J. Parasitol., 81(3), 1995, p. 368–383

GENETIC CHARACTERIZATION OF ISOLATES OF *GIARDIA DUODENALIS* BY ENZYME ELECTROPHORESIS: IMPLICATIONS FOR REPRODUCTIVE BIOLOGY, POPULATION STRUCTURE, TAXONOMY, AND EPIDEMIOLOGY

B. P. Meloni, A. J. Lymbery*, and R. C. A. Thompson

WHO Collaborating Centre for the Molecular Epidemiology of Parasitic Infections, School of Veterinary Studies, Murdoch University, Murdoch, Western Australia, Australia 6150

ABSTRACT: The nature and extent of genetic variation in *Giardia* was used to infer its mode of reproduction, population structure, taxonomy, and zoonotic potential. Ninety-seven isolates of *Giardia duodenalis*, from a defined area in Western Australia and throughout Australia and overseas, were obtained from humans, cats, cattle, sheep, dogs, goat, beaver, and rats. Enzyme electrophoresis revealed extensive genetic variation with 47 different zymodemes. The widespread occurrence of certain zymodemes and the similarity of relationships among isolates inferred from independent genetic markers suggests a clonal population structure for *G. duodenalis*, although occasional bouts of genetic exchange may occur. The 47 zymodemes clustered similarly in phenetic (UPGMA) and phylogenetic (Fitch–Margoliash) analyses. The level of genetic diversity in isolates from a defined geographical area in Western Australia was similar to the level of diversity in isolates from throughout Australia. These data suggest that clonal lineages within *G. duodenalis* are evolutionarily independent. Although there was a significant overall correlation between genetic distance separating zymodemes and occurrence in different host species, we found genetically identical isolates from humans and other animals and extensive genetic diversity between isolates from humans. We interpret this as evidence for zoonotic transmission of the parasite.

Giardia is a flagellated protozoan in the phylum Sarcomastigophora, class Zoomastigophorea, and order Diplomonadida (Levine et al., 1980; Cox, 1981). The genus has a worldwide distribution and consists of at least 5 species based on morphology (Filice, 1952; Meyer, 1985; Erlandsen and Bemrick, 1987; Erlandsen et al., 1990) and over 40 species based on host specificity (Hegner, 1926; Levine, 1973; Kulda and Nohynkova, 1978). Species of *Giardia* inhabit the intestine of virtually all classes of vertebrates (Kulda and Nohynkova, 1978; Thompson et al., 1990). Members of the *Giardia duodenalis* (synonyms: *G. lamblia* and *G. intestinalis*) morphological group infect humans and other animals and may be associated with asymptomatic, acute, or chronic giardiasis (Thompson et al., 1993). Today, the ubiquity of species of *Giardia* throughout the world is well recognized, and giardiasis is one of the most prevalent intestinal diseases of humans (Warren, 1989; Meyer, 1990; Schantz, 1991).

Numerous surveys in different countries have also shown that infection with *Giardia* is common and widespread in domestic cats, dogs, birds, domestic rats, horses, rabbits, sheep, cattle, and goats, as well as a range of sylvatic mammals and birds (Davies and Hibler, 1979; Panigrahy et al., 1984; Wallis et al., 1984; Woo, 1984; Kirkpatrick and Skand, 1985; Gasser et al., 1987a, 1987b; Buret et al., 1990; Erlandsen et al., 1990). The zoonotic potential of *G. duodenalis* from both domestic and wild animals is uncertain, although these hosts are considered important reservoirs due to their close association with humans and potential for environmental contamination with cysts (Davies and Hibler, 1979; Wenman et al., 1986; Kirkpatrick, 1987; Buret et al., 1990; Schantz, 1991; Thompson, 1995).

In order to determine the significance of genetic variation in *Giardia* to aspects of epidemiological importance such as transmission, treatment, and control, a thorough understanding of the taxonomy, population structure, and reproductive biology of *Giardia* is required. For example, the present taxonomy of *Giardia* fails to reflect the phenotypic and genetic heterogeneity that exists within the species *G. duodenalis* and provides little or no predictive value for factors such as host specificity, infectivity, or virulence. Knowing the population structure of *Giardia* is crucial because it determines the: (1) likelihood of associations between important biological characters such as host occurrence, pathogenicity, and drug sensitivity; and (2) rate of adaptation to adverse environmental conditions, such as exposure to drugs. The mode of reproduction of an organism has important implications for both population genetic structure and taxonomy. In particular, organisms that are predominantly or solely clonal generally possess a distinctive population structure characterized by excess heterozygosity, associations between independent genes (linkage disequilibrium), and a greater proportion of genetic variation distributed between, than within, populations (Tait, 1985; Cibulskis, 1988; Tibayrenc and Ayala, 1991). Taxonomically, they are difficult to deal with because the most widely used species definition, the biological species concept, is not applicable to them (Thompson et al., 1990; Tibayrenc et al., 1990; Tibayrenc, 1994).

Genetic variation detected by enzyme electrophoresis can be used to assess genetic relationships within and between species and is therefore suitable for species-level taxonomy (Richardson et al., 1986; Hillis and Moritz, 1990) and for providing information on factors of epidemiological significance such as transmission patterns for a given organism (Miles and Cibulskis, 1986). In addition, data generated from enzyme electrophoresis can also be interpreted to provide information on the ploidy of an organism, its mode of reproduction, and population structure. This is achieved by the genetic interpretation of the observed electrophoretic banding patterns, the nature of the variant enzyme types, and the proportion of the variant types in a given population (Tait, 1983; Miles, 1985; Tibayrenc et al., 1990, 1991; Tibayrenc and Ayala, 1991).

In this study, enzyme electrophoresis was used to: (1) examine the extent of genetic variation between isolates of *G. duodenalis* from different geographic areas in order to provide further in-

Received 7 July 1994; revised 12 January 1995; accepted 12 January 1995.

* Western Australian Department of Agriculture, Bunbury, Western Australia, Australia 6230.

formation on the genetic structure, ploidy, and taxonomy of *G. duodenalis*; and (2) compare isolates from different species of hosts in order to provide information on the zoonotic potential of *Giardia*.

MATERIALS AND METHODS

Sampling of isolates

Ninety-seven isolates of *G. duodenalis* of human, cat, sheep, rat, goat, cattle, dog, and beaver origin from throughout Australia and overseas were examined (Table I). Sixteen of the 97 isolates were from a localized endemic area (Meloni et al., 1993), Fitzroy Crossing (Table I), located in the north of Western Australia (Fig. 1). The 16 isolates of *Giardia* from Fitzroy Crossing were sampled from 4 communities, all within a 10-km radius of the town. These communities are provided with services in the town of Fitzroy Crossing and share a number of common facilities, including a shopping complex, hospital, sporting and recreational areas, school, and day care center. Individuals and families are very mobile and commonly move from community to community.

Enzyme electrophoresis

Methods for growing, harvesting, storing, and processing isolates for enzyme electrophoresis have been described (Meloni and Thompson, 1987; Meloni et al., 1988, 1992). Thirteen enzyme systems were used to characterize the 97 isolates of *G. duodenalis* and they are listed in Table II. Between 2 and 5 subcultures of each isolate were used for every enzyme. Occasionally, samples of culture media were electrophoresed to check for the presence of contaminating enzymes, but none were detected.

To examine the possibilities that isolates which produced multiple-banded enzyme patterns contained mixed populations of genotypically distinct organisms or were the result of posttranslational changes due to protease activity on primary gene products, a number of isolates were reexamined electrophoretically using stocks cloned from individual trophozoites and prepared using protease inhibitors in cell lysates.

Analysis of cloned stocks: Fourteen isolates were cloned using the microdrop method described by Binz et al. (1991), and 2 clonal stocks from each were reanalyzed by electrophoresis for enzymes that produced multiple-banded patterns. The isolates cloned and the enzymes used to examine clones are listed in Table III.

Analysis of stocks containing protease inhibitors: Stocks from 2 clones, BAH12C1 and BAH53C1, which produced multiple-banded patterns for 8/13 and 7/13 enzymes, respectively (Table III), were reanalyzed by enzyme electrophoresis after protease inhibitors had been added to lysates. A clone from isolate P1, which showed single-banded patterns for all enzymes, was used as a control.

A range of protease inhibitors was used, including those shown to be most effective in inhibiting protease activity in *G. duodenalis* (Hare et al., 1989). These included leupeptan (1 μg/ml), iodoacetamide (1 mM), *N*-a-*p*-tosyl-L-lysine chloromethyl ketone (1 mM), chymostatin (1 μg/ml), phenylmethylsulfonyl fluoride (1 mM), pepstatin (1 mM), and aprotinin (1 mM). All protease inhibitors were purchased from Sigma Chemical Co. (St. Louis, Missouri). Tubes containing packed trophozoites to which protease inhibitors were to be added were removed from the −70 C freezer, and while still frozen (to prevent protease activity), protease inhibitor and stabilizing solution were added. Trophozoites in tubes containing a specific inhibitor were lysed by homogenizing on an icebath and loaded onto gels.

Analysis of electrophoretic data

An allelic interpretation of banding patterns could not be made with confidence (Meloni et al., 1988), so enzyme patterns were treated as separate characters. For taxonomic studies, the percentage of enzyme showing fixed differences was used to calculate genetic distance between zymodemes. A fixed difference between 2 zymodemes occurs when they do not share a pattern for the particular enzyme in question. This ranges from 0 (0%), for identical enzyme profiles, to 1 (100%) for zymodemes with no shared enzyme patterns. This measure of genetic distance is identical to the fixed allelic differences of Andrews et al. (1989) for single-banded patterns but will underestimate distance for multiple-banded patterns, assuming their genetic interpretation of the banding patterns is valid. It is also highly correlated with other measures of genetic distance, either made with (Meloni et al., 1989) or without (Meloni et al., 1988) a genetic interpretation. Phylogenetic relationships between zymodemes were inferred from the genetic distance matrix by 2 techniques. First, genetic distances were clustered by the unweighted pair-group method with arithmetic means available in the computer package UPGMA2 (Constantine et al., 1994) to produce an ultrametric tree or phenogram. The cophenetic correlation coefficient (r_{cs}; Sneath and Sokal, 1973) was used to measure the agreement between distance values implied by the phenogram and those of the original distance matrix. Second, distances were analyzed phylogenetically using the Fitch–Margoliash method available in PHYLIP 3.5p (Felsenstein, 1993) to produce an additive tree or phylogram.

Mantel's test (Mantel, 1967) was used to correlate statistically pairwise estimates of genetic distances (percentage fixed enzyme differences) with pairwise descriptions of host occurrence between zymodemes of *Giardia*. Mantel's test determines the correlation between 2 distance matrices by comparing the sum of cross-products of analogous cells against an expected value calculated on the null hypothesis of random permutations between rows/columns of the second matrix. This approach avoids the problem of the lack of independence among cells of each test matrix, which is not taken into consideration using standard correlations (Douglas and Endler, 1982). Pairwise host occurrence was scored as 1 for zymodemes with isolates from different hosts and 0 for zymodemes containing isolates from the same host (see Appendix for data matrix).

The observed genotypic diversity (G_0) within populations was estimated from enzyme patterns as described by Stoddart and Taylor (1988):

$$G_0 = \frac{1}{\sum_{i=1}^{k} p_i^2},$$

where p_i is the observed frequency of the ith genotype (zymodeme), and k is the total number of zymodemes. The variance of G_0 was calculated as described by Stoddart and Taylor (1988):

$$\mathrm{Var}(G_0) = \frac{4}{N} G^2 \left(G^2 \sum_{i=1}^{k} p_i^3 - 1 \right).$$

RESULTS

Characterization of isolates of *G. duodenalis* using enzyme electrophoresis

Using 13 enzyme systems, 47 different zymodemes were found amongst the 97 isolates of *Giardia* examined (Tables I, IV). All 13 enzyme systems were variable (Table IV). The geographic distribution of the 47 zymodemes is shown in Figure 1.

Twenty-nine zymodemes contained single-banded patterns for all enzymes (M1–M6, M11–M13, M16, M18–M22, M27, M32–M41, M45–M47). Eighteen zymodemes contained isolates that produced multiple-banded patterns for 1 or more of the enzymes (M7–M10, M14, M15, M17, M23–M26, M28, M29–M31, M42–M44; Table III). Homozygote variants corresponding to the alternative alleles of presumed heterozygotes were found for 7 enzymes (EST, FDP, GOT, HK, ME, NP, and PGM, see Table II for abbreviations), but multiple-banded enzymes did not always fit expected heterozygote patterns.

Analysis of cloned isolates

The majority of clones produced multiple-banded patterns identical to their corresponding parent stock for all enzymes. However, on several occasions, patterns from 1 or more clones differed from the pattern displayed by the parent stock. Clone BAH30C2 did not express a fast-moving esterase band, which was present in the parent BAH30 and clone BAH30C1. Similarly, the clone BAH42C2 did not express a fast-moving esterase

TABLE I. Isolates of *Giardia duodenalis* characterized.

Code	Zymo-deme	Host	Clinical history*	Nature of sample	Geographical origin and year of ioslation†	Source‡
BAH1	4	Human	S	Cysts	Wyndham, W.A., 1986	SHL
BAH2	4	Human	S	Cysts	Woodanilling, W.A., 1986	SHL
BAH3	1	Human	NA	Cysts	Lockridge, W.A., 1986	SHL
BAH4	1	Human	A	Cysts	Derby, W.A., 1986	SHL
BAH5	1	Human	NA	Cysts	Marble Bar, W.A., 1986	SHL
BAH6	1	Human	S	Cysts	Warburton, W.A., 1986	SHL
BAH7	7	Human	A	Cysts	Katanning, W.A., 1986	SHL
BAH8	1	Human	S	Cysts	Wickham, W.A., 1986	SHL
BAH9	1	Human	S	Cysts	Marble Bar, W.A., 1986	SHL
BAH10	1	Human	NA	Cysts	South Hedland, W.A., 1986	SHL
BAH11	1	Human	S	Cysts	Kalgoorlie, W.A., 1986	SHL
BAH12	8	Human	S	Cysts	Wyndham, W.A., 1986	SHL
BAH13	1	Human	S	Cysts	Kelmscott, W.A., 1986	SHL
BAH14	3	Human	NA	Cysts	Kununurra, W.A., 1986	SHL
BAH15	12	Human	S	Cysts	Kununurra, W.A., 1986	SHL
BAH16	9	Human	S	Cysts	Derby, W.A., 1986	SHL
BAH17	18	Human	S	Cysts	Adelaide, S.A., 1986	UA
BAH18	33	Human	S	Cysts	Byford, W.A., 1987	SHL
BAH19	11	Human	S	Cysts	Kununurra, W.A., 1987	SHL
BAH20	10	Human	NA	Cysts	Kununurra, W.A., 1987	SHL
BAH21	2	Human	S	Cysts	Busselton, W.A., 1987	SHL
BAH22	1	Human	S	Cysts	Carnarvon, W.A., 1987	SHL
BAH23	3	Human	S	Cysts	High Wycombe, W.A., 1987	SHL
BAH24	1	Human	S	Cysts	Derby, W.A., 1987	SHL
BAH25	3	Human	S	Cysts	Bunbury, W.A., 1987	SHL
BAH26	1	Human	S	Cysts	Rockingham, W.A., 1987	SHL
BAH27	1	Human	NA	Cysts	Fitzroy Crossing, W.A., 1987	MU
BAH28	20	Human	NA	Cysts	Fitzroy Crossing, W.A., 1987	MU
BAH29	3	Human	NA	Cysts	Fitzroy Crossing, W.A., 1987	MU
BAH30	15	Human	S	Cysts	Derby, W.A., 1987	SHL
BAH31	21	Human	NA	Cysts	Broome, W.A., 1987	SHL
BAH32	12	Human	S	Cysts	Port Hedland, W.A., 1987	SHL
BAH33	16	Human	NA	Cysts	Broome, W.A., 1987	SHL
BAH34	17	Human	S	Cysts	Perth, W.A., 1987	PMH
BAH35	17	Human	S	Cysts	Perth, W.A., 1987	PMH
BAH36	3	Human	S	Cysts	Kununurra, W.A., 1987	SHL
BAH37	22	Human	NA	Cysts	Mundaring, W.A., 1988	SHL
BAH38	1	Human	S	Cysts	Esperance, W.A., 1988	SHL
BAH39	23	Human	NA	Cysts	Hong Kong, 1988	SHL
BAH40	21	Human	A	Cysts	Perth, W.A., 1988	SHL
BAH41	1	Human	S	Cysts	Perth, W.A., 1988	SHL
BAH42	24	Human	NA	Cysts	Nichol Bay, W.A., 1988	SHL
BAH43	21	Human	NA	Cysts	Fitzroy Crossing, W.A., 1988	MU
BAH44	25	Human	NA	Cysts	Fitzroy Crossing, W.A., 1988	MU
BAH45	33	Human	NA	Cysts	Fitzroy Crossing, W.A., 1988	MU
BAH46	22	Human	S	Cysts	Morley, W.A., 1988	SHL
BAH47	18	Human	S	Cysts	Port Hedland, W.A., 1988	SHL
BAH48	11	Human	S	Cysts	Rockingham, W.A., 1988	SHL
BAH49	26	Human	S	Cysts	Northam, W.A., 1988	SHL
BAH50	19	Human	NA	Cysts	Narrogin, W.A., 1988	SHL
BAH51	17	Human	S	Cysts	Broome, W.A., 1988	SHL
BAH52	22	Human	NA	Cysts	Broome, W.A., 1988	SHL
BAH53	28	Human	NA	Cysts	Fitzroy Crossing, W.A., 1988	MU
BAH54	29	Human	S	Cysts	Kununurra, W.A., 1988	SHL
BAH55	1	Human	S	Cysts	Halls Creek, W.A., 1988	SHL
BAH56	30	Human	NA	Cysts	Fitzroy Crossing, W.A., 1988	MU
BAH57	31	Human	S	Cysts	Carnarvon, W.A., 1988	SHL
BAH58	32	Human	S	Cysts	Wanneroo, W.A., 1989	SHL
BAH59	3	Human	S	Cysts	Carnarvon, W.A., 1989	SHL
BAH60	34	Human	S	Cysts	High Wycombe, W.A., 1989	SHL
BAH65	4	Human	NA	Cysts	Fitzroy Crossing, W.A., 1990	MU

TABLE I. Continued.

Code	Zymo-deme	Host	Clinical history*	Nature of sample	Geographical origin and year of ioslation†	Source‡
BAH66	3	Human	NA	Cysts	Fitzroy Crossing, W.A., 1990	MU
BAH67	4	Human	NA	Cysts	Fitzroy Crossing, W.A., 1990	MU
BAH92	1	Human	NA	Cysts	Fitzroy Crossing, W.A., 1991	MU
BAH93	1	Human	NA	Cysts	Fitzroy Crossing, W.A., 1991	MU
BAH94	42	Human	NA	Cysts	Fitzroy Crossing, W.A., 1991	MU
BAH95	43	Human	NA	Cysts	Fitzroy Crossing, W.A., 1991	MU
BAH97	44	Human	NA	Cysts	Fitzroy Crossing, W.A., 1991	MU
BAC1	6	Cat	NA	Cysts	Shenton Park, W.A., 1986	MU
BAC2	5	Cat	NA	Troph.	Murdoch, W.A., 1986	MU
BAC3	35	Cat	NA	Cysts	Murdoch, W.A., 1986	MU
BAC4	36	Cat	NA	Cysts	Murdoch, W.A., 1987	MU
BAC5	35	Cat	NA	Cysts	Murdoch, W.A., 1987	MU
BAC6	37	Cat	NA	Cysts	Murdoch, W.A., 1988	MU
BAC7	45	Cat	S	Cysts	Werribee, VIC., 1991	UM
BAG1	46	Goat	NA	Cysts	Werribee, VIC., 1991	UM
BAD1	14	Dog	A	Cysts	Murdoch, W.A., 1986	MU
BAS2	1	Sheep	S	Troph.	Albany, W.A., 1989	AD
Vanc/85/UBC/5	4	Human	S	Culture	British Columbia, CAN., 1985	UBC
Vanc/85/UBC/7	4	Beaver	NA	Culture	British Columbia, CAN., 1985	UBC
Mont/83/McG/12	4	Dog	NA	Culture	Quebec, CAN., 1983	UBC
P1 ATCC/30888	4	Cat	NA	Culture	Portland, U.S.A., 1970	UBC
Bris/83/HEPU/106	4	Human	S	Culture	Brisbane, QLD., 1983	QIMR
Bris/83/HEPU/120	4	Human	S	Culture	Brisbane, QLD., 1983	QIMR
Bris/83/HEPU/141	4	Human	S	Culture	Port Moresby, P.N.G., 1983	QIMR
P1 ATCC/30888	4	Cat	NA	Culture	Portland, U.S.A., 1970	QIMR
HP108	47	Human	NA	Culture	Poland, 1986	K
HP109	47	Human	NA	Culture	Poland, 1986	K
CH-H2	39	Human	NA	Culture	Zürich, SWIT., 1989	UZ
CH-H3	39	Human	NA	Culture	Zürich, SWIT., 1989	UZ
CH-C1	38	Dog	NA	Culture	Zürich, SWIT., 1989	UZ
CH-B1	38	Cattle	NA	Culture	Zürich, SWIT., 1986	UZ
CH-B2	39	Cattle	NA	Culture	Zürich, SWIT., 1989	UZ
CH-B3	40	Cattle	NA	Culture	Tessin, SWIT., 1989	UZ
CH-O1	38	Sheep	NA	Culture	Zürich, SWIT., 1987	UZ
BAR1	13	Rat	S	Troph.	Murdoch, W.A., 1986	MU
BAR3	41	Rat	A	Troph.	Murdoch, W.A., 1989	MU

* S, symptomatic; A, asymptomatic; NA, clinical information not available.
† W.A., Western Australia; S.A., South Australia; QLD., Queensland; VIC., Victoria; CAN., Canada; U.S.A., United States of America; SWIT., Switzerland; P.N.G., Papua New Guinea.
‡ SHL, State Health Laboratories of W.A.; UZ, University of Zurich, SWIT.; UBC, University of British Columbia, CAN.; AD, Agriculture Department of W.A.; UA, University of Adelaide, S.A.; K, Karol Marcinkowski Academy of Medicine, Poland; QIMR, Queensland Institute of Medical Research; UM, University of Melbourne, VIC.; PMH, Princess Margaret Hospital, W.A.; MU, Murdoch University, W.A.

band, whereas the parent BAH42 and clone BAH42C1 did so. The fast-moving esterase band in clones BAH44C1 and BAH44C2 moved more slowly than the corresponding band in the parent isolate. The final difference detected was in clones BAH49C1 and BAH49C2, which expressed only 2 of the 3 phosphoglucomutase enzyme bands that were present in the parent stock.

Analysis of stocks containing protease inhibitors

The addition of protease inhibitors to lysates from cloned isolates BAH12C1 and BAH53C1 did not result in the loss of multiple-banded enzyme patterns at any locus. However, on a number of occasions the mobility of enzyme patterns was altered in stocks containing specific protease inhibitors. This also occurred in the corresponding stock from the single-banded control and, therefore, probably reflects interference of the protease inhibitor with posttranslation of a number of *G. duodenalis* isoenzymes.

Genetic relationships between zymodemes

Differences in enzyme profiles between zymodemes, measured by percentage fixed enzyme differences, ranged from 7.7 to 100 (Appendix). The phenogram constructed from these data (cophenetic correlation $r_{cs} = 0.96$) is shown in Figure 2. There were 20 principal clusters, separated by at least 36% fixed enzyme differences. Group 1, consisting of 4 zymodemes, contained 3 isolates from humans in Western Australia and 2 from humans in Poland. Group 2, consisted of 5 zymodemes, containing 8 isolates from humans in Western Australia. Groups 3, 5–9, and 11–14 each contained single isolates from humans

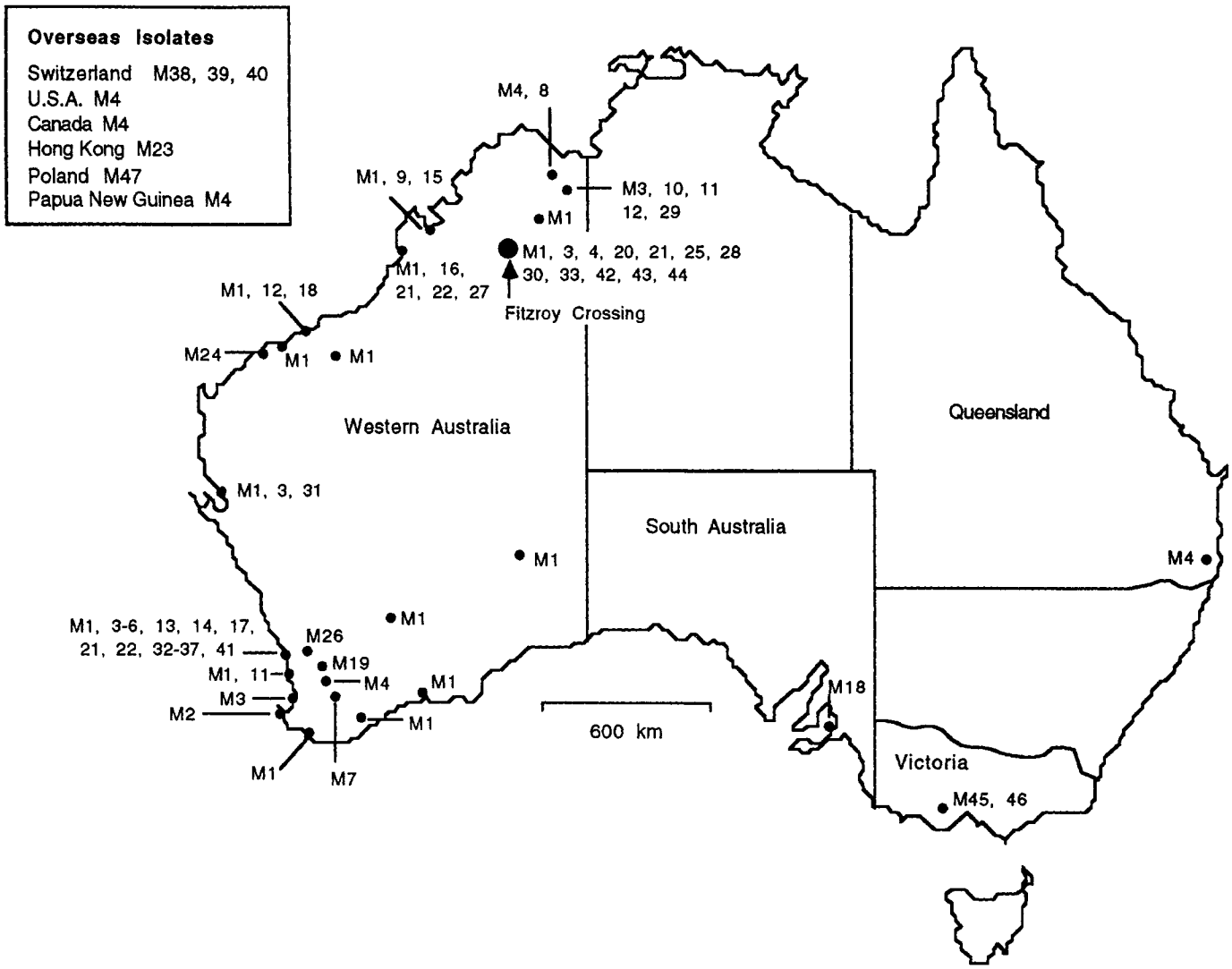

FIGURE 1. Geographical distribution of zymodemes of *Giardia duodenalis*.

in Western Australia. Group 4 consisted of a single isolate from a human in Hong Kong. Group 10 consisted of 2 zymodemes, each containing a single isolate from humans in Western Australia. Group 15 consisted of 2 zymodemes, each containing a single isolate from Western Australian rats. Group 16 consisted of a single goat isolate from the Australian state of Victoria. Group 17 consisted of 9 zymodemes containing 4 isolates from humans in Western Australia, 2 isolates each from humans in Switzerland and the Australian state of Queensland, 1 isolate each from a human in Papua New Guinea and Canada, 6 isolates from cats in Western Australia, 1 isolate from a dog in Canada, and 1 isolate from a dog in Switzerland, 3 isolates from cattle in Switzerland, 1 isolate from a sheep in Switzerland, 1 isolate from a beaver in Canada, and the Portland (P1) isolates. Group 18 consisted of 11 zymodemes containing 39 isolates from humans in Western Australia, 1 from a human in South Australia and 1 isolate from a Western Australian sheep. Groups 19 and 20 consisted of single isolates from a dog in Western Australia and a cat in Victoria, respectively.

These groupings were largely confirmed in the unrooted phylogram constructed from Fitch–Margoliash analysis of the distance matrix (Fig. 3). The only exceptions to the groupings implied by the phenogram in this tree are the closer relationships between zymodemes M9 and M26 and between M30 and M23. Maximum parsimony analysis using the enzyme banding patterns as character-state, rather than distance data, also produced congruent groupings (data not shown).

Despite the co-occurrence of isolates from different species of host in the same zymodeme and the same clusters in both the phenogram and phylogram, there was some overall structuring of genetic diversity along host lines, as shown by a significant correlation between genetic distance and host occurrence (Mantel's $g = 4.272$; $P < 0.001$; Appendix).

Genetic diversity within populations

A second phenogram consisting of only the 16 human isolates of *G. duodenalis* from Fitzroy Crossing (cophenetic correlation $r_{cs} = 0.98$) is shown in Figure 4. There were 7 principal clusters, corresponding to groups 8, 9, 10, 12, 14, 17, and 18 in Figure 2. Differences in enzyme profiles between zymodemes identified from Fitzroy Crossing, measured by percentage fixed enzyme

TABLE II. List of enzymes used to characterize *Giardia*.

Enzyme	Abbreviation	E.C. number
Acid phosphatase	ACP	E.C. 3.1.3.2
Esterase	EST	E.C. 3.1.1.1
Fructose 1-6-diphosphatase	FDP	E.C. 3.1.3.11
Glutamate dehydrogenase	GDH	E.C. 1.4.1.3
Glucose-6-phosphate dehydrogenase	G6PD	E.C. 1.1.1.49
Glutamate-oxaloacetate transaminase	GOT	E.C. 2.6.1.1
Glucose phosphate isomerase	GPI	E.C. 5.3.1.9
Hexokinase	HK	E.C. 2.7.1.1
Malate dehydrogenase	MDH	E.C. 1.1.1.37
Malic enzyme	ME	E.C. 1.1.1.40
Nucleoside phosphorylase	NP	E.C. 2.4.2.1
Phosphoglucomutase	PGM	E.C. 2.7.5.1
6-Phosphogluconate dehydrogenase	6PGD	E.C. 1.1.1.44

* Enzyme Commission.

differences, ranged from 7.7 to 100. The estimated genetic diversity was similar in isolates from Fitzroy Crossing ($G_0 = 9.78 \pm 0.68$) and other human isolates from throughout Western Australia ($G_0 = 8.64 \pm 0.34$), but lower than the overall genetic diversity in isolates from different host species from throughout Australia and overseas ($G_0 = 14.18 \pm 0.36$).

DISCUSSION

Mode of reproduction

Most isolates of *G. duodenalis* used in the present and in previous studies (Bertram et al., 1983; Baveja et al., 1986; Korman et al., 1986; Meloni et al., 1988, 1989, 1992; Andrews et al., 1989) show predominantly single-banded enzyme patterns, with different isolates often having different isoenzymes. This suggests that *G. duodenalis* is an asexual, functionally haploid organism, with variant enzymes the result of occasional mutations. However, 18 of the 47 different zymodemes identified in the present study contained isolates that produced multiple-banded patterns for between 1 and 8 of the enzymes (Table III). It is unlikely that the multiple-banded enzyme patterns are the result of mixed populations of genotypically distinct organisms or the result of posttranslational changes due to protease activity on primary gene products. Identical patterns occurred in all the subcultures of each isolate that were examined. Other isolates, prepared in exactly the same way, showed single-banded patterns for the same enzymes. In addition, multiple-banded patterns were retained in cloned isolates and in lysates of *G. duodenalis* trophozoites containing protease inhibitors. Although a number of clones produced slight differences in enzyme patterns from parent isolates, we believe these differences to be the result of either gene switching or mutations. Switching of genes in response to environmental factors has been reported in many organisms (Richardson et al., 1986; Bogliolo and Godfrey, 1987). The rapid growth rate and asexual division of *G. duodenalis* could provide the possibility of mutations or chromosomal rearrangements (Le Blancq, 1994) even over the time scale of observations.

If *G. duodenalis* is diploid or polyploid, which seems likely because trophozoites of *Giardia* have 2 nuclei and pulse field electrophoresis has provided evidence for multiple copies of similar chromosomes (Adam et al., 1988), the observed multiple-banded patterns could be explained by mutation. In diploid organisms reproducing solely asexually, the accumulation of mutations without recombination should produce heterozygosity at most loci. Thus, the pattern found in the few multiple-banded isolates would be expected, but not the single-banded enzyme profiles found in the majority of isolates. A number of explanations are possible. *Giardia duodenalis* may (1) reproduce sexually, as well as asexually; this has recently been shown to occur in a number of bacteria, yeast, and pro-

TABLE III. Zymodemes containing isolates of *Giardia* that produced multiple-banded enzymes.

Isolate*	Zymodeme	No. of multiple-banded enzymes	Enzymes
BAH7•	M7	2/13	GOT, G6PD
BAH12•	M8	8/13	EST, FDP, GDH, G6PD, ME, NP, PGM, 6PGD
BAH16	M9	3/13	ME, PGM, 6PGD
BAH20	M10	1/13	PGM
BAH30•	M15	2/13	EST, FDP
BAH34•, BAH35	M17	1/13	FDP
BAH39•	M23	3/13	GOT, NP, PGM
BAH42•	M24	1/13	EST
BAH44•	M25	8/13	EST, FDP, GDH, GOT, G6PD, HK, PGM, 6PGD
BAH49•	M26	4/13	FDP, ME, PGM, 6PGD
BAH53•	M28	7/13	FDP, GOT, G6PD, HK, ME, PGM, 6PGD
BAH54•	M29	3/13	G6PD, PGM, 6PGD
BAH56•	M30	2/13	ME, NP
BAH57	M31	1/13	EST
BAH94•	M42	4/13	EST, ME, NP, 6PGD
BAH95•	M43	5/13	GOT, ME, NP, PGM, 6PGD
BAH97•	M44	4/13	EST, GOT, NP, PGM
BAD1	M14	2/10	ME, PGM

* • Indicates isolates that were cloned and reexamined.

TABLE IV. Summary of enzyme patterns scored for the 47 zymodemes using 13 enzyme systems.

	Number of enzyme patterns detected for each enzyme system*												
Zymodeme	ACP	EST	FDP	GDH	G6PD	GOT	GPI	HK	MDH	ME	NP	6PGD	PGM
M1	4	5	4	4	6	9	6	6	4	5	3	7	2
M2	4	5	4	4	6	9	6	6	4	2	3	7	2
M3	4	5	4	4	6	9	6	6	4	6	3	7	2
M4	3	5	2	4	6	9	4	6	4	5	5	6	4
M5	2	5	4	4	6	9	4	6	4	5	5	7	4
M6	4	4	4	4	6	9	4	6	4	8	5	6	4
M7	7	7	5	5	8	5	1	2	3	7	3	1	3
M8	6	11	6	1	3	2	1	2	3	11	2	1	9
M9	6	3	1	5	4	6	3	2	3	11	3	2	9
M10	6	4	4	5	3	8	3	2	3	9	3	3	13
M11	6	6	4	5	3	7	3	5	3	9	3	4	3
M12	6	3	1	5	4	7	2	2	3	9	3	4	3
M13	−1	1	1	4	4	3	1	8	1	3	1	4	7
M14	−1	−1	−1	−1	7	9	4	4	4	1	5	7	8
M15	6	11	6	5	4	7	3	3	3	9	3	4	3
M16	6	3	5	5	3	1	3	2	3	9	3	4	3
M17	6	3	6	5	4	3	1	3	3	9	3	4	3
M18	5	5	3	4	6	9	6	6	4	5	3	7	2
M19	5	6	4	4	6	9	6	5	4	6	3	5	2
M20	4	5	5	4	6	9	6	6	4	5	3	7	2
M21	5	5	4	4	6	9	6	6	4	5	3	7	2
M22	4	5	3	4	6	9	6	6	4	5	3	7	2
M23	6	3	4	5	9	4	3	2	3	9	4	4	9
M24	6	11	4	5	3	7	2	5	3	9	3	4	3
M25	6	9	7	3	10	2	3	9	3	9	3	1	10
M26	6	6	6	5	4	1	3	2	3	12	3	1	6
M27	2	6	4	4	6	9	6	5	4	6	3	7	2
M28	6	8	8	5	11	2	1	10	3	13	3	1	11
M29	6	6	3	4	4	2	4	2	3	9	3	2	12
M30	5	6	4	5	2	7	3	2	3	14	4	4	3
M31	6	11	5	4	3	7	3	2	3	9	3	4	3
M32	4	5	4	4	6	9	5	6	3	5	3	4	2
M33	4	5	3	4	6	9	6	6	4	6	3	7	2
M34	6	3	5	4	3	6	1	2	3	9	3	4	3
M35	2	5	4	4	6	9	4	6	4	5	5	6	4
M36	3	5	4	4	6	9	4	6	4	5	5	6	4
M37	3	5	4	4	6	9	4	6	4	5	5	7	4
M38	3	5	1	4	6	9	4	6	4	5	5	7	4
M39	3	4	3	4	6	9	4	6	4	5	5	7	4
M40	3	4	4	4	6	9	4	6	4	5	5	7	4
M41	1	1	2	4	4	3	2	8	1	3	1	4	7
M42	6	10	5	5	1	3	4	1	3	11	2	1	7
M43	6	4	5	5	5	3	1	1	3	11	4	1	9
M44	5	10	2	5	5	6	4	2	3	10	2	4	10
M45	2	6	5	6	4	4	4	6	2	4	5	6	5
M46	2	2	1	2	6	9	2	7	4	10	3	8	1
M47	6	3	5	5	4	1	4	2	3	9	3	5	3

* A −1 indicates no enzyme bands detected. Different enzyme patterns may consist of one or more enzyme bands.

tozoan species (Tibayrenc and Ayala, 1991; Maynard Smith et al., 1993); (2) reproduce only asexually with single-banded isolates arising from mutations in both alleles, but this is unlikely because it would require the same mutation to occur twice (Tait, 1983); (3) be asexual with homozygosity maintained by selection (Asher and Mace, 1971) or the accumulation of nonfunctional alleles (Suomalainen et al., 1976; Cibulskis, 1988); and (4) reproduce asexually, but isolates differ in ploidy.

At present, the data do not allow an adequate distinction between possibilities 1, 3, and 4. The most interesting question to arise is the possibility of sexual reproduction. The best evidence of a sexual process in *G. duodenalis* was the finding of several presumed heterozygotes with both corresponding homozygous variants. The probability of the same mutation occurring in both alleles in the second homozygote by chance is extremely rare. Given the recent evidence for diploidy and ge-

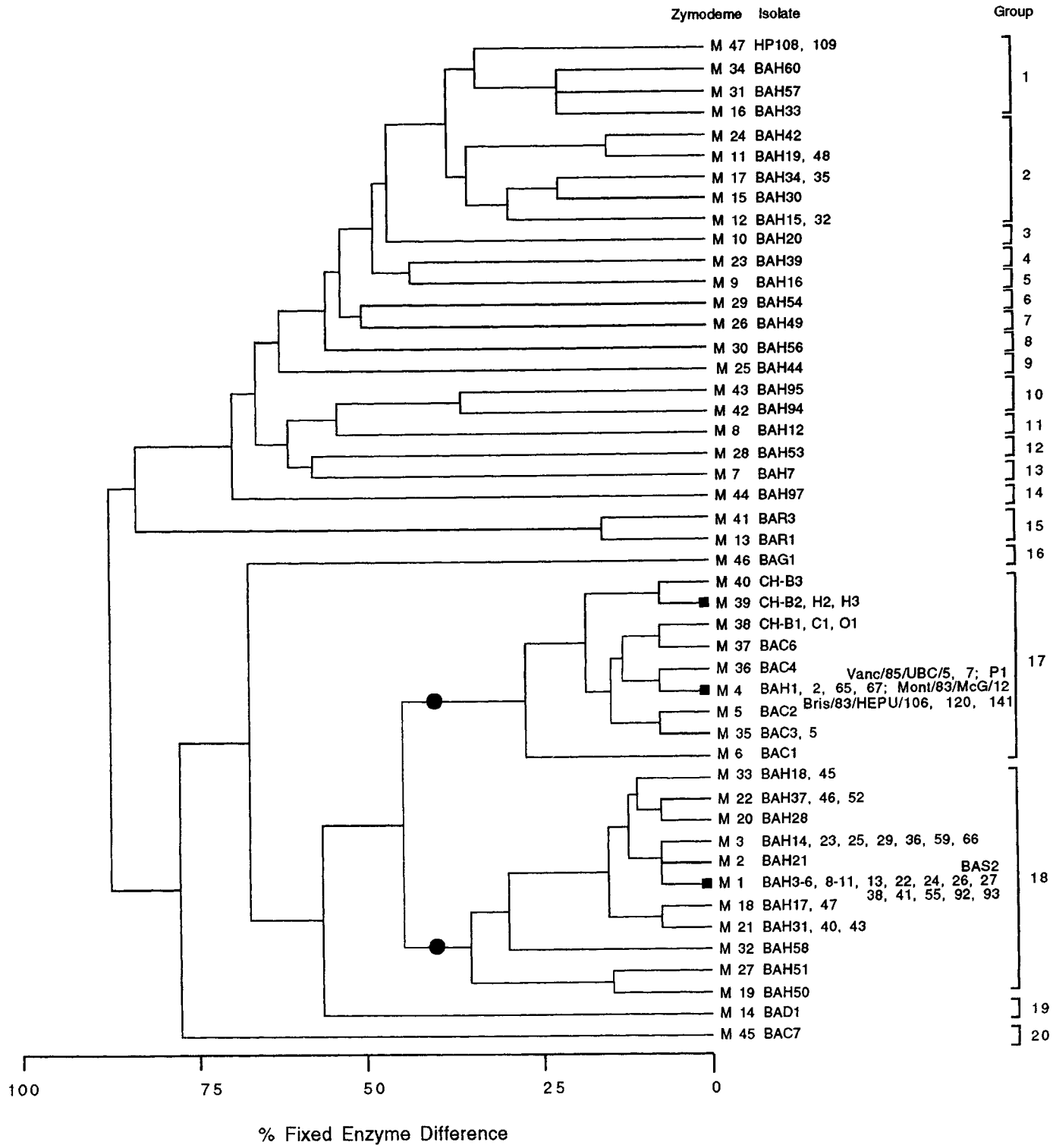

FIGURE 2. Phenogram of percentage fixed enzyme differences among zymodemes of *Giardia duodenalis*, clustered by the group average (UPGMA) strategy. ● Clusters with zymodemes containing isolates from human and nonhuman hosts. ■ Zymodemes containing isolates from human and nonhuman hosts.

netic exchange in protozoan genera such as *Trypanosoma* (Tait, 1980; Jenni et al., 1986), *Entamoeba* (Blanc et al., 1989), and *Naegleria* (Cariou and Pernin, 1987; Pernin et al., 1992), it is possible that *G. duodenalis*, at least occasionally, may undergo genetic recombination.

Population structure

Several criteria based on the significance of observed levels of segregation of alleles at a locus and their recombination between loci, which are the consequences of sexual reproduction,

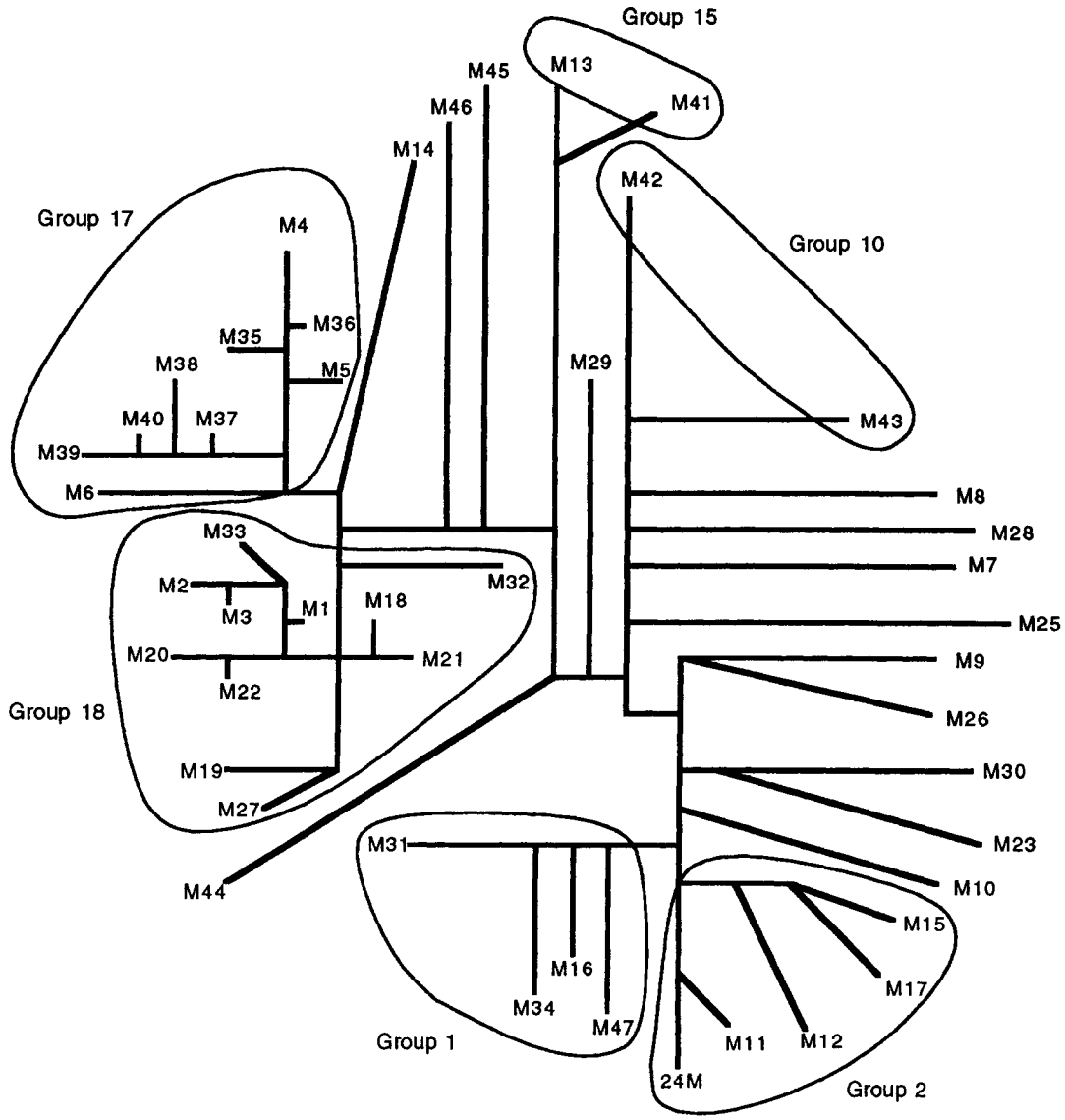

FIGURE 3. Unrooted phylogram of *Giardia duodenalis* zymodemes constructed from Fitch–Margoliash analysis of the distance matrix (Appendix). Circled zymodemes correspond to groups containing two or more zymodemes obtained from the phenogram in Figure 2.

have recently been applied to answer fundamental questions about the predominant mode of reproduction of parasitic protozoa, bacteria, and fungi (Ochman and Selander, 1984; Tait, 1985; Tibayrenc et al., 1990; Tibayrenc and Ayala, 1991; Maynard Smith et al., 1993). Four criteria exist based on recombination between loci: (1) overrepresented widespread identical genotypes, (2) absence of recombinant genotypes, (3) linkage disequilibrium, and (4) correlation between independent sets of genetic markers do not require prior knowledge of ploidy (Tibayrenc et al., 1990; Tibayrenc and Ayala, 1991) and, therefore, can be used to determine whether the population structure of *Giardia* follows the pattern expected for a clonal organism.

The results of enzyme electrophoretic characterization of isolates in the present study revealed the widespread occurrence of 3 genotypes sampled over a 5-yr period; M1, M3, and M4 (Fig. 1). Zymodeme M1 contained 18 human and 1 sheep isolate of *G. duodenalis* from a variety of locations in Western Australia; zymodeme M3 was sampled 7 times from humans throughout Western Australia, and zymodeme M4, which contains the Portland isolate (P1) was sampled from humans in Western Australia, Queensland, and New Guinea, and from a human, beaver, and dog in Canada (Fig. 2).

The results of a previous study (Meloni et al., 1989) showing a correlation between grouping of isolates on the basis of enzyme and restriction fragment length polymorphism (RFLP) patterns fulfilled criterion 4 and, therefore, provided population genetic evidence of a clonal population structure for *G. duodenalis*. This finding is supported by the correlation of enzyme groupings of 14 parent and clonal isolates used in this study with those obtained with RAPD (random amplified polymorphic DNA) analysis (Morgan et al., 1993). In addition, Homan et al. (1992), using enzyme electrophoresis, RFLPs, and RAPDs, found general agreement in the grouping of isolates obtained by the 3 methods.

Although the data suggest a clonal population structure in *G. duodenalis*, it should be noted that tests using linkage disequilibrium (with the exception of the criterion of overrepresented widespread genotypes) may be confounded by examining sam-

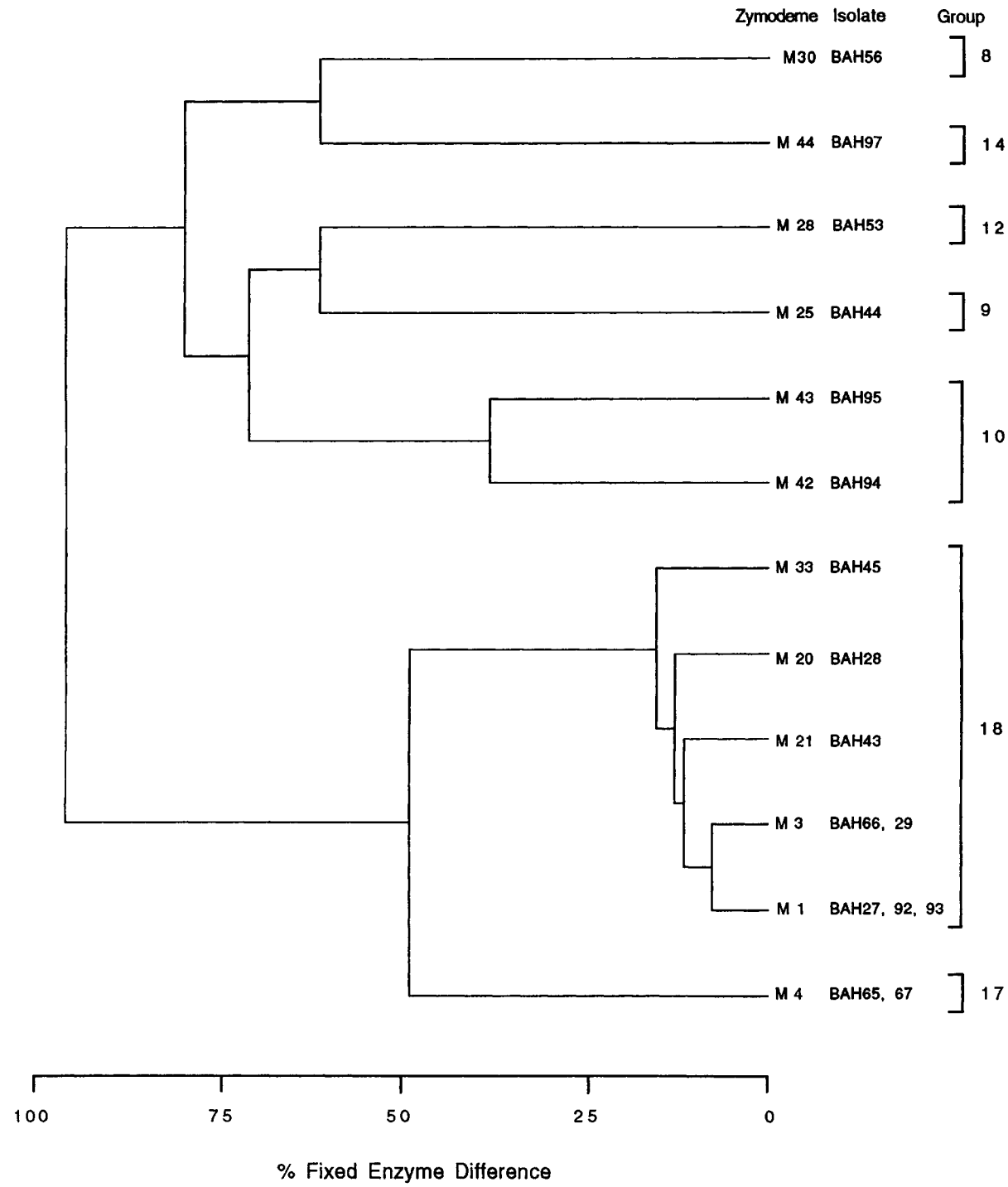

FIGURE 4. Phenogram of percentage fixed enzyme differences among 16 isolates of *Giardia duodenalis* from Fitzroy Crossing clustered by the group average (UPGMA) strategy.

ples from different populations and rare cyclic bouts of sexual reproduction, because 1 generation of sexual reproduction will fully restore equilibrium at the single-locus level but not at the multilocus level (Cibulskis, 1988; Dye, 1991). Therefore, to provide additional evidence on the population structure of *Giardia* (and other organisms), samples should be examined within a locally defined population. This was attempted in the present study by examining isolates from Fitzroy Crossing, but an accurate estimate of linkage disequilibrium was not possible because of the unknown ploidy of *G. duodenalis* and the small number of isolates in the 7 major groups detected, which may represent different species (Fig. 4). Until a proper species-level taxonomy (see below) can be devised for *G. duodenalis* quantitative measures of linkage disequilibrium for identifying clonal, panmictic, and epidemic population structures (Tibayrenc et al., 1990; Tibayrenc and Ayala, 1991; Maynard Smith et al., 1993) would not be meaningful because of uncertainty in the number of species of *Giardia* being analyzed.

The distribution of natural clones of *G. duodenalis* has revealed that many of the genotypes or zymodemes are unique,

as they were only sampled once or on a few occasions. In contrast, a limited number of major or dominant clones were repeatedly sampled from geographically different locations and various hosts. Three major clones represented by zymodemes M1, M3, and M4 contain 37 of the 97 stocks collected over a wide ecogeographical range and could be considered major clones. Therefore, as suggested for other protozoa (Keymer et al., 1990; Tibayrenc et al., 1990, 1991; Sibley and Boothroyd, 1992), it may be more useful to investigate characteristics of significant epidemiological importance such as host specificity, pathogenicity, virulence, and response to drugs in major clones common to an endemic area in order to maximize the control and treatment of giardiasis. This is especially significant with respect to *G. duodenalis* and giardiasis, because the mechanisms underlying variable clinical manifestations, host range, infectivity, and response to treatment are not fully known or are difficult to determine (Thompson et al., 1993).

Taxonomy

The taxonomic interpretation of genetic variation in *G. duodenalis* is hindered by uncertainty over an appropriate species concept for asexual organisms (see Thompson et al., 1990). Hence, 2 alternative criteria, host occurrence and phenetics, have been used to distinguish species of *Giardia*.

Occurrence in different species of host is expected to promote genetic divergence by subjecting populations to different selection pressures (Hegner, 1926; Kulda and Nohynkova, 1978; Woo, 1984). We found a significant positive correlation between genetic distance separating zymodemes and occurrence in different host species, which suggests that host associations do indeed promote genetic divergence. Despite this, there is little evidence for the rigid host specificity that would be required to equate host occurrence with specific status. This is true for *G. duodenalis* examined from different species of animals in this study (Fig. 2) and isolates examined by other workers (Nash et al., 1985; Proctor et al., 1989), which show levels of intrahost variation to be as great as those for interhost variation.

Species of *Giardia* have also been inferred from shared characteristics, usually morphological. Fixed differences in the shape of the median body, ventrolateral flange (Erlandsen and Bemrick, 1987), and caudal flagella (Erlandsen et al., 1990) have been used to divide the genus into 5 species. However, most controversy regarding the taxonomy of *Giardia* has centered on isolates within the *G. duodenalis* morphological group. To overcome the limitations of morphology, a phenetic approach using the degree of genetic similarity (estimated from biochemical or molecular techniques, particularly enzyme electrophoresis) has been applied to delimit species (Thorpe, 1982, 1983). Andrews et al. (1989) applied this genetic yardstick to identify 4 species among isolates of *G. duodenalis* from humans. Although we used fewer enzyme systems and analyzed our data differently than that of Andrews et al. (1989), if a similar genetic yardstick was applied to the isolates of *G. duodenalis* examined in this study, up to 27 species could be identified. This would obviously have severe ramifications for the systematics of the genus. Aside from such operational difficulties, the major problem with the phenetic approach to delimiting species is that the groups so delimited may not represent evolutionary units. That is, it makes no predictions about the extent of genetic or phenotypic divergence between species and may not necessarily be a good indicator of genetic cohesion (Lymbery, 1992).

The clonal population structure in *G. duodenalis* means that numerous clones have been evolving independently and the large genetic distance values detected by enzyme electrophoresis are a consequence of a long, separate evolution. The similarity in topology of the trees produced by UPGMA clustering and Fitch–Margoliash analysis attests to the stability of the clonal lineages identified in this study. As there is no, or limited, recombination or reassortment of genes in a population, these different clonal lineages may accumulate differences in a wide range of biological characters. However, evidence that different isolates are genetically heterogeneous does not necessarily warrant species designation. Studies with *Leishmania,* for example, which appear to have a basically clonal population structure have led to a multiplicity of strains, subspecies, and species names (Lainson and Shaw, 1987). This form of classification would, with the continual increase in the number of genetically characterized samples, eventually approach chaos and be devoid of any explanatory or predictive power. It may be more practical and informative to identify separate lineages or families of related lineages that behave similarly, to those that behave differently in factors of epidemiological significance. These lineages, if they exist, would represent "natural" groups with an existence independent of our attempts to classify them and, therefore, would be worthy of some formal taxonomic name.

Genetic variation and epidemiology

The nature of genetic variation detected in isolates from different geographic areas and host species also has important implications for understanding the transmission and variable symptomatology of giardiasis.

Zoonotic potential: the genetic similarity of isolates of *Giardia* from humans and other animals provides circumstantial evidence for zoonotic transmission, and a recent cross transmission experiment has clearly shown that humans are susceptible to *Giardia* of nonhuman origin in certain geographical areas (Majewska, 1994). In this study, human isolates of *G. duodenalis* grouped in identical or similar zymodemes containing isolates from sheep, cattle, cats, dog, and beaver (Fig. 1). The sheep isolate (BAS2) and a human isolate (BAH38) from a sheep shearer are of particular interest because they grouped in the same zymodeme and both originated from the south of Western Australia at similar times. Other studies using enzyme electrophoresis and RFLPs have also found little or no difference between the Portland (P1) isolate and human, rodent, sheep, cattle, dog, and beaver isolates of *G. duodenalis* from a variety of geographical areas (Bertram et al., 1983; Nash et al., 1985; Baveja et al., 1986; Uji et al., 1988; Andrews et al., 1989; Proctor et al., 1989; Strandén et al., 1990; Meloni et al., 1992). Although providing evidence of animal to human transmission of the parasite, the results must be interpreted with caution. Transmission patterns in 1 location may not be the same as those in other localities due to heterogeneity between isolates of *Giardia* from the same host species in different geographical areas (Meloni et al., 1988, 1989, 1992; Thompson et al., 1990; Thompson and Meloni, 1993).

A canine (BAD1) and rodent isolates (BAR1 and BAR3) from Western Australia and a feline (BAC7) and goat (BAG1) isolate from Victoria were genetically distinct from all others. However, they fell within the range of variation (measured by percentage fixed genetic differences) found between different human isolates, e.g., human isolates BAH3 (zymodeme M1) and BAH7 (zymodeme M7) (Fig. 2) and, therefore, their infectivity to humans cannot be ruled out. Alternatively, genetically distinct isolates could represent strains that are rigidly host specific, although we do not have any evidence to support this.

Variation in virulence and pathogenesis: The specific pathogenic mechanisms by which *Giardia* causes disease have not been identified. However, it is likely that a number of processes are involved and that

both parasite and host determine the clinical course of infection. It is possible that variable virulence between different isolates of *Giardia* may account for the wide range of symptomatology in giardiasis. Two studies have shown that different isolates may vary in the pattern of infection produced in animal models (Aggarwal et al., 1983; Aggarwal and Nash, 1987).

The extensive genetic variation detected between isolates of *G. duodenalis* in this study lends weight to the hypothesis that variable symptomatology of giardiasis is at least partly a reflection of differences between infective agents. The extent of genetic variation in 1 locality in Western Australia was as great as that in the whole of Australia and encompassed a number of independently evolving clonal lineages. We do not yet know the degree to which these lineages differ in epidemiologically significant characteristics, but recent research from our laboratory has demonstrated behavioral differences in vitro between clones of *Giardia* from this locality, which may be of clinical significance in vivo. In this respect, the challenge for the future with *Giardia* and other parasites must be to find molecular markers, for improved classification, and for the identification of clinically significant characters such as virulence and drug sensitivity (Thompson, 1994). Ideally, it should be possible that such characterization be performed directly on parasite material without culture amplification.

ACKNOWLEDGMENTS

This study was supported by the National Health and Medical Research Council of Australia and World Health Organization through research grants to R.C.A. Thompson. We thank Russell Hobbs and Claire Constantine for advice on the computer programs used in this study and Nicolette Binz for providing several cloned isolates. We also acknowledge the assistance of the community health nurses in Fitzroy Crossing for their help in obtaining *Giardia* samples.

LITERATURE CITED

ADAM, R. D., T. E. NASH, AND T. E. WELLEMS. 1988. The *Giardia lamblia* trophozoite contains sets of closely related chromosomes. Nucleic Acids Research **16:** 4555–4567.

AGGARWAL, A., A. BHATIA, S. R. NAIK, AND V. K. VINAYAK. 1983. Variable virulence of isolates of *Giardia lamblia* in mice. Annals of Tropical Medicine and Parasitology **79:** 163–169.

———, AND T. E. NASH. 1987. Comparison of two antigenically distinct *Giardia lamblia* isolates in gerbils. American Journal of Tropical Medicine and Hygiene **36:** 325–332.

ANDREWS, R. H., M. ADAMS, P. F. L. BOREHAM, G. MAYRHOFER, AND B. P. MELONI. 1989. *Giardia intestinalis*: Electrophoretic evidence for a species complex. International Journal for Parasitology **19:** 183–190.

ASHER, R. H. JR., AND G. W. MACE. 1971. The genetic structure and evolutionary fate of parthenogenetic amphibian populations as determined by Markovian analysis. American Zoologist **11:** 381–398.

BAVEJA, U. K., A. S. JYOTI, M. KAUR, D. S. AGARWAL, B. S. ANAND, AND R. NANDA. 1986. Isoenzyme studies of *Giardia lamblia* isolated from symptomatic cases. Australian Journal of Experimental Biology and Medical Science **64:** 119–126.

BERTRAM, M. A., E. A. MEYER, J. D. LILE, AND S. A. MORSE. 1983. A comparison of isozymes of five axenic *Giardia* isolates. Journal of Parasitology **69:** 793–801.

BINZ, N., R. C. A. THOMPSON, B. P. MELONI, AND A. J. LYMBERY. 1991. A simple method for cloning *Giardia duodenalis* from cultures and faecal samples. Journal of Parasitology **77:** 627–631.

BLANC, D. S., R. NICHOLLS, AND P. G. SARGEAUNT. 1989. Experimental production of new zymodemes of *Entamoeba histolytica* supports the hypothesis of genetic exchange. Transactions of the Royal Society of Tropical Medicine and Hygiene **83:** 787–790.

BOGLIOLO, A. R., AND D. G. GODFREY. 1987. Isoenzyme changes during the life cycle of *Trypanosoma cruzi*. Transactions of the Royal Society of Tropical Medicine and Hygiene **81:** 222–229.

BURET, A., N. DEN HOLLANDER, P. M. WALLIS, D. BEFUS, AND M. E. OLSON. 1990. Zoonotic potential of giardiasis in domestic ruminants. Journal of Infectious Diseases **162:** 231–237.

CARIOU, M. L., AND P. PERNIN. 1987. First evidence for diploidy and genetic recombination in free-living amoebae of the genus *Naegleria* on the basis of electrophoretic variation. Genetics **115:** 265–270.

CIBULSKIS, R. E. 1988. Origins and organization of genetic diversity in natural populations of *Trypanosoma brucei*. Parasitology **96:** 303–322.

CONSTANTINE, C. C., R. P. HOBBS, AND A. J. LYMBERY. 1994. FORTRAN programs for analysing population structure from multilocus genotypic data. Journal of Heredity **85:** 336–337.

COX, F. E. G. 1981. A new classification of the parasitic protozoa. Protozoological Abstracts **5:** 9–14.

DAVIES, R. B., AND C. P. HIBLER. 1979. Animal reservoirs and cross-species transmission of *Giardia*. *In* Waterborne transmission of giardiasis, W. Jakubowski, and J. C. Hoff (eds.). Environmental Protection Agency, Cincinnati, Ohio, p. 104–126.

DOUGLAS, M. E., AND J. A. ENDLER. 1982. Quantitative matrix comparisons in ecological and evolutionary investigations. Journal of Theoretical Biology **99:** 777–795.

DYE, C. 1991. Population genetics of nonclonal, nonrandomly mating malaria parasites. Parasitology Today **7:** 236–240.

ERLANDSEN, S. L., AND W. J. BEMRICK. 1987. SEM evidence for a new species, *Giardia psittaci*. Journal of Parasitology **73:** 623–629.

———, ———, C. L. WELLS, D. E. FEELY, L. KNUDSEN, S. R. CAMPBELL, H. VAN KEULEN, AND E. L. JARROLL. 1990. Axenic culture and characterization of *Giardia ardeae* from the great blue heron *Ardeae herodias*. Journal of Parasitology **76:** 717–724.

FELSENSTEIN, J. 1993. PHYLIP—Phylogeny inference package. Version 3.5p. University of Washington, Seattle, Washington.

FILICE, F. P. 1952. Studies on the cytology and life history of a *Giardia* from the laboratory rat. University of California Publications in Zoology **57:** 53–143.

GASSER, R. B., J. ECKERT, AND L. ROHRER. 1987a. Infectivity of Swiss *Giardia* isolates to jirds and mice, and *in vitro* cultivation of trophozoites originating from sheep. Parasitology Research **74:** 103–111.

———, ———, AND ———. 1987b. Isolation of *Giardia* from Swiss cattle and cultivation of trophozoites *in vitro*. Parasitology Research **73:** 182–183.

HARE, D. F., E. L. JARROLL, AND D. G. LINDMARK. 1989. *Giardia lamblia*: Characterization of proteinase activity in trophozoites. Experimental Parasitology **68:** 168–175.

HEGNER, R. W. 1926. The biology of host–parasite relationships among protozoa living in man. Quarterly Review of Biology **1:** 393–418.

HILLIS, D. M., AND C. C. MORITZ. 1990. An overview of applications of molecular systematics. *In* Molecular systematics, D. M. Hillis, and C. C. Moritz (eds.). Sinauer, Sunderland, Massachusetts, p. 502–515.

HOMAN, W. L., F. H. J. VAN ENCKEVORT, L. LIMPER, G. J. J. M. VAN EYS, G. J. SCHOONE, W. KASPRZAK, A. C. MAJEWSKA, AND F. VAN KNAPEN. 1992. Comparison of *Giardia* isolates from different laboratories by isoenzyme analysis and recombinant DNA probes. Parasitology Research **78:** 316–323.

JENNI, L., S. MARTI, J. SCHWEIZER, B. BETSCHART, R. W. F. LE PAGE, J. M. WELLS, A. TAIT, P. PAINDAVOINES, E. PAYS, AND M. STEINERT. 1986. Hybrid formation between African trypanosomes during cyclical transmission. Nature **322:** 173–175.

KEYMER, A. E., R. M. MAY, AND P. H. HARVEY. 1990. Parasite clones in the wild. Nature **346:** 109–110.

KIRKPATRICK, C. E. 1987. Giardiasis. Veterinary Clinics of North America: Small Animal Practice **17:** 1377–1387.

———, AND D. L. SKAND. 1985. Giardiasis in a horse. Journal of the American Veterinary Medical Association **187:** 163–164.

KORMAN, S. H., S. M. LE BLANCQ, D. T. SPIRA, J. EL ON, R. M. REIFEN, AND R. J. DECKELBAUM. 1986. *Giardia lamblia*: Identification of different strains from man. Zeitschrift für Parasitenkunde **72:** 173–180.

KULDA, J., AND E. NOHYNKOVA. 1978. Flagellates of the human intestine and of intestines of other species. *In* Protozoa of veterinary and medical interest, Vol. 2. J. P. Kreier (ed.). Academic Press, London, U.K., p. 69–104.

LAINSON, R., AND J. J. SHAW. 1987. Evolution, classification and geographical distribution. *In* The leishmaniases in biology and med-

icine, Vol. 1. W. Peters, and R. Killick-Kendrick (eds.). Academic Press, London, U.K., p. 1–120.

LE BLANCQ, S. M. 1994. Chromosome rearrangements in *Giardia lamblia*. Parasitology Today **10:** 177–179.

LEVINE, N. D. 1973. Protozoan parasites of domestic animals and man. Burgess, Minneapolis, Minnesota, 406 p.

———, J. O. CORLISS, F. E. G. COX, G. DEROUX, J. GRAIN, B. M. HONIGBERG, G. F. LEEDALE, A. R. LOEBLICH III, J. LOM, D. LYNN, E. G. MERINFEILD, F. C. PAGE, G. POLJANSKY, V. SPRAGUE, J. VAVRA, AND F. G. WALLACE. 1980. A newly revised classification of the protozoa. Journal of Protozoology **227:** 37–58.

LYMBERY, A. J. 1992. Interbreeding, monophyly and the genetic yardstick: Species concepts in parasites. Parasitology Today **8:** 208–211.

MAJEWSKA, A. C. 1994. Successful experimental infections of a human volunteer and Mongolian gerbils with *Giardia* of animal origin. Transactions of the Royal Society of Tropical Medicine and Hygiene **88:** 360–362.

MANTEL, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research **27:** 209–220.

MAYNARD SMITH, J., N. H. SMITH, M. O'ROUKE, AND B. G. SPRATT. 1993. How clonal are bacteria? Proceedings of the National Academy of Sciences USA **90:** 4384–4388.

MELONI, B. P., A. J. LYMBERY, AND R. C. A. THOMPSON. 1988. Isoenzyme electrophoresis of 30 isolates of *Giardia* from humans and felines. American Journal of Tropical Medicine and Hygiene **38:** 65–73.

———, ———, AND ———. 1989. Characterisation of *Giardia* isolates using a DNA probe, and correlation with the results of isoenzyme analysis. American Journal of Tropical Medicine and Hygiene **40:** 629–637.

———, AND R. C. A. THOMPSON. 1987. Comparative studies on the axenic *in vitro* cultivation of *Giardia* of human and canine origin: Evidence for intraspecific variation. Transactions of the Royal Society of Tropical Medicine and Hygiene **81:** 637–640.

———, ———, R. M. HOPKINS, J. A. REYNOLDSON, AND M. GRACEY. 1993. The prevalence of *Giardia* and other intestinal parasites in children, dogs and cats from aboriginal communities in the Kimberley. Medical Journal of Australia **158:** 157–159.

———, ———, A. M. STRANDÉN, P. KÖHLER, AND J. ECKERT. 1992. Critical comparison of *Giardia duodenalis* from Australia and Switzerland using isoenzyme electrophoresis. Acta Tropica **50:** 115–124.

MEYER, E. A. 1985. The epidemiology of giardiasis. Parasitology Today **1:** 101–105.

———. 1990. Taxonomy and nomenclature. *In* Giardiasis, E. A. Meyer (ed.). Elsevier, Amsterdam, Holland, p. 51–60.

MILES, M. A. 1985. Ploidy, 'heterozygosity' and antigenic expression of south American trypanosomes. Parassitologia **27:** 87–104.

———, AND R. E. CIBULSKIS. 1986. Zymodeme characterization of *Trypanosoma cruzi*. Parasitology Today **2:** 94–97.

MORGAN, U. M., C. C. CONSTANTINE, W. K. GREENE, AND R. C. A. THOMPSON. 1993. RAPD (random amplified polymorphic DNA) analysis of *Giardia* DNA and correlation with isoenzyme data. Transactions of the Royal Society of Tropical Medicine and Hygiene **87:** 702–705.

NASH, T. E., T. MCCUTCHAN, D. KEISTER, J. B. DAME, J. D. CONRAD, AND F. D. GILLIN. 1985. Restriction-endonuclease analysis of DNA from 15 *Giardia* isolates obtained from humans and animals. Journal of Infectious Diseases **152:** 64–73.

OCHMAN, H., AND R. K. SELANDER. 1984. Evidence for clonal population structure in *Escherichia coli*. Proceedings of the National Academy of Sciences USA **81:** 198–201.

PANIGRAHY, B., J. E. GRIMES, AND F. D. CLARKE. 1984. Zoonoses in psittacine birds. Journal of Infectious Diseases **149:** 123–124.

PERNIN, P., A. ATAYA, AND M. L. CARIOU. 1992. Genetic structure of natural populations of the free-living amoeba, *Naegleria lovaniensis*. Evidence for sexual reproduction. Heredity **68:** 173–181.

PROCTOR, E. M., J. L. ISAAC-RENTON, J. BOYD, Q. WONG, AND W. R. BOWIE. 1989. Isoenzyme analysis of human and animal isolates of *Giardia duodenalis* from British Columbia, Canada. American Journal of Tropical Medicine and Hygiene **41:** 411–415.

RICHARDSON, B. J., P. R. BAVERSTOCK, AND M. ADAMS. 1986. Allozyme electrophoresis. Academic Press, Sydney, Australia, 410 p.

SCHANTZ, P. M. 1991. Parasitic zoonoses in perspective. International Journal for Parasitology **21:** 161–170.

SIBLEY, L. D., AND J. C. BOOTHROYD. 1992. Virulent strains of *Toxoplasma gondii* comprise a single clonal lineage. Nature **359:** 82–85.

SNEATH, P. H. A., AND R. R. SOKAL. 1973. Numerical taxonomy. Freeman, San Francisco, California, 573 p.

STODDART, J. A., AND J. F. TAYLOR. 1988. Genotypic diversity: Estimation and prediction in samples. Genetics **118:** 705–711.

STRANDÉN, A. M., J. ECKERT, AND P. KÖHLER. 1990. Electrophoretic characterization of *Giardia* isolated from humans, cattle, sheep, and a dog in Switzerland. Journal of Parasitology **76:** 660–668.

SUOMALAINEN, E., A. SAURA, AND J. LOKKI. 1976. Evolution of parthenogenetic insects. Evolutionary Biology **9:** 209–257.

TAIT, A. 1980. Evidence for diploidy and mating in trypanosomes. Nature **287:** 536–538.

———. 1983. Sexual processes in the kinetoplastida. Parasitology **86:** 29–57.

———. 1985. Genetics of protein variation in populations of parasitic protozoa. *In* Ecology and genetics of host–parasite interactions, D. Rollinson, and R. M. Anderson (eds.). Academic Press, London, U.K., p. 185–203.

THOMPSON, R. C. A. 1995. Giardiasis. *In* Zoonoses, S. R. Palmer, L. Soulsby, and D. Simpson (eds.). Oxford University Press, Oxford (in press).

———, LYMBERY, A. J., AND B. P. MELONI. 1990. Genetic variation in *Giardia* Kunstler, 1882: Taxonomic and epidemiological significance. Protozoological Abstracts **14:** 1–28.

———, J. A. REYNOLDSON, AND A. H. W. MENDIS. 1993. *Giardia* and giardiasis. Advances in Parasitology **32:** 72–160.

THORPE, J. P. 1982. The molecular clock hypothesis: Biochemical evolution, genetic differentiation and systematics. Annual Review of Ecology and Systematics **13:** 139–168.

———. 1983. Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. *In* Protein polymorphism: Adaptive and taxonomic significance, G. S. Oxford, and R. D. Rollinson (eds.). Academic Press, London, U.K., p. 131–152.

TIBAYRENC, M. 1994. How many species of *Giardia* are there? *In* *Giardia*: From molecules to disease, R. C. A. Thompson, J. A. Reynoldson, and A. J. Lymbery (eds.). CAB International, Wallingford, U.K., p. 41–48.

———, AND F. J. AYALA. 1991. Towards a population genetics of microorganisms: The clonal theory of parasitic protozoa. Parasitology Today **7:** 228–232.

———, F. KJELLBERG, J. ARNAUD, B. OURY, S. F. BRENIÉRE, M. DARDÉ, AND F. J. AYALA. 1991. Are eukaryotic microorganisms clonal or sexual? A population genetics vantage. Proceedings of the National Academy of Sciences USA **88:** 5129–5133.

———, F. KJELLBERG, AND F. J. AYALA. 1990. A clonal theory of parasitic protozoa: The population structures of *Entamoeba, Giardia, Leishmania, Naegleria, Plasmodium, Trichomonas,* and *Trypanosoma* and their medical and taxonomical consequences. Proceedings of the National Academy of Sciences USA **87:** 2414–2418.

UJI, A., P. M. WALLIS, AND W. M. WENMAN. 1988. Comparison of *Giardia* isolates by DNA–DNA hybridization. *In* Advances in *Giardia* research, P. M. Wallis and B. R. Hammond (eds.). University of Calgary Press, Calgary, Canada, p. 165–167.

WALLIS, P. M., J. M. BUCHANAN-MAPPIN, G. M. FAUBERT, AND M. BELOSEVIC. 1984. Reservoirs of *Giardia* spp. in southwestern Alberta. Journal of Wildlife Diseases **20:** 279–283.

WARREN, K. S. 1989. Selective primary health care and parasite diseases. *In* New strategies in parasitology, K. P. W. McAdam (ed.). Churchill Livingstone, Edinburgh, Scotland, p. 217–231.

WENMAN, W. M., R. U. MEUSER, AND P. M. WALLIS. 1986. Antigenic analysis of *Giardia duodenalis* strains isolated in Alberta. Canadian Journal of Microbiology **32:** 926–929.

WOO, P. K. 1984. Evidence for animal reservoirs and transmission of *Giardia* infection between animal species. *In* *Giardia* and giardiasis, S. L. Erlandsen, and E. A. Meyer (eds.). Plenum Press, New York, New York, p. 341–364.

———, AND B. P. MELONI. 1993. Molecular variations in *Giardia*. Acta Tropica **53:** 167–184.

APPENDIX

Matrix of genetic distance measures, estimated from enzyme variation (fixed enzyme differences, below diagonal) and host occurrence (1 for zymodemes with isolate from different host and 0 for zymodemes containing isolates from the same host, above diagonal), between zymodemes of *Giardia*.

Zymodeme																
M4		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
	0.000	0.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M1	0.462		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	0.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M7	1.000	0.923		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M8	1.000	1.000	0.692		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M3	0.538	0.077	0.923	1.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M12	1.000	0.923	0.615	0.769	0.923		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M9	1.000	0.923	0.692	0.615	0.923	0.385		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M18	0.462	0.154	0.923	1.000	0.231	0.923	0.923		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M11	1.000	0.846	0.692	0.769	0.846	0.385	0.615	0.923		0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M10	1.000	0.846	0.692	0.692	0.846	0.538	0.538	0.923	0.385		0.000	0.000	0.000	0.000	0.000	0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M2	0.538	0.077	0.923	1.000	0.077	0.923	0.923	0.231	0.846	0.846		0.000	0.000	0.000	0.000	0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M15	1.000	0.923	0.692	0.692	0.923	0.308	0.538	0.923	0.308	0.538	0.923		0.000	0.000	0.000	0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M21	0.462	0.077	0.923	1.000	0.154	0.923	0.923	0.077	0.846	0.846	0.154	0.923		0.000	0.000	0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M16	1.000	0.923	0.538	0.692	0.923	0.308	0.462	0.923	0.308	0.385	0.923	0.385	0.923		0.000	0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M17	1.000	0.923	0.615	0.692	0.923	0.308	0.538	0.923	0.462	0.615	0.923	0.231	0.923	0.385		0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M22	0.462	0.077	0.923	1.000	0.154	0.923	0.923	0.077	0.923	0.923	0.154	0.923	0.154	0.923	0.923	
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M23	1.000	0.923	0.769	0.692	0.923	0.462	0.462	1.000	0.462	0.462	0.923	0.538	0.923	0.385	0.538	1.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
		1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

APPENDIX. Continued.

Zymodeme																
M24	1.000	0.846	0.692	0.692	0.846	0.308	0.692	0.923	0.154	0.462	0.846	0.308	0.846	0.385	0.462	0.923
	0.538		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M26	1.000	0.923	0.615	0.615	0.923	0.538	0.462	0.923	0.538	0.538	0.923	0.462	0.923	0.462	0.538	0.923
	0.615	0.692		0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M19	0.692	0.385	0.923	1.000	0.308	0.923	0.923	0.385	0.692	0.846	0.385	0.923	0.308	0.923	0.923	0.462
	0.923	0.769	0.846		0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M27	0.692	0.308	0.923	1.000	0.231	0.923	0.923	0.385	0.692	0.846	0.308	0.923	0.308	0.923	0.923	0.385
	0.923	0.769	0.846	0.154		0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M29	0.846	0.846	0.769	0.692	0.846	0.538	0.538	0.769	0.615	0.615	0.846	0.615	0.846	0.615	0.615	0.769
	0.692	0.692	0.538	0.769	0.769		0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M31	0.923	0.846	0.615	0.615	0.846	0.385	0.615	0.846	0.308	0.462	0.846	0.308	0.846	0.231	0.538	0.846
	0.538	0.308	0.615	0.846	0.846	0.538		0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M32	0.538	0.231	0.846	0.923	0.308	0.769	0.846	0.385	0.692	0.769	0.308	0.769	0.308	0.769	0.769	0.308
	0.769	0.692	0.846	0.538	0.538	0.769	0.692		0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M33	0.538	0.154	0.923	1.000	0.077	0.923	0.923	0.154	0.923	0.923	0.154	0.923	0.231	0.923	0.923	0.077
	1.000	0.923	0.923	0.385	0.308	0.769	0.846	0.385		0.000	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M34	0.923	0.846	0.538	0.615	0.846	0.385	0.538	0.846	0.462	0.538	0.846	0.538	0.846	0.231	0.385	0.846
	0.538	0.462	0.692	0.846	0.846	0.538	0.231	0.692	0.846		1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M6	0.308	0.462	1.000	1.000	0.462	1.000	1.000	0.615	0.923	0.846	0.462	1.000	0.538	1.000	1.000	0.538
	0.923	0.923	1.000	0.615	0.615	0.846	0.923	0.538	0.538	0.923		0.000	0.000	0.000	0.000	1.000
	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
M5	0.231	0.308	1.000	1.000	0.385	1.000	1.000	0.385	0.923	0.923	0.385	1.000	0.308	1.000	1.000	0.385
	0.923	0.923	1.000	0.615	0.462	0.846	0.923	0.462	0.462	0.923	0.308		0.000	0.000	0.000	1.000
	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
M35	0.154	0.385	1.000	1.000	0.462	1.000	1.000	0.462	0.923	0.923	0.462	1.000	0.385	1.000	1.000	0.462
	0.923	0.923	1.000	0.615	0.538	0.846	0.923	0.462	0.538	0.923	0.231	0.077		0.000	0.000	1.000
	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
M36	0.077	0.385	1.000	1.000	0.462	1.000	1.000	0.462	0.923	0.923	0.462	1.000	0.385	1.000	1.000	0.462
	0.923	0.923	1.000	0.615	0.615	0.846	0.923	0.462	0.538	0.923	0.231	0.154	0.077	0.000	1.000	1.000
	1.000	1.000	1.000	1.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		
M37	0.154	0.308	1.000	1.000	0.385	1.000	1.000	0.385	0.923	0.923	0.385	1.000	0.308	1.000	1.000	0.385
	0.923	0.923	1.000	0.615	0.538	0.846	0.923	0.462	0.462	0.923	0.308	0.077	0.154	0.077		1.000
	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
M13	0.917	0.917	0.917	0.917	0.917	0.750	0.833	0.917	0.917	1.000	0.917	0.833	0.917	0.917	0.667	0.917
	0.917	0.917	0.917	0.917	0.917	0.833	0.833	0.833	0.917	0.750	0.917	0.917	0.917	0.917	0.917	
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
M14	0.556	0.667	1.000	1.000	0.667	1.000	1.000	0.667	1.000	1.000	0.667	1.000	0.667	1.000	1.000	0.667
	1.000	1.000	1.000	0.778	0.667	0.889	1.000	0.889	0.667	1.000	0.556	0.444	0.556	0.556	0.444	1.000
		0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
M38	0.154	0.385	1.000	1.000	0.462	0.923	0.923	0.385	1.000	1.000	0.462	1.000	0.385	1.000	1.000	0.385
	1.000	1.000	1.000	0.692	0.615	0.846	0.923	0.538	0.462	0.923	0.385	0.154	0.231	0.154	0.077	0.833
	0.444		0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
M39	0.231	0.462	1.000	1.000	0.538	1.000	1.000	0.385	1.000	0.923	0.538	1.000	0.462	1.000	1.000	0.385
	1.000	1.000	1.000	0.692	0.615	0.769	0.923	0.615	0.462	0.923	0.308	0.231	0.308	0.231	0.154	0.917
	0.444	0.154		0.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

APPENDIX. Continued.

Zymodeme																
M40	0.231	0.385	1.000	1.000	0.462	1.000	1.000	0.462	0.923	0.846	0.462	1.000	0.385	1.000	1.000	0.462
	0.923	0.923	1.000	0.615	0.538	0.846	0.923	0.538	0.538	0.923	0.231	0.154	0.231	0.154	0.077	0.917
	0.444	0.154	0.077		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
M41	0.846	0.923	1.000	1.000	0.923	0.769	0.923	0.923	0.923	1.000	0.923	0.846	0.923	0.923	0.769	0.923
	0.923	0.846	0.923	0.923	0.923	0.846	0.846	0.846	0.923	0.846	0.923	0.923	0.923	0.923	0.923	0.167
	1.000	0.923	0.923	0.923		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
M45	0.692	0.923	0.923	1.000	0.923	0.923	0.923	0.923	0.923	1.000	0.923	0.923	0.923	0.923	0.923	0.923
	0.923	1.000	0.846	0.923	0.846	0.769	0.923	0.923	0.923	0.923	0.692	0.692	0.615	0.692	0.769	0.917
	0.778	0.769	0.769	0.769	0.923		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
M46	0.769	0.692	0.923	1.000	0.692	0.769	0.846	0.692	0.923	0.923	0.692	0.923	0.692	0.923	0.923	0.692
	1.000	0.846	0.923	0.692	0.615	0.923	0.923	0.769	0.692	0.923	0.769	0.692	0.692	0.769	0.769	0.917
	0.778	0.692	0.769	0.769	0.923	0.923		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
M47	0.923	0.923	0.538	0.769	0.923	0.308	0.462	0.923	0.538	0.538	0.923	0.462	0.923	0.231	0.385	0.923
	0.538	0.538	0.462	0.846	0.923	0.462	0.462	0.846	0.923	0.385	0.923	0.923	0.923	0.923	0.923	0.917
	0.889	0.923	0.923	0.923	0.923	0.769	0.923		0.000	0.000	0.000	0.000	0.000	0.000	0.000	
M20	0.462	0.077	0.846	1.000	0.154	0.923	0.923	0.154	0.923	0.923	0.154	0.923	0.154	0.846	0.923	0.077
	1.000	0.923	0.923	0.462	0.395	0.846	0.769	0.308	0.154	0.769	0.538	0.385	0.462	0.462	0.385	0.917
	0.667	0.385	0.462	0.462	0.923	0.846	0.692	0.846		0.000	0.000	0.000	0.000	0.000	0.000	
M42	0.923	1.000	0.692	0.615	1.000	0.769	0.692	1.000	0.769	0.769	1.000	0.769	1.000	0.692	0.692	1.000
	0.769	0.769	0.692	1.000	1.000	0.769	0.769	0.923	1.000	0.769	0.923	0.923	0.923	0.923	0.923	0.833
	0.889	0.923	0.923	0.923	0.846	0.846	1.000	0.615	0.923		0.000	0.000	0.000	0.000	0.000	
M43	1.000	1.000	0.615	0.538	1.000	0.769	0.615	1.000	0.769	0.692	1.000	0.769	1.000	0.692	0.615	1.000
	0.615	0.769	0.692	1.000	1.000	0.846	0.769	0.923	1.000	0.692	0.923	1.000	1.000	1.000	1.000	0.833
	1.000	1.000	0.923	0.923	0.923	0.923	1.000	0.692	0.923	0.385		0.000	0.000	0.000	0.000	
M25	1.000	0.923	0.769	0.692	0.923	0.692	0.692	0.923	0.615	0.615	0.923	0.615	0.923	0.615	0.692	0.923
	0.692	0.692	0.615	0.923	0.923	0.615	0.615	0.846	0.923	0.692	1.000	1.000	1.000	1.000	1.000	1.000
	1.000	1.000	1.000	1.000	1.000	1.000	0.923	0.692	0.923	0.769	0.769		0.000	0.000	0.000	
M28	1.000	0.923	0.615	0.615	0.923	0.692	0.692	0.923	0.692	0.692	0.923	0.692	0.923	0.692	0.615	0.923
	0.769	0.692	0.615	0.923	0.923	0.692	0.796	0.846	0.923	0.692	1.000	1.000	1.000	1.000	1.000	0.917
	1.000	1.000	1.000	1.000	1.000	1.000	0.923	0.692	0.923	0.692	0.615	0.615		0.000	0.000	
M44	0.846	1.000	0.769	0.769	1.000	0.692	0.692	0.923	0.769	0.769	1.000	0.769	0.923	0.692	0.769	1.000
	0.692	0.769	0.769	0.923	1.000	0.769	0.769	0.846	1.000	0.692	0.923	0.923	0.923	0.923	0.923	0.917
	0.889	0.923	0.923	0.923	0.846	0.923	0.923	0.692	1.000	0.615	0.769	0.846	0.846		0.000	
M30	1.000	0.923	0.692	0.846	0.923	0.538	0.692	0.923	0.385	0.615	0.923	0.538	0.846	0.538	0.692	1.000
	0.462	0.538	0.615	0.769	0.846	0.769	0.538	0.769	1.000	0.692	0.923	0.923	0.923	0.923	1.000	0.917
	1.000	1.000	1.000	0.923	0.923	0.923	1.000	0.692	1.000	0.846	0.769	0.846	0.846	0.615		

Zymodeme																
	M4	M1	M7	M8	M3	M12	M9	M18	M11	M10	M2	M15	M21	M16	M17	M22
	M23	M24	M26	M19	M27	M29	M31	M32	M33	M34	M6	M5	M35	M36	M37	M13
	M14	M38	M39	M40	M41	M45	M46	M47	M20	M42	M43	M25	M28	M44		