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## *Sfi*I and *Not*I polymorphisms in *Theileria* stocks detected by pulsed field gel electrophoresis

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DNAs of *Theileria parva parva*, *T. p. lawrencei*, *T. p. bovis* and *Theileria mutans* stocks, from Kenya, Uganda, Zanzibar and Zimbabwe were digested with either *Sfi*I or *Not*I and analysed using contour-clamped homogeneous electric field (CHEF) and field-inversion gel electrophoresis (FIGE). The *Sfi*I-digested *T. parva* genomic DNA resolved into approximately 30 fragments while the *Not*I digestion produced between 4–7 bands. The summation of the sizes of *Sfi*I fragments gave an estimate of  $9\text{--}10 \times 10^6$  base pairs for the size of the *T. parva* genome. Heterogeneity within *T. p. parva* Muguga, Pemba/Mnarani and Mariakani stocks was detected. All the *T. parva* stocks analysed showed *Sfi*I and *Not*I restriction fragment length polymorphisms (RFLP). Hybridisation of 5 *Sfi*I-digested *T. parva* DNAs with a *Plasmodium berghei* telomeric repeat probe suggested that most of the polymorphisms and heterogeneity occurred in the telomeric or sub-telomeric regions of the genome. The recognition by the *Plasmodium* telomeric probe of 7–8 strongly hybridising *Sfi*I bands indicates that the *T. parva* genome may possess at least 4 chromosomes. The *T. mutans* genome was cut frequently with the above enzymes resulting in large numbers of fragments predominantly below 50 kb, thus suggesting either a much higher G+C content than *T. parva* or the presence of highly reiterated G+C-rich regions.

Key words: *Theileria parva*; *Theileria mutans*; Polymorphism; CHEF; FIGE; Chromosome

### Introduction

East Coast fever (ECF) is a disease of cattle, caused by a tick-transmitted protozoan parasite *Theileria parva*. The parasite occurs in eastern, central and southern Africa and its presence coincides with the distribution of the tick *Rhipicephalus appendiculatus* which is its main vector. The disease is of economic importance because it causes high morbidity and mortality in *Bos taurus*

and their crosses with *Bos indicus*, and in improved *Bos indicus* cattle, thus inhibiting the introduction of genetically improved and more productive cattle into endemic areas.

An infection and treatment method of immunisation against the disease has been developed [1] and tested in several field trials with considerable success providing protection in over 90% of the immunised cattle [2–6]. However, one of the major problems with the immunity engendered in cattle is that it may be limited to certain strains of the parasite [7–9]. Thus, careful in vivo and in vitro characterisations of *T. parva* field isolates have to be performed before selecting immunising stocks in the infection and treatment method [10].

In vivo characterisation of the *T. parva* parasites, which involves cross-immunity tests in cattle, is useful in providing information on cross-protection between stocks, while in vitro tests,

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Abbreviations: CHEF, contour-clamped homogeneous electric field electrophoresis; ECF, East Coast fever; FIGE, field inversion gel electrophoresis; MAb, monoclonal antibody; PBL, peripheral blood lymphocytes; PFGE, pulsed-field gel electrophoresis; RFLP, restriction fragment length polymorphism; TAE, Tris-acetate/EDTA; TBE, Tris-borate/EDTA; TNE, Tris-HCl/NaCl/EDTA; SSC, saline sodium citrate.

such as isoenzyme analysis [11] monoclonal antibody (MAb) profiles [12], DNA probes [13,14], and two-dimensional gel electrophoresis [15], provide information on phenotypic and genomic diversity in parasite stocks. In addition, these tests are able to distinguish between stocks, providing a useful check on the identity of different parasites within a laboratory. Because of the complex epidemiology of theileriosis, involving wild Bovidae as reservoir hosts and several species of *Theileria*, it is necessary to have as many precise markers as possible to distinguish species, sub-species, stocks and strains. Such markers will have an important role in elucidating the epidemiology of theileriosis in Africa.

Pulsed-field gel electrophoresis (PFGE) has been successfully used in separating chromosome-sized DNA molecules from several protozoa [16–18] providing useful information on their genomic diversity at intra- and inter-specific level. In this paper we describe the use of two modified versions of PFGE, contour-clamped homogeneous electric field (CHEF) electrophoresis [19] and field inversion gel electrophoresis (FIGE) [20] to characterise *Theileria* species from eastern and southern Africa and demonstrate the usefulness of these techniques in defining polymorphism between and heterogeneity within various stocks of *T. parva*.

## Materials and Methods

### Cattle

The cattle used were Boran (*Bos indicus*) steers 3–18 months of age, born to dams on a farm free from ECF. The animals were raised under strict tick control and had no antibodies to *T. parva* or *T. mutans* in the indirect fluorescent antibody test [21] before experimentation.

### Parasites

Two species of *Theileria*, *T. parva* (comprising three sub-species *T. p. parva*, *T. p. lawrencei*, *T. p. bovis*) and *T. mutans*, were used in the study. Details of the various stocks of parasites used are given in Table I. The Kibarani stock was isolated from the Kilifi District of the Kenya Coast, and the Pemba/Mnarani stock from the island of Pemba, Tanzania (S.P. Morzaria and A.D. Irvin,

unpublished). The *T. p. lawrencei* 7014 stock was obtained from a naturally infected carrier buffalo captured in the Laikipia District of Kenya. The *T. mutans* Intona stock was isolated from Mara, Kenya (J. Mutugi and A.S. Young, unpublished) and the Kipange stock from the island of Unguja, Tanzania (S.P. Morzaria, T.T. Dolan and E. Flach, unpublished). The other stocks have been described elsewhere [12,22]. All the parasite stocks used in this study were uncloned and the Muguga and Marikebuni stocks have been identified as heterogeneous (refs. 13 and 14, and P. Toye, personal communication). Cattle were infected by inoculation with sporozoite stabilates as described by Cunningham et al. [23]. The schizont-infected cell-lines were obtained by in vitro infection of bovine peripheral blood lymphocytes (PBL) with sporozoites [24] and cultured to the required infected cell numbers.

Parasites from three stages of the life-cycle were examined; sporozoites from infected tick salivary glands, schizonts from established infected lymphoblastoid cell lines and piroplasms from infected erythrocytes. All these stages are assumed to be haploid [25].

### Purification of different parasite stages

**Piroplasms.** When the piroplasms parasitaemia in cattle was between 10–25%, 1–2 l of venous blood were collected in an equal volume of Alsever's solution containing 50 i.u. ml<sup>-1</sup> heparin. Following depletion of leukocytes, the erythrocytes were lysed in distilled water containing 1 mg ml<sup>-1</sup> concentration of saponin and piroplasms were purified as described [13]. The purified fraction was immediately processed for DNA preparation in low-melting-point agarose.

**Schizonts.** Approximately 10<sup>8</sup> infected cells were lysed with Ah-1 haemolysin [26] at room temperature for 25 min and the schizonts released were purified according to the method of Sugimoto et al. [27].

**Sporozoites.** *T. parva*-infected adult ticks were fed on rabbits for 4 days to induce maturation of sporoblasts to sporozoites [28]. The salivary glands of the pre-fed adults were then dissected and homogenized in RPMI-1640 medium (Flow Labo-

TABLE I  
*Theileria* stocks

Parasite	Stock	Parasite stage	Sporozoite stabilate number	Animal number	Cell line stabilate number
<i>T. p. parva</i>	Muguga	Piroplasms	3087	F290	–
		Piroplasms	2949	D642	–
		Sporozoites	1004	–	–
	Mariakani	Piroplasms	3029	D784	–
		Schizonts	3029	D212 <sup>a</sup>	3033
	Uganda	Piroplasms	3066	D786	–
		Schizonts	3066	D810	3086
	Pemba/Mnarani	Piroplasms	2913	E22	–
		Kibarani	Piroplasms	2448	E39
Marikebuni		Piroplasms	3014	F3	–
<i>T. p. lawrencei</i>	7014	Schizonts	3081	C577 <sup>a</sup>	3103
<i>T. p. bovis</i>	Boleni	Schizonts	3039	D768 <sup>a</sup>	–
<i>T. mutans</i>	Intona	Piroplasms	–	699	–
	Kipange	Piroplasms	–	E211	–

<sup>a</sup>In vitro infection of lymphocytes with sporozoites.

ratories, U.K.) before purification over a diethylaminoethyl cellulose column (Pharmacia Fine Chemicals, Uppsala, Sweden) following the method described for separation of sporozoites of *Plasmodium berghei* [29].

#### Parasite DNA preparation in agarose blocks

Purified parasite stages were resuspended in either TNE (for piroplasms) or RPMI-1640 medium (for schizonts and sporozoites) to give a concentration of between  $10^9$  and  $10^{10}$  parasites per ml. The parasite suspension was embedded with an equal volume of 1.5% low-melting-point agarose (BRL, Gaithersburg, MD, U.S.A.) and then lysed, deproteinated and washed as described [30].

#### Lambda ladders and *Trypanosoma brucei* chromosome markers

Concatemers of bacteriophage  $\lambda$  DNA CI 857 S7 ladders (48.5-kb monomer) and *Saccharomyces cerevisiae* DNA size markers were obtained commercially (Cambridge Bioscience, Cambridge, U.K.) and *Trypanosoma brucei* DNAs were prepared as described elsewhere [30].

#### Restriction enzyme digestion

Following equilibration in 1 ml of the appropriate restriction enzyme buffer for 2 h at 4°C, the agarose slice containing parasite DNA of 25  $\mu$ l volume was digested in 100  $\mu$ l of the enzyme buffer containing 40 units of either *Sfi*I or *Not*I for a minimum of 5 h at the temperature recommended by the supplier (New England Biolabs, Beverly, MA, U.S.A.). Following digestion the agarose slice was resuspended in 500  $\mu$ l of either 0.5  $\times$  TBE (90 mM Tris-HCl/90 mM boric acid/2.5 mM EDTA) or 1  $\times$  TAE (50 mM Tris-acetate/0.5 mM EDTA) and was used within 24 h.

#### CHEF and FIGE

Two modifications of PFGE, CHEF and FIGE were used to analyse *Theileria* DNAs. The CHEF apparatus was constructed at ILRAD according to the specifications of Chu et al. [19]. The FIGE apparatus was installed as described by Carle et al. [20]. The Programmable Power Inverter PPI-200 (MJ Research Inc., Cambridge, MA, U.S.A.) was used in association with either CHEF or FIGE to vary the pulse time intervals.

For CHEF electrophoresis, 1.5%, 10.5 cm × 14 cm agarose gels prepared in 0.5 × TBE were used and for FIGE 0.8% or 1%, 24 cm × 22 cm agarose gels prepared in either 0.5 × TBE or 1 × TAE were used. The buffer was recirculated and cooled to 12°C during electrophoresis. Gels were stained in ethidium bromide (0.5 µg ml<sup>-1</sup>) and photographed under ultra-violet transillumination.

#### Oligonucleotide hybridisation

A 28-mer oligonucleotide (CCCTGAACCC-TAAA)<sub>2</sub> corresponding to a sequence of a *P. berghei* telomeric clone [31] was made using an Applied Biosystems 381A Synthesiser. The oligonucleotide was labelled with <sup>32</sup>P as described [32], and was added to the hybridisation solution without removal of unincorporated [<sup>32</sup>P]dATP.

A Southern blot of *Sfi*I-digested *T. parva* DNA on a nylon filter (Amersham Hybond-N) was pre-hybridised for 6 h in 6 × SSC, 5 × Denhardt's (0.1% each of bovine serum albumin, Ficoll 400 and polyvinyl pyrrolidone), 0.05% sodium pyrophosphate and 0.5% SDS at 42°C and then hybridised with the labelled oligonucleotide in the same solution for 4 h at 42°C. After hybridisation, the filter was washed 4 times for 30 min in 5 × SSC at 45°C before overnight autoradiographic exposure.

#### Results

**CHEF gels of *Sfi*I and *Not*I digested *Theileria* DNA.** Separation of *T. p. parva* Muguga *Sfi*I-digested DNA on CHEF using a 10-s pulse switching time for 18 h predominantly resolved fragments of between 30–600 kb, while the 40-s pulse interval for the same period resolved fragments from 400 kb to 1000 kb. A combination of 2 pulse switching times (10 s for 16 h and 40 s for either 2.5 h or 4 h) gave optimum resolution of all the fragments between 40 and 1000 kb (Fig. 1A and B).

Using this combination of pulse-intervals and digestion with *Sfi*I, piroplasm DNAs from several stocks of *T. p. parva* were analysed. Fig. 1A shows 5 *T. p. parva* stocks and one *T. mutans* stock separated on CHEF. Except for minor distortions, the digested DNAs were resolved in dis-

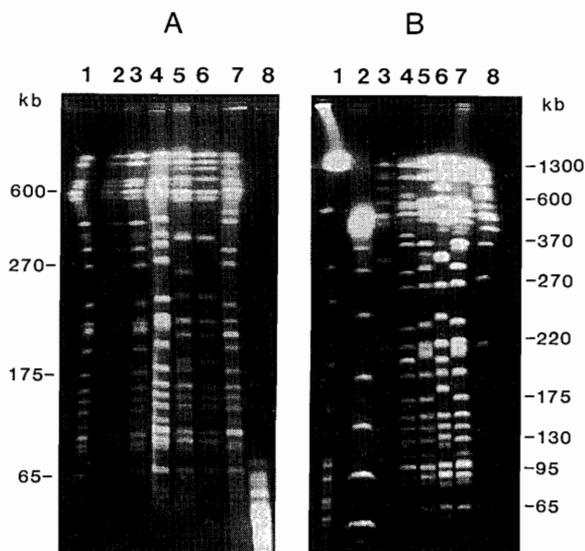


Fig. 1. Demonstration of polymorphism in 5 different *T. parva* stocks. (A) *Sfi*I-digested DNAs from (1) Muguga piroplasm (2949); (2) Muguga piroplasm (1004); (3) Muguga sporozoite (passage from 1004); (4) Pemba/Mnarani piroplasm (2913); (5) Mariakani piroplasm (3029); (6) Uganda piroplasm (3066); and (7) Kibarani piroplasm (2448). Lane 8 is the *Sfi*I-digested piroplasm DNA of *T. mutans* Intona stock. (B) Lanes 1, 2, and 8 show ILTat clone 3 *T. brucei* undigested DNA,  $\lambda$  concatemers and *S. cerevisiae* yeast as markers, respectively. Lanes 3–7 are *Sfi*I-digested piroplasm DNA of (3) Uganda (3066); (4) Kibarani (2448); (5) Marikebuni (3014); (6) Mariakani (3029); and (7) Muguga (3087) stocks. The CHEF in panel (A) was performed at a pulse frequency of 10 s for 16 h and 40 s for 2.5 h, while that in panel (B) was performed at a pulse frequency of 10 s for 16 h and 40 s for 4 h. Both gels were run at 200 V.

tinct numbers of discrete fragments on parallel lanes, allowing for accurate comparison between stocks. All 6 stocks of *T. p. parva*, Muguga; Pemba/Mnarani; Mariakani; Uganda; Kibarani (Fig. 1A, lanes 3–7) and Marikebuni (Fig. 1B, lane 5), analysed on CHEF showed restriction fragment length polymorphisms (RFLP). *T. p. parva* Muguga and Pemba/Mnarani stocks (Fig. 1A, lanes 2 and 4) were very similar except for an extra 350 kb fragment present in the Muguga stock. The Uganda stock was the most unusual in that 3 major bands of 270, 300 and 370 kb (Fig. 1A, lane 6), present in all other stocks except Mariakani, were missing. Varying degrees of ethidium bromide staining intensity of some fragments, e.g., 650–300 kb fragments in *T. p. parva* Muguga, Pemba/Mnarani and Mariakani stocks,

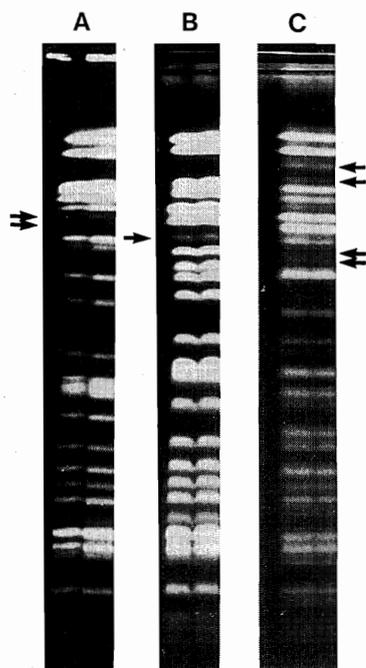


Fig. 2. CHEF separation of Pemba/Mnarani, Mariakani and Muguga *Sfi*I-digested DNAs (panels A, B and C, respectively) showing presence of DNA fragments stained less intensely with ethidium bromide than others of similar sizes (arrows). This may indicate that those stocks have mixed parasite populations.

indicated either the presence of heterogeneous parasite populations within stocks (Fig. 2A, B and C, respectively) or incomplete digestion of some of the *Sfi*I fragments. All these stocks were prepared from field isolates and have been maintained in the laboratory without cloning. The faint bands did not disappear by either increasing the period of digestion up to 10 h or increasing the concentration of the restriction enzyme up to 100 units. The total number of major *Sfi*I bands in the different stocks of *T. parva* examined varied between 28 and 33, the Uganda stock having the smallest number.

The summation of the sizes of *Sfi*I fragments, accounting for apparent doublets and excluding faint bands gave an estimate of  $9-10 \times 10^6$  bp for the size of the total genome of *T. parva* Muguga. The Uganda stock appeared to have the smallest genome.

In stark contrast to *T. parva* DNA, the genomic DNA of *T. mutans* was cut frequently by

*Sfi*I and the majority of the fragments were below 65 kb (Fig. 1A, lane 8). Various combinations of voltage and pulse intervals ranging from 10 to 3600 s in CHEF did not separate the undigested *T. parva* and *T. mutans* into more than 2 fragments.

*FIGE analysis of T. p. parva and T. mutans.* Fig. 3A (lanes 1, 2, 3 and 4) shows FIGE separation of *Not*I digested DNAs of 4 stocks of *T. p. parva* Mariakani, Muguga, Uganda and Kibarani respectively) and one stock of *T. mutans* (Intona). Polymorphism was again observed among the different *T. p. parva* stocks. As with *Sfi*I, the *T.*

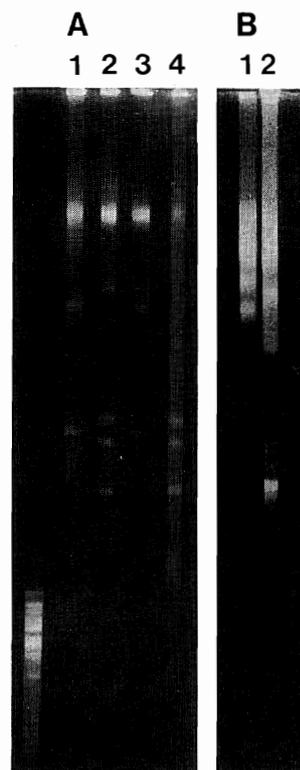


Fig. 3. FIGE of *T. parva* genome undigested and digested with *Not*I. (A) Lanes 1-4 are piroplasm DNAs of (1) *T. parva* Mariakani (3029); (2) Muguga (2949); (3) Uganda (3066); and (4) Kibarani (2448). (B) Lanes 1 and 2 show undigested piroplasm DNA of *T. parva* Kibarani and undigested DNA of ILTat clone 3 of *T. brucei*. The FIGE in panel (A) was performed in 1% agarose gel at forward pulse of 6.66 s and backward pulse of 2.22 s for 24 h at 300 V. The FIGE in panel (B) was performed in 0.8% agarose gel at forward pulse of 0.3 s and backward pulse of 0.1 s for 20 h at 240 V.

*mutans* DNA was cut into numerous fragments of less than 100 kb (Fig. 3A, unmarked lane). The undigested *T. parva* Kibarani (Fig. 3B, lane 1) and Marikebuni DNAs were separated into 4 fragments while the other stocks of *T. parva* and one stock of *T. mutans* separated into only 3 fragments.

**Analysis and comparison of DNA prepared from different developmental stages of *T. parva*.** Certain *T. p. parva* stocks do not produce high piroplasm parasitaemia, e.g. *T. p. bovis* Boleni, *T. p. lawrencei* 7014 and *T. p. parva* Marikebuni. Therefore, genomic DNAs were prepared from either schizonts derived from infected cell-lines or sporozoites obtained from infected tick salivary gland acini. The DNA was digested with *Sfi*I and then analysed by CHEF electrophoresis. Fig. 1A shows that no differences were apparent between *T. p. parva* Muguga piroplasm DNA (lane 2) and sporozoite DNA (lane 3) derived from the same stock. It is interesting to note that the *T. p. parva* Muguga sporozoites were passaged through the tick/cattle cycle 3 times while the piroplasms were passaged only once from the original reference stabilate.

The restriction profiles of the schizont-derived DNAs of Muguga and Uganda stocks were the same as those obtained from the piroplasm DNAs. The *Sfi*I restriction profiles of the *T. p. bovis* Boleni and *T. p. lawrencei* 7014 stocks were different from each other and from other *T. p. parva* stocks (results not shown). Although schizont-derived DNA showed evidence of bovine DNA contamination it was sufficiently pure to allow comparison with the piroplasm-derived DNA.

**Hybridisation of *Sfi*I-digested *T. p. parva* DNA to the *P. berghei* telomeric probe.** A Southern blot of *Sfi*I-digested DNA from 5 different *T. p. parva* stocks was probed with a <sup>32</sup>P-labelled oligonucleotide whose sequence was derived from the published sequence of cloned telomeric sequences from *P. berghei* [31]. Strong hybridisation signals were obtained on 7–8 *Sfi*I fragments (Fig. 4B). In addition, weak signals were observed, mostly corresponding to fragments staining weakly with ethidium bromide (e.g., in Fig. 4B, lanes 2 and 5), but sometimes to strongly

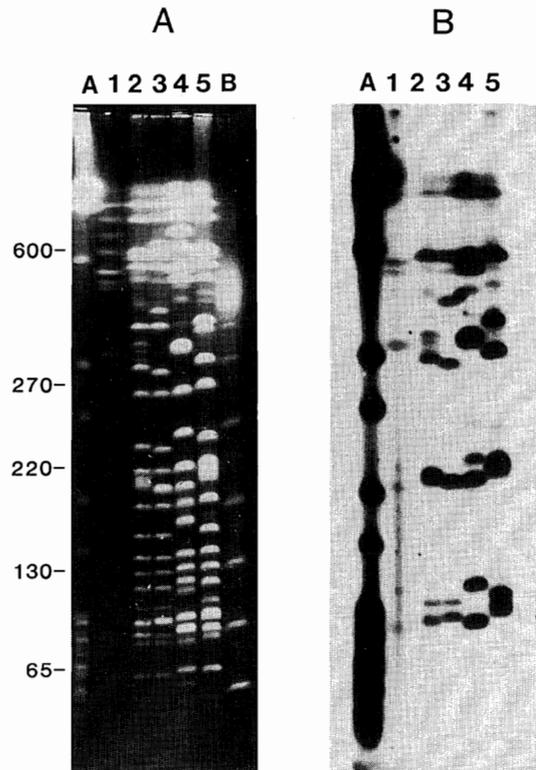


Fig. 4. Hybridisation of *Sfi*I fragments of *T. p. parva* DNA with a synthetic *P. berghei* telomeric repeat oligonucleotide. Panel (A) shows an ethidium bromide stained gel. Lanes 1–5 contained *Sfi*I-digested DNA from the *T. p. parva* stocks; (1) Uganda (3066); (2) Marikebuni (3014); (3) Kibarani (2248); (4) Mariakani (3029); and (5) Muguga (3087). Lane A contained undigested DNA from *T. brucei* ILTat 1.3 (ILRAD 430) and lane B contained concatemerised  $\lambda$ DNA. Sizes of fragments inferred from these markers are shown in kilobase pairs. Electrophoresis was carried out at 200 V using 10-s pulses for 16 h followed by 40-s pulses for 4 h. Panel (B) shows the results of hybridising a Southern blot of this gel with the oligonucleotide probe.

stained *Sfi*I fragments around 250 kb (Fig. 4B, lanes 4 and 5). Only the two largest fragments detected by this probe were of the same apparent size in all stocks. Many, but not all, of the *Sfi*I fragments differing between the stocks, hybridised with the probe. The pattern of *Sfi*I bands seen by the telomeric probe was unique in each of the 5 *T. p. parva* stocks examined except for Uganda (3066) and Mariakani (3029) which exhibited no obvious differences (Fig. 4B, lanes 1 and 4). The probe was also found to hybridise strongly with 7–8 bands on an *Eco*RI digest of

several stocks of *T. parva* DNA and these fragments are susceptible to BAL 31 digestion suggesting that they are situated at the ends of chromosomes (R.P. Bishop and B. Sohanpal, unpublished results).

The oligonucleotide probe hybridised strongly with all the *T. brucei* chromosomes (Fig. 4B, lane A) as has been observed with a different *Plasmodium* telomeric repeat probe [33].

## Discussion

*Sfi*I and *Not*I restriction enzymes cut the *T. parva* genome infrequently resulting in large-sized DNA fragments between 30 and 1000 kb. Both FIGE and CHEF resolved the majority of these fragments allowing genomic comparisons to be made between different species and stocks of *Theileria*.

Restriction digests using both enzymes revealed polymorphisms in different stocks of *T. parva*. In particular, *Sfi*I produced sets of fragments that could be discretely resolved by CHEF allowing informative comparisons to be made from ethidium bromide-stained gels. Of the electrophoresis methods used, CHEF was found to be the most useful. The straighter migration of DNA in this system allowed more accurate comparisons than could be made using OFAGE (ref. 13 and unpublished observations) and fragment resolution was superior to that obtained with FIGE.

The genome size of *T. parva* estimated from CHEF experiments agrees well with that deduced from  $C_0t$  analysis by Allsopp and Allsopp [14]. This agreement leads us to interpret the faint bands detected in some *T. parva* digests as originating from minor components within a mixed population, or from incomplete digestion at some sites. Otherwise, the stoichiometry of ethidium bromide staining would lead to a much larger estimate of the genome size.

The observation that the intensity of faint bands may vary on passage of a particular stock further supports the view that the parasite stocks are heterogeneous. For example, in *T. p. parva* Muguga stock, the two *Sfi*I fragments between 370 and 400 kb have been found to be more intensely stained in one passage than the ones described in this paper. However, the evidence that these faint bands

really belong to minor components of a mixed population must await analysis of cloned *T. parva* parasites. So far, cloned parasites are not available for such a study and due to the nature of *T. parva* life-cycle cloning may not be a trivial procedure. The presence of heterogeneous parasite populations in *T. parva* appears to be a common feature of field isolates and laboratory stocks. We were able to detect heterogeneity in Muguga, Pemba/Mnarani and Mariakani stocks. Similarly, heterogeneity has been detected in *T. p. parva* Muguga stocks by DNA probes [13,14] and in *T. p. lawrencei* by MAbs [12,13]. The heterogeneity in the Muguga stock is present despite its maintenance in the laboratory by several tick/cattle passages over 25 years.

Hybridisation of *Plasmodium* telomeric repeat sequences to some *T. p. parva Sfi*I fragments suggests that the telomeric repeats of these related Apicomplexan parasites are similar. Strong hybridisation of this probe with 7–8 *Sfi*I fragments in *T. parva* suggests that the genome of this organism contains at least four chromosomes. This is consistent with our observation of the presence of 4 discrete fragments in FIGE separations of undigested *T. parva* DNA (Fig. 3B).

Some weak hybridisation signals correspond to bands strongly stained by ethidium bromide and may represent internal genomic copies of telomeric repeats, as observed in *Plasmodium* [34]. However, most weak signals were associated with bands faintly stained with ethidium bromide, which we have supposed to be the result of genomic heterogeneity within these parasite stocks. Thus most of the heterogeneity within these stocks may be the result of alterations in telomeric or sub-telomeric regions (between 100 and 350 kb; Fig. 4).

Comparing different stocks, more than half of the polymorphic *Sfi*I fragments hybridise with the telomeric repeat probe. Thus, rearrangements in *Sfi*I telomeric fragments between 100 and 350 kb may account for the majority of the polymorphism between stocks. Further fine mapping of these *Sfi*I fragments needs to be performed to determine if this polymorphism is due to rearrangements in telomeric repeat or sub-telomeric regions. In *P. falciparum*, rearrangements within sub-telomeric regions [35,36] account for major

chromosome size polymorphisms. Changes within the *SfiI* telomeric fragments, however, cannot account for all the observed polymorphisms, as some polymorphic fragments do not hybridise with the telomeric probe. Whether the remaining differences are due to large scale rearrangements, or merely to mutations affecting *SfiI* sites, remains to be determined.

It is clear that *SfiI* RFLPs may be used in characterisation and differentiation of *T. parva* stocks and will form a useful supplement to the currently available tests such as antischizont MAB reactivities [12] and RFLPs revealed by radiolabelled probes [13,14]. The information gleaned about the parasite using the existing tests is limited, as the DNA probe hybridises mainly to one *SfiI* fragment and the panel of MAbs do not recognise more than 3 parasite specific antigens. This is in contrast to *SfiI* polymorphisms, which examine the whole genome.

The genome of *T. mutans* was cut more frequently with both *SfiI* and *NotI* than was that of

*T. parva*. This suggests that the genome of this parasite is either of much higher G+C content than those of related species, or that there may be highly reiterated G+C-rich regions in the genome of this parasite which are absent, or of different sequence in the others. Further genetic analysis of this parasite is necessary to establish its relationship to other *Theileria* species.

We conclude that, when purified parasites are available, *SfiI* RFLP analysis by CHEF electrophoresis provides a simple and useful addition to the battery of techniques available for the characterisation of *T. parva* parasites.

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