Genetic variability in parasites and host–parasite interactions

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SUMMARY

We have examined genetic variability in parasites in the context of ecological interactions with the host. Recent research on *Echinococcus*, *Giardia* and *Cryptosporidium* has been used to illustrate: (i) the problems that parasite variability and species recognition pose for understanding the complex and often controversial relationship between parasite and host occurrence; (ii) the need for accurate parasite characterization and the application of appropriate molecular techniques to studies on parasite transmission if fundamental questions about zoonotic relationships and risk factors are to be answered; (iii) our lack of understanding about within-host interactions between genetically heterogeneous parasites at the inter- and intraspecific levels, and the significance of such interactions with respect to evolutionary considerations and the clinical outcome of parasite infections. If advances in molecular biology and mathematical ecology are to be realized, we need to give serious consideration to the development of appropriate species concepts and *in vivo* systems for testing the predictions and assumptions of theoretical models.

Key words: *Echinococcus*, *Giardia*, *Cryptosporidium*, genetic variation, molecular characterization, host occurrence, species recognition, epidemiology, ecological interactions, evolution, virulence.

INTRODUCTION

Fifteen years ago, Price (1980) emphasised that 'the challenge of diversity' as coined by Mayr (1974), 'is still with us'. The challenge remains with us today but with the advent of sophisticated molecular tools for parasite characterization and increasing recognition by parasitologists and evolutionary biologists of the 'health' significance of parasite variability (*e.g.* see Ewald, 1988; Bull, 1994; Holmes & Garnett, 1994), more people have taken up this challenge. Putting this diversity into an ecological context is proving to be a fascinating and most rewarding area of research for both population biologists and epidemiologists. In what follows, we look at parasite variability in terms of ecological interactions with the host.

We have chosen three very different parasites to address this theme. This is because: (1) these illustrate how a greater knowledge of their genetic variability is helping us to understand more about their ecology, epidemiology, host interactions and transmission; but also (2) because such studies are revealing how little we really do know about the extent and nature of parasite variation, in particular, how dangerous it is to make generalizations or predictions about the genetic structure and molecular ecology of parasite populations. For example, as we will attempt to illustrate here, the considerable advances being made in our understanding of the genetic structure of parasite populations and the interactions of parasites at the community level are sometimes at variance with previously held views (*e.g.* Price, 1980) which undoubtedly reflects the increasing data that are being made available through molecular ecology and population genetic studies.

The parasites

The three parasites chosen show no close phylogenetic relationships, but are all ubiquitous parasites which colonize the small intestine of vertebrates and share the faecal/oral route as a major aspect of their transmission patterns (Fig. 1). In contrast to the direct one-host life cycles of *Giardia* and *Cryptosporidium*, *Echinococcus* has an obligate two-host life cycle, which involves the visceral development of a cystic larval stage that must be ingested by the definitive host. *Echinococcus* and *Cryptosporidium* exhibit a regular alternation of sexual and asexual (clonal) reproductive phases in their life cycles whereas *Giardia* is considered to be principally a clonal organism although occasional, irregular bouts of genetic exchange may occur.

GENETIC VARIABILITY, HOST OCCURRENCE AND THE SPECIES PROBLEM

From a taxonomic perspective, the current view of all three parasites is that each comprises a few species which are not particularly host specific and which exhibit variable degrees of intraspecific variation. However, observations on the extent and nature of genetic variation within these parasites necessitates a

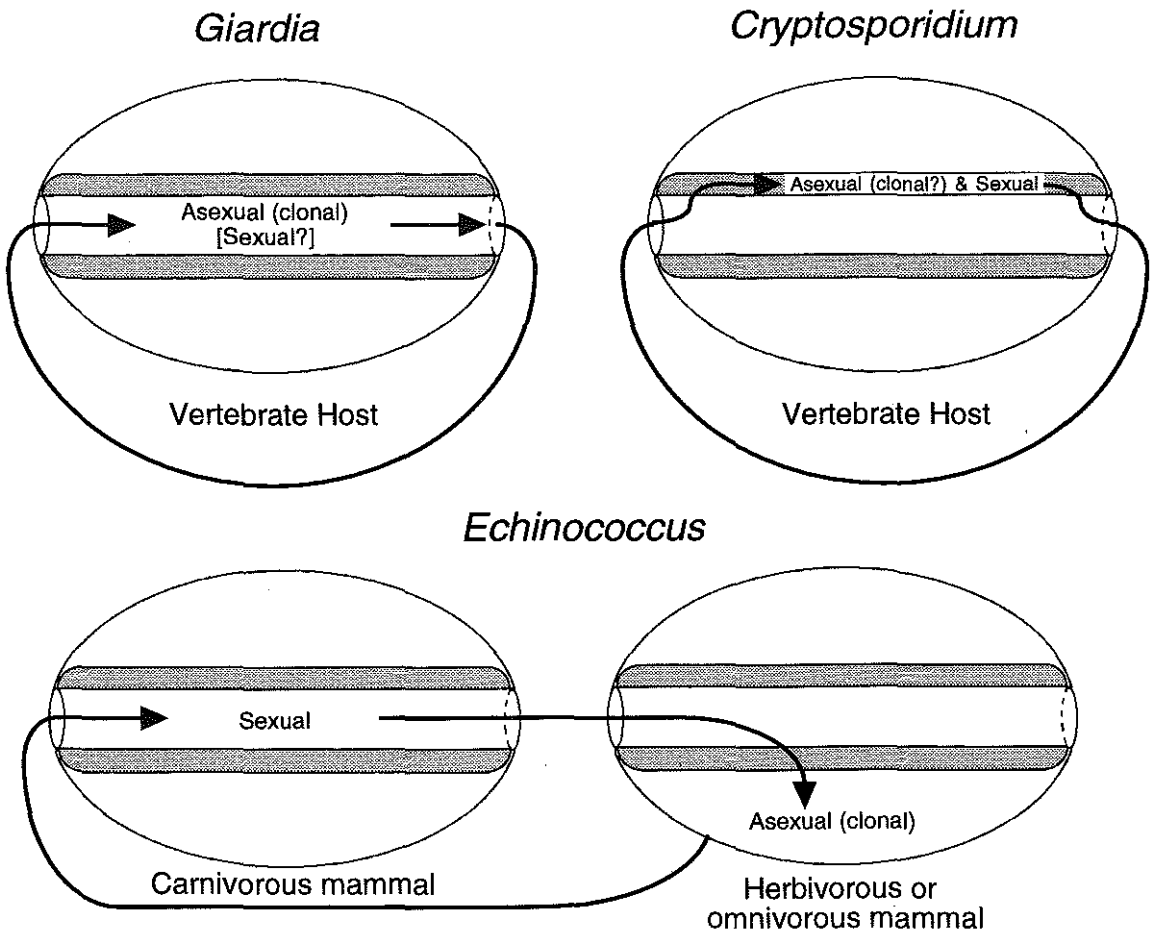

Fig. 1. Life cycles of *Giardia*, *Cryptosporidium* and *Echinococcus*.

reappraisal of both their taxonomy and genetic structure.

Echinococcus

Until recently, it was believed that the genus *Echinococcus* consisted of only 4 species and a large number of intraspecific variants, or strains, recognized principally on the basis of host occurrence. Molecular genetic studies, however, have show that many of these strains warrant species status (Table 1; Bowles, Blair & McManus, 1995; Thompson, Lymbery & Constantine, 1995) a situation that was recognized by many early authors (reviewed in Kumaratilake & Thompson, 1982) and predicted on the basis of available evolutionary theory (Price, 1980). The notion of a series of host-adapted species in the genus *Echinococcus* fits in nicely with our observations on host range, life cycle and transmission patterns in areas where hydatid disease (echinococcosis) is endemic. The maintenance of what were previously considered to be host-adapted strains of *E. granulosus*, in areas where definitive hosts potentially harbour mixed infections, is indicative of the existence of different species. For example, in the United Kingdom, the Middle East, and parts of Europe and Africa, *Echinococcus* may be perpetuated in the same geographical area in cycles that may involve horses, sheep, cattle or pigs, with the possibility that a given definitive host could therefore acquire infections from more than one species of intermediate host (Thompson & Lymbery, 1988; Thompson *et al.* 1995).

However, views about the ecology of *Echinococcus* populations must now be revised. In the only recent theory of genetic structure in parasites, Price (1980) predicted that parasite species would be split into many small, isolated populations, genetically uniform because of the effects of inbreeding and asexual reproduction, but genetically differentiated from other populations because of the effects of genetic drift and local adaptation in the absence of gene flow. Recent analyses of the genetic structure of *Echinococcus* populations do not conform to these predictions (Lymbery, 1995; Lymbery, Thompson & Hobbs, 1990; A. J. Lymbery, C. C. Constantine & R. C. A. Thompson, unpublished) and most genetic variation in *Echinococcus* occurs within, rather than between, local populations with little evidence of any spacial structuring in genetic variation. Our interpretation of the available data (Lymbery, 1995; A. J. Lymbery *et al.* unpublished) is that although self-fertilization predominates, at least in colonizing populations of *Echinococcus*, it occurs principally

Table 1. Proposed species and strains in the genus *Echinococcus*[1]

Possible taxonomic designation	Known definitive hosts	Known intermediate hosts	Probable[2] geographic distribution	Synonyms	Strains
E. sp.?	Dog	Pigs, humans?	Europe, Russia, South America	*E. granulosus* pig strain	Pig strain?
E. ortleppi	Dog	Cattle, buffalo, humans	Europe, Africa, India, Sri Lanka, Russia	*E. granulosus ortleppi*, *E. granulosus* cattle strain	
E. equinus	Dog	Horses and other equines	Europe, Middle East, South Africa (New Zealand? USA?)	*E. granulosus equinus*, *E. granulosus* horse strain	
E. multilocularis	Fox, dog, cat	Rodents, pigs, horses, humans	Europe, North America, Canada, Japan, China	*E. sibiricensis*	European strain, North American strain, Alaskan strain? Hokkaido strain?
E. vogeli	Bush dog	Rodents, humans	South America		
E. oligarthrus	Felines	Rodents, humans	South America	*E. pampeanus*, *E. cruzi*	
E. granulosus	Dog, fox, dingo, jackal, hyena	Sheep, Cattle, pigs, goats, buffalo, camels, macropods, humans	Australian, Europe, USA, New Zealand, Africa, China, Middle East, Asia, South America, Russia	*E. patagonicus*, *E. cepanazoi*, *E. granulosus*, *E. newzealandensis*	Common sheep strain, Tasmania sheep strain, Buffalo strain?

[1] For details see Thompson *et al.* (1995). [2] The geographic range of some species still needs to be fully defined.

through cross-insemination by genetically identical clone-mates in the intestine of the definitive host. Widespread dispersal via intermediate host movements and occasional outcrossing prevent both genetic differentiation between populations and substantial linkage disequilibrium, which are the normal concomitants of self-fertilization.

Outcrossing requires that a definitive host harbours worms derived from genetically different hydatid cysts. There are no studies which directly approach this question in natural populations, but Constantine *et al.* (1993) found that 27% of dingoes surveyed in south-eastern Australia contained worms derived, on their morphological characters, from different intermediate host species, and Lymbery & Thompson (1989) found that multiply-infected intermediate hosts commonly contained genetically different cysts.

Giardia

Giardia presents us with a confusing and controversial taxonomic interpretation of its extensive genetic diversity. Historically, we can define pre- and post-Filice eras. In the former, over 40 species were recognized primarily on the basis of host occurrence with little evidence of morphological discrimination. Following a detailed review by Filice (1952), and in the absence of evidence to support the concept of rigid host specificity, 5 morphologically defined species are currently recognized (Thompson, Lymbery & Meloni, 1990; Thompson, Reynoldson & Mendis, 1993). Such an apparent rationalization is, however, quite clearly an oversimplification and untenable in the light of recent molecular studies which have demonstrated extensive genetic diversity in just one species, *G. duodenalis*, which infects numerous mammals including humans (Meloni, Lymbery & Thompson, 1988, 1995; Andrews *et al.* 1989; Weiss, van Keulen & Nash, 1992; Morgan *et al.* 1993). Furthermore, recent comparative studies on what were considered to be species-specific morphological characters, such as the median bodies and flagella, have found considerable variation between isolates of *G. duodenalis* from humans (N. Binz, A. J. Lymbery & R. C. A. Thompson, unpublished).

The situation is far more complex than that thought to exist in the pre-Filice era when a series of presumed host-adapted species was recognized. Recent studies have found more variation between isolates of *G. duodenalis* from the same host species than between isolates from different species of host. This is true for isolates of *G. duodenalis* examined from different species of mammals by a number of workers (Nash *et al.* 1985; Proctor *et al.* 1989; Meloni *et al.* 1995), which show levels of intra-host variation to be as great as those for inter-host variation. Furthermore, a detailed study of the extent

of genetic variation in *G. duodenalis* undertaken in a defined, localized endemic focus found that the level of genetic diversity was similar to the level of diversity in isolates sampled from a broad geographical distribution (Meloni *et al.* 1995). Some clones seem to be restricted in their host range characteristics and geographical location whereas others are widely distributed and may occur in different hosts. A detailed analysis of this and other genetic data supports the concept of zoonotic transmission of *Giardia*, and suggests that clonal lineages of the parasite are evolutionary independent of one another (Meloni *et al.* 1995).

Cryptosporidium

As with *Giardia*, numerous species of *Cryptosporidium* have been described, principally on the basis of host occurrence (Dubey, Speer & Fayer, 1990; O'Donoghue, 1995). However, the demonstration of cross transmission between different species of vertebrate host has resulted in broad agreement that the genus comprises 6 species which are differentiated on the basis of oocyst morphology, infectivity to a particular class of vertebrate host and site of infection (Levine, 1984; O'Donoghue, 1995). *C. parvum* is the most widely distributed species, has the broadest host range and is a major cause of disease in humans and other mammals, particularly livestock (Dubey *et al.* 1990; Simmons, 1991).

Although few morphological differences have been found between isolates of *C. parvum* from different mammalian hosts, evidence of differences in characteristics such as virulence and antigenicity have been reported (Fayer & Ungar, 1986; McDonald *et al.* 1991; Nichols, McLauchlin & Samuel, 1991; Pozio *et al.* 1992; Nina *et al.* 1992). Unfortunately, until recently, it has been very difficult to compare the genetic characteristics of different isolates of *Cryptosporidium* because of the lack of appropriate animal models or *in vitro* cultivation procedures to amplify isolates of *C. parvum*. The advent of PCR-based technologies has, however, revolutionized the molecular characterization of parasites where material has been limiting. Random amplified polymorphic DNA (RAPD) analysis has been applied to the detection of genetic variation in *Cryptosporidium* (Morgan *et al.* 1995). This revealed at least 4 distinct genetic groups among a range of isolates of *Cryptosporidium* from mammals and snakes (Fig. 2). The snake isolates were grouped together but differed from the mammalian isolates, which supports their classification as a separate species, *C. serpentis*, based on host specificity, site of infection, parasite pathogenicity and morphology (Levine, 1984; O'Donoghue, 1995). *C. parvum* isolates from domestic animals were grouped together with minor differences between isolates of lamb and calf origin. The majority of the human *C. parvum* isolates were also found to be remarkably similar to each other apart from 2, which were genetically distinct. One of these isolates, which was from a child who lived in a rural area, was genetically very similar to calf isolates of *Cryptosporidium*. The genetic differences found between mammalian isolates raised questions about whether *C. parvum* is a uniform species. The results also suggest that isolates of *Cryptosporidium* may vary in their zoonotic potential. However, these are preliminary data, and further comparative studies are required in which isolates of *Cryptosporidium* from humans and other animals are examined from localized geographical areas.

Species concepts

An underlying problem with the species-level taxonomy of the 3 parasites considered here, is that there an no accepted concept of what constitutes a species. The most widely used definition of species is the biological species concept of Mayr (1942): 'Species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups'. This concept is obviously not applicable to organisms which reproduce exclusively (or perhaps even predominantly) by selfing or clonal multiplication. It has also been criticized because it cannot be applied to allopatric populations (Sokal & Crovello, 1970), because of the undue emphasis it places on reproductive isolating mechanisms (Paterson, 1985), for overstating the unifying power of gene flow (Ehrlich & Raven, 1969) and for ignoring the historical relationships of populations (de Queiroz & Donoghue, 1988).

The classical approach to the species problem in asexually reproducing organisms has been to apply a typological concept, which regards species as artificial constructs with their limits defined by arbitrary criteria. Species may be defined phenetically, on the basis of certain levels of genetic or morphological dissimilarity from other groups. Tibayrenc, Kjelberg & Ayala (1991) suggested that for clonal parasites, genetically distinct groups should be labelled as different species only when the genetic discontinuities correspond with differences in characters of medical significance.

Our preferred approach is to regard species, even in purely clonal organisms, as real units of evolution. From this standpoint, two different types of species concepts have been proposed in response to the perceived shortcomings of the biological species concept. Phylogenetic species concepts emphasize the pattern, rather than the processes of speciation and define species either on the possession of a unique combination of character states (Cracraft, 1983; Nixon & Wheeler, 1990) or on monophyletic origin (Rosen, 1979; de Querioz & Donoghue, 1990).

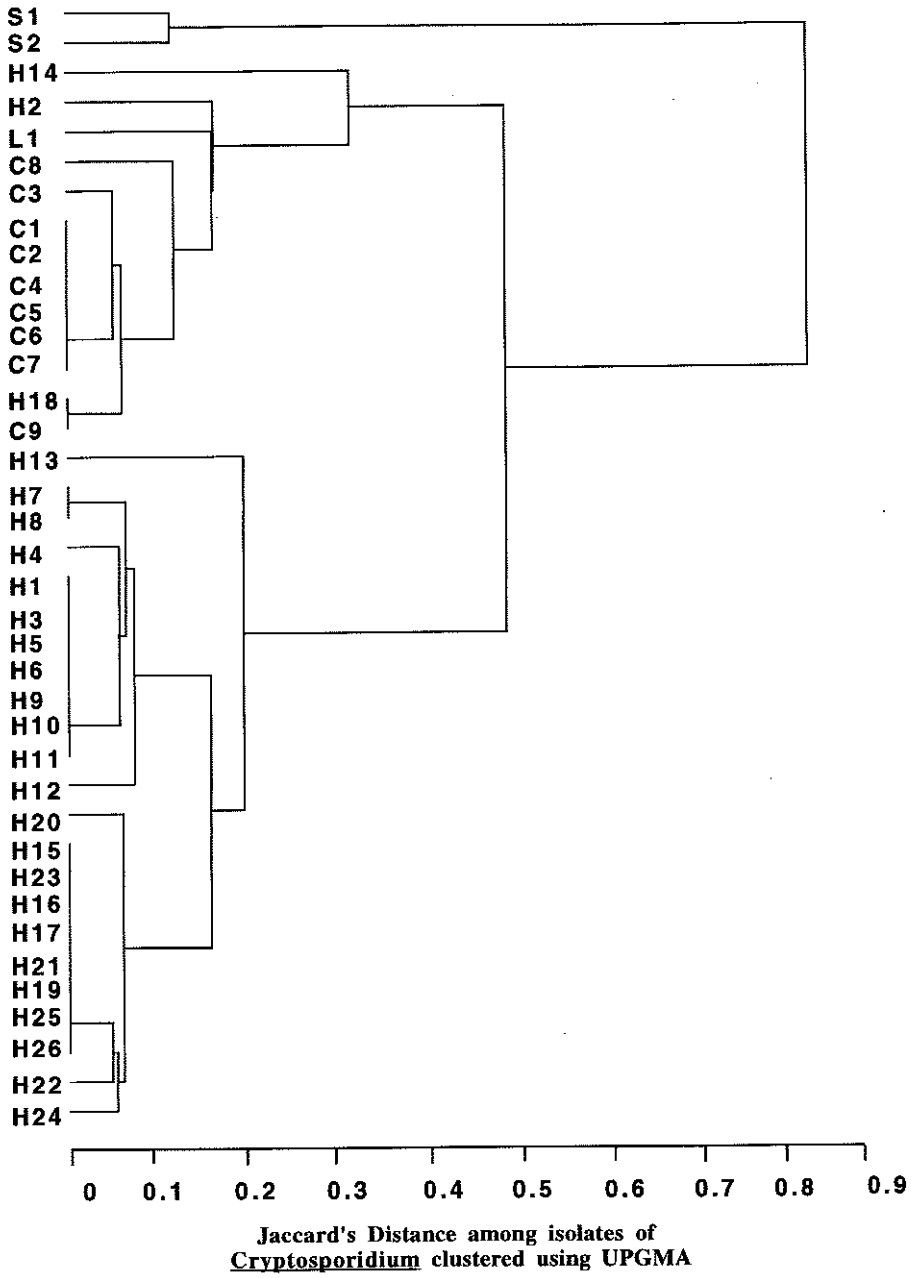

Fig. 2. Phenogram of Jaccard's distance among rapdemes of *Cryptosporidium*, clustered by the group average (UPGMA) strategy (after Morgan *et al.* 1995; Morgan, 1995). S = snake; H = human; L = lamb; C = cattle.

Evolutionary species concepts, by contrast, focus on the process of speciation, but consider processes such as shared history and ecological constraints to be as important as interbreeding in providing evolutionary cohesiveness to species (Wiley, 1978; Templeton, 1989).

We have documented elsewhere our preference for evolutionary, over phylogenetic species concepts (Lymbery, 1992, 1995), and provided operational rules for the implementation of an evolutionary species concept for the genus *Echinococcus* (Thompson *et al.* 1995). In brief, the procedure consists of identifying basal taxa or operational taxonomic units (OTUs), reconstructing the phylogeny of the OTUs and finally ranking as evolutionary species those monophyletic groups having the potential for genetic or demographic exchangeability (as defined by Templeton, 1989). For *Giardia* and *Cryptosporidium*, as for many other protozoan groups with a largely clonal population structure, the question of an appropriate species concept has hardly been addressed.

THE MOLECULAR ECOLOGY OF HOST-PARASITE INTERACTIONS

Giardia

Our research, and that of others (see above) has highlighted the extensive genetic heterogeneity that exists within the species *G. duodenalis*. Much of this variation is reflected in considerable phenotypic differences in characters such as host specificity,

growth rate, infectivity, virulence, drug sensitivity, antigenic characteristics and metabolism (reviewed in Thompson & Meloni, 1993). Such characters will have an obvious influence on the epidemiology and clinical features of giardiasis, and a major challenge for current research is to correlate genotypic and phenotypic characteristics; *i.e.* to find molecular markers for characters such as host preference, virulence and drug sensitivity. Because of the potential selectivity imposed by culture amplification and the fact that only a proportion of isolates of *Giardia* collected in the field are amenable in *in vitro* amplification, molecular characterization should ideally be carried out directly on parasite material isolated from naturally infected hosts. In addition, since the behavioural characteristics of *Giardia*, for example in zoonotic potential, may vary significantly from one endemic area to another, it is important to carry out detailed molecular epidemiological studies at a local level.

We have initiated such a study in remote Aboriginal communities in the north west of Western Australia. The frequency of *Giardia* transmission is high in these communities and effective control depends upon a better understanding of the biology of transmission. Infections are common, especially in children, and appear to persist throughout the year. As mentioned above, the parasite exhibits considerable clonal diversity in these communities and there are clinical data which indicate the existence of variability in drug sensitivity and in virulence, in terms of establishing acute or chronic infections. In addition, considerable phenotypic variability between isolates of *Giardia* from these communities has been demonstrated in the laboratory in characters such as growth rate *in vitro* and *in vivo*, predilection site in the intestine and duration of infection, pH preference, metabolism and sensitivity to drugs (Binz *et al.* 1992; Hall *et al.* 1992; Farbey, Reynoldson & Thompson, 1995; L. McInnes, J. A. Reynoldson & R. C. A. Thompson, unpublished). The aim is to develop genetic markers that may identify clones of the parasite which possess these features in the field. At a more fundamental level, however, we are trying to develop molecular tools which will allow us to follow clones of *Giardia* in these communities. Their application will provide data on the biology and nature of transmission in these communities so that questions such as: are all, or any, strains zoonotic? Is transmission principally dog to human or *vice versa*? How frequent are multiple infections? – can be answered. We know that young children in these communities are found to be repeatedly infected with *Giardia*. Is it with the same clone or are different clones found at different time intervals? Answers to such questions will provide valuable clues concerning host immunity and the dynamics of transmission.

In order to answer these questions there must be frequent sampling of the same individuals and the appropriate molecular tools for characterizing small quantities of parasite material directly from faecal samples. In order to meet the first requirement we have developed appropriate health education programmes which can be sustained by the communities themselves and upon which regular sampling programmes can be developed. This provides data that are fed back to individuals in the communities. The second requirement has not been easy to fulfil, involving: (1) the development of PCR-based techniques; and (2) finding the most appropriate level at which to study genetic diversity so that the movement of individual clones can be studied within communities. The latter will require a level of discrimination that is finer than that provided by isoenzyme electrophoresis and RAPD analysis, and which can differentiate between isolates presently grouped together within the same zymodemes or rapdemes.

Two broad approaches are being taken in our studies on the molecular epidemiology of giardiasis in Aboriginal communities. The first has concentrated on regions within the rDNA as well as the non-transcribed spacer, which separates the large and small rDNA subunits, in order to find variable regions of value in characterizing small quantities of cystic material directly from faeces using PCR. The aim has been to obtain a level of genetic discrimination which will place all isolates collected in the field into the broad genetic groups established using isoenzyme electrophoresis and RAPD analysis (Morgan *et al.* 1993; Meloni *et al.* 1995). Preliminary results from studies in which *Giardia*-specific primers have been used to amplify a 300 bp region of the small subunit rDNA do not support our hypothesis of frequent zoonotic transmission in these Aboriginal communities. Comparative sequence analysis of the 300 bp region suggests that dogs rarely harbour the isolates of *Giardia* that occur in humans from the same communities and *vice versa* with respect to isolates found in humans (R. M. Hopkins, B. P. Meloni, D. M. Groth, J. A. Reynoldson, J. D. Wetherall & R. C. A. Thompson, submitted).

A second approach has been to develop a PCR-based fingerprinting tool at least as sensitive as isoenzyme and RAPD analysis and, if necessary, with a level of genetic discrimination sufficient to differentiate between isolates/clones of *Giardia* currently grouped within a zymodeme or rapdeme, in order to study the transmission dynamics of *Giardia* on a fine scale in local communities. Initial studies have involved screening the *Giardia* genome to determine if either microsatellite or minisatellite loci are present. Results have shown that microsatellite repeat units are either not present or occur very infrequently in the *Giardia* genome (R. M. Hopkins *et al.* unpublished). This contrasts

with the situation in other flagellates, such as *Leishmania*, which possesses at least 3 microsatellites on all chromosomes of the parasite (Rossi *et al.* (1994). Subsequent studies have therefore concentrated on searching for minisatellite loci. Initial results obtained using the M13 and PER minisatellite loci as probes showed that a number of these regions were present within a range of *Giardia* isolates examined, and it was possible to fingerprint each of the isolates on the basis of the multilocus banding profiles exhibited. These observations support those of Upcroft, Mitchell & Boreham (1990) who also used the M13 minisatellite locus. Current studies are concerned with the isolation, cloning, sequencing and construction of primers so that we can evaluate single locus PCR-based minisatellite fingerprinting as a molecular epidemiological tool in the field.

Echinococcus

Because of the extensive genetic and phenotypic variation that has been demonstrated in *Echinococcus*, it is very important to characterize the aetiological agents in different endemic areas in order to determine transmission patterns, particularly where there is the possibility of interaction between cycles (Thompson, 1995; Thompson *et al.* 1994, 1995). For example, the two cycles of transmission which occur in Britain involve two quite distinct strains, probably species, of *Echinococcus* which have different intermediate host preferences and thus minimal opportunities for interaction (Thompson & Smyth, 1975; Thompson, 1991). Parasite characterization is particularly important in regions where there is more than one species of intermediate host and therefore the opportunity for different cycles of transmission and sources of infection for humans and livestock. This is the case in the Middle East, China, parts of Asia and Africa where numerous species of intermediate host harbour hydatid cysts (Thompson, 1995). For example, camels are commonly infected in the Middle East and Africa, yet opinions differ regarding the infectivity of *E. granulosus* of camel origin to humans (Eckert *et al.* 1989; Wachira *et al.* 1993; Thompson *et al.* 1995).

Developmental differences between strains of *Echinococcus*, such as variation in the onset of egg production, will affect transmission and impede control efforts when regular, adult cestodicidal treatment is the preferred control strategy for breaking the cycle of transmission (Kumaratilake, Thompson & Dunsmore, 1983; Thompson, Kumaratilake & Eckert, 1984; Eckert *et al.* 1989). In addition, the accurate surveillance of hydatid disease in humans and the future development of immunoprophylactic strategies may be jeopardised by demonstrated antigenic differences between isolates of *Echinococcus* (Thompson, 1995).

Variation in the pathogenicity of strains/species of *Echinococcus* will influence the prognosis in patients with hydatid disease, particularly the alveolar form for which early diagnosis is essential (see Thompson *et al.* 1995). There is also increasing epidemiological evidence that certain strains of *E. granulosus*, such as those adapted to horses and pigs, may not be infective to humans (Thompson & Lymbery, 1988, 1991; Shabovskaya *et al.*, 1989). Strains/species of *Echinococcus* may also differ in their response to particular chemotherapeutic regimes (Saimot *et al.* 1981; Schantz, van den Bossche & Eckert, 1982; Kammerer & Schantz, 1984). This is supported by detailed information being obtained on the biochemical differences between strains, including variation in the activity of individual enzymes (McManus & Smyth, 1982; McManus & Bryant, 1995).

As well as providing tools for characterizing species and strains of *Echinococcus* in different endemic areas, molecular genetic techniques can be used to provide information about population structure. Estimates of gene flow between populations in different hosts or geographic areas can have valuable epidemiological applications. For example, on the mainland of Australia where *E. granulosus* is maintained in different cycles of transmission involving either domestic or wild host assemblages, there is no evidence of restricted gene flow between the parasites found in these cycles (Lymbery, Thompson & Hobbs, 1990; Thompson & Lymbery, 1990; Hope, Bowles & McManus, 1991). This is significant for the control of hydatid disease in Australia, since wild and domestic cycles of transmission will interact in areas where they overlap (Thompson, 1992; Constantine *et al.* 1993). Gene flow is restricted between populations of the parasite on the mainland of Australia and in the island state of Tasmania, and these populations are often regarded as different strains (Lymbery & Thompson, 1988; Thompson & Lymbery, 1988). Despite the genetic differences between mainland and Tasmanian populations, however, Lymbery (1995) calculated that migration between the populations was of sufficient magnitude to be responsible for occasional breakdowns in the largely successful Tasmanian hydatid control campaign. Constantine *et al.* (1991) argued that the genetic distinctness of a population of *E. granulosus* on King Island, between mainland Australia and Tasmania, made it unlikely to have originated from a recent introduction from either area.

Cryptosporidium

An important prerequisite to determining the risk factors for AIDS patients with *Cryptosporidium* infections is characterization of the causative agents. In addition to the genetic diversity displayed between isolates of *C. parvum* from mammals, there is

increasing recognition of phenotypic variability between isolates of the parasite (Casemore, 1990; Ortega *et al.* 1991; Pozio *et al.* 1992). These include differences in virulence and pathogenesis, infectivity, antigenicity and drug sensitivity (Current & Reese, 1986; Fayer & Ungar, 1986; Mead *et al.* 1990; Nichols *et al.* 1991). Such heterogeneity is thought to explain differences in clinical picture, response to drugs and passive immunotherapy, and may also complicate immunological approaches to diagnosis. Indeed, recent research in Italy has demonstrated behavioural differences between isolates of *Cryptosporidium* from AIDS patients exhibiting mild and severe cryptosporidiosis, which are thought to reflect intrinsic characteristics of the parasites (Pozio *et al.* 1992). The genetic basis of such behavioural differences has still to be demonstrated but variability in virulence is thought to correlate with the source of infection, *i.e.* whether or not it was of zoonotic origin. Our results lend support to such an hypothesis (Morgan *et al.* 1995).

Since AIDS patients, and other immuncompromised groups are those most at risk from cryptosporidial infections, there is an urgent need to determine the extent of genetic diversity within isolates affecting humans, and to develop molecular characterization procedures to do this, so that sources of transmission can be identified and strain differences correlated with clinically important parameters such as severity of infection and drug sensitivity. As very little is known about the true extent of genetic diversity in *Cryptosporidium*, comparative studies should be undertaken in localised areas since they are likely to be more meaningful in terms of clarifying routes of transmission, the importance of zoonotic or human sources of infection, the spread of particularly virulent strains, and the clinical significance of parasite heterogeneity. In addition, appropriate and sensitive PCR techniques with the ability to detect and characterize isolates of *Cryptosporidium* at an early stage in HIV infection, when oocyst shedding may be very low, offer the potential to improve clinical management of the disease (U. M. Morgan, P. A. O'Brien & R. C. A. Thompson, unpublished). It may be possible to discriminate between strains of variable virulence, and allow chemotherapy to be initiated before the onset of symptoms which may result in fewer cases progressing to severe, and often chronic, cryptosporidiosis.

Outbreaks of cryptosporidiosis in child day-care centres are frequently reported (Alpert *et al.* 1986; Crawford *et al.* 1988; Diers & McCallister, 1989; Thompson, 1994; Hanna & Brookes, 1995). Although some family members are often affected during such outbreaks, a proportion of children remain asymptomatic and it is believed they could act as carriers of infection to relatives and the community. Thus the exclusion or physical separation of symptomatic from asymptomatic children may be important (Thompson, 1994) but will not contain an outbreak if there is asymptomatic excretion. In such circumstances, it would be useful to screen all children and family members to determine their infection status (Fig. 3), and characterise isolates of *Cryptosporidium* from infected individuals using appropriate PCR-based techniques, such as RAPD analysis or other fingerprinting approaches. Only in this way will it be possible to determine the dynamics of transmission in such epidemic situations, the risk factors associated with infection, and whether there is any correlation between parasite heterogeneity and overt disease.

Limitations and new approaches

One of the most exciting new developments in the molecular approach to epidemiology has been the contribution of population genetics to an understanding of parasite transmission and prospects for the control of parasitic disease (*e.g.* Tibayrenc & Ayala 1988; Lymbery *et al.* 1990; Day *et al.* 1991; Dame, Blouin & Courtney, 1993; Anderson, Romero & Jaenike, 1995; Meloni *et al.* 1995). Although such studies have produced estimates of important parameters such as outcrossing rate, effective population size and gene flow, they have not always provided convincing accounts of how these parameters, estimated from discrete molecular data, relate to genetic variation in more complex epidemiological traits.

Our problem lies with the way in which population genetic data are usually analysed. The standard method of analysis is to reduce the data to estimates of genotype and gene frequencies, and then to compute inbreeding coefficients, which can be thought of as probabilities that genes from the same or different populations (or individuals) are identical by descent, or as components of genetic variance within and between populations (or individuals). Given certain assumptions, inbreeding coefficients can be used to estimate gene flow between populations (Wright, 1951). Estimates made in this fashion, however, do not take into account the historical nature of the relationship between populations, and must therefore assume a specified set of equilibrium conditions. For species of parasites infecting humans or their domestic animals, and thereby subject to rapidly changing patterns of host movement, equilibrium conditions are unlikely and historical contingency may be all-important.

A general solution to this problem may lie with a coalescent approach to population genetic analysis. Coalescent models (Tavare, 1984; Hudson, 1990) differ from inbreeding models in using time to common ancestry of genes, rather than identity probabilities or variance in gene frequencies as a measure of genetic structure. They are much more

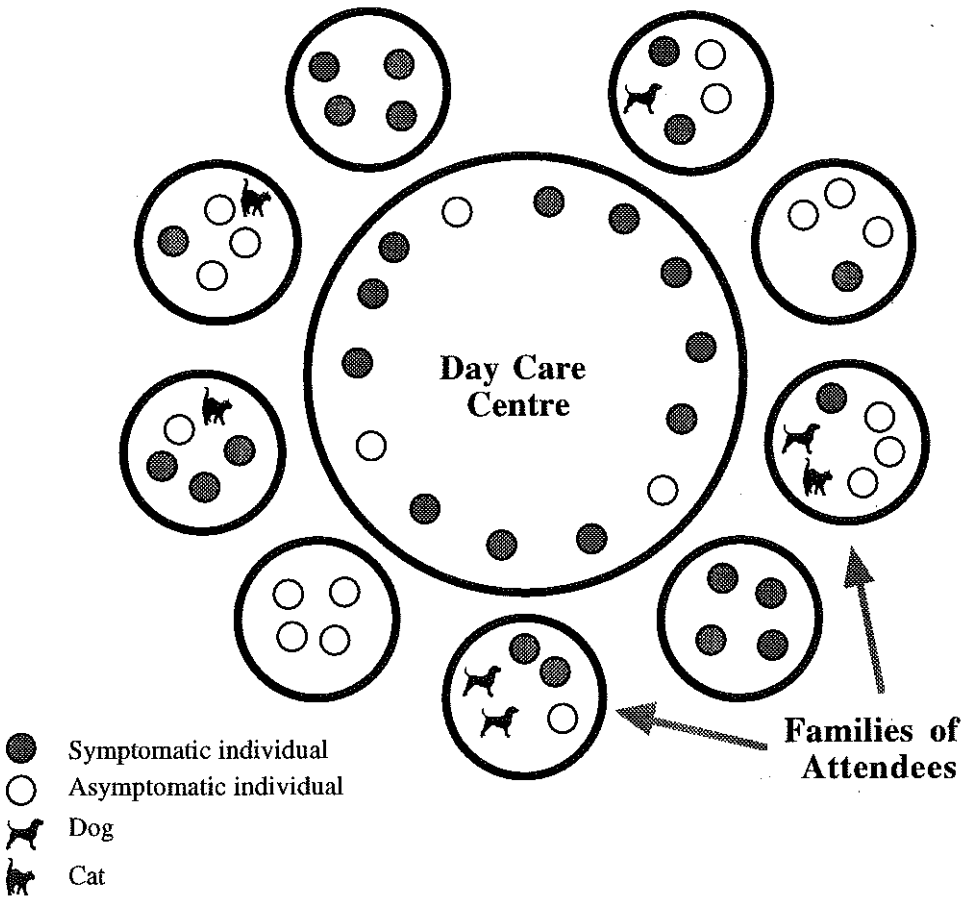

Fig. 3. Schematic diagram showing the links between symptomatic and asymptomatic individuals in a hypothetical outbreak of cryptosporidiosis associated with a day care centre.

powerful because they retain phylogenetic information and can be used to estimate genetic parameters under non-equilibrium conditions. They do, however, require data of sufficient resolution to allow accurate intra-specific gene phylogenies to be inferred. The coalescent approach has already found application in the epidemiological study of viral infections (Harvey & Nee, 1994; Holmes & Garnett, 1994).

Another problem is the lack of knowledge of the genetic control of most epidemiologically important traits in parasites. This is likely to be complex and polygenic (Severson, 1994; Thompson & Lymbery, 1994), but little is known about the number and magnitude of effects of the loci influencing them. There is little information about their heritability or of their genetic covariance with other traits. This may have far-reaching consequences for our understanding of genetic structures in parasite species, for genetic variation within and between populations may be very different for discrete genetic markers and complex quantitative traits (Bulmer, 1971).

For *Echinococcus*, variation in rostellar hook morphology has been used to differentiate populations in domestic and wild host cycles in Australia (Kumaratilake & Thompson, 1984). It has been interpreted as showing that there are genetically different strains of the parasite, an interpretation at odds with population genetic studies (Lymbery *et al.* 1990). Hobbs, Lymbery & Thompson (1990) found that most of the variance in metacestode hook measurements in Australian population of *E. granulosus* was accounted for by a contrast between hook number and length, and Lymbery (1995) found that while there was no significant genetic correlation between these traits, they had an environmental correlation of −0·38. The host in which the metacestode develops in fact has a major influence on hook number and hook length, and these host-induced changes are still recognizable in the definitive host, *i.e.* the adult worms bear an imprint of their larval origin in their rostellar hook traits. Constantine *et al.* (1993) used this imprint to determine predator–prey relationships in areas of Australia where sylvatic and domestic cycles of the parasite overlapped.

GENETIC VARIATION WITHIN THE HOST

In recent years, increasing attention has been given to the role of genetic variation in the dynamics of host–parasite associations. Nowhere has this been more evident than in theoretical studies of the transmission of parasite strains differing (genetically)

in virulence between hosts which differ in resistance (*e.g.* Anderson & May, 1992; Frank, 1994; Nowak & May, 1994). Only a few of these studies have considered the complications which arise from the co-infection of a single host with genetically different strains of a parasite, and these have been restricted to the case of superinfection, where one strain invariably out-competes another within the host (Bonhoeffer & Nowak, 1994; May & Nowak, 1994; Nowak & May, 1994).

The major conclusion from such studies has been that superinfection can lead to a stable polymorphism for virulence within the parasite supra-population and the evolution of higher levels of mean virulence than required to maximize the parasite reproductive rate. Although the insights provided by these theoretical studies can help us to answer basic questions such as the evolution of virulence (Bonhoeffer & Nowak, 1994) and applied problems such as vaccination strategies (Gupta *et al.* 1994), there is at present only a very narrow empirical base for either testing their predictions or determining the validity of their assumptions. We need better ecological/genetic data on questions such as: how frequently do multiple infections of a single host occur? is there a competitive dominance hierarchy among different parasite strains within a host? are more virulent strains competitively superior? can virulence be equated with reproductive rate? The advent of molecular characterisation techniques such as RFLP analysis, molecular karyotyping, PCR-based fingerprinting and direct sequencing have provided the tools for collecting such data (Hide & Tait, 1991; Bull, 1994; Thompson *et al.* 1994).

Intra-host competition

In asexually reproducing organisms, competition between clones for a restricted resource is expected to lead to monopolization by the competitively superior clone, and therefore a reduction in clonal diversity, in the absence of disturbing forces (Sebens & Thorne, 1985; Wilson & Hebert, 1992; Weider, 1992). We have argued that interference with the normal homogenizing influence of clonal competition may account for the greater than expected levels of genetic diversity reported in populations of *Giardia* from remote Aboriginal communities in Australia (Thompson & Meloni, 1993). In this situation, the disturbing force may be frequent exposure of the parasite to drugs, which is a feature of the situation in Aboriginal communities (Thompson, 1991). By interfering with clonal competition in *Giardi*, regular, sub-optimal application of chemotherapeutic regimes may serve to maintain genetic diversity through pre-emption of competitive exclusion (Thompson, 1991; Thompson & Meloni, 1993). For example, children in Western Australian Aboriginal communities often do not receive their full course of antigiardial chemotherapy with nitroimidazole drugs. This, combined with the well documented variable sensitivity of *Giardia* to these compounds (Boreham, Phillips & Shepherd, 1987; Majewska *et al.* 1991; Farbey *et al.* 1995), may inhibit competitive interactions between cohabiting clones of *Giardia*. Experimental studies have demonstrated that such interactions do occur *in vitro* between genetically distinct isolates of *G. duodenalis* with faster-growing isolates outcompeting those with slower growth rates (R. C. A. Thompson, A. J. Lymbery, D. A. Pearce, K. C. Finn, J. A. Reynoldson & B. P. Meloni, unpublished). The addition of sub-lethal concentrations of metronidazole to clonal mixtures *in vitro* prevented the competitive exclusion which was seen in normal culture. Probably because the drug reduced the growth rate, and presumably also the maximum stationary phase concentration, of the faster-growing but not the slower-growing clone.

Can these *in vitro* results be realistically extrapolated to the field situation? The answer is that we do not know. In endemic areas where extensive genetic heterogeneity exists within *Giardia* populations, mixed infections with more than one genetic variant (as reported by Weiss *et al.* 1992) are likely to occur, especially under conditions where the frequency of transmission is high, as in Aboriginal communities in the north of Australia (Meloni *et al.* 1992, 1995). The environment of a natural host, however, is much more complicated than that of a culture flask. Different strains of *G. duodenalis* have been shown to differ in their substrate requirements, pH preference and region of the small intestine they preferentially inhabit (Binz *et al.* 1992; Hall *et al.* 1992; McInnes, 1994; L. M. McInnes, J. A. Reynoldson and R. C. A. Thompson, unpublished; Fig. 4) and thus niche segregation may reduce competitive interactions inside the host. Further, Hassell, Comins & May (1994) recently showed that a mixture of coexisting genotypes may be spatially segregated even in a uniform environment, as a result of local dynamics and differences in dispersal rates. In addition, we need to consider that host factors such as immune status and/or nutrition, as well as concurrent infections (with other parasite species), may also have a significant environmental influence on competitive interactions in the intestine and a direct bearing on the maintenance of clonal diversity in *Giardia* and other clonal parasites.

The question of the extent to which parasite infracommunities are structured by competitive interactions is a vexed one. It is usual to think of infracommunities as ranged along a continuum from interactive (high rate of colonization, high density, high species diversity, intense interspecific competition) to isolationist (low rate of colonization, low density, low species diversity, little interspecific competition) although the empirical support for this linear view of community structure is mixed (Sousa,

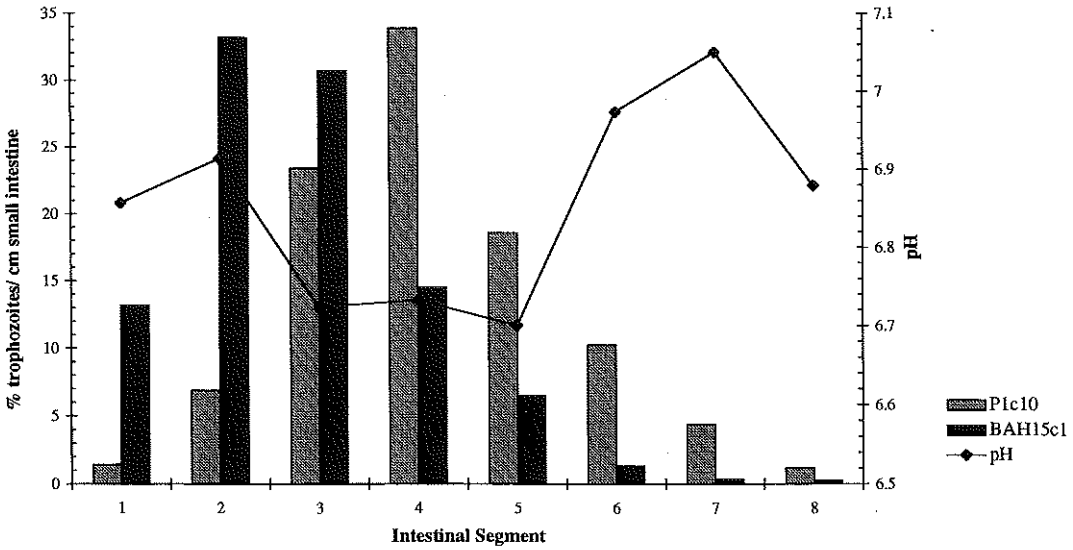

Fig. 4. Distribution of two isolates of *Giardia duodenalis*, P1c10 and BAH15c1, in the small intestine in mice 5 days post infection (after L. M. McInnes *et al.* unpublished).

1994). In interactive communities, intraspecific competition will extend niche width, while interspecific competition will reduce niche width or produce niche displacement (Holmes, 1990). For example, experimental studies have shown that different species of *Echinococcus* can coexist in the same region of the definitive host intestine (Thompson & Eckert, 1983), and Smyth (1987) suggested that potentially cohabiting strains of *E. granulosus* would not compete for the same nutritional resources in the gut of a dog because of their demonstrated differences in metabolic requirements. The situation in nature, however, is likely to be much more complicated. We have found considerable variation in site distribution, worm density and development in different dogs infected with *Echinococcus granulosus* (Fig. 5; C. C. Constantine, A. J. Lymbery, D. J. Jenkins, E. M. Bennet-Jenkins, C. Behm & R. C. A. Thompson, unpublished). The source of such variation, whether of host and/or parasite origin is not clear. Genetically identical worms in different regions of the gut of the same dog also have distinct metabolic characteristics which may relate to the worm density in different parts of the small intestine (Constantine *et al.* unpublished). It is not clear if worms select sites in the small intestine which provide the most favourable conditions for development or whether there is a relationship between site selection and parasite interaction with host immune responses. Recent research has demonstrated an association between heightened, local immune responsiveness in the gut of dogs and infection with *E. granulosus* (Deplazes, Thompson & Penhale, 1994). However, any interpretation of the role of such localized immune responses is confounded by the great variability in worm burden and stage of parasite development which precludes any correlation with immune reactivity. Elucidating the source of such variability in developmental parameters must be an important consideration when constructing predictive models of the transmission dynamics of *Echinococcus* which require data on reproductive potential. Considering the lack of appropriate and reproducible laboratory models for this parasite, it may not be possible at present to answer these fundamental questions about host–parasite interactions. There is a need, therefore, to evaluate further the potential of rodent models by studying interactions of the adult parasite with its host, following the encouraging results of Kamiya & Sato (1990*a*,*b*).

Virulence

Theoretical models of microparasite superinfection and transmission define virulence in terms of reproductive rate; strains of the parasite with the greater reproductive rate are more virulent and always superior in within-host competitive interactions (Bremermann & Pickering, 1983; Nowak & May, 1994). Virulence polymorphisms arise in these models because of the relationship specified between virulence and transmissibility. Although there is no universally acceptable definition of virulence (Bull, 1994), equating virulence with reproductive rate misses many features of the parasite/host relationship. From the few studies which have simultaneously measured parasite reproductive rate and disease severity, there is little evidence of a positive relationship between the two (Ebert, 1994).

In *Giardia duodenalis*, we have some evidence that slower growing strains (in standard culture conditions) may be less sensitive to antigiardial agents such as metronidazole, and have a longer duration of infection in rodent models. In this

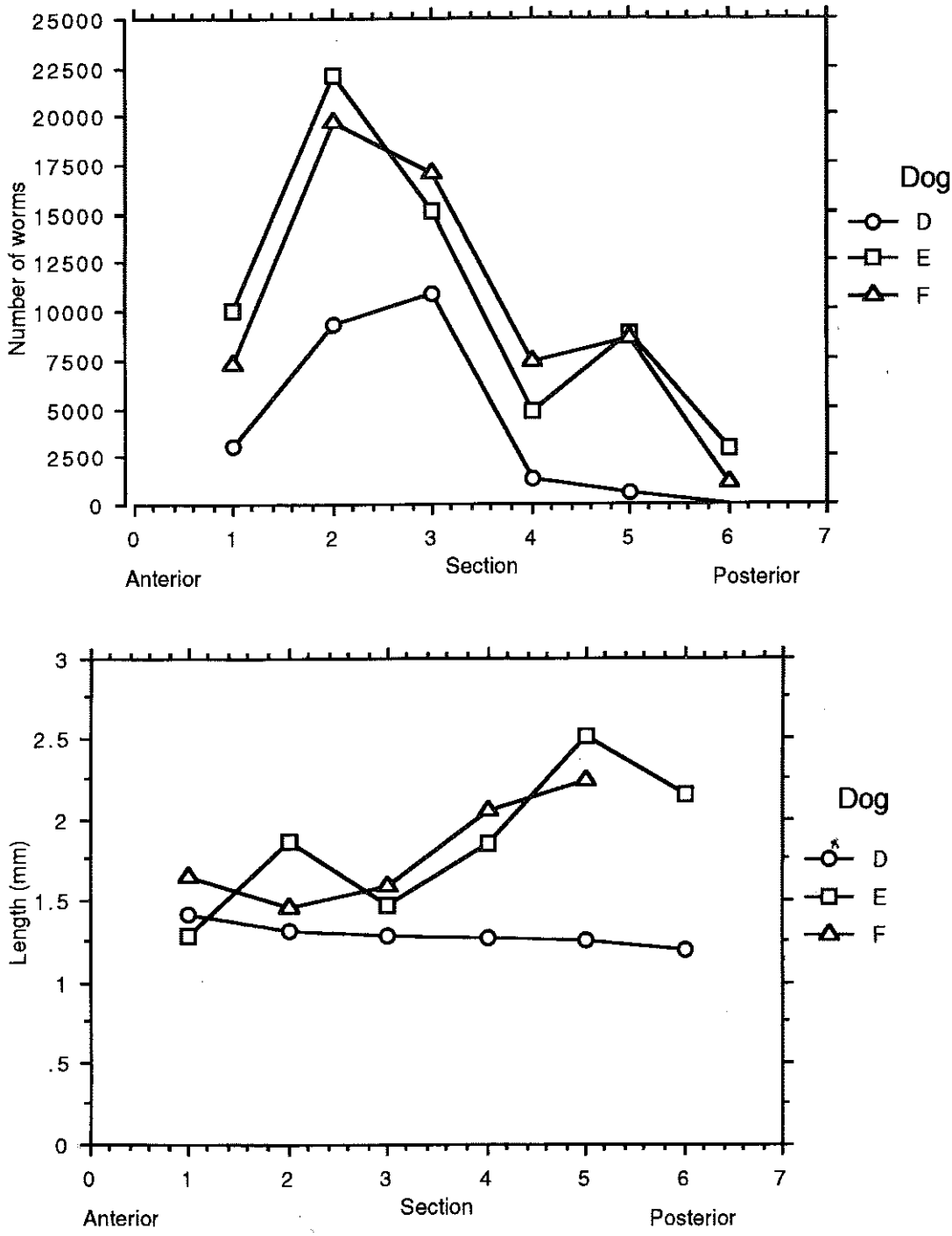

Fig. 5. Distribution and total length of *Echinococcus granulosus* in the small intestine of 3 dogs 35 days post infection. Dogs were siblings infected with identical doses of protoscoleces from the same hydatid cyst. (After C. C. Constantine *et al.* unpublished).

context, it may, in future, be possible to categorize different strains of *G. duodenalis* as being more likely to be associated with acute or chronic infections. This will require the collection of reliable clinical data on the nature of *Giardia* infections in endemic communities. This is an area fraught with problems because of the lack of specific symptomatology, and difficulties with correlating diarrhoea or failure to thrive with the presence of *Giardia*, especially when concurrent infections may be present and nutritional deficiencies are common. If we assume, however, that those strains of *Giardia* with a longer duration of infection are likely to be associated with chronic giardiasis, how do we define virulence in this organism? Under circumstances where the frequency of transmission is high and hosts are disadvantaged nutritionally and harbour concurrent infections, strains of *Giardia* that establish chronic, persistent infections will undoubtedly compromise the host to a greater degree clinically than strains with a quick turn around. Bull (1994) has enumerated the various models of virulence evolu-

tion, many of which rely on different definitions of the concept. Giardiasis offers an excellent host/parasite system for the study of these models.

CONCLUSIONS

Parasites exhibit a complex variety of genetic structures and defy any attempts to make generalizations about their ecological interactions or evolutionary potential. This has been clearly demonstrated over the last 15 years with the development of increasingly discriminatory molecular characterization techniques. The problems that we now face are those of interpreting the genetic data that are being generated. This will not be difficult with epidemiological problems and the transmission dynamics of parasitic infections. In these cases, we have outstanding questions that should readily be answered by the application of molecular epidemiological procedures. In contrast, we have yet to pose the appropriate questions with respect to taxonomic considerations and there is uncertainty as to how we should interpret the extensive data on parasite genetic diversity. On a finer scale, we have established elegant theoretical proposals about the consequences of within host interactions between genetically heterogeneous assemblages of parasites. However, their significance awaits testing in appropriate laboratory or field situations where the new molecular tools can be used to determine the consequences of parasite/host interactions. We need more empirical data, not only to test the predictions of theoretical models, but to improve the biological realism of their assumptions. In particular, we need information on the genetic architecture, heritability and genetic correlations between complex epidemiological traits such as virulence and transmissibility.

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