PCR-based DNA fingerprinting of *Giardia duodenalis* isolates using the intergenic rDNA spacer

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SUMMARY

The potential for the non-coding intergenic rDNA spacer (IGS) to DNA fingerprint *Giardia duodenalis* isolates was investigated. Conserved PCR primers, specific for the flanking large and small rDNA genes, were used to amplify the IGS from 52 *in vitro*-cultured *Giardia* isolates. Four distinct IGS–PCR size groups (1·35–1·6 kb) were observed, which correlated closely with the major genetic assemblages established previously for the same isolates using isoenzyme analysis. IGS–PCR size groups A (1·42 kb), C (1·4 kb) and D (1·35 kb) corresponded to isoenzyme assemblage A, and IGS–PCR group B (1·6 kb) to isoenzyme assemblage B. Amplified products from IGS–PCR size groups A and B, which contained 50/52 isolates, were subsequently digested with 8 different restriction enzymes and their profiles compared. Analysis separated isolates within each IGS–PCR size group into 2 distinct clusters which correlated almost exactly with the same genetic groups established previously using isoenzyme electrophoresis. Within each cluster, both methods exhibited a similar capacity to distinguish between *Giardia* genotypes although they established different genetic relationships between individual isolates. Much of the variability associated with the IGS was attributed to isolates harbouring multiple IGS-sequence types. Restriction analysis of IGS–PCR products amplified from cloned and parent lines of a human isolate BAH 39, which contains multiple IGS variants, showed that trophozoite populations are homogeneous with respect to the types of IGS-variants they maintain. Furthermore, *in vitro* culture of the cloned isolate BAH39c9 over a 6-year period also failed to reveal variation in IGS–PCR digestion profiles. These results suggest that IGS–PCR RFLP profiles are inherently stable. IGS–PCR analysis was successfully applied to 11 *Giardia* cyst samples highlighting the potential for this approach to genotype *Giardia* isolates without the need for *in vitro* culture.

Key words: *Giardia duodenalis*, protozoa, intergenic rDNA spacer, PCR, DNA fingerprinting.

INTRODUCTION

Giardia duodenalis is the most commonly reported intestinal parasite of humans in developed countries (Meyer, 1985; Acha & Szyfres, 1987) although it is in developing and socially disadvantaged regions of the world, where the clinical impact of *Giardia* infections are felt most acutely (Islam, 1990). In Australia, the most important endemic foci of *Giardia* infections are concentrated in Aboriginal communities where they represent an important cause of morbidity (Welch & Stuart, 1975; Roberts, Gracey & Spargo, 1988). Implementation of effective control and prevention strategies in these areas is dependent upon our understanding of the epidemiology of *Giardia* infections. With such knowledge it becomes possible to identify and target the most common sources of infection, assess the efficacy of chemotherapy and determine the potential for zoonotic transmission.

In a recent study we compared the small subunit (SSU) rDNA sequences of *Giardia* isolates collected from humans and dogs living in the same Aboriginal communities (Hopkins *et al*. 1997). Using a similar strategy, where isolates are recovered from a defined focus of infection, it should also be possible to examine *Giardia* transmission between individuals of the same host species. However, to be epidemiologically informative, a molecular tool is required which offers greater resolution than the 4 broad genetic groups identifed by SSU rDNA sequencing. We therefore, evaluated the potential of a non-coding ribosomal DNA region to differentiate between *Giardia* isolates. The structure of the rRNA repeat unit in *Giardia* is typically eukaryotic, containing 3 genes (SSU, 5·8S and large subunit rRNA) separated by 2 internal non-coding transcribed spacers (ITS) (Healey *et al*. 1990). The rRNA repeats are arranged in head-to-tail arrays separated by an intergenic spacer (IGS) region. The non-coding IGS and ITS sequences represent the fastest

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evolving ribosomal regions (Hillis & Davis, 1986; Hillis & Dixon, 1991) and have been used to detect inter- and intraspecific level variation in several protozoan species (Dietrich *et al*. 1990; Cupolillo *et al*. 1995; Didier *et al*. 1995) including *Giardia* (Upcroft, Healey & Upcroft, 1994).

We PCR amplified the IGS from 52 *in vitro*-cultured *Giardia* isolates using primers specific for the highly conserved SSU and LSU rDNA genes which flank the spacer. PCR products were subsequently digested with a range of restriction enzymes and the profiles compared to determine the extent of sequence variation. The results of PCR–RFLP analysis were compared to those obtained for the same *Giardia* isolates using isoenzyme electrophoresis (Meloni *et al*. 1995) to determine if the genetic relationships established by different molecular techniques could be correlated. Finally, IGS–PCR analysis was also undertaken using *Giardia* cysts in order to evaluate its potential to genotype *Giardia* samples purified directly from faeces.

MATERIALS AND METHODS

Isolates of Giardia *examined*

DNA extracted from 52 *Giardia duodenalis* isolates grown using *in vitro* culture was used for analysis. Details regarding their source, host of origin and isoenzyme genotype have been described previously (Meloni *et al*. 1995). Cloned lines from 3 *Giardia* isolates were obtained from Binz *et al*. (1991). Analysis was also undertaken using 11 *Giardia* cyst samples purified from the faeces of 9 individuals living in 3 Aboriginal communities in the Kimberley region of Western Australia (Table 1). For more detailed community information and cyst concentration procedures see Hopkins *et al*. (1997).

DNA isolation and PCR

DNA was isolated from all samples using a modified CTAB extraction method (Hopkins *et al*. 1997). The IGS was PCR amplified using primers specific for conserved sequences at the 3′ end of the LSU (RH8; 5′-CTGCGCTGACCGCCTCTAAG) and 5′ end of the SSU (RH3; 5′-GGCATGCATGGCTTCGTCCTTGGG) rDNA genes respectively (Healey *et al*. 1990). Amplifications were performed in 50 μl using 5–50 ng DNA, 12·5 pmol of each primer, 1 unit *Tth* plus DNA polymerase (Biotech International, Perth, Australia), 200 μM of each dNTP, 2 mM $MgCl_2$, 67 mM Tris–HCl (pH 8·8), 16·6 mM $(NH_4)_2SO_4$, 0·45 % Triton X–100, 0·2 mg/ml gelatin and H_2O. The final PCR mix also included 5 % dimethylsulfoxide and 1 unit of *Taq* Extender (Stratagene, California, USA) as these increased the specificity and yield of the IGS–PCR product. Reactions were heated to 96 °C for 2 min followed by 5 cycles of 96 °C for 20 sec, 59 °C for 30 sec and 72 °C for 2 min, 30 cycles of 96 °C for 20 sec, 55 °C for 30 sec and 72 °C for 2 min and 1 cycle of 72 °C for 7 min.

Digestion and electrophoresis of PCR fragments

For each isolate, 100 μl of PCR product was concentrated to a final volume of 50 μl after purification through PCR spin columns (Qiagen, Hilden, Germany). Digestions were performed in 25 μl volumes containing 3 μl of DNA and using one of the following restriction enzymes; *Apa*I, *Bam*HI, *Ban*II, *Bst*NI, *Fok*I, *Hin*fI, *Pst*I and *Sma*I (New England Biolabs, Maryland, USA). Restriction fragments were separated by horizontal electrophoresis, through 1·8–2 % Metaphor™ (FMC, Maryland, USA) agarose gels in Tris-borate buffer, stained with ethidium bromide and visualized under UV.

Distance calculations and molecular trees

A similarity index among *Giardia* isolates was created using the formula $F = 2n_{XY}/(n_X + n_Y)$ (Nei & Li, 1979), where F is an estimate of the proportion of fragments shared between 2 populations, n_{XY} is the number of fragments shared between 2 isolates and n_X and n_Y are the total number of fragments in each isolate. The F value was converted to P, the probability for a randomly chosen sequence to become a new restriction site, using the iterative formula $P = [F(3-2P1)]^{\frac{1}{4}}$. A value for the mean number of nucleotide substitutions per site (d) was calculated from $d = -(2/r)\ln(P)$ where r is the length of the restriction site. Phenograms were constructed from genetic distance matrices using the unweighted pair-group method (UPGMA) and DRAWGRAM programs available in PHYLIP 3.5p (Felsenstein, 1989). For bootstrap analyses, SEQBOOT of PHYLIP was used to construct 100 bootstrapped data sets which were each analysed using the similarity index formula and UPGMA. A consensus tree was computed from the 100 inferred trees using CONSENSE.

RESULTS

PCR amplification of the IGS

Amplification of the IGS from 52 *in vitro*-cultured *Giardia* isolates produced a single major IGS–PCR product for all isolates although some samples contained minor bands approximately 50–100 bp smaller and larger in size than the main PCR product (data not shown). Comparison of IGS–PCR fragments revealed 4 distinct size groups: 1420 bp (Group A; 34 isolates), 1600 bp (Group B; 16 isolates), 1400 bp (Group C; isolate BAG 1) and

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Table 1. Origin of *Giardia*-positive human faecal samples

Individual*	D.O.B.†	Community	Sample collection date
1A	1987	Bayulu	5 Feb 96
1B	1987	Bayulu	6 Aug 96
2	1989	Bayulu	6 Aug 96
3	1989	Bayulu	26 Feb 96
4	1991	Joy Springs	6 Aug 96
5	1993	Junjuwa	6 Aug 96
6	1994	Junjuwa	6 Aug 96
7	1992	Junjuwa	6 Aug 96
8	1991	Junjuwa	6 Aug 96
9A	1991	Junjuwa	6 Aug 96
9B	1991	Junjuwa	Oct 95

* Letters delineate samples collected from the same individual at different times.
† D.O.B. = date of birth.

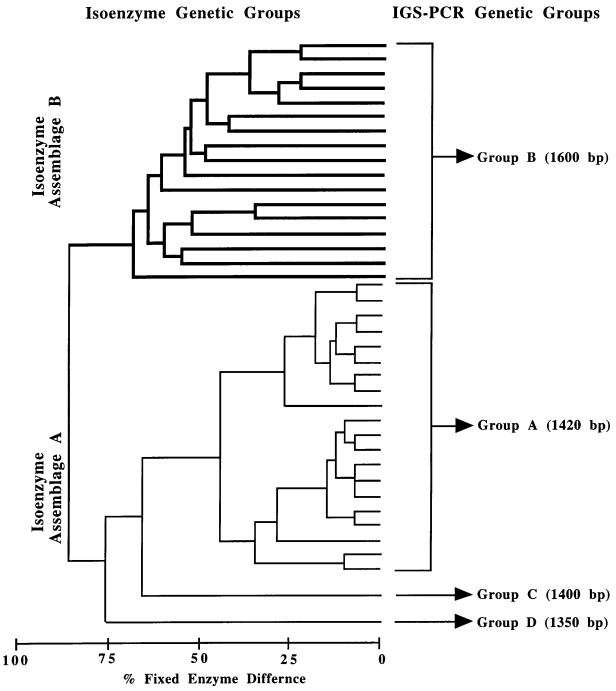

Fig. 1. Comparison between the IGS–PCR size groups and the major genetic assemblages characterized for the same isolates using isoenzyme electrophoresis (Meloni *et al*. 1995).

1350 bp (Group D; isolate BAC 7) which correlated strongly with the major genetic assemblages identified previously for the same 52 isolates using isoenzyme electrophoresis (Fig. 1; Meloni *et al*. 1995). Isoenzyme assemblage A corresponds to IGS–PCR groups A, C and D, and isoenzyme assemblage B to IGS–PCR group B.

RFLP analysis of IGS–PCR products

Amplified products from 34 IGS–PCR group A and 16 IGS–PCR group B isolates were digested with 6 and 7 different restriction enzymes, respectively, and their profiles compared (Fig. 2; Table 2). The numbers of digestion products generated differed depending upon the enzyme used (Table 2). Generally, enzymes generating larger numbers of distinct restriction fragments exhibited a greater capacity to differentiate between isolates (data not shown). For many isolates, the sum of the digestion fragment sizes exceeded that of the original IGS–PCR product suggesting that multiple IGS sequence variants are presented (Table 2). IGS–PCR group B isolates exhibited larger values for both the total number and sum of RFLP fragment sizes (Table 2), a feature which increased the complexity of their digestion profiles and meant that interpretation of gels for this group of isolates was more difficult than their IGS–PCR group A counterparts (Fig. 2).

Analysis of molecular trees

Consensus RFLP molecular trees for each IGS–PCR size group were constructed by combining the digestion results from individual restriction enzymes. These trees were then compared to phenograms generated for the same isolates using isoenzyme electrophoresis (Meloni *et al*. 1995). For IGS–PCR genetic group A, digestion profiles from 6 restriction enzymes representing a total of 60 distinct restriction fragments (Table 2), were used to constrict a consensus phenogram for 33 *Giardia* isolates (Fig. 3A). The consensus tree for 16 IGS–PCR group B isolates, generated using 7 restriction enzymes and representing 103 distinct restriction fragments, is shown in Fig. 3B. For both IGS–PCR size groups distance analysis separated isolates into 2 major clusters which correspond almost exactly to the same genetic clusters established using isoenzyme electrophoresis. The only exception occurred with the IGS–PCR group B isolate BAH 39 which fell into isoenzyme cluster B1 and IGS–PCR cluster B2 (Fig. 3B). Bootstrap analysis supported the genetic divisions between clusters within each IGS–PCR size group although higher values were obtained for the IGS–PCR group A clusters (96%) compared to those in IGS–PCR group B (80–89%). Within each cluster, IGS–PCR RFLP analysis and isoenzyme electrophoresis exhibited a similar capacity to distinguish between *Giardia* genotypes although they differed in terms of the genetic relationships established between individual isolates.

Both techniques were also in agreement with regard to the relative distances that each method used to separate isolates into different genetic clusters. For IGS–PCR group A isolates the distance between the 2 genetic clusters corresponded to a fixed enzyme difference of 45% and 0·0161 substitutions/site when using isoenzyme electrophoresis and IGS–PCR RFLP analysis respectively (Fig. 3A). For IGS–PCR group B isolates, the

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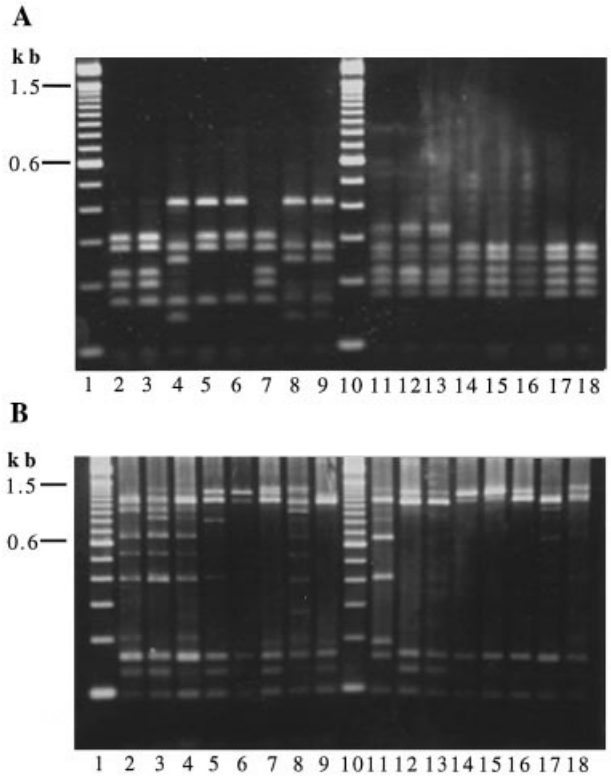

Fig. 2. Ethidium bromide-stained 2% Metaphor™ agarose-TBE gels showing restriction profiles of (A) IGS–PCR group A PCR products digested with *Sma*I and (B) IGS–PCR group B PCR products digested with *Hin*fI. (A) Lane 1 = 100 bp ladder (Gibco), 2 = BAH 92, 3 = BAH 66, 4 = BAH 46, 5 = BAH 59, 6 = BAH 40c11, 7 = BAH 36, 8 = BAH 28, 9 = BAH 26c2, 10 = 100 bp ladder, 11 = H3, 12 = H2, 13 = BAC 5, 14 = CH-C1, 15 = 120, 16 = UBC 5, 17 = BAH 2c2 and 18 = 106. (B) 1 = 100 bp ladder, 2 = BAH 97, 3 = BAH 95, 4 = BAH 94, 5 = BAH 60, 6 = BAH 56, 7 = BAH 54, 8 = BAH 53, 9 = BAH 42, 10 = 100 bp ladder, 11 = BAH 39c9, 12 = BAH 34c8, 13 = BAH 33c1, 14 = BAH 15c1, 15 = BAH 16, 16 = BAH 30, 17 = BAH 12c14, and 18 = BAH 7c5.

genetic clusters were separated by larger fixed enzyme values (65–69%) which corresponded to a greater number of substitutions/site (0·0221; Fig. 3B).

IGS–PCR–RFLP analysis of cloned Giardia *isolates*

The finding that isolates appear to harbour multiple IGS variants raises questions regarding how the different IGS types are distributed amongst individuals within trophozoite populations. Are all trophozoites homogeneous with regard to the types of IGS sequence variants they maintain or do they habour distinct IGS sequence types? To examine this, IGS–PCR products were amplified from parent and cloned lines of isolate BAH 39 (IGS–PCR cluster B2) and then digested with *Sma*I (data not shown). Previous restriction analysis with this enzyme had shown that BAH 39 contained multiple IGS sequence types. A comparison of *Sma*I digestion profiles failed to reveal any variation in banding patterns or the relative ratios of different restriction fragments either between cloned and parent stocks or amongst the cloned isolates themselves. Furthermore, *Sma*I digestion of IGS–PCR products amplified from stocks of the parent BAH 39 isolate, grown *in vitro* in 1988 and again in 1991 also revealed identical restriction profiles. Similarly, no variation in banding patterns was observed between stocks of the BAH39c9 isolate which had been recovered from *in vitro* cultures in 1991, 1995 and 1997. This isolate had been cultured almost continually in our laboratory from the date it was initially cloned in 1991 (Binz *et al*. 1991). Analysis undertaken using an additional 2 *Giardia* isolates, Portland 1 (cluster A1) and BAH 40 (cluster A2) which contain only a single IGS sequence type, again failed to reveal any heterogeneity in banding profiles between parent and cloned lines.

RFLP analysis of IGS–PCR products amplified from Giardia *cyst samples*

The potential for IGS–PCR to genotype isolates recovered directly from the environment was investigated using *Giardia* cysts purified from human faeces. Fig. 4 compares the IGS–PCR *Sma*I digestion profiles of 11 *Giardia* cyst samples obtained from 9 individuals living in the same endemic area (Table 1). Also included are 3 reference *Giardia* isolates representing 3 of the 4 major *Giardia* IGS–PCR RFLP genetic clusters (Fig. 3). The gel contains 2 examples of samples collected from individuals at different times. Cyst samples collected 6 months apart from individual 1 were found to exhibit distinct *Sma*I RFLP patterns with both profiles corresponding to IGS–PCR group B *Giardia* genotypes. Records show this individual was treated twice during this period with metronidazole. Differences in restriction profiles were also observed between 2 *Giardia* cyst samples recovered from individual 9. The first sample (9A) contains mixed *Giardia* genotypes corresponding to IGS–PCR groups A2 and B whereas a sample collected 10 months later (9B) contains only the IGS–PCR group A2 genotype.

A comparison of IGS–RFLP patterns from *Giardia* isolates collected at the same time from 4 members of the same Junjuwa household shows that all were infected with the same IGS–PCR group A2 *Giardia* genotype (individuals 6, 7, 8 and 9). All infected individuals were less than 5 years of age (Table 1). The same digestion profiles found in the family group were also observed in *Giardia* samples collected at the same time from children living in Junjuwa (individual 5) and Joy Springs (individual 4). Finally, examination of 4 cyst samples recovered

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Table 2. Number of distinct restriction fragments and the sum of IGS digestion fragment sizes generated using different restriction enzymes

Enzyme	Number of distinct restriction fragments	Range of the sum of IGS–RFLP fragment sizes
IGS–PCR group A*		
*Apa*I	12	1060–2262 bp
*Bam*HI	5	1420–2840 bp
*Ban*II	12	1180–1975 bp
*Fok*I	10	1420–2780 bp
*Hin*fI	6	1420–2510 bp
*Sma*I	15	1350–2210 bp
Combined RFLP fragments	60	
IGS–PCR group B†		
*Apa*I	13	1580–3900 bp
*Ban*II	14	1630–4491 bp
*Bst*NI	17	2420–6500 bp
*Fok*I	9	1570–4040 bp
*Hin*fI	18	1700–7225 bp
*Pst*I	17	1590–4600 bp
*Sma*I	15	1560–4215 bp
Combined RFLP fragments	103	

* Size of original IGS–PCR product = 1420 bp.
† Size of original IGS–PCR product = 1600 bp.

from 3 individuals living in the Bayulu community were all found to contain different *Giardia* genotypes. Individuals 1 and 2 were infected with isolates containing restriction profiles corresponding to IGS–PCR group B genotypes whereas individual 3 contained the same IGS–PCR group A2 genotype observed in the Junjuwa and Joy Spring communities.

DISCUSSION

In this study, PCR and RFLP analysis were used to determine the extent of genetic heterogeneity within the IGS region of *G. duodenalis* isolates. On the basis of the size of the IGS–PCR product, *Giardia* isolates were separated into 4 groups which correlated almost exactly with the 2 major genetic assemblages established using isoenzyme analysis (Meloni *et al.* 1995). IGS–PCR size groups A, C and D corresponded to isoenzyme assemblage A, and IGS–PCR group B to isoenzyme assemblage B. The distinction between IGS–PCR groups A, C and D agrees with analysis of the complete SSU rDNA gene which revealed small but significant sequence differences between each of the three groups (R. M. Hopkins, unpublished observations). Furthermore, the genetic partition between IGS–PCR groups A and B, which contain the majority of *Giardia* isolates examined, is consistent with previous RFLP (Meloni, Lymbery & Thompson, 1989), RAPD (Morgan *et al.* 1993), and SSU rDNA sequence analyses (Hopkins *et al.* 1997) which all support the idea of a fundamental genetic division between the 2 isoenzyme groups. When one considers the range of loci examined by these techniques it suggests these differences extend throughout the whole *Giardia* genome.

In an earlier study examining the IGS in *Giardia*, Upcroft *et al.* (1994) also reported finding both length and sequence variation in this region. These authors observed that the IGS in isolate 1279 was approximately 100 bp longer than in isolate 106, which is similar to the size difference found between IGS–PCR groups A and B. Furthermore, 106 which was included in the present IGS–PCR study, was grouped with isolates from IGS–PCR group A while examination of partial SSU-rDNA sequences from the GS isolate showed that it was identical to those from IGS–PCR group B (data not shown). This suggests that the isolates described by Upcroft and co-workers are genetically equivalent to those in IGS–PCR groups A and B. In the same study, Upcroft *et al.* (1994) compared IGS sequences from isolates 1279 and 106 and observed variation at greater than 40% of nucleotide positions. The authors noted that this exceeded the level of IGS sequence divergence required to distinguish between *Xenopus* and *Drosophila* sp. and argued, on this basis, that the 2 *Giardia* isolates could also be regarded as separate species. Although such comparisons may not be valid given the differences in reproductive strategies used by these organisms and also the fact that individual *Giardia* isolates appear to harbour multiple IGS-types, these findings are

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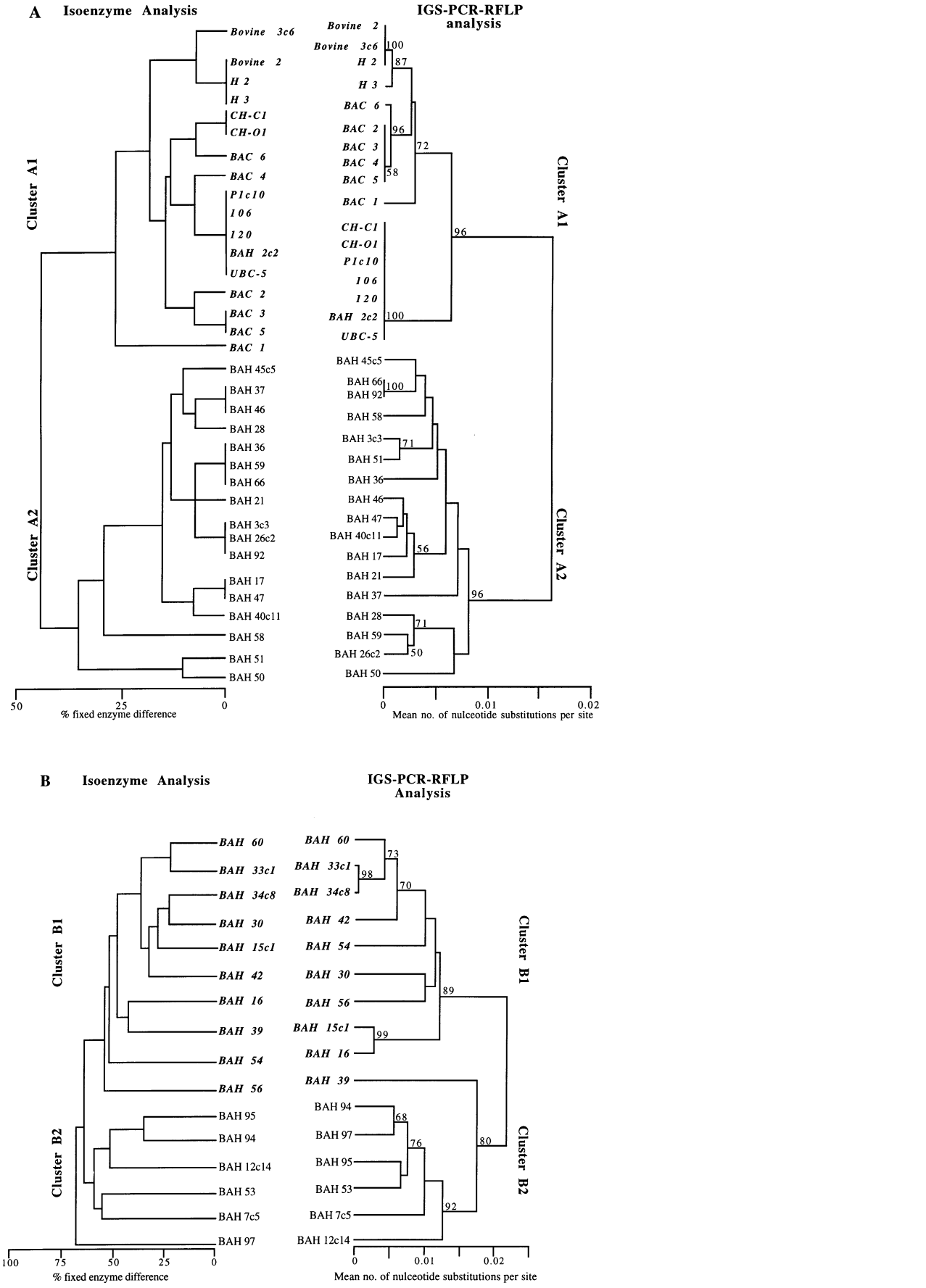

Fig. 3. Phenograms comparing genetic groups established for (A) IGS–PCR group A and (B) IGS–PCR group B *Giardia* isolates using isoenzyme electrophoresis and IGS–PCR RFLP analysis. On the left, the phenograms show the percentage fixed enzyme difference among zymodemes clustered by the UPGMA strategy using 13 enzyme loci

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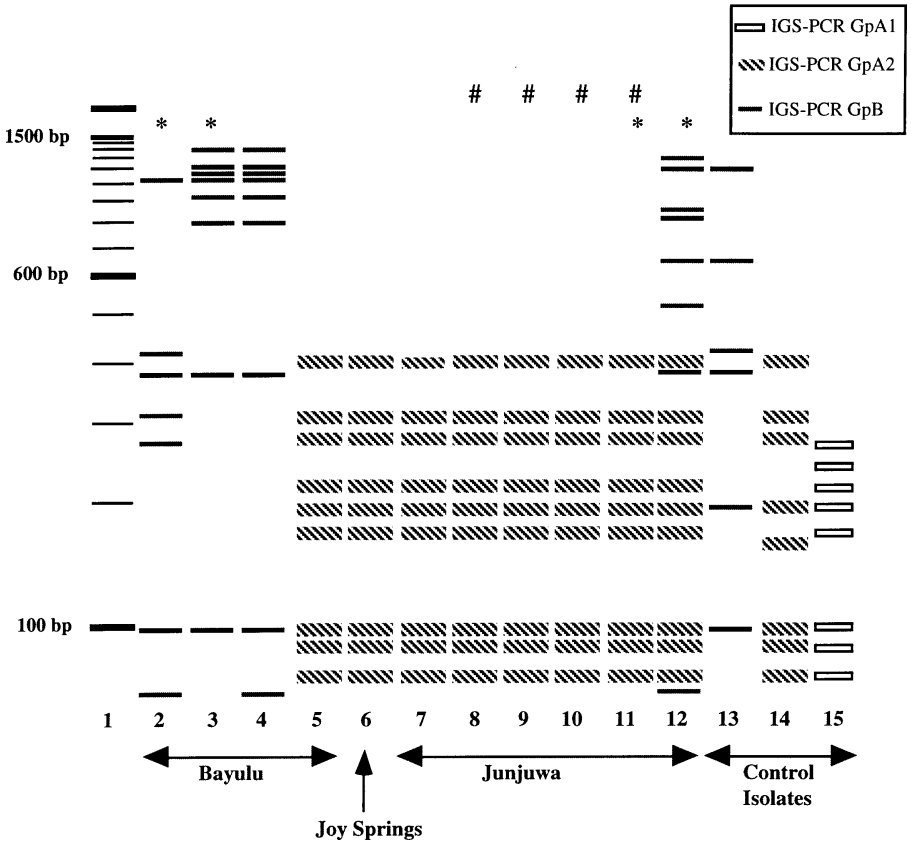

Fig. 4. Schematic representation of an ethidium bromide-stained 1·5% Metaphor-agarose gel comparing IGS–PCR *Sma*I digestion profiles of 11 *Giardia* cyst samples recovered from 9 different individuals. Lane 1 = 100 bp ladder, 2 = sample 1A, 3 = 1B, 4 = 2, 5 = 3, 6 = 4, 7 = 5, 8 = 6, 9 = 7, 10 = 8, 11 = 9A, 12 = 9B, 13 = BAH 60 (IGS–PCR Gp B), 14 = BAH 50 (IGS–PCR Gp A2), 15 = P1c10 (IGS–PCR Gp A1). * Denotes samples collected from the same individual at different times and # shows samples collected from members of the same family at the same time.

consistent with an accumulating body of evidence showing there is a fundamental genetic division between populations of *G. duodenalis* isolates worldwide. Studies using a range of phenotypic and genotypic criteria, including surface antigens (Ey *et al*. 1993*a*, *b*; Nash, 1995), isoenzymes (Andrews *et al*. 1989; Homan *et al*. 1992; Mayrhofer *et al*. 1995; Meloni *et al*. 1995) and DNA sequencing (Weiss, van Keulen & Nash, 1992; van Keulen *et al*. 1995; Baruch, Isaac-Renton & Adam, 1996; Monis *et al*. 1996), have consistently distinguished between at least 2 major groups of *G. duodenalis* isolates.

The finding that the size of IGS–PCR products amplified from goat (BAG 1, IGS–PCR group C) and cat (BAC 7, IGS–PCR group D) *Giardia* isolates, were distinct from other human and animal isolates raises questions regarding their zoonotic potential. Isoenzyme analysis has previously placed BAG 1 and BAC 7 into isoenzyme assemblage A (Meloni *et al*. 1995), which contains both human and animal isolates, although they represent the most genetically diverse genotypes within this assemblage. Recently, Ey *et al*. (1997) described a sublineage of *Giardia* isolates recovered exclusively from hoofed animals that were also affiliated with a broader genetic group of human and animal isolates referred to as Assemblage A. It is possible that the goat-derived BAG 1 and the Novel livestock genotype described by Ey *et al*. (1997) are equivalent although comparative studies are needed to confirm this. Further analysis is also needed to determine if the unique IGS–PCR product amplified from BAC 7 represents another genetic sublineage whose distribution is confined to cats and/or related hosts.

In addition to variability in the length of the IGS, the extent of sequence divergence between isolates within each IGS–PCR size group was examined using RFLP analysis. Within both IGS–PCR groups

(Meloni *et al*. 1995). On the right, the consensus molecular trees depict the genetic distance between isolates, calculated using UPGMA and based on RFLP profiles generated using (A) 6 restriction enzymes (*Apa*I, *Bam*HI, *Ban*II, *Fok*I, *Hin*fI and *Sma*I) and (B) 7 restriction enzymes (*Apa*I, *Ban*II, *Bst*NI, *Fok*I, *Hin*fI, *Pst*I and *Sma*I) respectively. Bootstrap values (%) are presented above each internode. Isolates presented in either ***bold italic*** or plain text are derived from isoenzyme clusters A1/B1 and A2/B2 respectively.

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A and B, two RFLP genetic clusters were identified which correlated almost exactly with those established using isoenzyme electrophoresis. The ability to resolve differences between these major genetic groups is similar to the results of other PCR-based studies aimed at genotyping *Giardia* isolates (Weiss *et al*. 1992; Ey *et al*. 1993*a*; van Keulen *et al*. 1995; Baruch *et al*. 1996; Monis *et al*. 1996) although IGS–PCR analysis exhibits an additional capacity to distinguish between isolates within each major genetic group. The level of discrimination was comparable to isoenzyme analysis although the 2 techniques differed in the genetic relationships established between individual isolates.

A significant factor driving much of the variability between individuals within each IGS–PCR group was the presence of multiple IGS sequence classes in the same isolate. While such intra-individual variation in IGS sequences clearly adds to the discriminatory power of the IGS–PCR, it is important to consider the mechanisms underlying how these sequence variants are maintained and what effect this has on the stability of RFLP profiles. This is particularly true, when one considers the potential impact of concerted evolution (Elder & Turner, 1995) a process that leads to the homogenization of sequences within repetitive DNA families such as the rDNA genes. If the rate of homogenization is fast, as in some species of parthenogenetic lizards (Hillis & Dixon, 1991), it would preclude the use of the IGS as a fingerprinting tool for *Giardia*. Fortunately, our evidence suggests this is not the case. Analysis of the IGS enabled the reconstruction of genetic groups which were similar to those established using other molecular criteria (Meloni *et al*. 1995; Hopkins *et al*. 1997; Morgan *et al*. 1993) implying there is a significant level of evolutionary conservation within families of IGS sequences. Furthermore, no variation in IGS restriction profiles were observed between parent and cloned *Giardia* isolates which suggests that populations of trophozoites are homogeneous with respect to the classes of IGS sequences they contain. Additionally, temporal analysis of DNA samples collected from *in vitro* cultures of BAH 39 and one of its clones, BAH 39c9 at varying times since 1988, showed that all samples maintained the same IGS digestion profiles. Although these studies do not take into account the selective pressures operating on the IGS *in situ* they do suggest that IGS–PCR RFLP profiles are inherently stable.

To further investigate its potential as a DNA fingerprinting tool, the IGS–PCR was successfully applied to *Giardia* cyst samples recovered from Aboriginal communities in which *Giardia* was endemic. Although preliminary in nature, these results provided an insight into different aspects of *Giardia* transmission. The potential for intra-familial transmission was highlighted by the fact that *Giardia* samples collected from 4 family members at the same time all contained identical genotypes. Furthermore, the same genotype present in the family group was distributed amongst other individuals of similar age living in the same as well as different communities, possibly indicative of an outbreak of transmission. IGS–PCR analysis was also able to detect mixed infections and showed that, in some individuals the genotype of *Giardia* isolates changed over time, whereas in others the same genotype was maintained.

As a fingerprinting tool analysis of the IGS region offers several features likely to be useful for studying *Giardia* transmission. Its ability to achieve a fine-scale resolution of *Giardia* genotypes in addition to its capacity to group isolates into conserved genetic clusters should enhance the prospect of (i) studying the movement of individual isolates within a defined geographical area and (ii) identifying phenotypic or biological markers which are common to genetically related groups of *Giardia* isolates. Studies are currently underway to apply this molecular tool to larger numbers of *Giardia* samples recovered from endemic areas.

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