Comparative evolutionary genetics of trematode parasites (Plagiorchiidae) and their anuran hosts

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A study of five trematode species of the genera *Glypthelmins* and *Haplometrana* inhabiting anurans produced allozyme-based estimates of Nei's (1978) genetic distance ranging from 0.126 to 1.867. An estimate of trematode phylogeny is in agreement with earlier morphologically based phylogenetic hypotheses and is similar to the phylogeny for associated host ranid and hylid frogs based on rDNA and morphology. A comparison of the genetic distances observed among trematodes with those of their respective hosts suggests that host and parasite genetic distances are not significantly correlated. This is counter to expectations based on a molecular clock for protein evolution in hosts and parasites. These results may reflect (*i*) the effects of sampling error on genetic distance estimates, (*ii*) a non-clocklike rate of protein evolution in hosts and (or) parasites, (*iii*) temporally independent speciation events in associated host and parasite lineages, or (*iv*) the effects of host hybridization.

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L'étude de cinq espèces de trématodes des genres *Glypthelmins* et *Haplometrana*, parasites d'anoures, a donné lieu à une estimation des distances génétiques de Nei (1978) basées sur les allozymes variant entre 0,126 et 1,867. La phylogénie des trématodes établie selon cette étude s'accorde avec les hypothèses phylogénétiques classiques basées sur la morphologie et s'accorde aussi avec la phylogénie des grenouilles (ranidés et hylidés) hôtes fondée sur la morphologie et sur l'étude de l'ADNr. La comparaison entre les distances génétiques observées chez les trématodes et celles observées chez leurs hôtes respectifs semble indiquer que les distances génétiques des parasites et de leurs hôtes ne sont pas en corrélation significative. Cette observation contredit l'hypothèse de l'existence d'une « horloge moléculaire » responsable de l'évolution des protéines chez les hôtes et les parasites. Les résultats peuvent refléter (*i*) les effets d'une erreur d'échantillonnage lors de l'estimation des distances génétiques, (*ii*) l'existence d'un rythme d'évolution des protéines chez les hôtes et (ou) les parasites qui soit de nature autre que celle d'une « horloge », (*iii*) des étapes de spéciation indépendantes du temps chez les lignées des hôtes et des parasites, (*iv*) ou alors les effets d'une hybridation chez les hôtes.

[Traduit par la rédaction]

Review

Parasitologists have frequently noted a correspondence between the patterns of evolutionary divergence for hosts and those for their parasites. These observations prompted a number of early workers to formulate rules for host–parasite coevolution. Fahrenholz's rule suggested that natural classifications for hosts and parasites should correspond with one another (Eichler 1948), and Szidat's rule proposed that a more primitive host will harbour a more primitive parasite (Brooks 1985). The first of Manter's rules (Manter 1955) suggested that parasites typically evolve "more slowly" than their hosts (see Brooks 1985 for a review).

Either selection or neutral genetic drift and mutation may be supposed to play the primary role in generating patterns of host and parasite genetic variation. Price (1980) has speculated about the possible effects of host environments on parasite genetic variability, and one hypothesis suggests that a higher level of host specificity, or fewer intermediate hosts, may result in selection for a narrower range of phenotypes, and hence a lower level of average heterozygosity among parasites; a larger number of hosts may result in selection for a wider range of phenotypes, and higher levels of heterozygosity (see Price 1980; Nadler 1987). If selection is of only minor importance in determining parasite allelic distributions, then mutation and genetic drift are likely to be the principal factors influencing genetic evolution, with levels of heterozygosity then a function of effective population size and average mutation rate.

The explanation chosen for observed levels of heterozygosity among parasites is usually contingent upon whether selection, or drift, is considered to be the evolutionary mechanism of principal importance. A low level of heterozygosity in a parasite population might be interpreted as being due to a small (or fluctuating) effective population size (for a neutral model) or, instead, as being the result of directional selection for a restricted range of phenotypes (for a selection model).

Another approach to evaluating host–parasite evolutionary genetics involves comparing the overall degree of genetic divergence among pairs of associated host and parasite taxa. The basis for such a comparison is the molecular-clock hypothesis, which suggests that proteins evolve, or consolidate mutations, at a relatively constant rate (see Wilson et al. 1977). If hosts and parasites display rates of protein evolution that are equal, or constant, it may be expected that cospeciating hosts and parasites will show similar or correlated amounts of genetic divergence. This might be evidenced by a similarity in the branch lengths of associated nodes of host and parasite phenograms (e.g., Hafner and Nadler 1988) or, more quantitatively, by an overall correlation for genetic distances among associated hosts and parasites (Hafner and Nadler 1990).

Recently, allozyme data have been used in an attempt to evaluate a number of hypotheses concerning the effects of long-term association between populations of hosts and parasites (see Nadler 1990 for a review). From a selectionist perspective, Bullini et al. (1986) suggested that the variation

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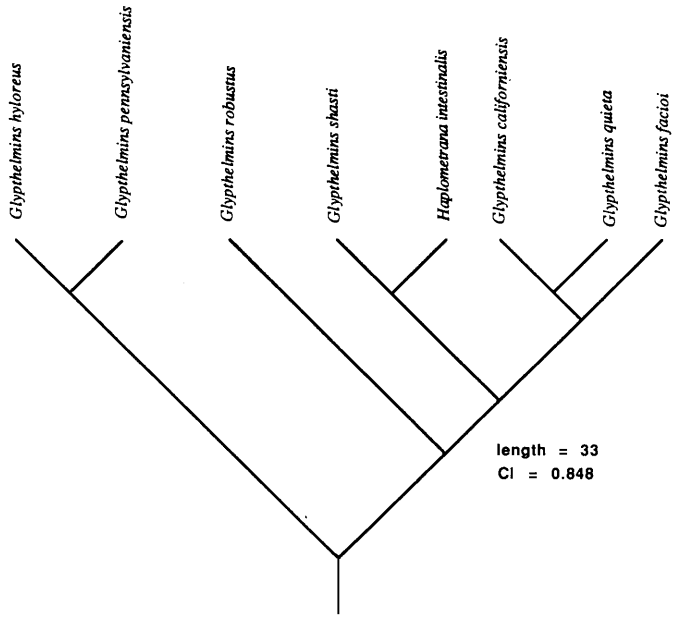

FIG. 1. The most parsimonious tree obtained by O'Grady (1987) based on morphological data for trematodes. CI, consistency index.

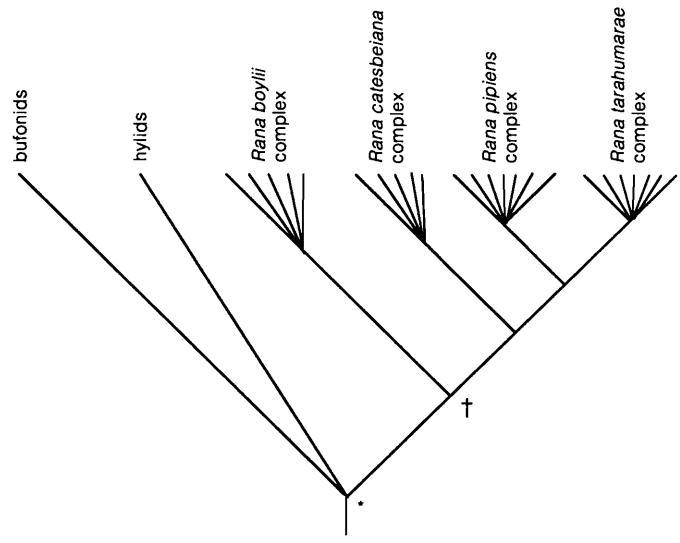

FIG. 2. Partial phylogeny for the ranid frogs based on rDNA data of Hillis and Davis (1986) (†) and postulated relationship to the bufonids and hylids (*). Modified from Hillis and Davis (1986).

they observed in levels of heterozygosity among several species of ascaridoid worms might be a direct result of the worms' differing life histories; some had direct life cycles, and others life cycles involving two or more intermediate hosts (but see Nadler 1987 for a critical evaluation).

From a neutralist perspective, Baverstock et al. (1985) compared interspecific genetic divergence among cestodes of the *Progamotaenia festiva* complex and their host marsupials and attempted to "assess possible cospeciation and host switching." They concluded that "the cestodes are far more divergent genetically than the hosts" and suggested this might be due to either "choice of loci" (some loci appear to evolve more rapidly than others (see, e.g., Sarich 1977), "host-switching in the past," speciation of the "common ancestor of the *Progamotaenia*...long before the evolution of the contemporary Macropodids," or a higher "rate of molecular evolution in the parasite...than in the host."

Merenlender et al. (1987) compared the genetic distance between Chinese and Philippine strains of the blood fluke *Schistosoma japonicum* with that for their molluscan intermediate hosts. They suggested that the similar genetic distances observed, 0.54 for schistosomes and 0.63 for molluscs, "support an evolutionary scenario where parasite and host diverge at similar rates."

Hafner and Nadler (1988) compared phenograms generated from allozyme data for three genera of pocket gophers, *Thomomys*, *Geomys*, and *Orthogeomys*, and the two genera of chewing lice, *Thomomydoecus* and *Geomydoecus*, that they host. They suggested that speciation events at four nodes in host and parasite trees "represent hypothesized cospeciation events in the history of the assemblage." They further concluded that "speciation events represented by...(these nodes) were approximately contemporaneous, and that the host and parasite lineages involved have accumulated protein differences *at equal rates*" (emphasis mine). Hafner and Nadler 1990) reexamined these gopher and lice associations by means of the statistical method of Platnick and Nelson (1978) to compare host and parasite phylogenies, and applied the Mantel test (Mantel 1967) to estimate the degree of correlation between matrices of host and parasite genetic distances. They obtained a significant correlation among host and parasite genetic distances for these comparisons. Page (1990, 1991) also reevaluated Hafner and Nadler's (1988) louse and gopher data, using component analysis to compare topologies and a pseudorandomization approach to compare branch lengths and concluded that the "timing" of speciation events (e.g., branch lengths) among hosts and parasites is not significantly correlated if the influence of topology is factored out.

Introduction

Approximately 20 species of trematodes have been placed in the genus *Glypthelmins*. These worms are exclusively parasites in the small intestine or bile duct of frogs and toads and are widely distributed throughout the Americas, Eurasia, and southeast Asia. Based on morphology, Brooks (1977) recognized four lineages of *Glypthelmins*. Several *Glypthelmins* species of lineages I and II occur in North American frogs and toads. North American *Glypthelmins* species belonging to lineage I include *Glypthelmins californiensis* (Cort 1919) Miller 1930 in western ranid hosts *Rana aurora* and *Rana boylii*; *Glypthelmins shastai* Ingles 1936 in western bufonid host *Bufo boreas*; and *Glypthelmins quieta* (Stafford 1900) Stafford 1905 in eastern ranids of the leopard frog (*Rana pipiens*) complex, as well as bullfrogs (*Rana catesbeiana*) and green frogs (*Rana clamitans*). *Haplometrana intestinalis* Lucker 1931 (=*Haplometrana utahensis* Olsen 1937), of the monotypic genus *Haplometrana*, has also been suggested as a member of the *Glypthelmins* lineage I based on similarities in life cycle, morphology (O'Grady 1987), and allozyme genetics (Rannala 1990*a*) to other lineage I *Glypthelmins*. The host for *H. intestinalis* is the western ranid *Rana pretiosa*.

North American *Glypthelmins* of lineage II include *Glypthelmins hyloreus* Martin 1969 in hosts *Hyla regilla* and *Pseudacris triseriata* in the Midwest, and *Glypthelmins pennsylvaniensis* Cheng 1961 in host *Hyla crucifer* on the East Coast. The proposed phylogenetic relationships (based on the morphological data of O'Grady 1987) among eight species of *Haplometrana* and *Glypthelmins* in the intestines of anurans in North, Central, and South America, including the five species examined in this study, are shown in Fig. 1. The most parsimonious tree

TABLE 1. Electrophoresis conditions and enzymes used to study nine allozyme loci among *Glypthelmins* and *Haplometrana* parasites

Enzyme	EC No.	Running buffer	Voltage (V/cm)	Time (h)
Esterase (EST-2)	3.1.1.1	Tris-citrate II	6.1	12
Fructose-bisphosphatase (FBP)	3.1.3.11	Tris-EDTA-lithium	5.8	12
Glucose-phosphate isomerase (GPI)	5.3.1.9	Tris-citrate II	6.1	7
Glucose-6-phosphate dehydrogenase (G6PDH)	1.1.1.49	Poulik pH 8.7	11.0	7
Isocitrate dehydrogenase (IDH)	1.1.1.42	Tris-citrate II	6.1	12
Malate dehydrogenase (MDH-1)	1.1.1.37	Tris-citrate II	6.1	12
Malate dehydrogenase (MDH-2)	1.1.1.37	Tris-citrate II	6.1	12
Peptidase A (glycyl-L-leucine) (PEP-A)	3.4.13.11	Tris-EDTA-lithium	5.8	12
Peptidase B (L-leucylglycylglycine) (PEP-B)	3.4.13.11	Tris-EDTA-lithium	5.8	12

TABLE 2. Electrophoresis conditions and enzymes used to study 17 allozyme loci among host ranids

Enzyme	EC No.	Running buffer	Voltage (V/cm)	Time (h)
Aconitate hydratase (ACOH-1)	4.2.1.3	Tris-citrate-EDTA	4.4	12
Aconitate hydratase (ACOH-2)	4.2.1.3	Tris-citrate-EDTA	4.4	12
β-glucuronidase (βGLUR)	3.2.1.31	Tris-HCl/borate	11.0	6
Creatine kinase (CK)	2.7.3.2	Tris-HCl/borate	11.0	6
Fructose-bisphosphatase (FBP-1)	3.1.3.11	Tris-citrate-EDTA	4.4	12
Fructose-bisphosphatase (FBP-2)	3.1.3.11	Tris-citrate-EDTA	4.4	12
Glucose-phosphate isomerase (GPI)	5.3.1.9	Tris-citrate II	6.1	7
Isocitrate dehydrogenase (IDH-1)	1.1.1.42	Tris-citrate II	6.1	12
Isocitrate dehydrogenase (IDH-2)	1.1.1.42	Tris-cirtate II	6.1	12
L-lactate dehydrogenase (LDH-1)	1.1.1.27	Tris-citrate III	3.8	18
L-lactate dehydrogenase (LDH-2)	1.1.1.27	Poulik pH 9.5	11.0	8
Malate dehydrogenase (MDH-1)	1.1.1.37	Poulik pH 9.5	11.0	8
Malate dehydrogenase (MDH-2)	1.1.1.37	Tris-citrate-EDTA	4.4	12
Malic enzyme (ME-1)	1.1.1.40	Tris-citrate-EDTA	4.4	12
Malic enzyme (ME-2)	1.1.1.40	Tris-citrate-EDTA	4.4	12
Mannose-phosphate isomerase (MPI)	5.3.1.8	Tris-citrate III	3.8	18
Phosphogluconate dehydrogenase (PGDH)	1.1.1.44	Tris-citrate-EDTA	4.4	18

has 33 steps and a consistency index of 0.848. A phylogenetic analysis of the ranids, based on rDNA data (Hillis and Davis 1986), suggests that the western ranids form a sister-group to the bullfrogs and green frogs plus the eastern ranids. Hylids and bufonids probably represent basal sister-groups to the ranids (Duellman and Trueb 1986) (Fig. 2).

Materials and methods

Sample collection

Anuran hosts for five *Glypthelmins* and *Haplometrana* species were collected during the summer, fall, and spring of 1989 and 1990. Fifty-five *Glypthelmins hyloreus* were obtained from 154 *Pseudacris triseriata* collected near Ogallala, Nebraska; 79 *Glypthelmins pennsylvaniensis* were obtained from 16 *Hyla crucifer* collected at Haliburton, Ontario; 41 *Glypthelmins quieta* were obtained from 41 *Rana clamitans* collected near Battle Creek, Michigan; 76 *Glypthelmins californiensis* were obtained from 28 *Rana aurora* collected near Victoria, British Columbia; and 58 *Haplometrana intestinalis* were obtained from 8 *rana pretiosa* collected at Trail, British Columbia. Parasites were removed from the host's upper small intestine, rinsed in distilled water, placed individually in labelled cryovials, and frozen in liquid nitrogen. Heart, liver, and muscle tissues were taken from hosts. Samples were transported at −60°C on dry ice to the Laboratory of Analytical Systematics of the Royal Ontario Museum and stored in an ultracold freezer at −80°C for 4−12 months.

Electrophoresis

Prior to electrophoresis, individual worms were homogenized in 5 μL of distilled deionized water, using a ground-glass pestle on a frosted depression slide and a "blue ice" stage. Worm homogenates were absorbed directly to 1 × 9 mm Whatman No. 3 filter paper wicks and inserted into chilled (4°C) 11% horizontal starch gels (lot No. 451-1; Connaught Laboratories Ltd., Toronto, Ontario). Individual host liver, muscle, and heart tissue samples were combined and homogenized by hand in an equal volume of distilled deionized water (at 4°C), using a ground-glass homogenizer. Host homogenates were stored from 1 to 4 weeks at −80°C prior to electrophoresis and then loaded to horizontal starch gels using 4 × 9 mm wicks. Host and parasite samples were subjected to horizontal starch gel electrophoresis in a cold room at 4°C (Murphy et al. 1990). Blue ice packs were placed on gels during electrophoresis to conserve enzyme activity. Six slices (1.5 mm thick) were obtained from each 10 mm thick gel following electrophoresis, and each slice was histochemically stained for a different enzyme system. A maximum of six enzymes could be scored for each individual worm. Host tissue samples were included alongside parasite samples as controls on all gels to ensure that host enzymes, which might be present on the parasite epithelium or in the gut, were not mistaken for parasite allozymes. Apparent host allozymes were observed on several gels (Rannala 1991).

Sequential electrophoresis of host samples allowed each individual to be scored for most of the enzyme systems examined. Gel slices were incubated in staining solutions at 37°C for periods ranging from 10 min to 4 h. The staining procedures are described by Murphy et al. (1990).

Eight specific enzyme systems, representing nine putative loci, could be reliably scored for the five trematode species examined. The combinations of enzymes and electrophoretic conditions that provided consistent resolution for the trematodes are shown in Table 1. Twelve specific enzyme systems, representing 17 putative loci, were scored for the hosts. The combinations of enzymes and electrophoretic conditions that provided consistent resolution for the hosts are shown in Table 2.

TABLE 3. Distribution of alleles at nine loci in five species of trematodes

Locus	Allele	Taxon 1	2	3	4	5
Est-2		(2)	(9)	(3)	(8)	(7)
	a	1.000	1.000	0.000	0.000	0.000
	b	0.000	0.000	0.667	0.875	0.000
	c	0.000	0.000	0.000	0.000	1.000
	d	0.000	0.000	0.333	0.125	0.000
Fbp		(4)	(9)	(6)	(12)	(10)
	a	1.000	1.000	0.000	1.000	0.000
	b	0.000	0.000	1.000	0.000	0.000
	c	0.000	0.000	0.000	0.000	1.000
Gpi		(4)	(9)	(7)	(12)	(11)
	a	0.000	0.000	0.000	1.000	0.000
	b	1.000	1.000	0.000	0.000	0.000
	c	0.000	0.000	0.000	0.000	1.000
	d	0.000	0.000	1.000	0.000	0.000
G6pdh		(2)	(7)	(6)	(5)	(2)
	a	1.000	1.000	1.000	1.000	1.000
Idh		(2)	(13)	(7)	(13)	(7)
	a	0.000	0.000	0.000	0.000	0.929
	b	0.000	0.115	0.000	0.192	0.071
	c	0.500	0.731	0.000	0.538	0.000
	d	0.000	0.000	1.000	0.000	0.000
	e	0.500	0.154	0.000	0.269	0.000
Mdh-1		(4)	(7)	(4)	(9)	(8)
	a	1.000	1.000	0.000	0.000	0.000
	b	0.000	0.000	1.000	1.000	1.000
Mdh-2		(4)	(10)	(8)	(13)	(12)
	a	1.000	1.000	0.000	0.000	0.000
	b	0.000	0.000	1.000	0.000	0.000
	c	0.000	0.000	0.000	1.000	0.000
	d	0.000	0.000	0.000	0.000	1.000
Pep-A		(2)	(6)	(2)	(4)	(6)
	a	1.000	0.000	1.000	0.750	0.000
	b	0.000	1.000	0.000	0.000	0.000
	c	0.000	0.000	0.000	0.000	1.000
	d	0.000	0.000	0.000	0.250	0.000
Pep-B		(2)	(5)	(3)	(5)	(5)
	a	0.500	0.700	0.000	0.000	0.000
	b	0.500	0.300	1.000	1.000	1.000

NOTE: Taxa are designated as follows: 1, *Glypthelmins hyloreus*; 2, *Glypthelmins pennsylvaniensis*; 3, *Glypthelmins quieta*; 4, *Glypthelmins californiensis*; 5, *Haplometrana intestinalis*. Numbers in parentheses are sample sizes.

Phylogenetic analysis

Nei's unbiased genetic distances (Nei 1978) were estimated among hosts and parasites based on allozymes by means of the BIOSYS-1 computer program (Swofford and Selander 1981). Parasite phylogeny was estimated by UPGMA clustering using genetic distances (Nei 1987) and parsimony analysis based on gene frequency (with explicit enumeration), using the FREQPARS computer program (Swofford and Berlocher 1987; Swofford 1988). A nonparametric permutation test based on the Mantel (1967) statistic and computer pseudorandomization (a program in PASCAL is available from the author) was used to compare pairwise genetic distances among hosts and parasites for correlation.

Results

The distribution of alleles for nine loci scored among the five trematode species are shown in Table 3. The alleles for 17 loci scored among the three ranid host species are shown

TABLE 4. Distribution of alleles at 17 loci in three species of ranids

Locus	Allele	Taxon 1	2	3
Aco-1		(3)	(8)	(10)
	a	1.000	1.000	1.000
Aco-2		(10)	(8)	(10)
	a	1.000	1.000	0.000
	b	0.000	0.000	1.000
B-Glur		(22)	(7)	(11)
	a	1.000	1.000	1.000
Ck		(17)	(6)	(12)
	a	1.000	1.000	1.000
Fbp-1		(25)	(8)	(10)
	a	1.000	1.000	0.000
	b	0.000	0.000	1.000
Fbp-2		(25)	(7)	(11)
	a	1.000	0.714	1.000
	b	0.000	0.286	0.000
Gpi		(8)	(8)	(14)
	a	0.000	0.000	1.000
	b	0.000	0.750	0.000
	c	1.000	0.250	0.000
Idh-1		(9)	(7)	(7)
	a	0.000	1.000	0.000
	b	0.000	0.000	1.000
	c	1.000	0.000	0.000
Idh-2		(8)	(7)	(5)
	a	1.000	1.000	1.000
Ldh-1		(26)	(8)	(12)
	a	0.000	0.000	1.000
	b	0.000	1.000	0.000
	c	1.000	0.000	0.000
Ldh-2		(25)	(8)	(12)
	a	0.000	0.000	1.000
	b	0.000	1.000	0.000
	c	1.000	0.000	0.000
Mdh-1		(26)	(8)	(3)
	a	1.000	1.000	1.000
Mdh-2		(25)	(4)	(12)
	a	0.000	1.000	0.000
	b	0.000	0.000	0.792
	c	0.000	0.000	0.000
	d	0.560	0.000	0.000
	e	0.440	0.000	0.208
Me-1		(26)	(8)	(14)
	a	0.000	0.000	0.571
	b	1.000	0.000	0.429
	c	0.000	1.000	0.000
Me-2		(25)	(8)	(10)
	a	1.000	0.000	0.000
	b	0.000	1.000	1.000
Mpi		(23)	(8)	(14)
	a	0.000	0.000	0.250
	b	0.022	0.500	0.000
	c	0.174	0.000	0.000
	d	0.804	0.500	0.750
Pgdh		(25)	(8)	(12)
	a	0.000	1.000	0.000
	b	1.000	0.000	1.000

NOTE: Taxa are designated as follows: 1, *Rana aurora*; 2, *Rana pretiosa*; 3, *Rana clamitans*. Numbers in parentheses are sample sizes.

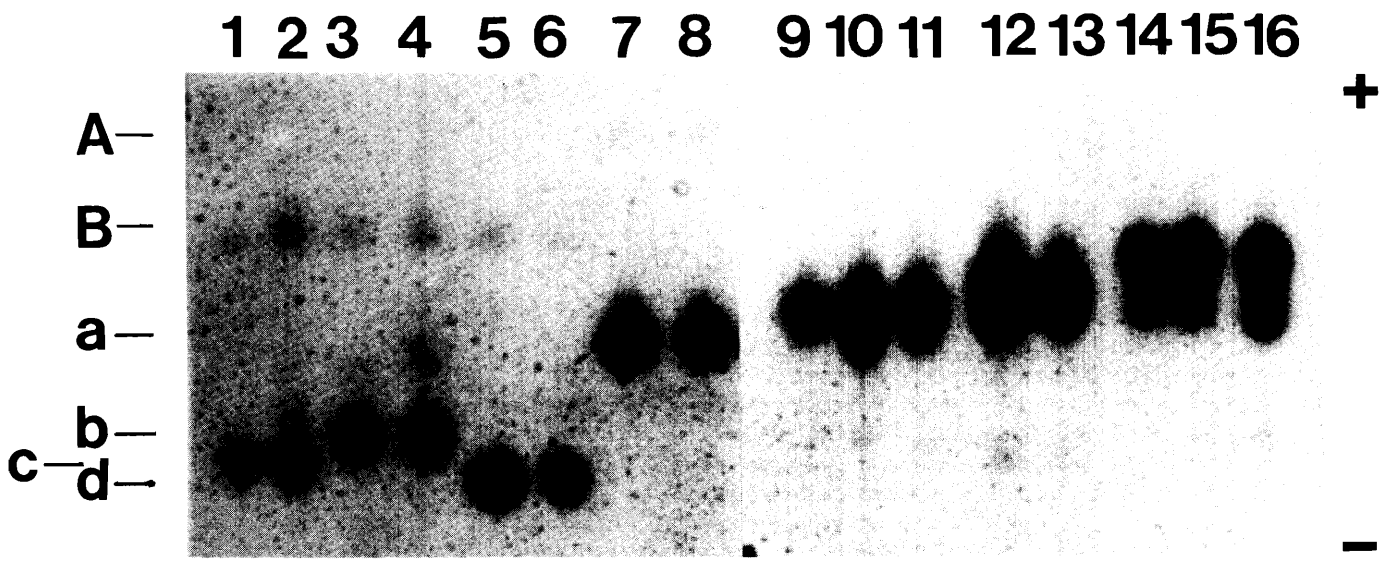

FIG. 3. Photograph of electrophoretic gel stained for malate dehydrogenase (Mdh) enzyme system (Tris-citrate II buffer). Samples are designated as follows: 1, 2 = *Glypthelmins californiensis*; 3, 4 = *Glypthelmins quieta*; 5, 6 = *Haplometrana intestinalis*; 7, 8 = *Glypthelmins pennsylvaniensis*; 9, 10, 11 = *Rana pretiosa*; 12, 13 = *Rana aurora*; 14, 15, 16 = *Rana clamitans*. Putative loci and alleles are designated as follows: A, *Mdh-1* [*a*]; B, *Mdh-1* [*b*]; *a*, *Mdh-2* [*b*]; *c*, *Mdh-2* [*c*]; *d*, *Mdh-2* [*d*]. Note that host allozymes are not adequately resolved using Tris-citrate II buffer.

TABLE 5. Nei's (1978) unbiased genetic distances among five *Glypthelmins* and *Haplometrana* parasites (above diagonal) and Nei's (1987) estimates of variance for genetic distance (below diagonal)

Species	1	2	3	4	5
1. *Glypthelmins hyloreus*		0.126	1.170	0.744	1.704
2. *Glypthelmins pennsylvaniensis*	0.015		1.851	1.052	1.867
3. *Glypthelmins quieta*	0.247	0.596		0.614	1.060
4. *Glypthelmins californiensis*	0.123	0.207	0.094		1.009
5. *Haplometrana intestinalis*	0.522	0.608	0.210	0.194	

in Table 4. A photograph of allozyme patterns for the trematode *Mdh-1* and *Mdh-2* loci is provided in Fig. 3. Unbiased Nei genetic distances (Nei 1978) among parasites, as well as their associated estimates of variance (Nei and Roychoudhury 1974), are shown in Table 5. A comparison, using the *t*-test, of the genetic distance (D) between *Haplometrana intestinalis* and *Glypthelmins californiensis* obtained for this study (D = 1.009; variance = 0.194; average n = 8.278) with that of an earlier analysis (Rannala 1990*a*) considering a partially different subset of loci for these same populations (D = 0.70; variance = 0.10; average n = 15), suggested that the two estimates of genetic distance for these species are not significantly different (α = 0.05). The genetic distances obtained between hosts *Rana aurora* versus *Rana pretiosa*, *Rana aurora* versus *Rana clamitans*, and *Rana pretiosa* versus *Rana clamitans* were 0.640 (variance = 0.053), 0.675 (variance = 0.057), and 0.795 (variance = 0.071), respectively. The genetic distance between *Hyla crucifer* and *Pseudacris triseriata*, based on the allozyme data of Hedges (1986), is 0.719 (variance = 0.033).

An analysis of parasite allozymes, by explicit enumeration, using FREQPARS (Swofford 1988), produced two most parsimonious trees of length 33.018 and a third tree only marginally longer, of length 33.019 (Fig. 4). The tree obtained by UPGMA clustering of trematode genetic distances is shown in Fig. 5 and supports relationships identical to those predicted based on morphology (see Fig. 1). An analysis, using the nonparametric Mantel test, of four pairwise genetic distances among hosts and parasites (10 000 randomizations) resulted in a nonsignificant probability of P = 0.537.

Discussion

If a group of hosts and parasites has cospeciated it may be expected that, if rates of protein evolution are relatively constant for both, similar or correlated genetic distances may be observed among them. If rates are equal and constant among hosts and parasites (e.g., a universal molecular clock) then similar distances should be observed. If rates are constant within parasite and host lineages but differ between the two groups, then correlated, but not necessarily equal, distances should be an outcome (Hafner and Nadler 1990).

Two components are involved in a study of genetic divergence among hosts and parasites. First, a phylogenetic analy-

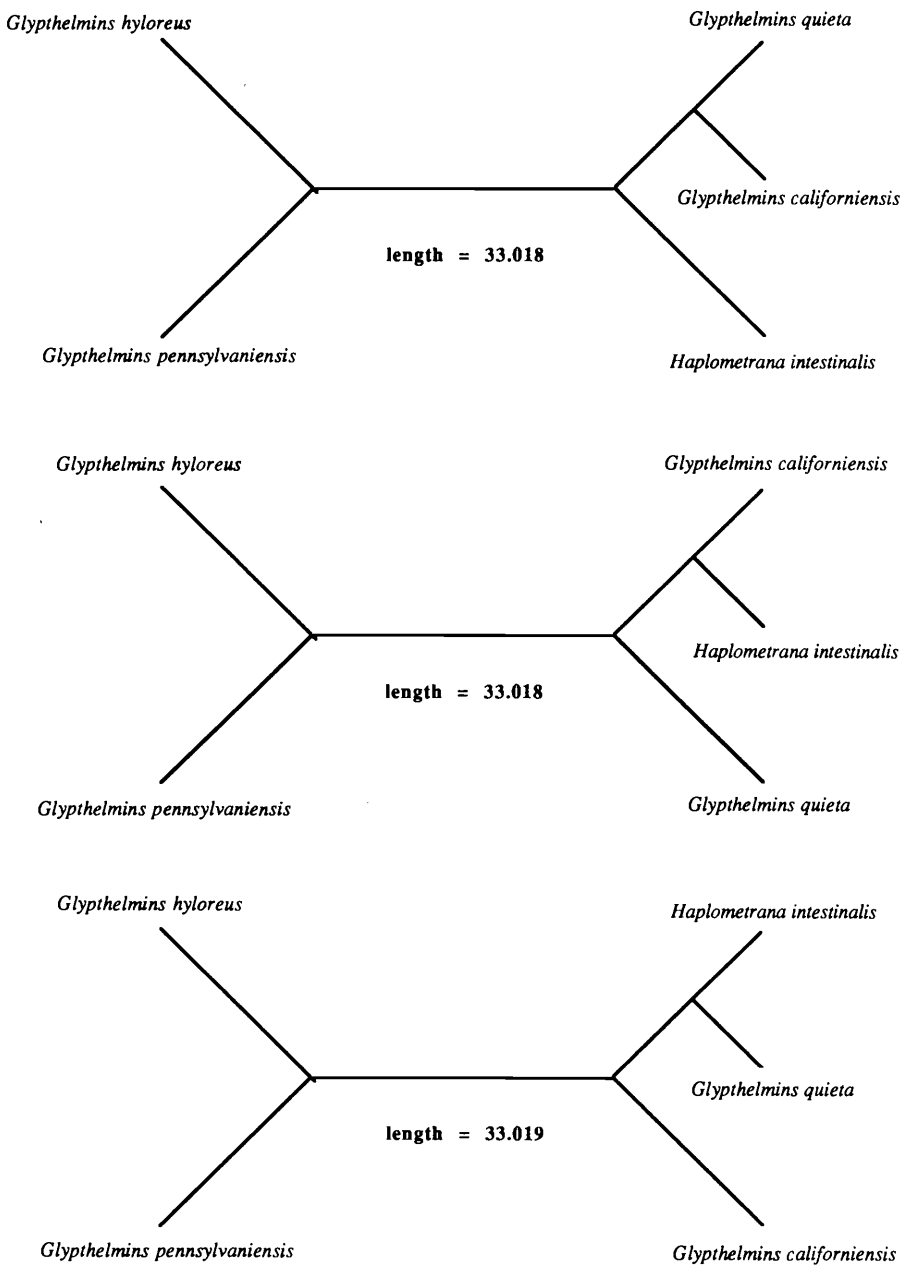

FIG. 4. The three most parsimonious (shortest) trees produced for trematode allozyme data by means of FREQPARS computer program.

sis may indicate whether hosts and parasites have cospeciated (i.e., have similar phylogenies). Second, if hosts and parasites appear to have cospeciated, a matrix correspondence test may determine whether pairwise genetic distances among associated hosts and parasites are correlated.

In this study, a phylogenetic analysis using FREQPARS and UPGMA clustering methods provided differing levels of resolution of the relationships among the flatworm species examined (Fig. 4 and 5). The three most parsimonious unrooted trees obtained by a FREQPARS analysis differentiate *Glypthelmins hyloreus* + *Glypthelmins pennsylvaniensis* and *Glypthelmins quieta* + *Glypthelmins californiensis* + *Haplometrana intestinalis* as comprising two independent lineages but fail to resolve relationships within the latter group. The UPGMA tree (Fig. 5) supports relationships that are in agreement with existing morphologically based phylogenetic estimates. A parsimony based comparison of host and parasite phylogenies, using a morphological phylogenetic estimate for the parasites that included the taxa examined in this study (O'Grady 1987), suggested that the five parasite taxa examined are all cospeciated with their current hosts, with the exception of *G. californiensis*, which appears to have evolved with host *Rana montezumae* in Mexico and to have been secondarily acquired in host *Rana aurora*. Hence, there is independent evidence for host–parasite cospeciation among four of the five taxa examined.

Genetic distances, based on allozyme data, among trematodes inhabiting ranids typically appeared greater than those for their hosts; distances among trematodes of hylids appeared either smaller or greater (see Table 5). In addition, no overall correlation was observed between host and parasite genetic distance matrices: the Mantel (1967) test probability value of 0.537 was not significant.

There are several possible explanations for an observation of concordant host and parasite phylogenies (based on mor-

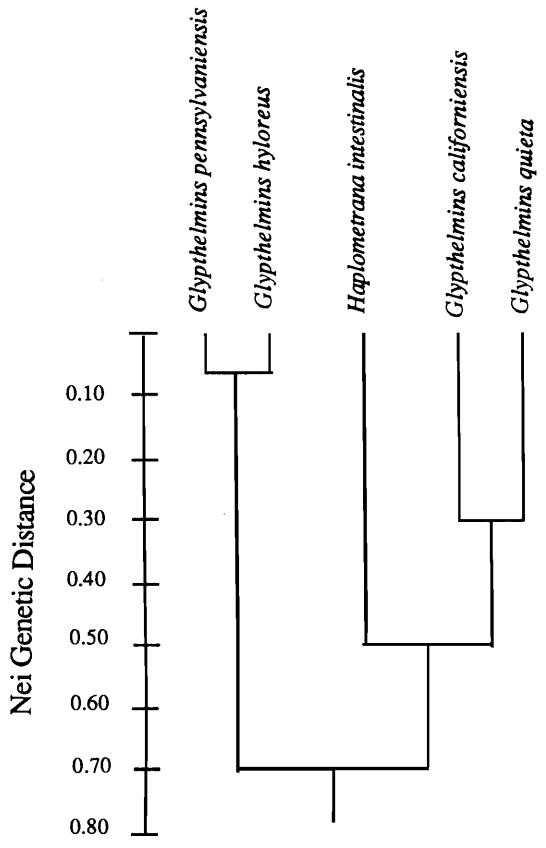

FIG. 5. Tree obtained by UPGMA clustering of Nei (1978) genetic distances for trematodes based on allozyme data.

phology) and noncorrelated genetic distances. First, rates of protein evolution may be highly variable in one, or both, lineages. Second, parasite speciation events may not be strictly temporally linked to host speciation events. If host populations experience greater gene flow than parasites (e.g., have a greater vagility), gene introgression among parapatrically speciating host populations might be greater than among their parasites, resulting in less host genetic divergence. If the degree of linkage among host and parasite speciation events varies for each host–parasite population-level association, this might produce a heterogeneity of distances that is independent of the rate of protein evolution. This potential mechanism is, in some respects, a stochastic counterpart to Hafner and Nadler's (1990) "delayed cospeciation." A number of species of ranid frogs are known to hybridize, and *R. aurora* and *Rana pretiosa* have been reported in sympatry in British Columbia (Licht 1974). Hybridization might also tend to reduce host genetic distances.

In addition, Nei's (1978) genetic distance is not strictly linearly related to time for most evolutionary genetic models beyond about a D value of 1.0 (Nei 1987, pp. 236–238). In this study, 7 of 10 genetic distances for parasites are greater than 1.0 (Table 5), and the nonlinearity of these large distances might be expected to influence correlations among host and parasite matrices. Another confounding factor is the absence of genetic distances for the ranids versus the hylids; these two families are simply too distantly related for an allozyme comparison to be meaningful. As well, the estimate of genetic distance between hylid species *Pseudacris triseriata* and *Hyla crucifer* is based on data from Hedges' (1986) study, and the individuals sampled are not from the same region as the hylids sampled for parasites *G. hyloreus* and *G. pennsylvaniensis* in this study; this might also distort the results of a correlation analysis.

More importantly perhaps, sampling error may play a significant role in generating the above observations. Interspecific variance for Nei's (1978) genetic distance depends primarily on the degree of divergence and number of loci examined among individuals (see Nei 1987). Typically, allozyme studies of parasites have included fewer loci than studies of free-living organisms, and the resulting estimates of genetic distance are therefore subject to a greater variance and standard error (Rannala 1990*b*). In this study, the small number of loci scored for the parasites, and the large genetic distances observed, have resulted in large variances for the genetic distance estimates (lower half of Table 5). These variances might also be expected to influence estimates of the correlation among host and parasite matrices and should be taken into account when considering the results.

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