Characterization of *Megatrypanum* trypanosomes from European Cervidae

R. BÖSE[1], K. PETERSEN[1], H. POSPICHAL[2], N. BUCHANAN[3] *and* A. TAIT[3]

[1] *Institute of Parasitology, School of Veterinary Medicine, Bünteweg 17, 3000 Hannover 71, Germany*
[2] *Swiss Tropical Institute, Socinstrasse 57, 4002 Basel, Switzerland*
[3] *Wellcome Unit of Molecular Parasitology, Department of Veterinary Parasitology, University of Glasgow, Bearsden Road, Glasgow G61 1QH, UK*

(*Received 26 August 1992; revised 11 January 1993; accepted 11 January 1993*)

SUMMARY

Megatrypanum trypanosomes have been isolated from a number of different European Cervidae, but on the basis of morphology it has not been possible to define the species to which these isolates belong. We isolated *Trypanosoma* (*Megatrypanum*) *theileri* from 10 cattle, and *Megatrypanum* trypanosomes from 11 fallow deer (*Cervus dama*), 9 red deer (*Cervus elaphus*), and 4 roe deer (*Capreolus capreolus*) by blood culture on a biphasic medium (NNN agar slopes). Trypanosomes were propagated in Schneider's Drosophila medium and characterized by isoenzyme analysis and molecular karyotyping. Isocitrate dehydrogenase and phosphoglucomutase were visualized after starch gel electrophoresis of trypanosome lysates. By cluster analysis of this data all isolates from deer were clearly separated from the *T.* (*M.*) *theileri* isolates from cattle. Isolates from roe deer were different not only from *T.* (*M.*) *theileri* but also from the other deer isolates. Isolates from fallow deer and red deer were grouped together. Thus, there are probably at least two different species of *Megatrypanum* trypanosomes in the three Cervidae. One parasitizing roe deer, the other, apparently less host specific species, infecting red deer and fallow deer. Separation of the chromosomes of *Megatrypanum* trypanosomes by pulsed-field gradient gel electrophoresis (PFGE) showed that each isolate contained a large number (> 18) of chromosomes ranging in size from 300 to > 2200 kb. The molecular karyotypes were similar for all isolates, although no isolate was identical to another.

Key words: *Megatrypanum* trypanosomes, *Trypanosoma theileri*, isoenzyme analysis, molecular karyotype, Cervidae.

INTRODUCTION

Trypanosomes of the subgenus *Megatrypanum* are common parasites of ruminants. Typically these parasites are apathogenic with very low parasitaemias in the vertebrate hosts (Hoare, 1972). In deer, two species have been described, *T.* (*M*). *mazamarum* occurring in South American deer (Mazza, Romaña & Fiora, 1932; Deane, 1961) and *T.* (*M.*) *cervi* infecting North American Cervidae (Kingston & Morton, 1975). In Europe, *Megatrypanum* trypanosomes have been demonstrated in several deer species (Friedhoff *et al.* 1984; Hoffmann, Büscher, & Friedhoff, 1984; Kingston & Bobek, 1985; Kingston & Nikander, 1985; Kingston, Dróżdż & Rutkowska, 1985; Dirie *et al.* 1990*a*). Morphometric data of blood stages of trypanosomes isolated from fallow deer, red deer, and roe deer did not show any significant differences between isolates and so did not allow a species description (Hoffmann *et al.* 1984). Transmission studies provided evidence that the trypanosomes from fallow deer (*Cervus dama*) are not identical with *T.* (*M.*) *theileri* from cattle (Böse, Friedhoff & Olbrich, 1987). One study involving isoenzyme analysis of *Megatrypanum* trypanosomes in deer has been published (Dirie *et al.* 1990*a*), and isolates from 2 Swedish reindeer (*Rangifer tarandus*) differed significantly from an isolate from a Swedish moose (*Alces alces*) and from 4 isolates from North American Cervidae which are probably *T.* (*M.*) *cervi*. No further studies have been undertaken and it remains unclear how many species of *Megatrypanum* trypanosomes occur in deer.

We isolated *Megatrypanum* trypanosomes from fallow deer (*Cervus dama*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and *T.* (*M.*) *theileri* from cattle (*Bos taurus*), and examined the molecular karyotype and isoenzyme profile to determine whether the trypanosomes from different hosts could be distinguished.

MATERIALS AND METHODS

Trypanosome isolates

Isolates of *T.* (*M.*) *theileri* were obtained from cattle examined at the Veterinary Clinic in Hannover. Cattle from 6 months to 7 years old, all originating from farms located within a distance of 150 km from Hannover, were bled from the jugular vein. From deer, blood was collected shortly after killing from one of the large body veins or from the *vena lienalis*. All deer were shot in areas within the vicinity of Hannover (< 100 km distance). In all cases blood

was collected under aseptic conditions into 1/10 volume of 0·9% saline containing 200 units of heparin/ml as anticoagulant.

Culture methods

A sample of 1–2 ml of blood with anticoagulant was inoculated on to NNN agar slopes (Herbert, 1961) and incubated at 28 °C. After 5 and 10 days part of the culture was removed at the level of the buffy coat using a Pasteur pipette and transferred to a micro-haematocrit tube. Agar slopes were examined for trypanosomes using the method of Woo (1969). Blood was removed from positive agar slopes, suspended in 10 ml of Schneider's Drosophila medium (Serva Feinbiochemica, Heidelberg, Germany), and centrifuged (200 ***g***, 10 min, 4 °C). The supernatant fraction was transferred to a fresh tube and centrifuged at 1200 ***g*** (10 min, 4 °C). Using a Pasteur pipette the top layer of the resulting pellet was removed and resuspended in 1 ml of Schneider's Drosophila medium containing 20% foetal calf serum (Biochrom, Berlin, Germany) and 50 μg/ml gentamycin. Cultures were held in 24-well tissue-culture plates (No. 143982, Nunc, Wiesbaden, Germany) at 28 °C. The first subcultures were made after 2–3 days by 3-fold dilution of a growing culture in fresh medium, thereafter subcultures were made daily until sufficient material was obtained. After an initial adaptation phase of 2–3 days all isolates grew well in Schneider's Drosophila medium with a population doubling time of 14 h. Epimastigote forms with a free flagellum were most frequently observed.

Isoenzyme analysis

Trypanosomes harvested in the log phase of growth were washed in phosphate-buffered saline with glucose (PSG 6:4, Lanham & Godfrey, 1970) and treated as described previously (Betschart, Wyler & Jenni, 1983). Starch gel electrophoresis was carried out and isocitrate dehydrogenase (ICD, EC 1.1.1.42) and phosphoglucomutase (PGM, EC 2.7.5.1) visualized according to the method described by Tait, Babiker & Le Ray (1984). Using the data obtained a cluster analysis was performed and a dendrogram was generated (Le Blancq, Cibulskis & Peters, 1986). The computer programme SIM (supplied by Dr R. E. Cibulskis, Liverpool School of Tropical Medicine) was used to calculate the similarity between the iso-enzyme profiles of the different stocks and then the data entered into a clustering programme 4 M. A simple matching coefficient was calculated as the mean for both enzymes between each individual of

$$\frac{a+d}{a+b+c+d}$$

where a = number of bands common to each individual; b = number of bands present in the first individual but absent in the second; c = number of bands present in the second individual but absent in the first individual; d = number of bands absent in both individuals.

This analysis produces a similarity matrix comparing each sample with every other sample, giving values between 0 (complete dissimilarity) and 1 (complete identity). This matrix was then entered into the group average clustering programme 4 M which represents such data graphically by grouping the individuals as a cluster diagram based on their degree of similarity.

Pulsed-field gradient gel electrophoresis

Washed parasites were counted in a haemocytometer and 2×10^7 trypanosomes resuspended in 100 μl of 0·5% (w/v) low-melting agarose (Sigma Chemicals, Deisenhofen, Germany). Agarose blocks were cast in a mould, allowed to solidify for 10 min at 4 °C, transferred to a tube containing 0·5 M EDTA, pH 9·0, with 1% sodium lauryl sarcosine and 500 μg proteinase K/ml and incubated for 20 min at room temperature and a further 48 h at 50 °C (Van der Ploeg *et al.* 1989). Subsequently tubes were washed 3 times with 50 mM EDTA, pH 8·0, and stored at 4 °C until used. Chromosomes were separated by pulsed-field gradient gel electrophoresis (Schwarz & Cantor, 1984) using an LKB Pulsaphor apparatus. Agarose gels (1%; 15 × 15 cm) were cast in TB (0·1) E buffer (89 mM Tris, 89 mM borate, 0·2 mM EDTA) and each sample block inserted into the gel using a sample comb. The gels were subjected to electrophoresis at 82 V or 100 V, and 12 °C for 4–5 days using three different pulse rate/time schedules which are specified in the legend to Fig. 3.

After gel runs were complete, the gel was stained for 1–6 h in 1 μg/ml ethidium bromide in 0·5 × TBE and then destained in the same buffer over the next 6–8 h. The stained gels were viewed and photographed under u.v. (302 nm wavelength). Sizes of the chromosome bands were estimated relative to those of *Saccharomyces cerevisiae* strain YNN 295 (BioRad Laboratories Ltd, Herts, UK) with chromosome sizes of 2200; 1600; 1125; 1020; 945; 850; 800; 770; 700; 630; 580; 460; 370; 290 and 245 kb.

RESULTS

Isoenzyme analysis

The results of this analysis are illustrated diagrammatically in Fig. 1. All *T.* (*M.*) *theileri* isolates showed an identical enzyme pattern. Although there was some variation between isolates derived from the same deer species it is clear, that all patterns observed were different from those of *T.* (*M.*) *theileri*. The

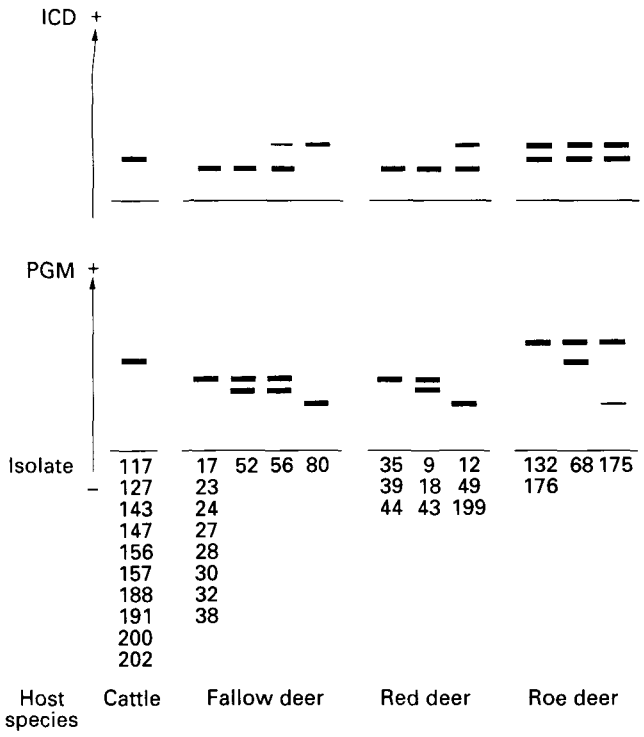

Fig. 1. Diagrammatic representation of the isoenzyme analysis of *Trypanosoma* (*Megatrypanum*) *theileri* from cattle and *Megatrypanum* trypanosomes from European Cervidae. Trypanosome lysates were separated on starch gels, and bands for isocitrate dehydrogenase (ICD, EC 1.1.1.42) and phosphoglucomutase (PGM, EC 2.7.5.1) visualized as described in the Materials and Methods section. The numbers represent individual isolates from each host species. Isolates showing an identical banding pattern are represented by one lane only.

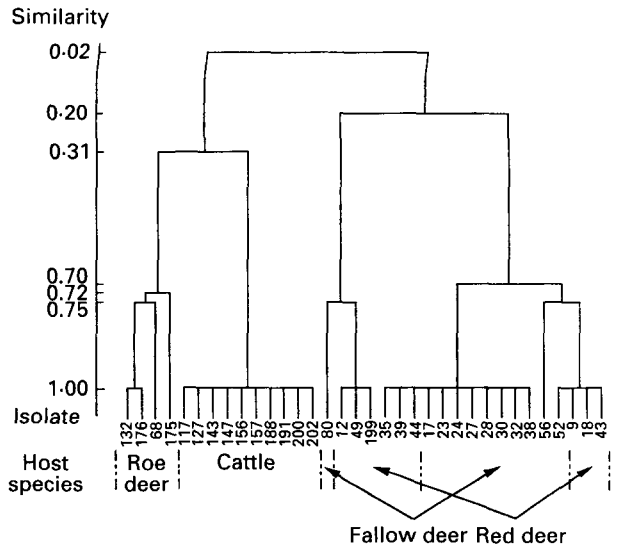

Fig. 2. Dendrogram of *Trypanosoma* (*Megatrypanum*) *theileri* and *Megatrypanum* trypanosomes from European Cervidae. Data are based on the banding pattern of isocitrate dehydrogenase and phosphoglucomutase and have been analysed using the computer programme kindly provided by Dr R. E. Cibulskis (Liverpool School of Tropical Medicine). The similarity is measured as a simple matching coefficient and the resulting matrix represented as a group average cluster dendrogram.

isolates from roe deer were distinct not only from *T.* (*M.*) *theileri*, but also from the other deer isolates. In order to examine these general conclusions on a more defined and quantitative basis, a cluster analysis was undertaken and the resulting dendrogram illustrated in Fig. 2. The *T.* (*M.*) *theileri* isolates (cattle) were grouped together based on an identical isoenzyme pattern. There was a greater degree of inter-isolate variation within the deer isolates. The roe deer isolates were separated from all other deer isolates. All fallow deer and red deer isolates were also different from *T.* (*M.*) *theileri* but not from each other. Some isolates from these two deer species were grouped together on the basis of identical isoenzyme patterns and at a level of 70% similarity all fallow and red deer isolates were grouped together.

Molecular karyotype

In preliminary experiments using a range from 2×10^8 to 5×10^6 parasites per lane, we found that a concentration of 2×10^7 was suitable. Higher concentrations resulted in overloaded gels and no clearly defined chromosome bands. Using blocks containing 2×10^7 *T.* (*M.*) *theileri*, conditions for the resolution of the chromosomes were determined empirically based on previous experience with *Trypanosoma* (*T.*) *brucei*. It is clear (Fig. 3) that *T.* (*M.*) *theileri* contains chromosomes of a range of sizes from 400 kb to > 2000 kb and that one set of conditions was unlikely to resolve all the chromosome bands simultaneously. Consequently, three sets of conditions were determined which gave resolution in the ranges 2200–1400 kb (Fig. 3A, Fig. 4), 900–1800 kb (Fig. 3B) and 400–900 kb (Fig. 3C). As the objective of this study was a comparative analysis of the molecular karyotype of different isolates, attempts to resolve the chromosomes further have not been made. However, comparison of the staining intensities between different resolved bands (within a track) suggests that some bands contained more than one chromosome.

The overall features of the karyotype were that a maximum of 17–18 chromosome bands were resolved (isolate 202) which ranged in size from 450 kb to > 2200 kb, and that there were no mini-chromosomes (40–200 kb) similar to those observed in *T. brucei*. Three size classes of chromosomes were observed, firstly 1–2 chromosomes at around 2·0 Mb, secondly a group of 9 chromosomes ranging in size from 1·8 to 1·0 Mb, and thirdly a group of intermediate size chromosomes ranging in size from 900 to 400 kb. Comparison of the molecular karyotype between the four isolates of the same species

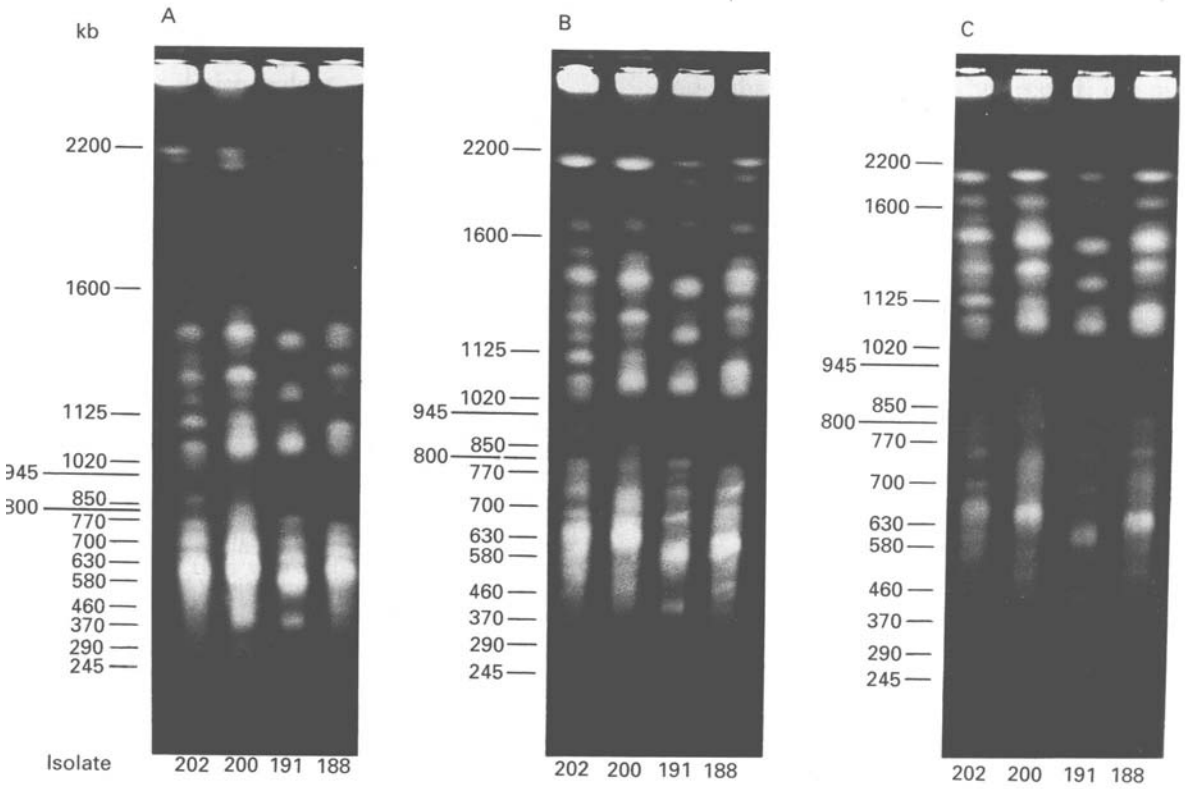

Fig. 3. Karyogram of *Trypanosoma* (*Megatrypanum*) *theileri* generated by pulsed-field gradient gel electrophoresis. Chromosomes were separated in 1 % agarose gels using pulse times of (A) 700, 600, 500, 400, and 300 s for 24 h at 82 V, (B) 600, 500, 400, 300, and 200 s for 24 h at 82 V, (C) 400 and 300 s for 18 h each, and 200 and 150 s for 24 h each at 100 V. Figures on the left indicate the position of *Saccharomyces cerevisiae*, strain YNN 295 chromosome markers in kb.

(Fig. 3) shows a considerable degree of karyotype diversity in all three size classes of chromosomes, with each isolate being unique. Further comparisons with another 6 isolates of *T.* (*M.*) *theileri* again showed variation (data not shown). Using the conditions shown in Fig. 3A, isolates from fallow deer, red and roe deer were compared together with isolates from *T.* (*M.*) *theileri*, and representative patterns are shown in Fig. 4 for one set of conditions. In total 11 isolates from fallow deer, 9 isolates from red deer and 4 isolates from roe deer have been examined. Overall the molecular karyotypes of all isolates, irrespective of the host from which they were isolated, showed a broad similarity with the same set of three size classes of chromosomes. Due to the variation between isolates from a single host species, it was difficult to define specific differences between the isolates from different host species.

DISCUSSION

Megatrypanum trypanosomes typically cause very low parasitaemias in their vertebrate hosts (Hoare, 1972). As these trypanosomes cannot be propagated in common laboratory animals, *in vitro* cultures provide the only method to obtain large numbers of parasites for biochemical and molecular studies. *Megatrypanum* trypanosomes can be grown at vector or vertebrate host temperatures. Growing the trypanosomes at 37 °C is slightly more laborious as it requires the presence of suitable feeder cells (Dirie *et al.* 1990*a*, *b*). Transferring trypanosomes to feeder cell cultures under field conditions would have been impractical, so isolates were grown under culture conditions presumably similar to the growth conditions in the vector.

We characterized a total of 24 isolates from the 3 most common deer species in Western Europe. The data from the isoenzyme and cluster analyses show that none of the *Megatrypanum* isolates from deer are identical with *T.* (*M.*) *theileri* from cattle. A difference between *Megatrypanum* trypanosomes from fallow deer and *T.* (*M.*) *theileri* has also been demonstrated by transmission studies using tabanid vectors (Böse *et al.* 1987). As the isolates from different deer species were made from similar geographical locations, it is unlikely that the differences in electrophoretic pattern which distinguish the isolates from the different host species are due to allelic differences between the same species of trypanosome. Among the deer trypanosomes the isolates from fallow deer were grouped with the red deer isolates. Thus, the trypanosomes from the three deer species probably represent two different species of *Megatrypanum* trypanosomes, i.e. one species of roe deer and another species of fallow and red deer.

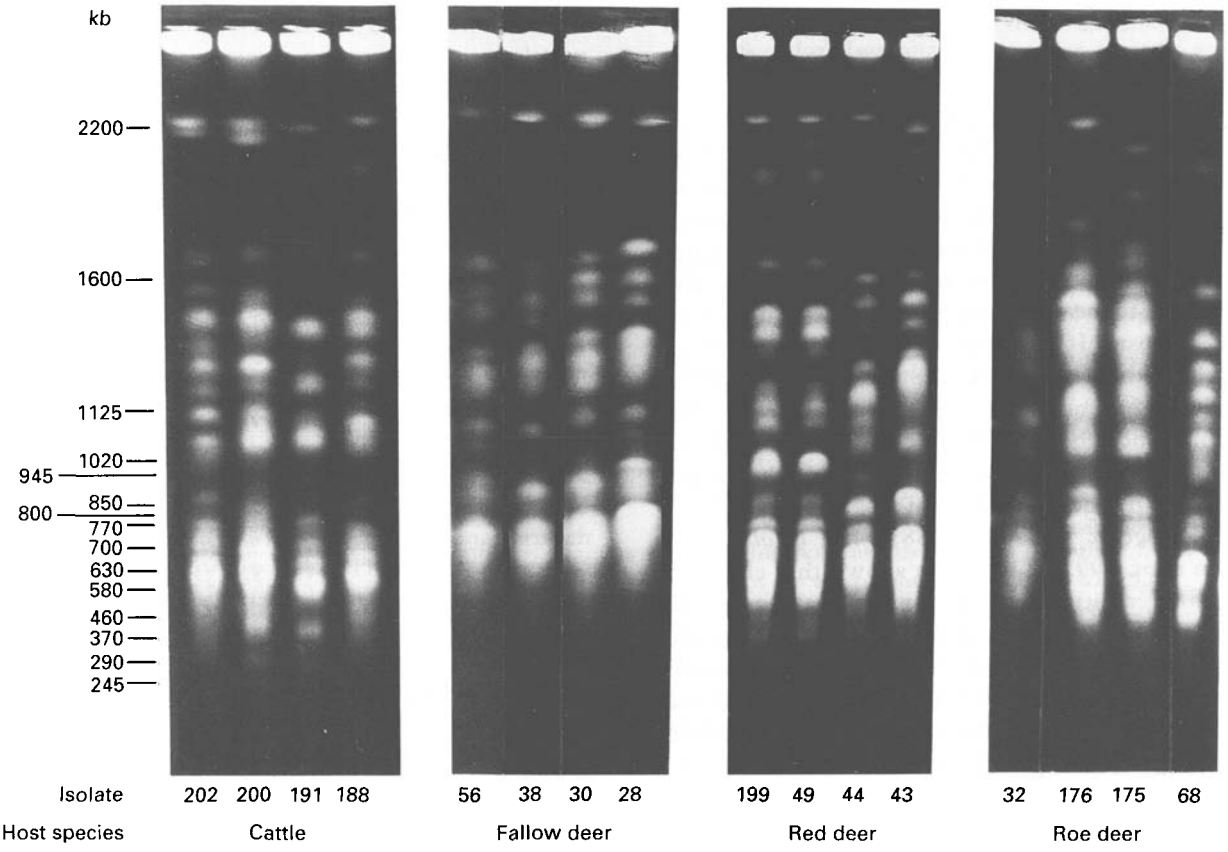

Fig. 4. Molecular karyotypes of *Trypanosoma* (*Megatrypanum*) *theileri* from cattle and *Megatrypanum* trypanosomes from European Cervidae, generated by pulsed-field gradient gel electrophoresis. Chromosomes of *T.* (*M.*) *theileri* and *Megatrypanum* isolates from fallow deer (*Cervus dama*), red deer (*Cervus elaphus*), and roe deer (*Capreolus capreolus*) were separated using pulse times of 700, 600, 500, 400, and 300 s for 24 h each under the conditions described in the Materials and Methods section.

It has been demonstrated previously that the trypanosome isolates from 2 Swedish reindeer (*Rangifer tarandus*) were significantly different from an isolate from a Swedish moose (*Alces alces*) (Dirie *et al.* 1990*a*). These results, coupled with those presented here, suggest that there are several *Megatrypanum* species infecting European deer. These species appear to exhibit a narrow host range or even host-specificity. This conclusion is in contrast to the species description for *T.* (*M.*) *cervi* (Kingston & Morton, 1975). This species has been reported to infect a wide range of deer species ranging from wapitis (*Cervus canadensis*) (Kingston & Morton, 1975) to mule deer (*Odocoileus hemionus*) (Matthews, Kingston & Morton, 1977), white tailed deer (*Odocoileus virginianus*) (Kingston & Crum, 1977), reindeer (*Rangifer tarandus*) (Kingston, Morton & Dieterich, 1982), and moose (*Alces alces*) (Kingston *et al.* 1981; Kingston, Franzmann & Maki, 1985).

In order to extend and confirm the conclusions on speciation of *Megatrypanum* provided by the isoenzyme data, an analysis of the molecular karyotypes of the deer and cattle isolates was undertaken. In contrast to the salivarian trypanosomes (Gottesdiener *et al.* 1990; Gibson, 1989; Gibson, Garside & Bailey, 1992) the chromosomes of *Megatrypanum* species range in size from 2·2 Mb to 300 kb and do not appear to have an abundant class of minichromosomes. Although we cannot exclude the presence of chromosomes of much larger size than 2·2 Mb due to the retention of DNA in the sample slot of the PFGE-gels, all attempts to resolve chromosomes above this size using methods which have proved successful with chromosomes from salivarian trypanosomes have been unsuccessful (data not shown).

In overall size range, the molecular karyotype of the *Megatrypanum* isolates is more similar to that of *Leishmania* sp. (Lighthall & Giannini, 1992) than to that of *Trypanosoma*. In *Leishmania* around 20 bands of DNA can be resolved ranging in size from 300 kb to 3 Mb. However, in general terms, the high level of *Megatrypanum* karyotype polymorphism is more characteristic of *Plasmodium* (Corcoran *et al.* 1988; Foote & Kemp, 1989; Ravetch, 1989) or *Trypanosoma* (Gibson *et al.* 1992; Tait & Buchanan, unpublished observations) where most cloned isolates have a unique karyotype, in contrast to the relatively conserved karyotypes found in *Leishmania*, as illustrated by *L. major* (Giannini *et al.* 1990). However, with *T. evansi* (Lun, Brun & Gibson, 1992) an intermediate situation is observed where

there is considerable conservation in karyotype between isolates from one geographical area, but considerable divergence when isolates from different areas are examined.

The overall karyotype pattern is similar between the *Megatrypanum* isolates from different host species in terms of size range and size classes of chromosomes. The high level of polymorphism between isolates coupled with the overall similarity in general pattern does not allow any specific distinction to be made between the isolates from different hosts. If gene probes from these parasites were available, it would be possible to define homologous chromosomes which might define specific differences between the isolates. The origins of this high level of karyotypic polymorphism are unknown. In other parasite protozoa (Corcoran *et al.* 1988; Pologe & Ravetch, 1988; Shea *et al.* 1986; Gibson *et al.* 1992) it has been shown that chromosome size variation can be generated during both mitosis and meiosis. It is interesting to note that the species of parasite in which high levels of interstrain karyotype polymorphism have been observed are those in which the existence of genetic exchange has been established. No information is available on the stability of the karyotype of *Megatrypanum* in *in vitro* culture.

In summary, the results presented here demonstrate that there is a considerable level of polymorphism between isolates of *Megatrypanum* as demonstrated by both isoenzyme analysis and molecular karyotyping. The isoenzyme data suggest that there are at least two *Megatrypanum* species infecting European deer, one of which is limited to infecting roe deer while the other is a parasite of fallow and red deer. A third distinct species infects cattle.

The project was supported by grants from The British Council (A. T., N. B.) the Swiss National Science Foundation (H. P.) and the German Academic Exchange Service (DAAD) (R. B., K. P.) and the Wellcome Trust (A. T., N. B.). We wish to thank Mr W. Gleber, Forstamt Peine and Mr H. Tylinski, Städt. Forstamt Hannover, for enthusiastic support in the collection of blood samples from deer, Mrs K. Daemen and Mr M. Wolfhagen for technical support, Professor Dr K. T. Friedhoff, Dr C. Schelp and Mrs J. Jenkins for critical review of the manuscript, Dr G. Hide for assistance with the cluster analysis and Dr R. E. Cibulskis, Liverpool School of Tropical Medicine, for providing the computer programme for the cluster analysis.

REFERENCES

BETSCHART, B., WYLER, R. & JENNI, L. (1983). Characterization of *Trypanozoon* stocks by isoelectric focusing and isoenzyme analysis. *Acta Tropica* **40**, 25–8.

BÖSE, R., FRIEDHOFF, K. T. & OLBRICH, S. (1987). Transmission of *Megatrypanum* trypanosomes to *Cervus dama* by Tabanidae. *Journal of Protozoology* **34**, 110–13.

CORCORAN, L. M., THOMPSON, J. K., WALLIKER, D. & KEMP, D. J. (1988). Homologous recombination within subtelomeric repeat sequences generates chromosome size polymorphism in *P. falciparum*. *Cell* **53**, 807–13.

DEANE, L. M. (1961). Tripanosomideos de mamíferos da Regiao Amazônica. I. Alguns flagelados encontrados no sangue de mamíferos silvestres do Estado do Pará. *Revista do Instituto de Medicina Tropical* (São Paulo) **3**, 15–28.

DIRIE, M. F., BORNSTEIN, S., WALLBANKS, K. R., MOLYNEUX, D. H. & STEEN, M. (1990*a*). Comparative studies on *Megatrypanum* trypanosomes from cervids. *Tropical Medicine and Parasitology* **41**, 198–202.

DIRIE, M. F., BORNSTEIN, S., WALLBANKS, K. R., STILES, J. K. & MOLYNEUX, D. H. (1990*b*). Zymogram and life-history studies on trypanosomes of the subgenus *Megatrypanum*. *Parasitology Research* **76**, 669–74.

FOOTE, S. J. & KEMP, D. J. (1989). Chromosomes of malaria parasites. *Trends in Genetics* **5**, 337–42.

FRIEDHOFF, K. T., PETRICH, J., HOFFMANN, M. & BÜSCHER, G. (1984). Trypanosomes in Cervidae in Germany. *Zentralblatt für Bakteriologie und Hygiene*, A **256**, 286–7.

GIANNINI, S. H., CURRY, S. S., TESH, R. B. & VAN DER PLOEG, L. H. T. (1990). Size-conserved chromosomes and stability of molecular karyotype in cloned stocks of *Leishmania major*. *Molecular and Biochemical Parasitology* **39**, 9–22.

GIBSON, W. C. (1989). Analysis of a genetic cross between *Trypanosoma brucei rhodesiense* and *T. b. brucei*. *Parasitology* **99**, 391–402.

GIBSON, W. C., GARSIDE, L. & BAILEY, M. (1992). Trisomy and chromosome size changes in hybrid trypanosomes from a genetic cross between *Trypanosoma brucei rhodesiense* and *T. b. brucei*. *Molecular and Biochemical Parasitology* **52**, 189–200.

GOTTESDIENER, K., GARCÍA-ÃNOVEROS, J., LEE, M. G. & VAN DER PLOEG, L. H. T. (1990). Chromosome organisation of the protozoan *Trypanosoma brucei*. *Molecular and Cellular Biology* **10**, 6079–83.

HERBERT, I. V. (1961). Bovine trypanosomiasis due to *Trypanosoma theileri*, Laveran, 1902 and its occurrence in Eire. *Irish Veterinary Journal* **15**, 230–6.

HOARE, C. A. (1972). *The Trypanosomes of Mammals*. Oxford, Edinburgh: Blackwell Scientific Publications.

HOFFMANN, M., BÜSCHER, G. & FRIEDHOFF, K. T. (1984). Stercorarian trypanosomes from deer (Cervidae) in Germany. *Journal of Protozoology* **31**, 581–4.

KINGSTON, N. & BOBEK, B. (1985). A Trypanosome in roe deer, *Capreolus capreolus*, in Southern Poland. *Proceedings of the Helminthological Society, Washington* **52**, 143.

KINGSTON, N. & CRUM, J. (1977). *Trypanosoma cervi* Kingston and Morton, 1975 in white-tailed deer, *Odocoileus virginianus*, in the Southeastern United States. *Proceedings of the Helminthological Society, Washington* **44**, 179–84.

KINGSTON, N., DRÓŻDŻ, J. & RUTKOWSKA, M. (1985). *Trypanosoma* sp. in red deer (*Cervus elaphus*) and elk (*Alces alces*) in Poland. *Proceedings of the Helminthological Society, Washington* **52**, 144–5.

KINGSTON, N., FRANZMANN, A. & MAKI, L. (1985). Redescription of *Trypanosoma cervi* (Protozoa) in

moose, *Alces alces*, from Alaska and Wyoming. *Proceedings of the Helminthological Society, Washington* **52**, 54–9.

KINGSTON, N. & MORTON, J. K. (1975). *Trypanosoma cervi* sp. n. from elk (*Cervus canadensis*) in Wyoming. *Journal of Parasitology* **61**, 17–23.

KINGSTON, N., MORTON, J. K. & DIETERICH, R. (1982). *Trypanosoma cervi* from Alaskan reindeer, *Rangifer tarandus*. *Journal of Protozoology* **29**, 588–91.

KINGSTON, N. & NIKANDER, S. (1985). Poron, *Rangifer tarandus*, *Trypanosoma* sp. Suomessa (*Trypanosoma* sp. in reindeer (*Rangifer tarandus*) in Finland). *Suomen Eläinlääkärilehti* **91**, 1.

KINGSTON, N., THORNE, E. T., THOMAS, G. M., McHOLLAND, L. & TRUEBLOOD, M. S. (1981). Further studies on trypanosomes in game animals in Wyoming II. *Journal of Wildlife Diseases* **17**, 539–46.

LANHAM, S. M. & GODFREY, D. G. (1970). Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. *Experimental Parasitology* **28**, 521–34.

LE BLANCQ, S. M., CIBULSKIS, R. E. & PETERS, W. (1986). Leishmania in the Old World: 5. Numerical analysis of isoenzyme data. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 517–24.

LIGHTHALL, G. K. & GIANNINI, S. H. (1992). The chromosomes of *Leishmania*. *Parasitology Today* **8**, 192–9.

LUN, Z.-R., BRUN, R. & GIBSON, W. C. (1992). Kinetoplast DNA and molecular karyotypes of *T. evansi* and *T. equiperdum* from China. *Molecular and Biochemical Parasitology* **50**, 189–96.

MAZZA, S., ROMAÑA, C. & FIORA, A. (1932). Algunos hemoparásitos de mamíferos del norte. VI. *Reunión de la Sociedad Argentina de Patológica Regional del Norte* (Buenos Aires), **2**, 990–7.

MATTHEWS, M. J., KINGSTON, N. & MORTON, J. K. (1977). *Trypanosoma cervi* Kingston and Morton, 1975 from mule deer, *Odocoileus hemionus*, in Wyoming. *Journal of Wildlife Diseases* **13**, 33–9.

POLOGE, L. P. & RAVETCH, J. V. (1988). Large deletions results from breakage and healing of *P. falciparum* chromosomes. *Cell* **55**, 869–74.

RAVETCH, J. V. (1989). Chromosomal polymorphism and gene expression in *Plasmodium falciparum*. *Experimental Parasitology* **68**, 121–5.

SHEA, C., GLASS, D. J., PARANGI, S. & VAN DER PLOEG, L. H. T. (1986). Variant surface glycoprotein gene expression site switches in *Trypanosoma brucei*. *Journal of Biological Chemistry* **261**, 6056–63.

SCHWARTZ, D. C. & CANTOR, C. R. (1984). Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell* **37**, 67–75.

TAIT, A., BABIKER, E. A. & LE RAY, D. (1984). Enzyme variation in *Trypanosoma brucei* spp. I. Evidence for the sub-speciation of *Trypanosoma brucei gambiense*. *Parasitology* **89**, 311–26.

VAN DER PLOEG, L. H. T., SMITH, C. L., POLVERE, R. I. & GOTTESDIENER, K. (1989). Improved separation of chromosome-size from *T. brucei* stock 427–60. *Nucleic Acids Research* **17**, 3217–27.

WOO, P. T. K. (1969). The haematocrit centrifuge technique for the detection of trypanosomes in blood. *Canadian Journal of Zoology* **47**, 921–3.