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Lack of evidence for co-speciation in a parasitic nematode of grey kangaroos

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Abstract

Multilocus enzyme electrophoresis was used to compare specimens of the parasitic nematode *Cloacina obtusa* from the stomach of the eastern grey kangaroo, *Macropus giganteus* and the western grey kangaroo, *M. fuliginosus*. Allelic variation among nematodes was detected at 17 (85%) of 20 loci, but there was only a single fixed genetic difference (at the locus for isocitrate dehydrogenase, IDH) between *C. obtusa* from *M. fuliginosus* and those from *M. giganteus* in areas where each host occurred in allopatry. However, this fixed difference was not apparent within the zone of host sympatry. Although electrophoretic data indicate genetic divergence among allopatric populations of *C. obtusa* in the two host species, the magnitude of the electrophoretic difference (5%) between these populations does not refute the hypothesis that *C. obtusa* represents a single species. The 'usual' situation for parasitic helminths of grey kangaroos is that pairs of parasite species occur in the two host species. This situation differs for *C. obtusa*, where there has been a lack of speciation following a speciation event in its macropodid marsupial hosts. This finding suggests that a speciation event in the host does not necessarily lead to a speciation event for all its parasites and further highlights our lack of understanding of which processes drive speciation in parasites.

Introduction

Host–parasite associations represent useful models to investigate evolutionary processes (Price, 1977). It is generally assumed that the principal mode of speciation for parasitic organisms occurs following geographical separation of the hosts (and hence its parasites) into two or more isolated populations (Inglis, 1971; Price, 1977; Combes, 2001). Evidence for co-speciation has been provided by numerous studies conducted over the last 20 years on a variety of host–parasite associations (see examples in review by Paterson & Banks, 2001 and in Page, 2003). Comparisons of the phylogenetic relationships of parasites and their hosts have often revealed other co-phylogenetic events (e.g. host-switching, extinction, duplication and 'missing the boat'; Paterson & Banks, 2001). Such events have been proposed to explain the incongruence between host and parasite trees (Paterson & Banks, 2001; Johnson & Clayton, 2003; Johnson *et al.*, 2003; Page, 2003).

There have been few attempts to compare whether the co-phylogenetic events in one group of parasites are the same as those for a second group of parasites which parasitize the same group of hosts. However, in the recent study by Johnson & Clayton (2003), comparisons were made of the phylogenetic relationships of two groups of ischnocercan feather lice and their avian hosts. They found little concordance between the co-speciation events in wing lice and those in body lice, and several instances

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of where these ectoparasites had failed to speciate with their avian hosts. It has been suggested that the frequency of some co-phylogenetic events (e.g. duplications and 'missing the boat') may be over-estimated because the algorithms of the analytical methods used to compare host and parasite phylogenies (e.g. TreeMap; Page, 1994) do not consider the failure of parasites to speciate in response to host speciation (defined as 'inertia' by Paterson & Banks, 2001) as an event leading to the incongruence between host and parasite trees (Johnson & Clayton, 2003; Johnson *et al.*, 2003). In the present paper, we examine whether there has been co-speciation or a failure to speciate in different genera of endoparasites (helminths) within the same species-pair of host (grey kangroos).

The eastern grey kanagroo (*Macropus giganteus*) and the western grey kangaroo (*M. fuliginosus*), represent an exemplary host species-pair for the study of host–parasite co-speciation. These macropodid marsupials harbour a range of parasitic helminths, most of which do not occur in any other species of macropodid marsupial (Beveridge & Arundel, 1979; Spratt *et al.*, 1991). The grey kangaroos are very closely related based on morphological (Kirsch & Poole, 1972; Caughley *et al.*, 1987), serological (Kirsch & Poole, 1972) and electrophoretic data (Richardson & McDermid, 1978; Baverstock *et al.*, 1985). They have speciated recently after being separated into eastern and western populations, probably due to geographical separation by a marine incursion in the Nullarbor region (Maynes, 1989). The extent of the electrophoretic differences between *M. fuliginosus* and *M. giganteus* (Nei's D = 0.03, Baverstock *et al.*, 1985) also suggests, by analogy with other Australian mammals (Baverstock *et al.*, 1986), that their separation has occurred only within the last 1 million years. Subsequently, a decline in sea level provided the opportunity for *M. fuliginosus* to migrate eastwards and come into contact with *M. giganteus* (see Oliver *et al.*, 1979; Maynes, 1989). Evidence for *M. fuliginosus* having evolved in Western Australia and subsequently migrating eastwards is based on its tolerance to the poison, fluoracetate, as fluoracetate-containing leguminous plants are most abundant in south-western Australia (Oliver *et al.*, 1979). Currently, there is a broad zone of sympatry between the two grey kangaroo species (Caughley *et al.*, 1987). However there is no evidence of hybridization between them in the wild (Kirsch & Poole, 1972; van Oorschot *et al.*, 1986), although unconfirmed hybrids have been reported occasionally (Coulson & Coulson, 2001).

This scenario of host speciation should lead to the formation of sister species pairs of parasites (i.e. co-speciation). For example, there are sister species pairs of anoplocephalid cestode (*Progamotaenia*) and strongyloid nematode (*Macropostrongyloides*) within grey kangaroos (Baverstock *et al.*, 1985; Beveridge *et al.*, 1993). Furthermore, a combined multilocus enzyme electrophoretic and morphological study (Chilton *et al.*, 1993) demonstrated that parasitic strongyloid nematodes of the genus *Paramacropostrongylus* occurring in the stomachs of grey kangaroos represented a species pair (*P. typicus* in *M. fuliginosus* and *P. iugalis* in *M. giganteus*). A subsequent electrophoretic study has also demonstrated that, in the zone of host sympatry, there is host-switching by the two *Paramacropostrongylus* species and subsequent hybridization (Chilton *et al.*, 1997a).

This finding for *Paramacropostrongylus* raises questions as to whether other parasites which exclusively occur in grey kangaroos also represent species pairs, and if so, whether they hybridize in the zone of host sympatry, or are reproductively isolated from one another. Several species of strongylid nematodes are found exclusively in grey kangaroos, many of which are shared by both host species (Beveridge & Arundel, 1979). For instance, of the 116 described species of the nematode genus *Cloacina* from kangaroos and wallabies (Beveridge, 1998, 1999, 2002; Beveridge & Speare, 1999), 12 are restricted to grey kangaroos (Beveridge, 1998). One such species, *C. obtusa*, occurs in both *M. giganteus* and *M. fuliginosus* throughout most of their geographic ranges (Beveridge, 1998). The present study used multilocus enzyme electrophoresis to examine whether *C. obtusa* represents a single species or a species-pair (i.e. one species of parasite in each species of host). This biochemical technique has been used previously to detect the presence of cryptic (i.e. genetically distinct but morphological similar) species within the genus *Cloacina* from other species of macropodid marsupial (Chilton *et al.*, 1997b).

Materials and methods

Live specimens of *C. obtusa*, identified according to Beveridge (1998), were obtained from the stomach of seven kangaroos, which had been killed recently by motor vehicles, in southern and eastern Australia (fig. 1). The hosts examined were three *M. giganteus* and one *M. fuliginosus* from areas of host allopatry, and one *M. giganteus* and two *M. fuliginosus* from the zone of host sympatry (near Bourke, New South Wales). Kangaroos in the zone of sympatry were identified by coat colour (Kirsch & Poole, 1972), and photographs taken of most carcasses to provide a permanent record of the pelage. Samples of host tissue, used as controls in the electrophoretic analyses, were placed in Eppendorf tubes and frozen in liquid nitrogen. Nematodes were washed in saline, their anterior and posterior ends were excised, placed on a microscope slide and fixed in glacial acetic acid then stored in 70% ethanol as reference specimens. The remaining part of each nematode was placed in a separate Eppendorf tube, frozen in liquid nitrogen and subsequently stored at −70°C until used for electrophoresis.

Thawed nematodes were prepared for electrophoresis by adding an equivalent volume of lysing solution (100 μl β-mercaptoethanol, 10 mg nicotinamide-adenine dinucleotide phosphate and 100 ml distilled water) to the thawed nematode prior to homogenization by hand. Samples of host tissue, used on electrophoretic gels as controls (i.e. to distinguish between parasite-specific and host-specific loci in nematode samples), were prepared by adding an equivalent volume of lysing solution to the thawed sample which was then sonicated and centrifuged at 5000 *g* for 10 min at 4°C. Electrophoresis was conducted on cellulose acetate, 'Cellogel' (Malta s.r.l., Milan) according to the methods described by Richardson *et al.* (1986). Electrophoretic gels were run at 200 v for 2–2.5 h, then stained histochemically for specific enzymes

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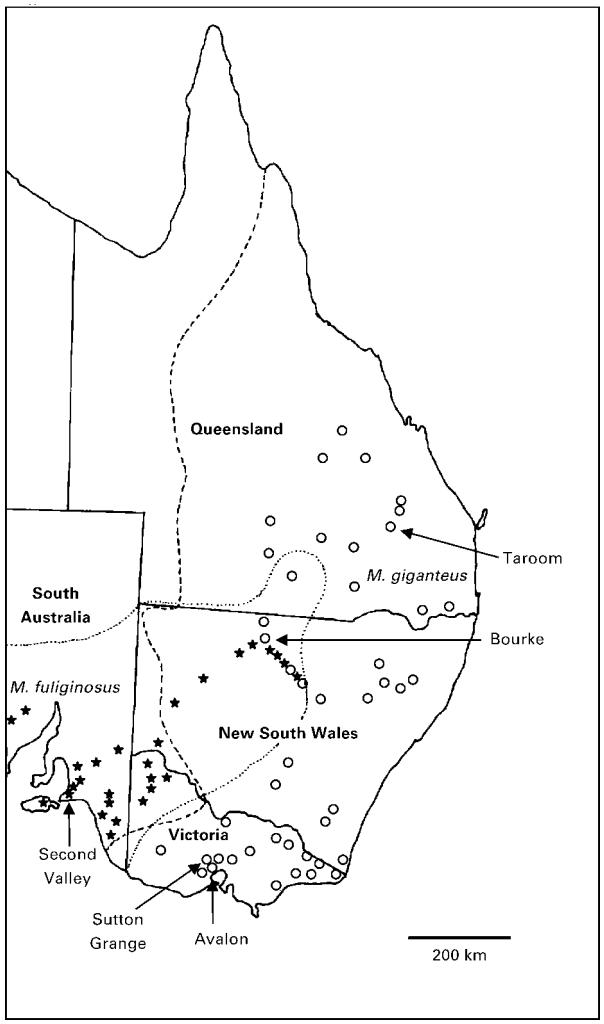

Fig. 1. Distributions of grey kangaroos, *Macropus fuliginosus* and *M. giganteus* in eastern Australia (based on Caughley *et al.*, 1987) and the localities at which *Cloacina obtusa* has been recorded (from Beveridge, 1998) in *M. fuliginosus* (★) and *M. giganteus* (○). Specimens used for electrophoresis were obtained from Taroom in Queensland, Sutton Grange and Avalon in Victoria, Second Valley in South Australia and near Bourke in New South Wales.

(as described in Harris & Hopkinson, 1976; Richardson *et al.*, 1986). The electrophoretic banding patterns of each enzyme locus were interpreted allozymically (i.e. allele with the least electrophoretic mobility from the cathode designated as allele *a*; Andrews & Chilton, 1999).

Results

A total of 18 enzymes, encoded by 20 presumptive loci, gave sufficient staining intensity and resolution to allow reliable genetic interpretation of the nematode samples. These enzymes and their corresponding Enzyme Commission (E.C.) numbers were: aldolase (ALD, E.C. 4.1.2.13), citrate synthase (CS, E.C. 4.1.3.7), enolase (ENOL, E.C. 4.2.1.11), glyceraldehyde 3-phosphate dehydrogenase (GA3PD, E.C. 1.2.1.12), glutamate dehydrogenase (GDH, E.C. 1.4.1.3), aspartate aminotransferase (GOT, E.C. 2.6.1.1), glucose-phosphate isomerase (GPI, E.C. 5.3.1.9), alanine aminotransferase (GPT. E.C. 2.6.1.2), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), malate dehydrogenase (MDH, E.C. 1.1.1.37), mannose-phosphate isomerase (MPI, E.C. 5.3.1.8), nucleoside diphosphate kinase (NDPK, E.C. 2.7.4.6), peptidase valine-leucine (PEP A, E.C. 3.4.13.11), peptidase lysine-leucine (PEP C, E.C. 3.4.13.11), phosphoglycerate mutase (PGAM, E.C. 5.4.2.1), phosphoglucomutase (PGM, E.C. 5.4.2.2), sorbital dehydrogenase (SORDH, E.C. 1.1.1.14) and triose-phosphate isomerase (TPI, E.C. 5.3.1.1). Two enzymes, MDH and PGM were each encoded by two presumptive loci; the locus with the least electrophoretic mobility from the cathode is referred to as locus-1 (i.e. *Mdh-1* and *Pgm-1*). In addition, for each enzyme screened, there was no evidence on the electrophoretic gels that parasite samples were contaminated by host enzymes.

The allelic profiles of each nematode collected from *M. giganteus* and *M. fuliginosus* are shown in table 1. Three enzyme loci, *Enol*, *Ga3pd* and *Tpi*, were invariant among all nematodes. Allelic variation among nematode samples was detected at 17 (85%) of the 20 loci. The multiple-banding patterns at these polymorphic loci were consistent with those expected for heterozygous individuals at loci for monomeric (two bands; *Mpi*, *PepA*, *Pgm-1* and *Pgm-2*), dimeric (three bands; *Cs*, *Got*, *Gpi*, *Gpt*, *Idh*, *Mdh-1*, *Mdh-2*, *PepC* and *Pgam*) and tetrameric enzymes (five bands; *Ald* and *Sordh*). The number of bands for heterozygous individuals at one enzyme locus (*Gdh*) could not be reliably determined. No heterozygous individuals were detected at the polymorphic *Ndpk* locus. One unusual electrophoretic pattern was detected at the *Pgm-2* locus for one female *C. obtusa* (Avalon1-9, table 1) which had four bands compared with all other nematodes, in which a single band (=homozygotes) or two bands (=heterozygotes) were present. The *Pgm-2* banding pattern for this female could be interpreted as evidence of multiple matings resulting in the production of eggs with different combinations of four alleles.

The 16 *C. obtusa* collected from areas in eastern Australia where *M. giganteus* occurs in allopatry, had variable allelic profiles at 13 loci. There were also allelic differences of *C. obtusa* from localities in Queensland (Taroom) and from Victoria (Avalon and Sutton Grange). For example, one *C. obtusa* from Taroom possessed the allele '*c*' for *Gdh* which was not detected in the 14 individuals from Victoria. Similarly, the ten *C. obtusa* from the site in South Australia where *M. fuliginosus* occurs in allopatry, had variable profiles at ten loci. Comparison of *C. obtusa* from localities at which each host species occurs in allopatry showed that there was one fixed genetic difference (i.e. where groups of samples do not share any alleles in common at a locus) between *C. obtusa* from *M. fuliginosus* and *M. giganteus* (for *Idh*, table 1). Other allelic differences were detected between nematodes from areas where both host species occurred in allopatry. For instance, *C. obtusa* from *M. fuliginosus* lacked allele *a* for *Cs*, *Gdh*, *PepA*, *PepC* and *Sordh*, allele *b* for *Gpt*, alleles a and b for *Pgm-2*, and allele *c* for *Got* and *Mdh-1*, which were detected in *C. obtusa* from *M. giganteus* (table 1).

Within the zone of host sympatry, the fixed genetic difference detected at the *Idh* locus between allopatric populations of *C. obtusa* from the two host species, was no

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Table 1. Allelic profiles of individual *Cloacina obtusa* from locations of host allopatry and sympatry.

	Ald	*Cs*	*Enol*	*Ga3pd*	*Gdh*	*Got*	*Gpi*	*Gpt*	*Idh*	*Mdh1*	*Mdh2*	*Mpi*	*Ndpk*	*PepA*	*PepC*	*Pgam*	*Pgm1*	*Pgm2*	*Sdh*	*Tpi*
M. giganteus: in allopatry																				
Avalon1-1	b	b	a	a	a	b	b	a	bd	b	b	d	b	a	ab	bd	ab	c	a	a
Avalon1-2	b	b	a	a	a	ab	b	a	b	b	b	b	b	a	b	b	a	c	a	a
Avalon1-3	b	b	a	a	a	b	b	a	d	b	b	b	b	b	ab	b	ab	a	bc	a
Avalon1-4	b	b	a	a	a	b	b	a	b	b	b	cd	b	a	ab	d	ab	ac	bc	a
Avalon1-5	b	b	a	a	a	b	b	b	bd	b	b	d	b	b	ab	d	ab	cd	c	a
Avalon1-6	b	b	a	a	a	bc	b	ab	b	bc	b	d	b	a	ab	d	a	bc	c	a
Avalon1-7	b	b	a	a	b	b	b	a	b	b	b	d	b	ab	b	bd	a	c	c	a
Avalon1-8	b	b	a	a	ab	ab	b	b	d	b	b	d	b	ab	a	bc	ab	c	ab	a
Avalon1-9	b	a	a	a	a	b	b	ab	ad	b	b	cd	b	ab	ab	d	a	abcd	b	a
Avalon1-10	b	ab	a	a	b	b	b	ab	ab	ab	b	bc	b	ab	b	bc	a	c	ac	a
Sutton1-1	b	b	a	a	b	b	b	a	bd	b	b	bd	b	b	ab	a	a	c	b	a
Sutton1-2	b	b	a	a	a	b	b	a	b	b	b	cd	b	a	b	d	ab	b	bc	a
Sutton1-3	b	–[1]	a	a	b	b	b	a	bd	b	b	d	b	a	a	b	a	c	b	a
Sutton1-4	b	b	a	a	a	ab	b	a	ab	b	b	cd	b	a	b	bd	ab	bc	c	a
Taroom1-1	b	b	a	a	a	b	b	a	b	b	b	c	b	ac	b	ac	b	c	–	a
Taroom1-2	b	b	a	a	bc	b	b	a	ab	b	b	c	b	a	b	c	b	bc	–	a
M. fuliginosus: in allopatry																				
Second1-1	b	b	a	a	c	b	b	a	c	ab	b	c	b	b	b	bc	a	c	b	a
Second1-2	b	b	a	a	c	b	b	a	c	a	b	b	b	b	b	c	a	c	b	a
Second1-3	b	b	a	a	bc	b	b	a	c	b	b	bc	b	c	b	–	b	c	bc	a
Second1-4	–	–	a	a	c	ab	b	–	c	b	b	–	b	b	b	c	b	c	b	a
Second1-5	b	b	a	a	c	b	b	–	c	b	b	–	b	b	b	d	–	c	bc	a
Second1-6	b	b	a	a	c	b	b	a	c	b	b	ab	b	bc	b	c	b	c	b	a
Second1-7	b	b	a	a	c	ab	b	a	c	b	b	bc	b	c	b	c	b	c	b	a
Second1-8	b	b	a	a	c	b	b	a	ce	ab	b	bc	b	b	b	cd	b	c	b	a
Second1-9	b	b	a	a	c	b	b	a	c	b	b	bc	b	b	b	ce	b	–	bc	a
Second1-10	b	b	a	a	c	b	b	a	c	b	b	bd	b	b	b	c	b	cd	b	a
M. giganteus: zone of sympatry																				
Bourke1-1	b	b	a	a	ac	b	b	a	bd	b	b	bc	b	a	ab	bd	bc	bc	b	a
Bourke1-2	b	ab	a	a	–	b	b	a	bd	b	b	bc	b	a	ab	d	bc	ac	–	a
Bourke1-3	ab	b	a	a	ac	b	b	ab	bd	ab	b	bc	b	a	a	ad	bc	bc	b	a
Bourke1-4	b	b	a	a	a	c	b	a	b	ab	b	ab	b	a	b	ad	c	ab	b	a
Bourke1-5	ab	b	a	a	c	ab	b	b	b	b	b	b	b	b	a	c	c	ab	b	a
Bourke1-6	b	ab	a	a	ac	b	b	a	cd	b	ab	bc	b	a	ab	ad	bc	ab	–	a
Bourke1-7	b	b	a	a	a	b	b	a	bd	b	b	d	b	a	ab	d	c	ab	–	a
Bourke1-8	b	b	a	a	ac	b	b	–	bd	b	b	bc	b	a	ab	ad	bc	ab	–	a
Bourke1-9	b	ab	a	a	ac	b	b	b	bd	b	b	–	b	a	ab	d	b	ab	–	a
M. fuliginosus: zone of sympatry																				
Bourke2-1	b	b	a	a	b	ab	b	b	bc	b	b	bc	b	a	a	a	b	–	b	a
Bourke2-2	b	b	–	a	c	b	b	a	b	b	b	cd	b	a	ab	ad	–	–	b	a
Bourke2-3	b	b	a	a	c	ab	b	a	ab	b	b	c	b	a	ab	ad	ab	–	–	a
Bourke2-4	b	b	a	a	bc	b	b	b	c	b	ab	cd	b	a	ab	ad	ac	b	–	a
Bourke2-5	b	–	a	a	b	–	a	b	–	–	b	ab	a	a	ab	d	bc	b	–	a
Bourke2-6	b	b	a	a	a	b	b	b	bc	b	b	bc	b	a	b	bd	bc	–	–	a
Bourke3-1	b	b	a	a	ac	b	b	–	b	b	b	bc	b	a	a	b	bc	–	b	a
Bourke3-2	b	b	a	a	ac	b	ab	a	b	–	b	bc	b	a	ab	d	c	–	ab	a
Bourke3-3	b	–	–	a	ac	b	b	a	–	–	b	bc	b	a	ab	–	–	–	–	a

[1] –, Insufficient staining activity.

longer evident (table 1). For instance, one nematode from *M. giganteus* (Bourke1-6, table 1) possessed the *c* allele, present in *C. obtusa* from the allopatric population of *M. fuliginosus*. Several nematodes from *M. fuliginosus* possessed the *a* and/or *b* allele, which was present in *C. obtusa* from the allopatric populations of *M. giganteus*. Also of interest was the detection of additional alleles for four loci (i.e. alleles *a* for *Ald*, *Gpi*, *Mdh-2* and *Ndpk*) in nematodes from the zone of host sympatry that were not detected in individuals from areas where each host occurred in allopatry.

Discussion

Co-speciation of hosts and parasites has been proposed as a major mechanism of evolution for parasitic organisms, whereby speciation of the parasites follows speciation of their hosts (Manter's rule). This is one of a number of parasitological rules that have been formulated to explain host–parasite coevolution (see Inglis, 1971; Brooks, 1979). Grey kangaroos represent an ideal host species-pair in which to investigate whether speciation in the host always results in the formation of species-pairs of parasites. This is because grey kangaroos have speciated recently (within < 1 million years) and harbour a number of parasitic helminths (Beveridge & Arundel, 1979; Spratt *et al.*, 1991), many of which, including *C. obtusa*, do not occur in any other species of macropodid marsupial (Beveridge, 1998).

Electrophoretic results for *C. obtusa* revealed no significant genetic difference among individuals collected from *M. giganteus* in Victoria (Sutton Grange and Avalon) and in Queensland (Taroom), a distance of 1350 km. By contrast, of the 20 enzyme loci examined, a single fixed genetic difference at the *Idh* locus was detected between nematodes collected from *M. fuliginosus* and *M. giganteus* in areas where each host occurs in allopatry. All *C. obtusa* from allopatric populations of *M. fuliginosus* had the *c* and/or *e* alleles while those from allopatric populations of *M. giganteus* had the *a*, *b* and/or *d* alleles. Fixed genetic differences in the order of 15% between allopatric populations is generally indicative of the existence of more than one species, provided that a sufficient number of enzyme loci has been examined (Richardson *et al.*, 1986; Andrews & Chilton, 1999). This has been the case in other electrophoretic studies of morphologically distinct species of strongyloid nematode (Beveridge *et al.*, 1993; Chilton *et al.*, 1993; Chilton & Smales, 1996; Smales & Chilton, 1997). Based on this premise, the single fixed genetic difference (=5%) between allopatric populations of *C. obtusa*, does not refute the null hypothesis that *C. obtusa* represents a single species. However, if the fixed difference at the *Idh* locus were maintained in the zone of host sympatry, such that no heterozygous individuals existed, then the single fixed genetic difference would establish the absence of gene flow between two sympatric populations of sexually reproducing diploid organisms (Richardson *et al.*, 1986), therefore indicating the presence of two species. Examination of electrophoretic data for *C. obtusa* from the zone of host sympatry revealed that one individual from *M. giganteus* possessed an allelic profile for *Idh* (*cd*) intermediate between the *C. obtusa* from the allopatric populations of *M. giganteus* and *M. fuliginosus*. The same situation occurred for two *C. obtusa* from *M. fuliginosus* in the zone of host sympatry. Also, several individuals had an allelic profile for *Idh* typical of *C. obtusa* from allopatric populations of *M. giganteus*. These data therefore support the null hypothesis that *C. obtusa* represents one species, but that there is genetic divergence between populations from the two host species in allopatric parts of their distributions.

Comparison of electrophoretic data for *C. obtusa* with those of congeners further support the null hypothesis that *C. obtusa* represents a single species. For example, no fixed genetic difference was detected at 19 enzyme loci among populations of *C. communis* from three species of macropodid marsupial host, *M. robustus*, *M. antilopinus* and *M. agilis* (see Chilton *et al.*, 1997b), with overlapping distributions (Strahan, 1995). In the study by Chilton *et al.* (1997b), *C. communis* was collected over an equivalent geographical distance to that for *C. obtusa*. Similarly, no significant genetic differences were detected at 18 enzyme loci among different populations of *C. similis* from four hosts, *M. dorsalis*, *M. eugenii*, *Petrogale assimilis* and *Thylogale billardierii* (see Chilton *et al.*, 1997b), collected from geographical localities up to 2000 km apart. Two of these hosts, *M. eugenii* and *T. billardierii*, are each geographically isolated on islands (Strahan, 1995). Results for *C. obtusa*, *C. communis* and *C. similis* stand in contrast to those for the *C. petrogale* complex, in which fixed differences at 18–44% of loci were recorded between species (Chilton *et al.*, 1997b).

The genetic divergence detected between allopatric populations of *C. obtusa* from *M. giganteus* and *M. fuliginosus* (Nei's $D = 0.18$) is, nonetheless, greater than that between the two host species (Nei's $D = 0.03$; Baverstock *et al.*, 1985). This suggests that evolutionary change in *C. obtusa* populations may be occurring at a faster rate than in the hosts (which is consistent with the findings for other parasites; e.g. Hafner *et al.*, 1994; Page *et al.*, 1998; Paterson *et al.*, 2000; Johnson *et al.*, 2003), even though the different *C. obtusa* populations have not diverged sufficiently to become separate species. This lack of parasite speciation following a host speciation event has also been reported for the louse, *Austrogoniodes cristati*, an ectoparasite of all six species of crested penguin (see details in Paterson & Banks, 2001) and for several species of feather lice of pigeons and doves (Johnson & Clayton, 2003; Johnson *et al.*, 2003).

Johnson *et al.* (2003) have suggested that a failure to speciate is likely to occur when gene flow among parasite populations is much higher than that of their hosts. In their study of wing lice, one species (*Columbicola theresae*) failed to speciate following allopatric speciation in their avian hosts (*Streptophelia vinacea* and *S. capicola*), which are sister taxa (see Johnson *et al.*, 2003). However, in this case, there were no barriers to gene flow among *C. theresae* on the host sister taxa because the parasites also occur on other avian hosts which are sympatric with *S. vinacea* and *S. capicola* (see Johnson *et al.*, 2003). A similar situation was reported for a second louse species, *C. macrourae* (see Johnson *et al.*, 2003). Therefore, Johnson *et al.* (2003) concluded that lice which are host-specific are unlikely to

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fail to speciate following allopatric speciation in their host. This is not the case for *Cloacina obtusa*, as this nematode is host-specific (Beveridge, 1998), even though its hosts (grey kangaroos) occur in sympatry with other species of macropodid marsupial, but failed to speciate following speciation in grey kangaroos. Furthermore, the lack of co-speciation of *C. obtusa* with its hosts is markedly different to that for other parasitic helminths of grey kangaroos examined thus far. For example, there are two morphologically distinct species of intestinal nematode, *Macropostrongyloides baylisi* and *M. yamaguti*, that are found in the large intestine of *Macropus giganteus* and *M. fuliginosus*, respectively (Beveridge *et al.*, 1993), although occasional host-switching has been recently detected (Morris *et al.*, unpublished data). Comparison of the allelic profiles showed fixed genetic differences between the two species of *Macropostrongyloides* at 33% of loci in areas of host sympatry. Comparison of the genetic data with other species in the genus *Macropostrongyloides* further suggests that these nematodes represent a species-pair, having co-speciated with their hosts (Beveridge *et al.*, 1993). Another species-pair of strongylid nematode in grey kangaroos, *Paramacropostrongylus typicus* in the stomach of *Macropus fuliginosus* and *P. iugalis* in the stomach of *M. giganteus*, are morphologically and genetically distinct (Chilton *et al.*, 1993). In the zone of sympatry between *M. giganteus* and *M. fuliginosus*, host-switching occurs. However, unlike the situation for *Macropostrongyloides*, there is also evidence of hybridization between *P. iugalis* and *P. typicus* in the zone of host sympatry (Chilton *et al.*, 1997a). There is also genetic evidence to show that the morphologically distinct anoplocephalid cestodes, *Progamotaenia effigia* and *P. festiva*, represent a species-pair in grey kangaroos (Beveridge & Spratt, 1996), even though there are morphologically similar species within the *P. festiva* species complex in other macropodid and vombatid marsupials (Baverstock *et al.*, 1985).

The results obtained in the present study, and in previous electrophoretic studies on parasites of grey kangaroos (Baverstock *et al.*, 1985; Chilton *et al.*, 1993, 1997a), suggest that the chance events or the processes which drive speciation may not be the same for different parasite taxa, even when they parasitize the same host species. In the case of *C. obtusa*, speciation of the host appears not to have led to speciation of the parasite (based on electrophoretic data), whereas in other nematode genera, there has been host–parasite co-speciation, resulting in species-pairs of nematodes (e.g. *M. baylisi* and *M. yamaguti*, *P. typicus* and *P. iugalis*), with varying degree of reproductive isolation in the zone of host sympatry. It is uncertain as to the reasons why *C. obtusa* failed to speciate following host speciation. These findings further highlight the problems associated with the universality of the 'rules' of parasite evolution.

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