

The *Leishmania* genome comprises 36 chromosomes conserved across widely divergent human pathogenic species

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ABSTRACT

All the physical linkage groups constituting the genome of *Leishmania infantum* have been identified for the first time by hybridization of specific DNA probes to pulsed field gradient-separated chromosomes. The numerous co-migrating chromosomes were individualised using the distinctive size polymorphisms which occur among strains of the *L.infantum/L.donovani* complex as a tool. A total of 244 probes, consisting of 41 known genes, 66 expressed sequence tags (ESTs) and 137 anonymous DNA sequences, were assigned to a specific linkage group. We show that this genome comprises 36 chromosomes ranging in size from 0.35 to ~3 Mb. This information enabled us to compare the genome structure of *L.infantum* with those of the three other main *Leishmania* species that infect man in the Old World, *L.major*, *L.tropica* and *L.aethiopica*. The linkage groups were consistently conserved in all species examined. This result is in striking contrast to the large genetic distances that separate these species and suggests that conservation of the chromosome structure may be critical for this human pathogen. Finally, the high density of markers obtained during the present study (with a mean of 1 marker/130 kb) will speed up the construction of a detailed physical map that would facilitate the genetic analysis of this parasite, for which no classical genetics is available.

INTRODUCTION

The protozoan parasite *Leishmania* causes a broad spectrum of diseases affecting ~15 million people, mainly in tropical and subtropical areas. Increased incidence of the disease leishmaniasis can be related to various causes, including HIV/*Leishmania* co-infection. The genetic study of this parasite has long been hampered by the lack of any manipulable recombination system. The construction of a physical map for the *Leishmania* genome and the localization on this map of a large set of markers would be of great help for the definition of chromosome structure and

subsequently for the study of the genetics of this parasite. As the chromosomes of this organism do not condense significantly during its life cycle, they could only be separated with the advent of pulsed field gel electrophoresis (PFGE).

Attempts at separating all the chromosomes of *Leishmania* by PFGE have only been partially successful, due to a lack of knowledge about chromosome number and ploidy and also because of the concentration of numerous chromosomal bands in a limited size class interval. Due to these limitations, estimates of the chromosome number of different *Leishmania* species have varied from 23 to 96 (1–4), giving estimated genome sizes between 23 and 134 Mb. This confusion was also due to a lack of comparative mapping analysis between different *Leishmania* species, which vary widely in geographic distribution and pathogenic behaviour. The nucleotide divergence between the main species of this genus has been estimated at 13–25% (5). This corresponds to a long evolutionary time and questions the degree of synteny conservation among different species.

The structure of the chromosomes of *Leishmania* seems comparable with that of other protozoa, with a central core of conserved single or low copy sequences and long stretches of subtelomeric and telomeric repeated sequences (6). These subtelomeric regions are responsible for size variations between homologous chromosomes, which appear so frequent that virtually every *Leishmania* isolate displays a distinctive karyotype (7). Therefore, the identification of each chromosome relies upon obtaining a specific set of single copy markers. Such markers have recently been developed for the six smallest chromosomes of *L.infantum* (8). They allowed a comparison of the organization of these chromosomes between distant *Leishmania* species. Surprisingly, this analysis revealed a conservation of the linkage groups for these six chromosomes, without any evident rearrangement (8). It remained to be investigated if the results obtained for these chromosomes, which represent only ~6% of the total nuclear DNA, were applicable to the whole genome.

In this study we used the systematic hybridization of specific markers onto the molecular karyotypes to identify all the chromosomes of *Leishmania*. Besides their usefulness in future mapping analyses of individual chromosomes, these markers allowed us to compare the chromosome contents of different species of this parasite.

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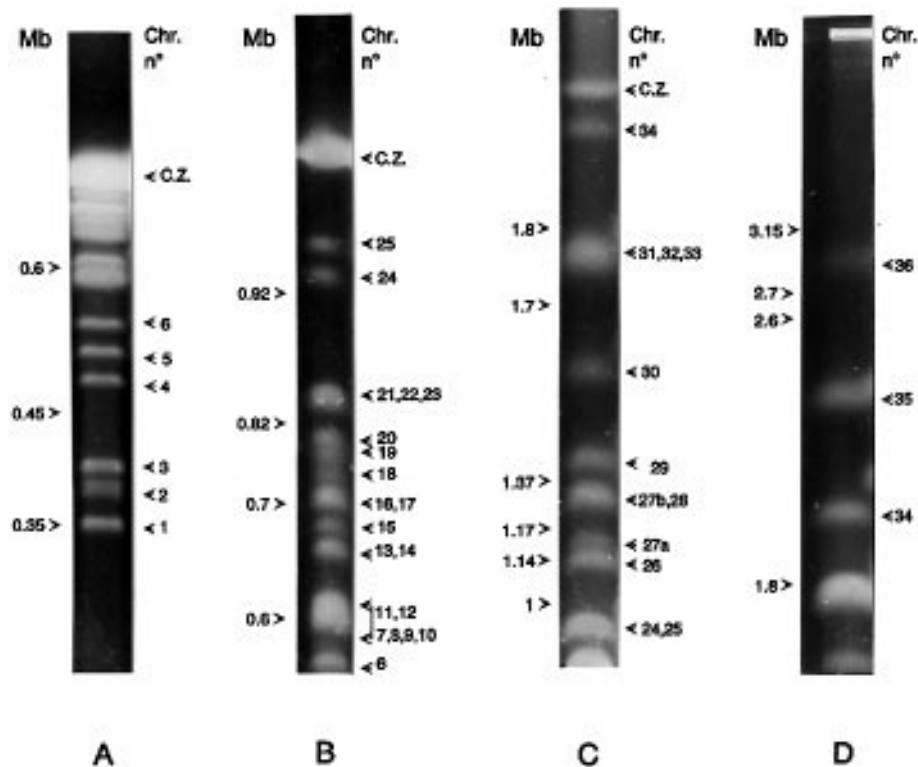


Figure 1. Complete resolution of the karyotype of *L. infantum* LEM1317 by PFGE. The sizes of *S. cerevisiae* and *H. wingei* chromosomes are indicated on the left in megabase pairs (Mb) and the position and number of the *Leishmania* chromosomes identified in the present study are shown on the right of each lane. C.Z., compression zone. (A) Separation of the smallest chromosomes was achieved using pulse/migration times of 30 s/46 h and 50 s/30 h in a 1.5% agarose gel at 7.5 V/cm. (B) Separation of the intermediate sized chromosomes was achieved using 90 s/24 h, 85 s/32 h and 80 s/32 h in a 1.5% agarose gel at 7.5 V/cm. (C) Separation in the megabase range was achieved using 220 s/32 h, 180 s/32 h and 150 s/24 h in a 1.5% agarose gel at 7.5 V/cm. (D) Separation above 2 Mb was achieved with 600 s/48 h, 400 s/48 h and 250 s/38 h in a 1% agarose gel at 5 V/cm.

MATERIALS AND METHODS

Parasite strains

The *Leishmania* strains used for the definition of the linkage groups in the *L. donovani* complex were: *L. infantum* LEM1317, a clone of LEM356 (MHOM/FR/82/LEM356), LEM1163, a clone of strain LEM75 (MHOM/FR/78/LEM75), LEM1284, a clone of LEM251 (MCAN/FR/81/LEM251), and LEM267 (MCAN/FR/81/LEM267); *L. donovani* LEM1651, a clone of LEM138 (MHOM/IN/00/DEVI), and LEM1448, a clone of LEM536 (MHOM/SA/81/JEDDAH-KA). The inter-species comparison was made with: *L. major* LEM1958, a clone of LEM62 (MHOM/YEM/76/LEM62), and LV39-Clone5 (MRHO/SU/00/LV39-CL5); *L. tropica* LEM1661, a clone of LEM579 (MHOM/GR/00/LEM579), and LEM1909, a clone of LEM408 (MHOM/AF/82/K0061); *L. aethiops* LEM1660, a clone of LEM144 (MHOM/ET/72/L100). All strains were cultivated on blood agar (NNN) medium supplemented with RPMI 1640 (Gibco). Their identity was checked by the examination of isoenzyme profiles (15 isoenzyme systems) immediately prior to this study, at the reference Laboratoire d'Ecologie Médicale et Pathologie Parasitaire in Montpellier.

PFGE and hybridizations

Chromosomal DNA agarose blocks were prepared and processed for PFGE on home-made devices as described previously (7). PFGE was performed at 15°C in 1 or 1.5% agarose gels with 0.5× TBE

(1× TBE = 89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA, pH 7.4) running buffer. The voltages and pulse conditions necessary to obtain a fine resolution for every chromosomal size class are described in the legend to Figure 1. The size markers were chromosomes from *Saccharomyces cerevisiae* strain AB1380, *Hansenula wingei* (BioRad) or *Schizosaccharomyces pombe* (BioRad). After staining with ethidium bromide gels were blotted onto nylon filters (Hybond N⁺; Amersham) by alkaline transfer (9). The probes were labelled by random primed synthesis and hybridised as described previously (8).

DNA probes

Three different sources of probes were used to establish the linkage groups. First we constructed a series of genomic libraries consisting of pBluescript vectors carrying inserts of small size (100–500 bp), in order to limit the presence of repeated sequences. The clones from these libraries were called ISA (for the libraries made from *L. infantum* LEM75 DNA) or ST (for the libraries made from *L. infantum* LEM1317 or LEM189). The source DNA was either the total nuclear content or gel-purified chromosomes. The latter was done to avoid under-representation of the smallest chromosomes in a total DNA library or elsewhere to increase the number of markers in a specific size class. A total of 10 different libraries were used, made from the six smallest chromosomes (ST1–ST6 series), a mixture of the eight intermediate sized chromosomal bands (ST7–ST9 series) and the total DNA (STP and ISA series). To avoid repeated cloning of the same inserts these libraries were made with

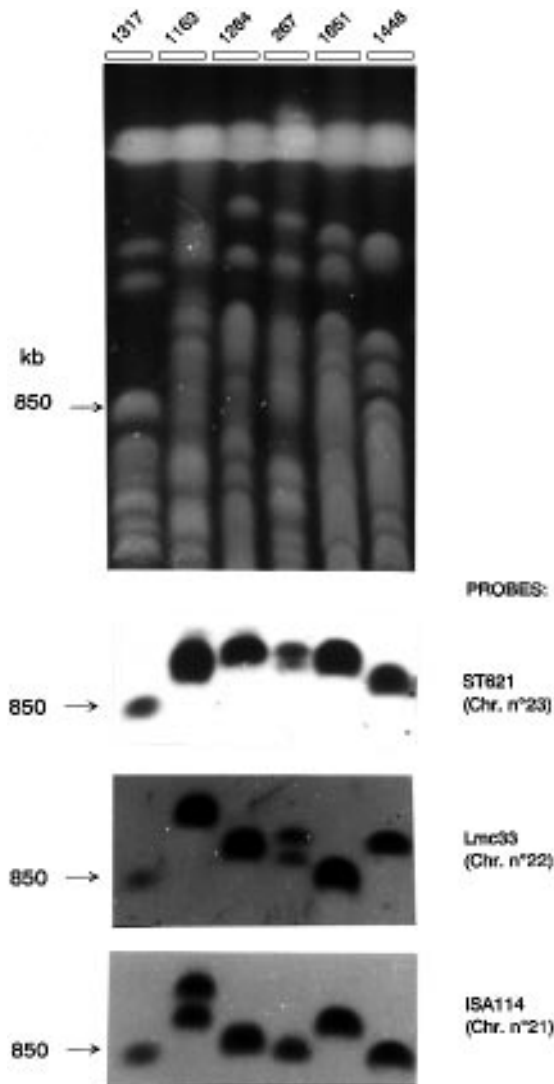


Figure 2. Use of inter-strain chromosome size polymorphisms to define the contents of an 850 kb chromosomal band in LEM1317. The upper photograph shows part of a pulsed field separation of intermediate sized chromosomes of four *L. infantum* strains (LEM1317, 1163, 1284 and 267) and two *L. donovani* strains (LEM1651 and 1448). The migration conditions were as in Figure 1B. The gel was blotted and successively hybridized with the chromosome-specific probes ST621, Lmc33 and ISA114. The region of the blots highlighted by the probes is shown aligned with the UV picture. Every probe hybridizing to the 850 kb band in LEM1317 gave rise to one of three patterns with the other karyotypes, thus identifying three distinct physical linkage groups. In particular, different sized homologs seen in one particular strain for each of these three chromosomes (e.g. chromosome 22 in LEM267) hybridised respectively with all the probes of the corresponding linkage group.

a series of different restriction enzymes (*Pst*I, *Hinf*II, *Taq*I, *Hha*I and *Clal*) and a limited number of clones were isolated from each library. Either PCR-amplified products or inserts obtained from restriction digests were used for labelling.

A second source of probes was ESTs sequenced from a *L. major* cDNA library made in λ ZAP and provided by J.Ajioka and J. Blackwell (Cambridge University, UK; M. Levick *et al.*, submitted for publication). The inserts were purified after PCR amplification with M13/M13 reverse primers.

Finally, we used 41 gene probes isolated in different laboratories from different *Leishmania* species. The corresponding genes are listed thereafter by order of increasing size of the identified chromosomes: *L. major* mini-exon (10); *L. major* actin (D. F. Smith, Imperial College, London, UK, unpublished data); minisatellite sequence LiSTIR1 (11); the B729 fragment of dihydrofolate reductase-thymidylate synthase (DHFR-TS) from *L. major* (12); the *L. major* β -tubulin gene family (13); *L. mexicana* cysteine protease lmc33 (14); *L. donovani* phosphoribosyl pyrophosphate synthetase (PRS) (15); surface protease gp63 from *L. major* (16); histone H2B from *Leishmania* (17); *L. major* surface antigen PSA-2 (18); *L. donovani* ornithine decarboxylase (ODC) (19); α -tubulin from *Leishmania* (20); *L. chagasi* kinesin (21); *L. major* developmentally regulated cDNAs 2, 7, 14 and 16 (22); *L. donovani* S-adenosylhomocysteine hydrolase (SAH) (23); *L. donovani* inosine 5'-monophosphate dehydrogenase (IMPDH) (24); *L. mexicana* cysteine protease lmc33 (25); *L. infantum* histone H2A (26); *L. mexicana* CDC2-related kinase lmmcrk1 (27); hypoxanthine-guanine phosphoribosyl transferase (HGPRT) from *L. donovani* (28); the *L. tarentolae* H region implicated in drug resistance (29); *L. major* ribosomal protein S8 (30); the *L. major* homolog of yeast Silent Regulator 2 (hSIR2) (31); *L. major* heat shock protein 70-related gene hsp70.4 (32); adenine phosphoribosyl transferase (APRT) from *L. donovani* (33); the *Trypanosoma brucei* ribosomal DNA cluster (34); *Drosophila melanogaster* hsp70 (35); *L. mexicana* cysteine protease lmc33 (36); the *L. major* homolog of hsp100 (clpB) (37); *L. major* hsp70-related hsp70.1 (38); *L. donovani* S-adenosylmethionine decarboxylase (SAMdc) (B. Ullman, unpublished results); *L. amazonensis* hsp83 (39); the P-glycoprotein implicated in multi-drug resistance (MDR) from *L. donovani* (40); the E13 fragment from the frequently amplified LD1/CD1 region from *L. donovani* (41); *L. mexicana* pyruvate kinase (PK) (42); *Leishmania* Pro-1 glucose transporter (43); *L. amazonensis* N-acetylglucosamine 1-phosphate transferase (NAGT) (44); *L. mexicana* CDC2-related kinase lmmcrk3, homologous to the *T. brucei* tbcrc3 gene (45).

RESULTS

The complete molecular karyotype of *L. infantum* LEM1317

Clone LEM1317 of *L. infantum* was previously selected for these studies because of its relatively simple karyotype. To gain the maximal information from the PFGE separation of its chromosomes we had to use four different running conditions (Fig. 1). A total of 25 chromosomal bands, ranging in size from 0.35 to ~3 Mb, were resolved with very variable staining intensities, suggesting the presence of more than one chromosome in many bands, as well as of different sized homologues of the same chromosome. The number of bands may be approximate, since some wide bands may be more or less resolved according to the electrophoretic conditions. Two facts indicate that no chromosome of a larger size exists in LEM1317. First, under the conditions shown in Figure 1D the *S. pombe* chromosomes were able to enter the gel, with the 3.5 Mb chromosome 3 being separated from the two others (data not shown) and no compression zone was seen in this region for LEM1317. Second, we never found any DNA probe tested against the LEM1317 karyotype that would hybridise only to the gel slot. We concluded that the 25 bands observed under these four electrophoretic conditions contain all the nuclear DNA molecules of *L. infantum*.

Use of inter-strain polymorphisms to define linkage groups

We have previously defined six physical linkage groups for the six smallest chromosomes of Old World *Leishmania* (8). This was possible because of the clear separation of the six chromosomes in most strains. This approach proved impracticable above 550 kb, as the patterns observed were more complex in this size class, probably due to the presence of many chromosomes of a similar size. The use of densitometric measurements to estimate the number of chromosomes per band was also impossible, because of the size variations between homologous chromosomes in the same strain (6) and of the as yet uncertain ploidy of *Leishmania*. To define the chromosomal contents of each band in the LEM1317 karyotype we selected five strains (three of *L.infantum* and two of the closely related species *L.donovani*) previously shown to display highly polymorphic karyotypes due to widely divergently sized homologues in all size classes (7). In all cases previously analysed these size variations were attributed to amplification/deletion mechanisms and not to inter-chromosomal rearrangements (6,8; our unpublished results). In none of these strains was the karyotype clearer than in LEM1317 (Fig. 2). The chromosome size polymorphisms were used here as a tool, allowing identification of each chromosome from its distinctive inter-strain hybridization pattern with specific probes. An example is shown in Figure 2, where a chromosomal band of 850 kb in

LEM1317 was resolved as three different patterns corresponding in the final numbering to chromosomes 21, 22 and 23. The pairs of different sized homologues observed in some strains both hybridized with all the probes defining the corresponding linkage groups.

A total of 244 probes were hybridised on these six karyotypes, consisting of 41 genes, 66 ESTs and 137 anonymous DNA markers. Using this method we identified 36 different physical linkage groups (Table 1). All these chromosomes were clearly identifiable by their specific hybridization patterns on the six karyotypes. One exception was chromosomes 32 and 33, which remain closely associated in all six strains. The fact that they constitute independent linkage groups was established using polymorphic *L.braziliensis* strains, where these chromosomes migrate at close but different positions (data not shown). Subtle but consistent differences between these two chromosomes were also visible in *L.tropica* and *L.aethiopic* strains (data not shown). The existence of additional unidentified chromosomes is very unlikely for two reasons: first, individual chromosomes in the karyotype always consist of a band with a low staining intensity and all the intensely staining bands have been resolved using a high number of probes (e.g. 30 for the band containing chromosomes 21, 22 and 23); second, no band remained unidentified in the karyotypes of the six polymorphic *L.infantum*/*L.donovani* strains used for definition of the linkage groups (not shown).

Table 1. List of the DNA markers identifying each chromosome of *L.infantum*

Chromosome	Size (Mb) ^a	Genes/known sequences ^b	ESTs ^c	Anonymous probes ^b
1	0.35	LiSTIR1*		ST113, ST114, ST124, ST 126, ST132, ST143, ST242, ISA132
2	0.37	Mini-exon		ST111, ST127, ST638, ISA150
3	0.4			ST137, ST204, ST211, ST238, ST249, ST254, ISA112, ISA139b, ISA156
4	0.47	Actin		ST116, ST420, ST431, ST445, ST669, ISA106h
5	0.5	LiSTIR1*		ST105, ST429, ST444, ST506
6	0.55	DHFR-TS	g12, j31*	ST210, ST602, ST606, ST612, ST613, ST618, ST632, ST641, ST671
7	0.6			ST601, ST815, ISA142b
8	0.6	β -Tubulin*, lmcpb, PRS		ST807, ST5103
9	0.6			ST656, ST706, ISA139h, ST904
10	0.6	gp63		ST715, ST5062, ISA113, ST729
11	0.6	Histone H2B*	c38	ST108, ST666*, ST911
12	0.6	PSA-2, ODC		ST716, ST811, ST905
13	0.65	α -Tubulin	b09, e18, e47	ST708
14	0.65	Kinesin		ST416, ST603, ST702, ST803, ST903, ST5074, ISA151
15	0.68		f30, i46*	ST809, ST901, ST5059
16	0.7	Histone H2B*, cDNA14		ST707, ST710, ST912, ST5053
17	0.7		a44, f24	ST604, ST724, ST731, ST806, ST808, ST810
18	0.75			ST403, ST704, ST816, ST917
19	0.8	Histone H2B*, SAH, IMPDH, lmcpa, LiSTIR1*	c23, f14, g11	ST714, ST726, ST817, ISA140b
20	0.8		c08	ST711, ST712, ST719, ST723
21	0.85	Histone H2A*, β -tubulin*, lmmcrk1, HGPRT		ST255, ST5083, ISA114, STP116
22	0.85	LiSTIR1*	a37, c33, c47, g06, j31*	ST145, ST649, ST668, STP3
23	0.85	cDNA16, H region	a45, c14, c35, f39,	ST109, ST621, ST666*, ST902,
24	0.93	Ribosomal protein S8	a40, h13 g35, i28	ST814, ISA145 ST5005

Table 1. continued

25	0.96		c01	ST433, ST5184, ISA157, ISA159, ST999
26	1.1	hSIR2, hsp 70.4, APRT	h34	
27	1.15 + 1.3	ribosomal DNA	e19, h29	ST505, ST5136
28	1.3	cDNA7, hsp70	e16	
29	1.4	Histone H2A*, lmcpc, clpB	a35, b18, c45, f42, i48	<i>ST110</i> , STP2, ISA107*
30	1.55	cDNA2, hsp70.1, SAMdc		ST223, ST5163
31	1.75		c29, f26, f35, g01, g17	<i>ST208</i> , ST426, ST701, ST5141, STP6, STP25, STP82, ISA107*
32	1.75	β -Tubulin*, hsp83	e28, e48*, h32, i46*	ST217, ST727, STP23
33	1.75		e14, g30, h37	ST408, ST705, STP41
34	2.0	MDR	b03, f16, g13, h19, i38, j12	STP67, ISA155
35	2.2	LD1/CD1, PK	b14, c04, e02, e20, e39, e48*, f43, j08	ST424, STP45
36	3.0	Pro-1 glucose transporter, NAGT, Immcrk3	b12, b15, b22, g04, g15, g27	ISA143, ISA153

^aSizes defined in the reference strain LEM1317.

^bSee Materials and Methods for the gene abbreviations and anonymous probes.

^cESTs codes given here are those given by J. Ajioka and J. Blackwell (Department of Pathology, Cambridge University) and are preceded by the prefix Lm.

*Indicates a probe present in more than one chromosome.

The probes that fail to hybridize with DNA of species others than *L.infantum/L.donovani* are italicized.

These results also enabled us to establish the haploid genome size of *L.infantum* by summing up the sizes of the 36 heterologous chromosomes in LEM1317: this gave a value of 35.5 Mb.

Comparison of the *L.infantum* linkage groups among pathogenic species

In order to compare the physical linkage groups across different species, we selected representative cloned strains of the species *L.tropica*, *L.major* and *L.aethiopica*. We chose two *L.major* and two *L.tropica* strains with polymorphic karyotypes and a single *L.aethiopica* strain (Fig. 3). Of the 244 loci defined in *L.infantum*, 241 gave a good hybridisation signal on these three species, the difference arising from three probes with a low level of inter-species sequence conservation (indicated in italics in Table 1). We observed that all probes belonging to a specific chromosome defined in *L.infantum* remained linked to the same chromosomal band in these three species. Moreover, specific size variations of most chromosomes were observed among the two *L.major* and between the two *L.tropica* strains (Fig. 3B). This enabled us to conclude that the linkage groups defined in each band correspond to specific chromosomes. Although this comparison was not made between *L.aethiopica* isolates, it seems highly unlikely that the intensely staining bands containing more than one linkage group in this species may be the product of an inter-chromosomal sequence exchange giving rise to two chromosomes of exactly the same size. We concluded that the *L.infantum* linkage groups were conserved in these four species. Figure 4 shows a diagrammatic representation of the molecular karyotypes of one representative clone of each of these four species, with localization of each of the 36 linkage groups. The extensive inter-strain chromosomal size polymorphisms should be noted, as well as the shuffling in the chromosome order to which they lead. The haploid genome size estimates were comparable in all species examined (35.7 Mb for LEM1958, 36.5 Mb for LEM1909 and 35 Mb for LEM1660).

DISCUSSION

For the first time, we have resolved the complete karyotype of several species of *Leishmania* into 36 chromosomes using specific single or low copy DNA probes. This enabled us to define unambiguously the haploid genome size of this organism as 36 ± 1 Mb (depending on the strains considered). The approach we followed made use of the size variations that affect homologous chromosomes in different strains of the same species. This proved essential for the correct assignment of probes to a specific linkage group. Many imprecise localizations were noted when we compared our data with published results. For example, the existence in a *L.tropica* strain of two widely different sized bands for the PSA-2/gp46 antigen family was interpreted as suggesting the presence of these genes on two heterologous chromosomes (46). We actually found that they mapped on a single chromosome (no. 12 in Table 1) whose linkage group is perfectly conserved but which varies greatly in size, so that many strains contain two different versions of it. Another example is the localization of hsp70, a β -tubulin cluster, a histone H2A cluster and the ribosomal DNA region on a single 1.3 Mb chromosome in *L.infantum* (47). We found that they mapped to four different linkage groups of similar sizes (chromosomes 27, 28, 29 and 32). Thus the use of a single strain to identify a linkage group may lead to the confusion of a chromosome with a chromosomal band or of two homologues with two different chromosomes. It can be expected that the availability of a set of probes for each of the 36 chromosomes identified in this study and of reference karyotypes will allow a definitive chromosomal assignment for any new locus to be cloned.

The method used in the present work did not allow consideration of the chromosomal regions containing highly dispersed repetitive DNA present on many chromosomes. Nevertheless, we were able to map repeated loci when they were present on two to four heterologous chromosomes. We found nine such probes, defining a total of 22 loci (indicated by an asterisk in Table 1). It is noteworthy that when a single chromosome bears several of these markers

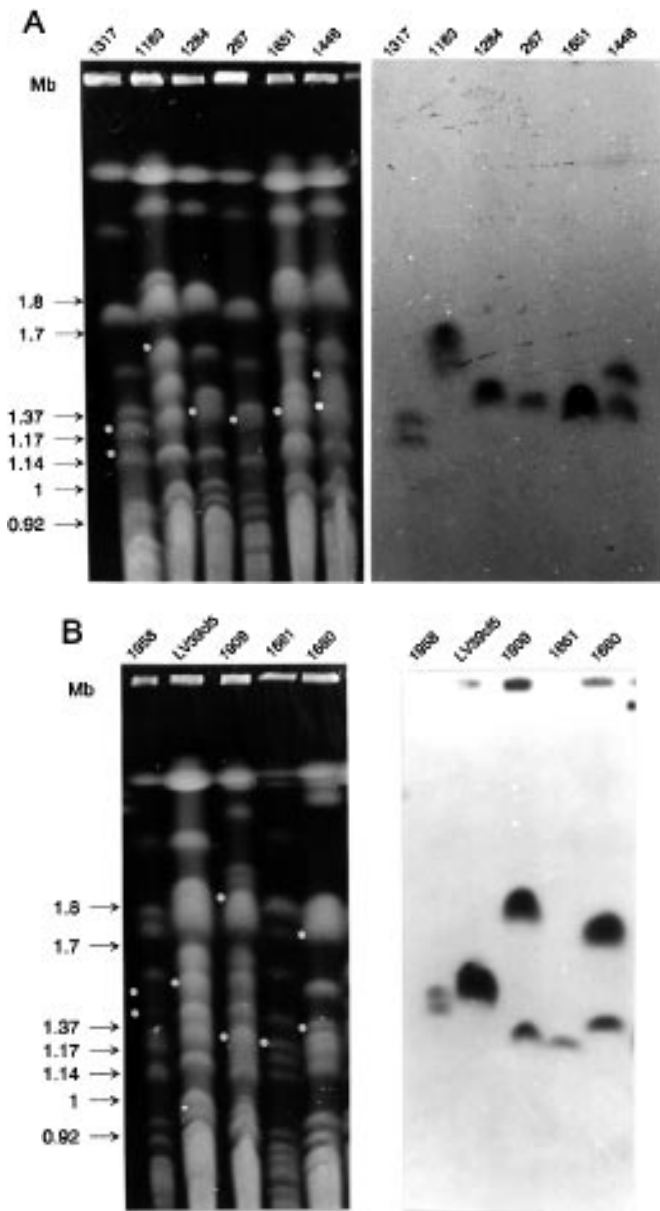


Figure 3. Hybridization of Lme19, a marker specific for chromosome 27, onto the karyotypes of different *Leishmania* species. (A) Ethidium bromide stained pulsed field gel of the six *L. infantum*/*L. donovani* strains used for chromosome identification (left) and the corresponding hybridization pattern of Lme19 (right). (B) Ethidium bromide stained gel of the five strains of *L. major*, *L. tropica* and *L. aethiopia* used for inter-species comparison (left) and the corresponding location of chromosome 27 revealed by hybridization of Lme19 (right). The migration conditions were as in Figure 1C. The position of chromosome 27 is indicated on the UV picture by a white circle. The size markers indicated on the left are from *H. wingei* chromosomes.

(chromosomes 11, 19, 21, 22, 29 and 32) the other copies of each of these markers are borne by different chromosomes (see Table 1). This suggests that these duplications are not large and that the *Leishmania* genome is probably not very redundant.

An important result of the present work was the finding of complete conservation in linkage group constitution in four different *Leishmania* species. These four taxa were studied previously in terms of isoenzyme variation (48) and nuclear restriction site divergence (5). Both techniques showed that they were separated by

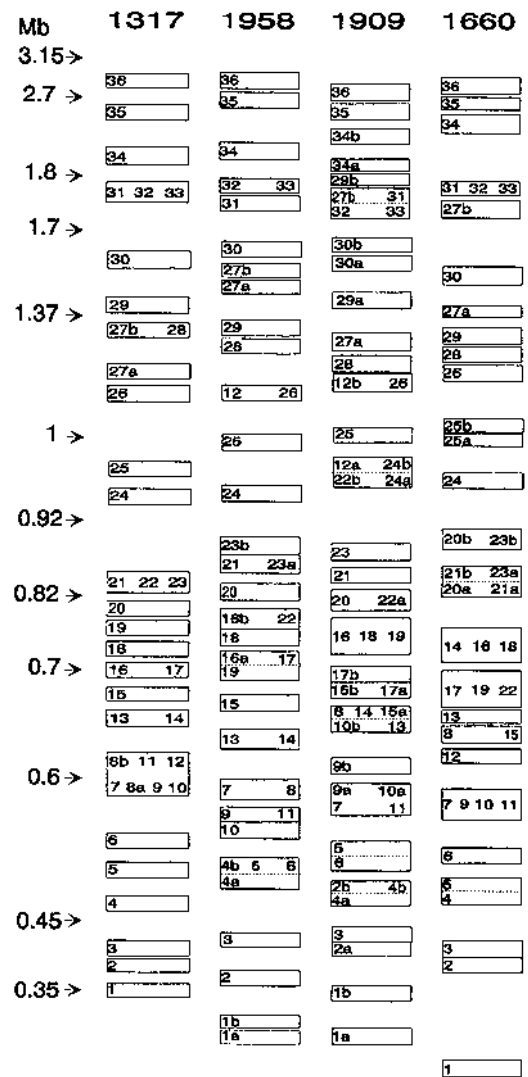


Figure 4. Schematic representation of the complete karyotypes of four representative cloned strains of the species *L. infantum* (LEM1317), *L. major* (LEM1958), *L. tropica* (LEM1909) and *L. aethiopia* (LEM1660). Each box represents a chromosomal band, with the number of the corresponding linkage group(s) indicated inside it. When two homologs differing in size are present in the same strain they are indicated as a and b. The scale is not directly proportional, but tries to represent the actual resolution seen on different gels. Molecular size markers from *S. cerevisiae* and *H. wingei* chromosomes are indicated on the left.

significant genetic distances. These biochemical estimates agreed with extrinsic criteria, such as the geographic distribution and the clinical disease, which served as the initial criteria for species separation. The conservation in linkage group constitution, for which we did not observe any exception, is therefore surprising. It could be explained either by a very low level of recombination between heterologous chromosomes or by specific constraints on the chromosomal contents. In this respect one can speculate that it may be related to the transcriptional mechanism in *Leishmania* and related trypanosomatids, which seems to be essentially polycistronic (49). It is possible that new transcription units, such as those expected from a chromosome translocation, would not be selectively advantageous. Indeed, a few examples of translocations have recently been shown among rodent malaria parasite species (for

which transcription does not appear to be polycistronic), although the density of probes used was only about a third of those obtained in the present study (50).

A consequence of these results is that the extreme genome plasticity observed in *Leishmania* (51) is not caused by large inter-chromosomal rearrangements. It has been shown that amplification/deletion in subtelomeric repetitive regions are responsible for the variation in chromosome 1 and 5 in *L. infantum* and chromosome 2 in *L. major* (6,10,11; our unpublished results). The present work suggests that this may be a general mechanism for chromosome size variability in *Leishmania*, as observed in other protozoan parasites (reviewed in 52).

The strategy used and the number of probes localized in this study are essential for the future construction of a detailed map of the *Leishmania* genome. With the genome size that we have defined we can estimate a density of 1 marker/130 kb (with a mean of 7 markers/chromosome and assuming a random distribution of the probes). This estimate may be even higher if we consider the size of the subtelomeric repeated regions, which can constitute up to 25% of the chromosome length (6). These markers should help to speed up the construction of long range restriction maps of individual chromosomes and to validate the construction of cloned contigs. The four karyotypes shown in Figure 4 may serve as a reference for future mapping studies. It is noteworthy that several of the remaining clones we examined exhibited much more complex karyotypes with diffuse or compressed bands and are therefore less helpful in this respect. Moreover, since all chromosomes are now characterized by their marker contents, we suggest that the numbering of the chromosomes of *Leishmania* established in this study also serve as a reference for future studies.

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