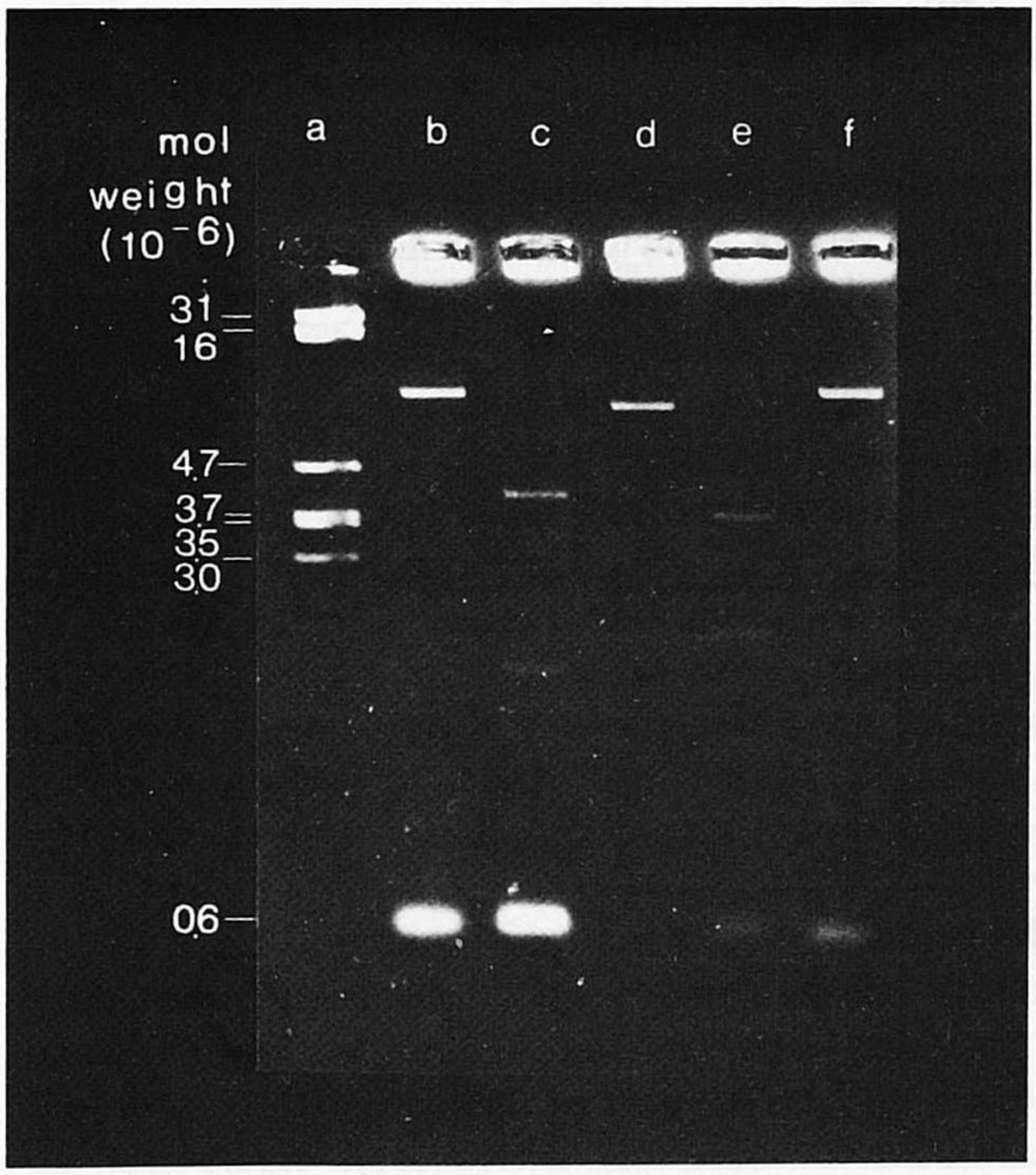

Fig. 3. Agarose gel electrophoresis of maxi-circle fragments from *T. equiperdum*. Kinetoplast DNA from *T. equiperdum* was digested with HapII (lane b), HapII + EcoRI (c), EcoRI (d), EcoRI + HindIII (e) and HindIII (f) and electrophoresed in a 0.7% agarose gel. Lane a is phage PM2 DNA digested with HindIII and used as molecular weight marker.

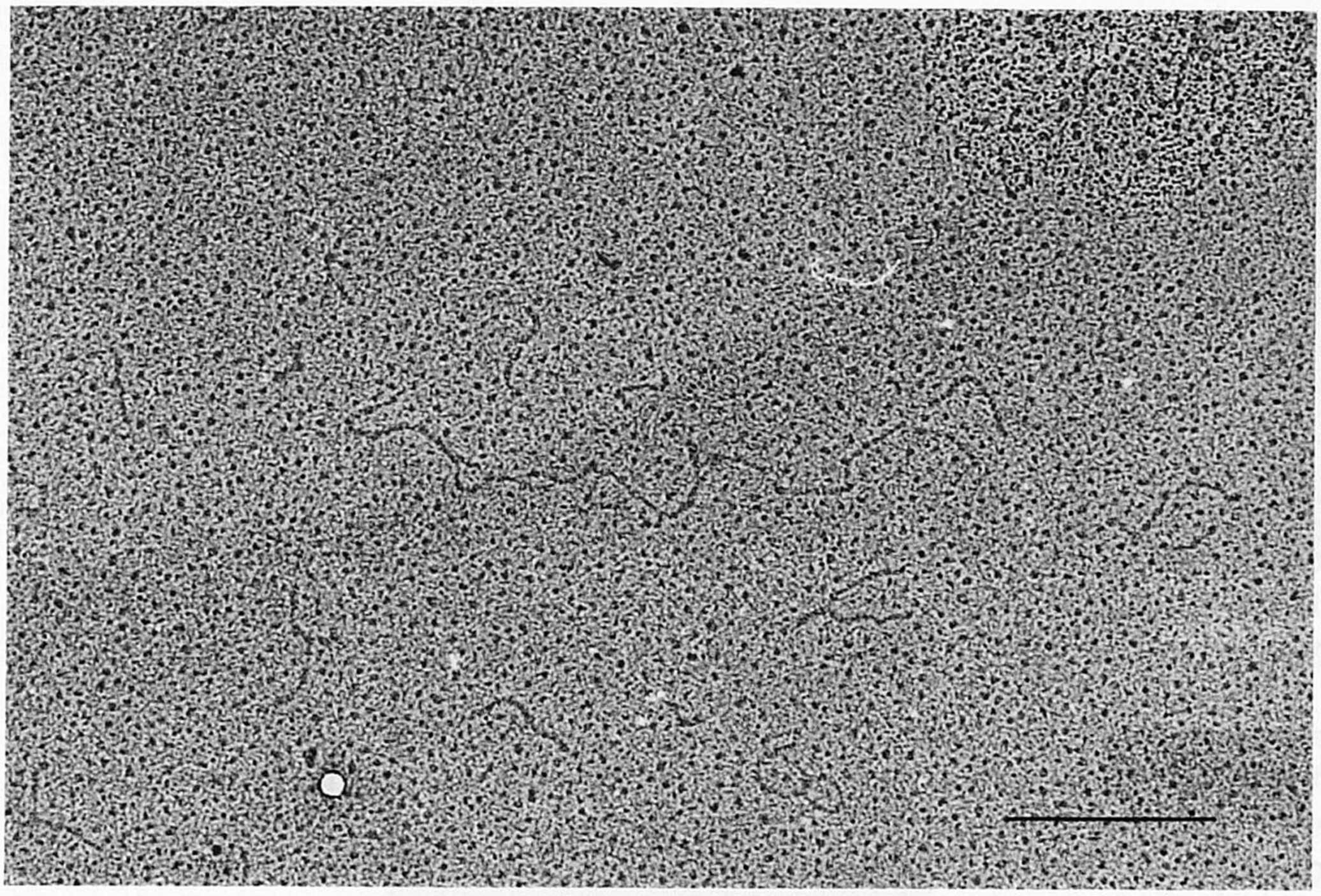

Fig. 4. Electron micrograph of a heteroduplex DNA molecule present in co-renatured kinetoplast DNA of *T. brucei* and *T. equiperdum*. Kinetoplast DNA of both organisms was digested with EcoRI and undigested network remnants were removed by centrifugation. The supernatants were mixed to give a 1 : 1 ratio of *T. equiperdum* and *T. brucei* DNA. The mixture was heat-denatured, renatured, spread in a protein monolayer and analyzed as described [8]. The bar is 0.5 μm.

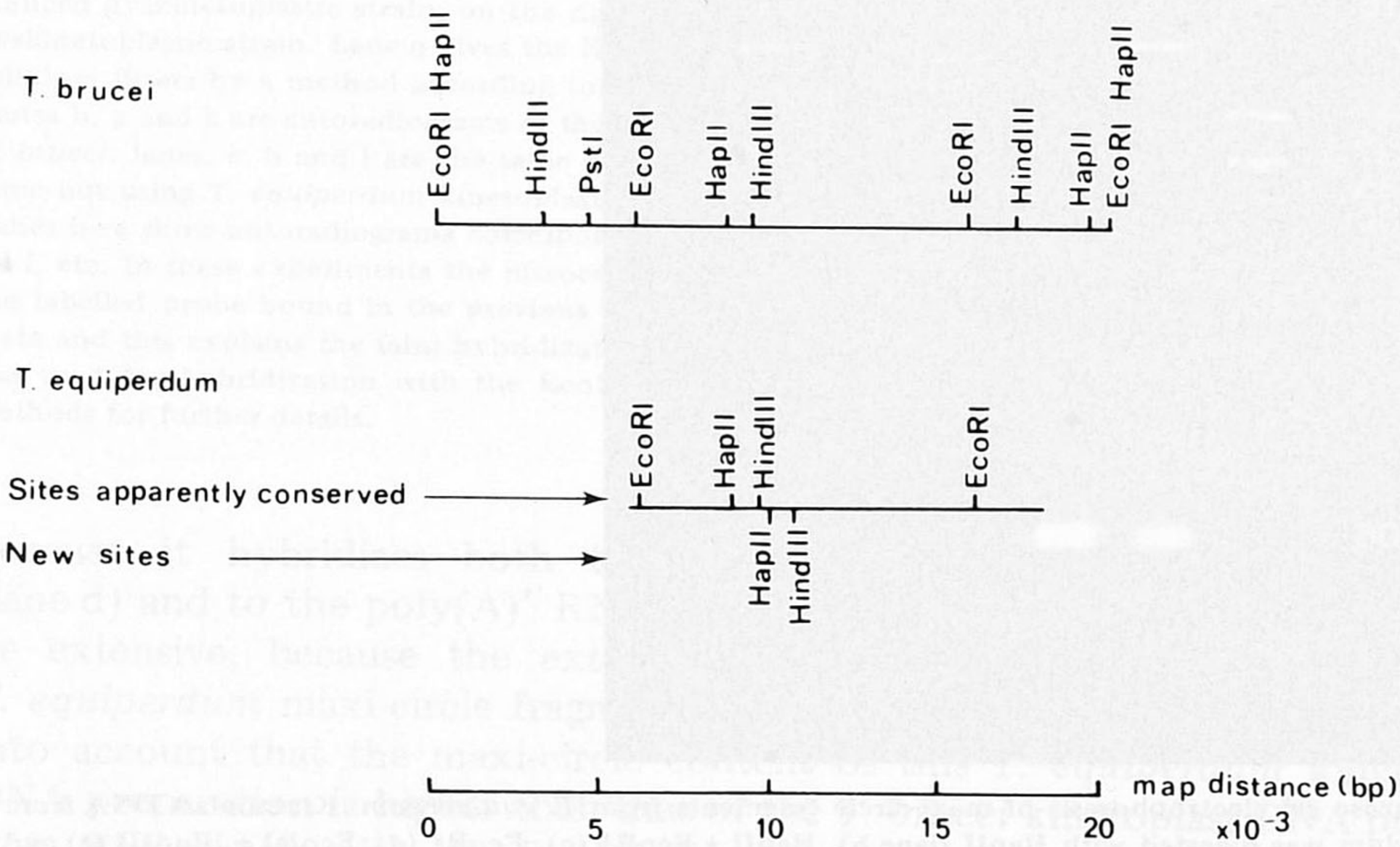

Fig. 5. A tentative map for the maxi-circle of *T. equiperdum* and its relation to the maxi-circle of *T. brucei*. The data for *T. brucei* are from Ref. 7.

HapII sites within this fragment (Fig. 3) and the hybridization results presented in Fig. 2. The sequence corresponding to Eco2 (RR2) of *T. brucei* is largely absent, resulting also in the absence of the single PstI site (see Fig. 1, lane b). The remainder of this Eco2 (RR2) fragment is joined with sequences from the Eco3 (RR3) fragment in the small EcoRI fragment of *T. equiperdum*. Fig. 3 shows that this small EcoRI fragment does not contain HindIII or HapII sites and, therefore, we have no indication as to which part of the Eco3 (RR3) fragment of *T. brucei* is contained in it.

Although the hybridization experiments and the apparent conservation of restriction sites suggest an overall similarity of sequence between the large EcoRI fragments from *T. equiperdum* and *T. brucei*, the differences in detail are substantial. From the presence of partial digestion products we infer that the *T. equiperdum* maxi-circle gives rise to a small HindIII fragment (see lane c of Fig. 1) and a small HapII fragment (see faint partial digestion product in lane f of Fig. 2). These fragments have no counterpart in *T. brucei* and they can only be positioned by postulating the presence of new HapII and HindIII sites in the *T. equiperdum* maxi-circle as indicated in Fig. 5. Moreover, there are large differences in the size of the TaqI fragments derived from Eco1 (RR1) of *T. brucei* and *T. equiperdum* as judged from the hybridization experiments in Fig. 2. The limited results from our heteroduplex analyses are in agreement with this. It should be noted that the EcoRI (RR1) fragment spans the only region in the maxi-circle of *T. brucei* which varies in size in different strains. The maximal size difference is $0.7 \cdot 10^6$ daltons, the Eco1 (RR1) fragment from 427 being the largest [4,5]. The Eco1 (RR1) fragment from *T. equiperdum* falls in this range.

Analysis of mini-circle sequence heterogeneity

The mini-circles of *T. brucei* show extensive heterogeneity in sequence. The results of the restriction endonuclease digestions in Figs. 1 and 2 suggest that this heterogeneity is much less marked in *T. equiperdum* and this has been confirmed by two additional experiments. Fig. 6 shows kinetoplast DNAs digested with TaqI or MboI and run on 2% agarose gels, which permit a better resolution of mini-circle fragments than the 0.7% gels used in Figs. 1—3. The result shows that most mini-circles of *T. equiperdum* are cut once by TaqI and twice by MboI whereas the digestion of *T. brucei* mini-circles with these enzymes yields a more diverse set of fragments. The mini-circles of *T. equiperdum* still have some homology with the mini-circles of *T. brucei*, as shown by their hybridization with the cloned *T. brucei* mini-circles (Fig. 6) and the hybridization of labelled *T. equiperdum* kinetoplast DNA with the mini-circles of *T. brucei* (Fig. 2).

The high degree of sequence homogeneity of the mini-circles of *T. equiperdum* was confirmed by an experiment in which the mini-circles cut with TaqI were denatured and renatured and analysed by electron microscopy. The majority of the molecules present in Fig. 7 are fully-matched, linear duplexes of mini-circle size. Under the same conditions, the renatured molecules from *T. brucei*, *Trypanosoma cruzi* or *Crithidia* contain single-stranded tails and/or internal single-stranded loops (Refs. 5, 8 and unpublished results).

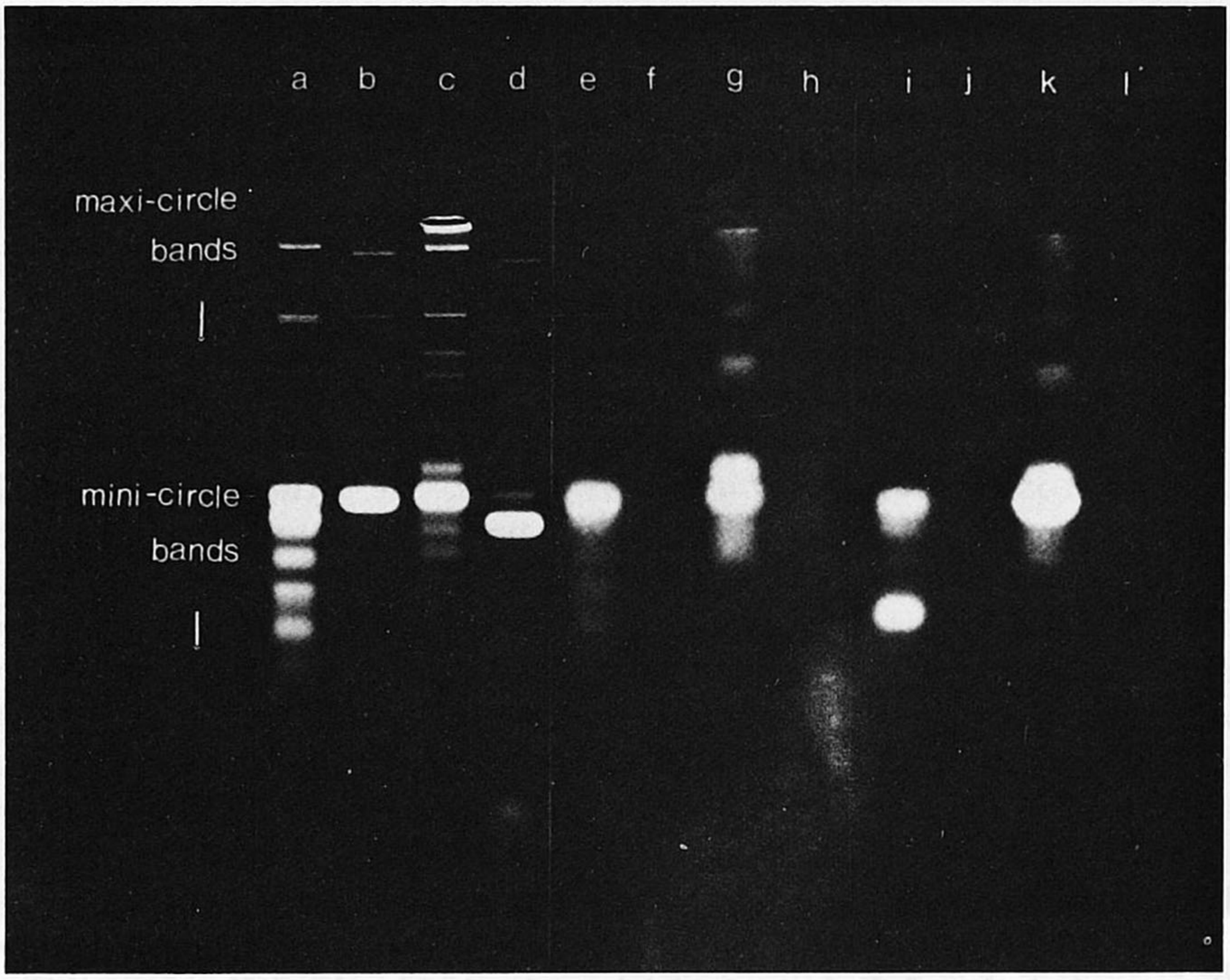

Fig. 6. Characterization of the mini-circles of *T. equiperdum* by cleavage with restriction endonucleases and hybridization experiments. Kinetoplast DNA of *T. brucei* (lanes a and c) and *T. equiperdum* (lanes b and d) was digested with TaqI (a and b) and MboI (c and d) and electrophoresed in a 2% agarose gel. The gel from lanes a—d was blotted onto nitrocellulose filters and hybridized with two different, nick-translated cloned mini-circles from *T. brucei* (lanes e—h and i—l).

A search for kinetoplast DNA sequences in dyskinetoplastic T. equiperdum strains

From this kinetoplastic *T. equiperdum* strain, two dyskinetoplastic strains have been derived that have apparently lost kinetoplast DNA networks [6]. Hajduk and Cosgrove [6] have previously found that both strains still contain DNA of the same density as kinetoplast DNA (cf. Ref. 20) and, moreover, both contained DNA circles in the same size range as the maxi-circles in the kinetoplastic *T. equiperdum* strain, but no mini-circle. To see if these strains still contain kinetoplast DNA sequences, we have hybridized restriction digests of the DNA of both strains with labelled kinetoplast DNA of the *T. equiperdum* kinetoplastic parent strain. To increase the sensitivity of the experiment, the DNA from the spontaneously dyskinetoplastic strain was fractionated in an NaI equilibrium gradient and the DNA in the region corresponding to the density of kinetoplast DNA was used for restriction endonuclease digestion and hybridization.

The analytical CsCl gradient of this DNA, presented in Fig. 8 together with the unfractionated total DNA from the acriflavin-induced dyskinetoplastic strains, shows a major band at 1.690 g/cm^3, the density of kinetoplast DNA. The hybridization experiments with both DNAs are shown in lanes o—s of Fig. 2. Neither DNA shows significant hybridization with the kinetoplast DNA probe: the proper transfer of the digests to the nitrocellulose filter is

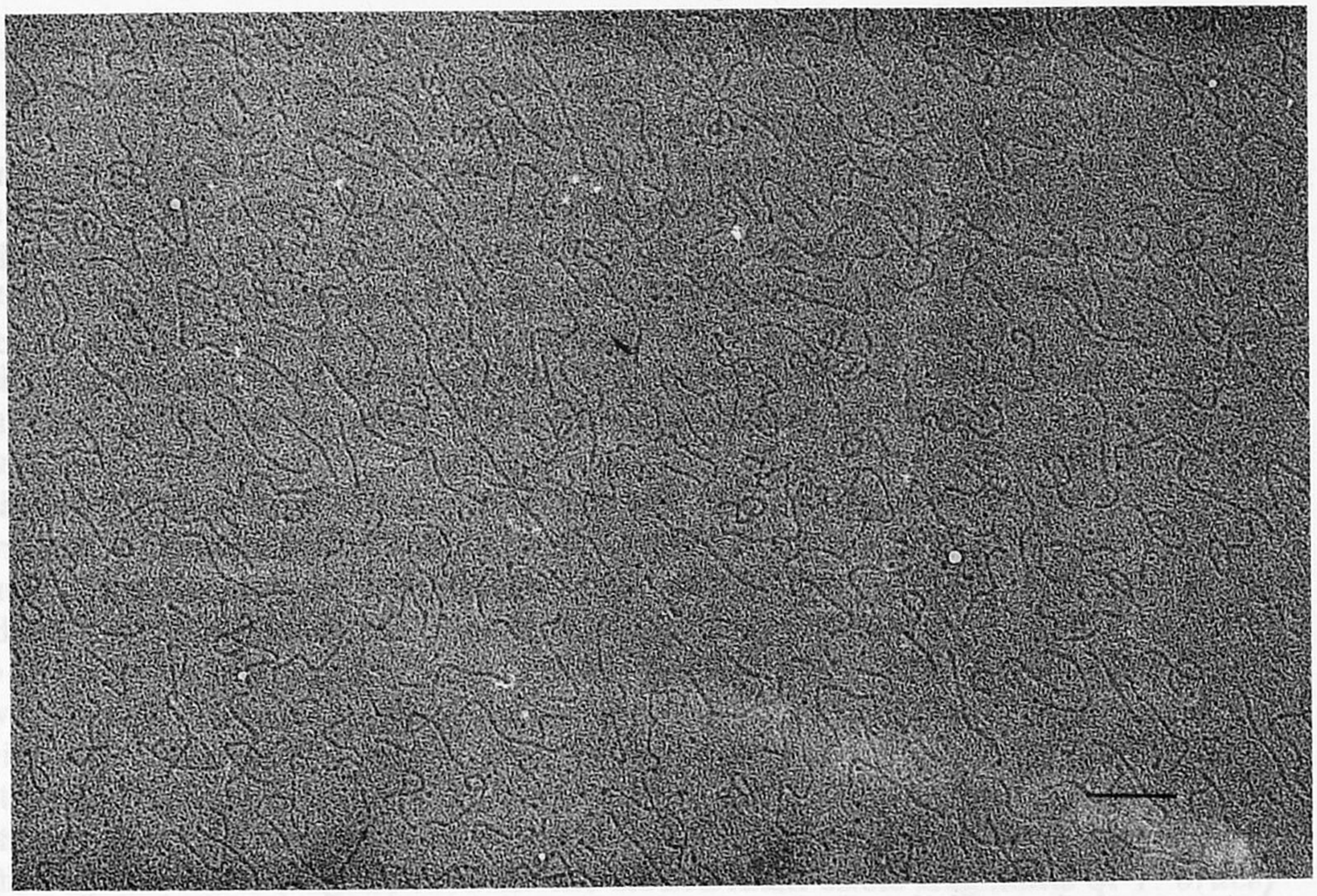

Fig. 7. Electron micrograph of renatured mini-circles from *T. equiperdum*. Kinetoplast DNA from *T. equiperdum* was digested with TaqI, denatured, renatured and spread as described [8]. The bar is 0.2 μm.

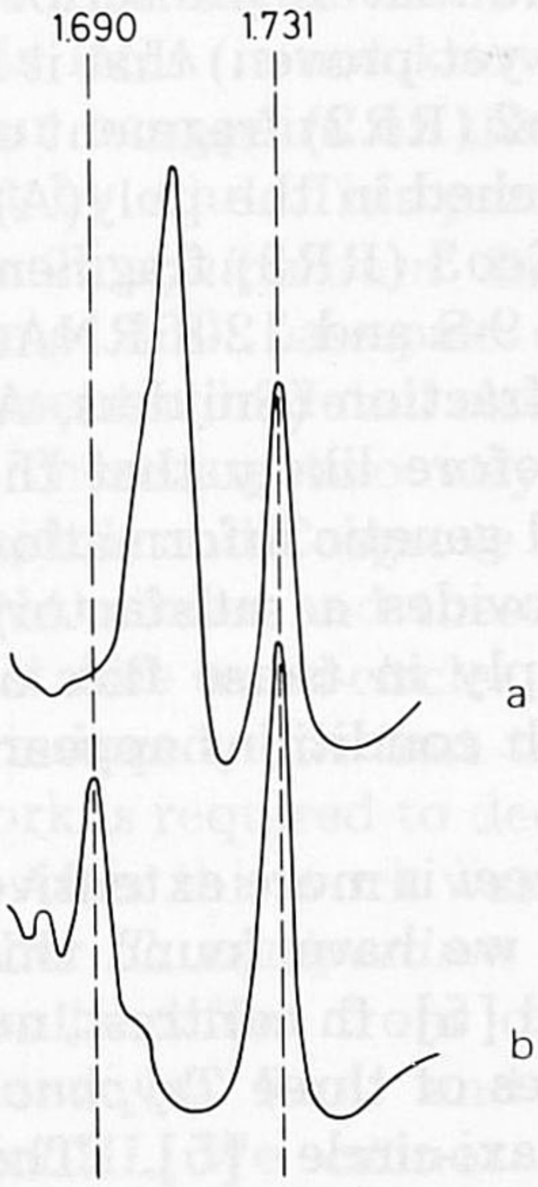

Fig. 8. Microdensitometer tracings of ultraviolet light photographs of *T. equiperdum* DNA at equilibrium in CsCl. The tracings are matched at the *M. luteus* marker at 1.731 g/cm^3. (a) Total cell DNA from the acriflavin-induced *T. equiperdum* strain. (b) A low-density DNA fraction obtained by fractionating the DNA from the spontaneously dyskinetoplastic *T. equiperdum* strain in NaI as described in Methods. The numbers are densities in g/cm^3.

illustrated in panel s by the hybridization with poly(A)$^+$ RNA which shows the characteristic two bands derived from the nuclear ribosomal RNA genes of the organisms of the *T. brucei* group (unpublished observations).

Two unusual features of the gels of the 'low-density' DNA from the spontaneously dyskinetoplastic strain should be noted: firstly, the DNA contains a rather prominent band in the EcoRI digest (lane q) at $33.5 \cdot 10^6$ daltons. In additional experiments, we have found that this band is present in the input DNA and that it is not cleaved by HapII, TaqI or HaeIII (not shown). This band disappears after digestion with HindIII (lane o). The second remarkable point is the presence of a regular 'ladder' in the HindIII digest of this DNA with a spacing of approx. 180 base-pairs.

Electron microscopy of the 'low-density' fraction of the DNA of the dyskinetoplastic strain showed a high proportion of circular DNA molecules, heterogeneous in size. Since similar results have recently been obtained with another dyskinetoplastic *T. equiperdum* strain by Riou and Pautrizel [18] we refrain from a more detailed presentation of our data.

Discussion

At first sight the kinetoplast DNA from the kinetoplastic *T. equiperdum* strain studied by us looks similar to that of *T. brucei*. The networks are of about equal size and they both contain mini-circles and maxi-circles. The more detailed analysis presented here has uncovered two major differences: the maxi-circle of *T. equiperdum* contains a 7—8 kilo-base-pair deletion, relative to its counterpart in *T. brucei* and the mini-circles do not have the sequence heterogeneity which is so pronounced in *T. brucei*.

The maxi-circles are the only known DNA component that is transcribed in trypanosomes (see Ref. 5) and it is likely (though not yet proven) that it is the equivalent of the mtDNA of other organisms. The Eco2 (RR2) fragment of *T. brucei* hybridizes to at least two RNAs that are enriched in the poly(A)$^+$ fraction of total RNA from bloodstream *T. brucei*; the Eco3 (RR3) fragment hybridizes with the two major mitochondrial RNAs, the 9-S and 12-S RNAs, and with two other RNAs found in the poly(A)$^+$ RNA fraction (Snijders, A. and Hoeijmakers, J.H.J., unpublished results). It is therefore likely that the deletion in the maxi-circle of *T. equiperdum* has removed genetic information essential for mitochondrial biogenesis. This finding provides a satisfactory explanation for the inability of *T. equiperdum* to multiply in tsetse flies or defined culture media at 26°C because growth under both conditions appears to require functional mitochondria.

The sequence heterogeneity in the mini-circles of *T. brucei* is more extensive than in any other trypanosomatid studied thus far, and we have found this sequence heterogeneity in each of the five strains analyzed [5]. In contrast, no sequence heterogeneity at all was found in the mini-circles of three *Trypanosoma evansi* strains, which contain no trace of maxi-circle [5]. The *T. equiperdum* strain studied here still has a (defective) maxi-circle and shows limited sequence heterogeneity. It remains possible, therefore, that sequence heterogeneity is related to the recombination between maxi-circles and mini-circles which is presumably required to keep the maxi-circles catenated in the

network. It is intriguing that the mini-circles of our *T. equiperdum* strain give a similar digestion pattern with MboI (with two closely-spaced sites, see Fig. 6) as the mini-circles of three *T. evansi* strains (Borst, P., unpublished results). This suggests that there is a basic mini-circle sequence that tends to predominate in all representatives of the *T. brucei* group, but that is obscured by the extensive mini-circle heterogeneity in *T. brucei* itself.

We have previously reported that another kinetoplastic *T. equiperdum* strain of poorly-defined origin had completely lost its maxi-circles [3,4]. We have since found that the restriction digests of the nuclear DNA of this strain differ from those of the *T. equiperdum* strains studied here and are indistinguishable from the digests obtained with nuclear DNAs from authentic *T. evansi* strains (Borst, P., unpublished results). It seems likely, therefore, that this strain without maxi-circles is not a *T. equiperdum* strain but a mislabelled *T. evansi* strain.

The dyskinetoplastic *T. equiperdum* strains studied here were found by Hajduk and Cosgrove [6] to contain DNA of kinetoplast DNA density in CsCl: 5-μm circles were prominent in this 'low-density' DNA but no mini-circle was found. The simple interpretation (cf. Ref. 5) that these circles are maxi-circles retained as free circles after the total loss of the mini-circles from these strains, is not supported by our present analysis. We find no maxi-circle in these strains by restriction enzyme analysis or by hybridization with *T. equiperdum* kinetoplast DNA (Fig. 2) or with the cloned maxi-circle fragments of *T. brucei* (not shown). Instead, we find a low-density satellite, at least in the spontaneously dyskinetoplastic strain, which has the same properties as a simple sequence DNA found in all of the five *T. brucei* and three *T. evansi* strains studied in this lab (Borst, P., unpublished results). This DNA is not cut by restriction endonucleases EcoRI, HapII, HaeIII, TaqI, PstI or MboI, but it is cut into oligomers of a 180 base-pair repeat by HindIII. The cellular location of this DNA is not known. Since restriction endonuclease AluI cleaves the inner four base-pairs of the hexamer recognized by HindIII, the satellite should be cut by AluI. This prediction has recently been verified both for *T. brucei* and for *T. equiperdum*. In both cases we find mainly 180 base-pair monomers and some 360 base-pair dimers after cleavage with AluI. These results will be presented in detail elsewhere.

There are two ways in which these results can be reconciled with those of Hajduk and Cosgrove [6]. 5-μm circles could be a predominant class of satellite DNA circles and these were the ones observed by them. The other possibility is that free maxi-circles may be retained in the first period after dyskinetoplasty is induced, but that these maxi-circles are lost in subsequent transfers. Further work is required to decide between these alternatives.

After this work was completed, an analysis of the kinetoplast DNA from two other *T. equiperdum* strains was published by Riou and Saucier [19]. Their results differ from ours in two major points. Firstly, the low-density DNA found by Riou and Saucier in their dyskinetoplastic strain is not cut by HindIII. We cannot account for this difference. Secondly, the maxi-circle found in their kinetoplastic strain is 15% larger than that of *T. brucei*, rather than 35% smaller. The map presented for this maxi-circle is different from that of *T. brucei*, but the size of the fragments obtained with several restriction enzymes is not [19]. The only real differences, that cannot be attributed to

calibration or the loss of small fragments, are the presence in *T. equiperdum* of one extra HapII and one extra BamHI site and the loss of one HaeIII site. Moreover, we can also fit the data of Riou and Saucier [19] into a map with the same fragment order as that established for *T. brucei* rather than the fragment order deduced by the authors. The maps differ only in one out of eight hexamer and two out of six tetramer sequences. This suggests that the *T. equiperdum* strain studied by Riou and Saucier is a very close derivative of *T. brucei* with kinetoplast DNA that is much less altered than the kinetoplast DNA in our strain. Unfortunately, there is no laboratory test to distinguish *T. equiperdum* from a *T. brucei* strain that has lost the ability to make functional mitochondria. It remains possible, therefore, that a strain thought to be *T. equiperdum* is *T. brucei*.

Acknowledgements

We thank Mrs. F. Fase-Fowler and Miss J. Van den Burg for their help in some of these experiments and Mr. J. Hoffschlag and Mr. F.P.J.M. Leijse for running the ultracentrifuge. We are indebted to the Staff of the Laboratory of Electron Microscopy (University of Amsterdam) for their generous provision of facilities. A.C.C.F. gratefully acknowledges a research training grant from the World Health Organization (No. M8/181/4/F.78). This work was supported in part by a grant to P.B. from the Foundation for Fundamental Biological Research (BION) which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO) and a grant from the North Atlantic Treaty Organization (No. 559).

References

1 Borst, P. and Hoeijmakers, J.H.J. (1979) Plasmid 2, 20—40
2 Hajduk, S.L. (1978) in Progress in Molecular and Subcellular Biology (Hahn, G.E. ed.), pp. 158—200, Vol. 6, Springer-Verlag, Berlin
3 Borst, P. and Fairlamb, A.H. (1976) Abstract 2nd International Symposium on the Biochemistry of Parasites and Host-Parasite Relationships, p. 31, Janssen Research Foundation, Beerse
4 Fairlamb, A.H., Weislogel, P.O., Hoeijmakers, J.H.J. and Borst, P. (1978) J. Cell Biol. 76, 293—309
5 Borst, P. and Hoeijmakers, J.H.J. (1979) in Extrachromosomal DNA: ICN-UCLA Symposia on Molecular and Cellular Biology (Cummings, D.J., Borst, P., Dawid, I.B., Weissman, S.M. and Fox, C.F., eds.), Vol. 15, pp. 515—531, Academic Press, New York
6 Hajduk, S.L. and Cosgrove, W.B. (1979) Biochim. Biophys. Acta 561, 1—9
7 Borst, P. and Fase-Fowler, F. (1979) Biochim. Biophys. Acta 565, 1—12
8 Leon, W., Frasch, A.C.C., Hoeijmakers, J.H.J., Fase-Fowler, F., Borst, P., Brunel, F. and Davison, J. (1980) Biochim. Biophys. Acta, in the press
9 Steinert, M., van Assel, S., Borst, P. and Newton, B.A. (1976) in The Genetic Function of Mitochondrial DNA (Saccone, C. and Kroon, A.M., eds.), pp. 71—81, North-Holland, Amsterdam
10 Donelson, J.E., Majiwa, P.A.O. and Williams, R.O. (1979) Plasmid 2, 572—588
11 Cunningham, M.R. and Vickerman, K. (1962) Trans. R. Soc. Trop. Med. Hyg. 56, 48—59
12 Southern, E.M. (1975) J. Mol. Biol. 98, 503—517
13 Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) J. Mol. Biol. 113, 237—251
14 Goldbach, R.W., Borst, P., Bollen-de Boer, J.E. and van Bruggen, E.F.J. (1978) Biochim. Biophys. Acta 521, 169—186
15 Brunel, F., Davison, J., Merchez, M., Borst, P. and Weijers, P.J. (1980) FEBS Symposium on DNA, Pergamon, Oxford, in the press
16 Schildkraut, C.L., Marmur, J. and Doty, P. (1962) J. Mol. Biol. 4, 430—443
17 Sueoka, N. (1961) J. Mol. Biol. 3, 31—40
18 Riou, G. and Pautrizel, R. (1977) Biochem. Biophys. Res. Commun. 79, 1084—1091
19 Riou, G. and Saucier, J.-M. (1979) J. Cell Biol. 82, 248—263
20 Renger, H.C. and Wolstenholme, D.R. (1971) J. Cell Biol. 50, 533—540