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GENETIC VARIATION IN THE *HETERODOXUS OCTOSERIATUS* GROUP (PHTHIRAPTERA): A TEST OF PRICE'S MODEL OF PARASITE EVOLUTION

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Abstract—BARKER S. C., BRISCOE D. A., CLOSE R. L. and DALLAS P. 1991. Genetic variation in the *Heterodoxus octoseriatus* group (Phthiraptera): a test of Price's model of parasite evolution. *International Journal for Parasitology* **21**: 555–563. Most of the genetic variation in the *H. octoseriatus* group is present as fixed gene differences between species which have been described on morphological criteria. Based on allozymes, the taxonomic status of some species was challenged. There was insufficient evidence, however, to demonstrate that these were not 'good' biological species. Overall, the limited intraspecific variation was present as fixed gene differences among lice from different hosts and from different colonies of hosts; heterozygotes were rare. Two predictions derived from Price's model of parasite evolution were met: populations of lice were genetically homogeneous and, where genetic markers were present, we found substantial genetic variation among populations. These data contrast with those for endoparasitic helminths, where, in general, the amount of genetic variation is similar to that of free-living invertebrates.

INDEX KEY WORDS: Allozyme electrophoresis; genetic variation; heterozygosity; alleles; polymorphic loci; lice; *Heterodoxus octoseriatus* group; parasite evolution; rock-wallabies.

INTRODUCTION

PRICE (1977, 1980) has proposed a general model for the evolution of parasites. From this model he predicted that species of parasites are comprised of "small, relatively homozygous populations with little gene flow between populations, which result in many specialized races, rapid evolution and speciation without geographic isolation, and an abundance of sibling species". These predictions contrast with earlier axioms of parasite evolution, particularly those for phthirapterans (lice) (Metcalf, 1929; Hopkins, 1949), that, compared with other animals, evolution is slower in parasites. The axioms arose from observations that many groups of parasites are morphologically conservative. However, conservative morphology does not necessarily reflect conservatism in other facets of evolution. Recent studies, using allozyme electrophoresis, have revealed considerable genetic variation, including cryptic species, within some species of parasites described on morphological criteria (e.g. Baverstock, Adams & Beveridge, 1985 for *Progamotaenia* spp.; Adams, Andrews, Robinson, Christy, Baverstock, Dobson & Blackler, 1989 for *Naegleria* spp.).

Price's predictions also contrast with data from endoparasitic helminths. With few exceptions the genetic variability of species of endoparasitic helminths is apparently similar to those of free-living species of invertebrates (Nadler, 1990). In the present study, Price's predictions are tested for a second group of parasites; the lice (Phthiraptera). Three questions are considered for species in the *Heterodoxus octoseriatus* group:

1. Are the 11 species in the *H. octoseriatus* group 'good' biological species? This question was addressed in two ways. Firstly, are some species described on morphological criteria conspecific, and secondly, do some of the species contain cryptic species?

2. Are populations of species in the *H. octoseriatus* group genetically homogeneous? A population is defined as the lice infecting the rock-wallabies in a single colony; and

3. Is there much genetic variation among populations of lice in the *H. octoseriatus* group?

An answer to question 1 is required to define a species in the *H. octoseriatus* group. Questions 2 and 3 were derived directly from Price's model.

MATERIALS AND METHODS

Collection and identification of lice. Forty-nine hosts from

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TABLE 1—ALLOZYMES OF LICE (*Heterodoxus* SPECIES) FROM ROCK-WALLABIES

Species Locality	Host No. *N*	ACP-C	ADA-C	ADA-A	MDH-C	MDH-A	IDH-C	IDH-A	GOT-C	GOT-A	ALDOL	αGPD-C	ENOL-A	FUM	EST	GDA-C	PK	ME	PEP	G6PD	AK-A	AK-C
H. harrisoni																						
Ironhurst	S697 12	–	b	b	b	a	b	-	-	b	b	b	-	-	b	b	b	b	-	-	c	-
Ironhurst	S699 11	–	b	b	b	a	b	c	-	-	-	b	-	a	-	-	-	b	-	b	c	-
Maiden	S902 7	b	b	b	b	a	b	-	a	-	-	b	b	a	-	b	-	b	a	-	d	b
Watch Hill	S922 9	b	b	-	b	a	b	-	a	-	b	b	b	a	b	b	-	b	a	-	c	b
Blackdown	B171 8	b	b	-	b	a	b	-	a	-	-	b	b	a	b	b	-	-	a	-	c	b
H. hughendensis																						
Galah Ck	S727 13	-	b	b	b	a	-	-	-	-	-	-	-	-	-	-	b	a	-	-	-	-
H. closei																						
Alehvale	S878 11	b	b	b	b	a	b	-	-	-	-	-	-	-	b	-	b	-	-	-	-	-
H. lesouefi																						
Porc. Gorge	S721 13	-	-	b	-	-	-	-	-	-	-	-	-	-	-	-	-	c	-	-	-	-
Clyde Park	S726 10	-	b	b	b	a	b	c	-	-	-	b	-	a	a	-	-	c	-	b	-	-
Horse Mtn	S905 9	-	b	b	b	a	b	-	a	-	-	b	b	-	-	-	a	-	a	-	e	-
H. briscoei																						
Donnybrook	S736 20	-	b	b	b	a	b	-	-	-	-	b	-	a	b	-	c	a	-	b	-	-
H. insulatus																						
Alma Bay	B149 51	b	b	b	b	a	b	a	a	a	b	b	b	a	b	b	b	a	a	a	d	b
Maud Bay	B058 5	-	-	b	-	a	-	a	-	-	-	-	-	a	-	-	-	-	-	-	-	-
Maud Bay	B059 12	-	-	-	b	-	b	-	-	-	-	-	-	-	a	-	-	a	-	-	-	-
Cape Ferguson	S874 16	-	b	e	b	-	b	a	-	-	-	-	-	-	-	-	-	a	-	-	-	-
Cape Cleve.	S879 13	-	b	b	b	a	b	-	-	-	-	-	-	-	-	-	-	a	-	-	-	-
H. insularis																						
Mt Fullstop	S851 11	-	c	a	a	b	b	c	-	b	-	a	-	a	b	-	-	c	-	b	-	-
Mt Fullstop	S852 14	b	c	a	a	-	b	-	a	-	b	-	-	-	b	-	a	c	-	-	c	-
Tomahawk Ck	S853 12	b	c	a	a	b	b	-	a	b	b	-	-	-	-	-	a	c	-	b	c	-
Biscuit Ck	S854 13	-	c	a	a	-	b	-	-	b	b	a	-	-	b	b	a	-	-	-	c	-
Biscuit Ck	S855 14	b	c	a	a	b	b	-	a	b	-	-	-	-	b	-	a	c	-	b	c	-
Mt Claro	S860 10	-	-	a	a	b	b	-	a	b	b	-	-	-	b	-	a	c	-	b	-	-
H. insularis—Glen Harding form																						
Glen Harding	B182 7	-	c	-	a	a	d	-	a	-	-	a	b	-	b	-	a	-	d	-	c	-
H. orarius																						
Pinnacle	B185 11	-	c	c	a	a	b	-	a	-	b	a	b	a	b	b	-	-	d	-	a	a
Mt Mulgrave	S927 7	b	c	c	a	a	-	-	a	-	b	a	b	a	b	-	a	a	d	-	d	-
Mt Mulgrave	S929 13	-	c	c	a	a	b	-	a	-	-	a	b	a	b	-	-	a	d	-	d	-
Laura	S511 13	-	e	c	a	-	b	-	-	b	b	-	-	-	b	b	b	a	-	-	-	-
Musgrave	S865 10	-	c	b	a	a	a/b	c	-	-	-	a	b	-	b	-	a	a	-	-	-	-
Musgrave	S866 12	-	c	b	a	a	b	c	-	-	-	a	-	a	b	-	-	-	-	-	d	-
H. murrayi																						
Mitchell R	S930 8	-	c	b	b	a	b	-	a	-	b	a	b	a	b	-	-	a	d	-	c	-
Fall Ck	S868 12	-	c	b/c	-	-	b	-	a	b	-	-	b	-	b	b	b	a	-	-	-	-
H. octoseriatus—Northern form																						
Mt Sebastopol	S885 35	-	b	b	d	a	b	a	-	b	b	c	b	a	a	b	a	a	-	a	-	-
Mt Sebastopol	S886 10	-	b	-	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	b
Kinbombi	S678 6	-	-	-	d	b	b	a	-	b	b	c	b	a	-	-	b	-	-	a	-	-
Cooyar Ck	S679 11	-	-	a	b/d	c	b	a	-	b	b	c	b	a	-	-	b	a	-	a	-	-
Cooyar Ck	S682 13	-	b	a	d	b	b	a	-	-	b	c	b	-	-	-	b	a	-	-	-	-
Cooyar Ck	S681 5	-	-	-	b/d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Round Scrub	S684 11	-	b	b	d	c	b	a	-	b	b	c	c	a	-	-	a	b	-	b	-	-
Round Scrub	S685 5	-	b	b	d	b	-	-	-	-	-	-	-	a	-	-	a	b	-	a	-	-
H. octoseriatus—Southern form																						
Perseverance	S671 13	-	b	-	b	c	b	a	-	b	a	b	-	-	-	-	b	b	-	b	-	-
Perseverance	S672 9	-	b	-	b	c	b	a	-	c	-	-	b	a	-	-	b	b	-	b	-	-
Yarraman Ck	S675 3	-	-	-	-	-	b	b	-	-	-	-	-	a	-	-	b	b	-	b	-	-
Nukinenda	S688 4	-	b	d	b	c	b	-	-	-	-	-	-	-	-	-	b	b	-	b	-	-
H. maynesi																						
Flagstaff Bay	S694 10	-	b	b	b	-	b	-	-	-	-	-	b	a	a	-	b	b	-	-	-	-
Lowestoft	S735 19	-	b	b	b	a	-	-	-	-	-	-	-	a	-	-	b	b	-	-	-	-
Emu Plains	S739 28	-	-	b	b	a	b	-	-	-	-	b	-	a	-	-	-	b	-	-	-	-
Pelican Ck	S744 10	-	b	b	b	-	-	-	-	-	-	b	-	-	-	-	-	b	-	-	-	-

Continued

Table 1 *Continued*

H. maynesi—Whitsunday Is form																								
Joe's Beach	S713 93	b	b	b	b	a	b	a	a	b	b	b	b	a	a	b	a	a	b	a	c	a		
Outgroup																								
H. sp. 14																								
Gloucester Is	S769 49	a	d	-	c	b	c	b	a	c	a	d	a	a	-	a	d	d	d	b	b	a		

S Numbers are host collection numbers, N = Number of lice examined, dash indicates data not available.

38 localities (colonies) in Queensland, Australia were made available to us from a separate study of evolution in the genus *Petrogale* (Table 1). Seven hundred and twenty-one adult lice were collected with forceps from the 49 freshly shot hosts. Latitudes, longitudes and map positions of localities, and the taxa of hosts infected are shown in Tables 1–3 of Barker & Close (1990)—the localities and host collection numbers in Table 1 allow cross reference. Lice were frozen in a portable gas freezer and/or liquid N_2 in the field. Lice were identified to species based on the genitalia of males and females (Barker, in press a). Using dissecting needles (Brady, 1965) and a 50 × dissecting microscope the distal third of the abdomen (containing the genitalia) was removed from samples of frozen lice prior to electrophoresis; the abdomens were prepared as permanent slide specimens (Barker, in press a). Genitalia and/or intact lice were prepared as permanent slide specimens for all but five hosts: B171, B182, S672, S675 and S685 (Barker, in press a). For the latter five hosts, lice were identified to species using a dissecting microscope, and lice from at least one other host from that colony were prepared as permanent slide specimens. For 37 of the 49 hosts, additional series of males and females (up to 25 lice collected from skins held in 70% ethanol, see below) were prepared as permanent slide specimens (Barker, in press a). Forty-eight hosts (37 localities) were infected with species from the *H. octoseriatus* group; the other *P. persephone* from Gloucester Island, Queensland, was infected with *H.* sp. 14 from the *H. ampullatus* group (Baker, unpublished data). The latter was used for out-group reference in a companion paper (Barker, Briscoe and Close, submitted). All 49 hosts were infested with a single species of louse only. Similarly, single species of lice only (from the *H. octoseriatus* group) were collected from another 145 rock-wallabies (Barker & Close, 1990); thus, it is unlikely that more than one species of louse was included in samples of lice used for electrophoresis. The skins of all hosts were held in separate containers of 70% ethanol. A strong jet of water was used to dislodge lice from the fur of hosts and a 180 μm filter collected nymphs and adults. Skins were washed in 15 l of water three times and then until no adult lice were found in two consecutive washes.

Electrophoresis. Samples of frozen lice were homogenized in 5 ml of lysing solution (bromophenol blue 0.01 mg ml^{-1}, nicotinamide adenine dinucleotide phosphate (NADP) 0.1 mg ml^{-1}, nicotinamide adenine dinucleotide (NAD) 0.1 mg ml^{-1}), by grinding in plastic wells with a glass test tube and finely ground glass. Some samples were also vibrated with a sonicator (Sonifer B-12, Branson Sonic Power Co.) to aid homogenization of tissues. Cellulose acetate gels ('Cellogel', Chemetron, Milan) were run for 90 min at 15 V cm^{-1} at 4°C. In a pilot study: (i) none of the electromorphs detected in host blood corresponded with those in the louse homogenate, when fresh host blood was run adjacent to louse homogenate. Thus, blood meals homogenized with the lice did not confuse scoring of the gels; (ii) heterozygotes were absent—101 lice, representing seven of the 11 species in the *H. octoseriatus* group, were analysed individually (14 loci were screened); and (iii) sufficiently concentrated homogenate for only two to four gel applications could be extracted from a single louse (the lice were about 3 mm long and weighed approximately 0.4 mg, perhaps half of which was cuticle). Based on these data five to 10 lice were homogenized together in the main electrophoretic survey [fewer than 13 lice were available for most hosts—the median infestation was 12 adult lice per host (Barker, unpublished Ph.D. thesis, Macquarie University, 1988)]. If multi-banded electromorphs were found, half of the lice in the sample were assumed to be heterozygous and future treatments of lice from that locality for that enzyme would be individual lice. However, this assumption is relatively unimportant as heterozygotes were rare (see below).

OTUs (operational taxonomic units). To address part of question 1 (are the 11 species in the *H. octoseriatus* group 'good' biological species?) species were subdivided into OTUs if samples of lice differed by two or more fixed gene differences (f.g.d) and at least nine loci had been examined.

Analysis. Differences among OTUs and species were assembled as a matrix of per cent fixed gene differences (Richardson, Baverstock & Adams, 1986). The unweighted average linkage method (Sneath & Sokal, 1973) was used to generate cluster diagrams.

RESULTS

Thirty-five putative gene loci were screened. Electromorphs at 21 of these were reliably identified in species from the *H. octoseriatus* group and the outgroup (Tables 1,2). Data are not available for all localities, because insufficient lice were available. This reflects the problems associated with collecting lice from rock-wallabies. Rock-wallabies generally occurred in remote regions, were difficult to catch and were lightly infested [the median infestation of 92 rock-wallabies was 12 adult lice (Barker, 1988, thesis cited above)]. In this study, at least two electromorphs were detected at each of 19 of the 21 loci (91%) examined. Within the *H. octoseriatus* group, at least two electromorphs were detected at each of 17 of the 21 loci (81%) (Table 1).

Heterozygosity and quaternary structure

Heterozygotes were distinguished from homozygotes as multi-banded vs single-banded types. The presence of heterozygotes suggests that lice of the *H. octoseriatus* group are diploid. Heterozygotes were detected at three loci only, ADA-A, IDH-C and MDH-C. As in vertebrates (Richardson *et al.*, 1986), ADA-C is apparently monomeric, having two-banded heterozygotes. IDH-C and MDH-C are apparently dimeric, having three-banded heterozygotes; that is, both homopolymer mobilities and an intermediate heteropolymer band.

TABLE 2—ENZYMES STAINED FOR, THEIR ENZYME COMMISSION NUMBER AND THE ABBREVIATION USED

Enzyme	Enzyme commission number	Abbreviation
Acid phosphatase†	3.1.3.2	ACP
Adenosine deaminase*‡	3.5.4.4	ADA
Malate dehydrogenase*‡	1.1.1.37	MDH
Isocitrate dehydrogenase*‡	1.1.1.42	IDH
Aspartate aminotransferase*‡	2.6.1.1	GOT
Aldolase‡	4.1.2.1.3	ALDOL
Glycerol-3-phosphate‡	1.1.1.8	αGPD
Enolase‡	4.2.1.11	ENOL
Fumarate hydratase‡	4.2.1.2	FUM
Esterase‡	3.1.1.1	EST
Guanine deaminase‡	3.5.4.3	GDA
Pyruvate kinase‡	2.7.1.40	PK
Malic enzyme‡	1.1.1.40	ME
Peptidase‡	3.4.11	PEP
Glucose-6-phosphate dehydrogenase‡	1.1.1.49	G6PD
Adenylate kinase*‡	2.7.4.3	AK

Two loci (C and A) were examined for some enzymes (*). Buffers used: 0.01 M-citrate phosphate pH 6.4 (†), 0.1 M-Tris–EDTA-maleate-$MgCl_2$ pH 7.4 ‡ (see Richardson *et al.*, 1986, for recipes).

TABLE 3—PROPORTION OF LOCI POLYMORPHIC (*P*) IN THE 11 SPECIES OF THE *Heterodoxus octoseriatus* GROUP

Species	Number loci polymorphic/ number loci examined	Proportion (*P*)
H. harrisoni	0/21	0
H. hughendensis	0/6	0
H. closei	0/8	0
H. lesouefi	0/16	0
H. briscoei	0/11	0
H. insulatus	2/21	0.10
H. insularis	2/21	0.10
H. orarius	5/20	0.25
H. murrayi	1/17	0.06
H. octoseriatus	11/17	0.65
H. maynesi	2/21	0.10
Mean		0.11

Samples from lice from only four of 48 hosts infested with lice from the *H. octoseriatus* group contained heterozygotes; each at a single locus. All other lice were homozygous at all loci examined (Table 1). The samples that contained heterozygotes ranged in size from five to 12 lice (Table 1). The proportion of heterozygotes per locus (*H*) in *H. orarius* was 0.0069, in *H. murrayi* 0.0258 and in *H. octoseriatus* 0.0057. No heterozygotes were found in the remaining eight species. The overall mean for the 11 species was 0.0017.

Polymorphic loci

The proportion of loci polymorphic per species (*P*) varied from 0 to 0.65; the mean was 0.11 (Table 3). *H. octoseriatus* (*H* = 0.65) had many more variant loci than the other species; polymorphism in the remaining 10 species varied from 0 to 0.25 (mean 0.06).

Variation in lice from different hosts within individual host colonies

Electrophoretic variation among samples of lice was detected at four of the 10 colonies where lice from two or more hosts were examined: (i) at Musgrave, *H. orarius* from S865 were heterozygous (IDH-C^a and IDH-C^b), while those from S866 were homozygous for IDH-C^b; (ii) at Perseverance Dam, lice from S671 were fixed for Got-A^b, while lice from S672 were fixed for Got-A^c; (iii) at Round Scrub, *H. octoseriatus* from S684 were fixed for MDH-A^c and G6PD^b, while lice from S685 were fixed for MDH-A^b and G6PD^a; and (iv) at Cooyar Ck lice from S679 and S681 were heterozygous for MDH-C^b and MDH-C^d, while lice

TABLE 4—MATRIX OF PER CENT FIXED GENE DIFFERENCES (16 LOCI) AMONG GROUPS OF *Heterodoxus octoseriatus* FROM 10 HOSTS

Locality (host number)	S671	S672	S675	S678	S679	S682	S684	S685	S688	S886
Perseverance Dam (S671)	9	5	9	10	9	11	6	7	11	11
Perseverance Dam (S672)	11.1		6	9	10	8	11	7	7	11
Yarraman Ck (S675)	20.0	16.7		5	6	4	6	4	4	6
Kinbombi Falls (S678)	55.6	44.4	40.0		11	8	11	5	5	11
Cooyar Ck (S679)	40.0	30.0	50.0	9.1		10	13	7	7	13
Cooyar Ck (S682)	55.6	37.5	50.0	0.0	10.0		11	6	7	11
Round Scrub (S684)	36.4	36.4	33.3	36.4	38.5	45.5		8	8	14
Round Scrub (S685)	66.7	57.1	50.0	20.0	57.1	50.0	25.0		7	8
Nukinenda Falls (S688)	0.0	0.0	0.0	60.0	42.9	57.1	37.5	71.4		8
Target Hill (S886)	63.6	54.5	66.7	18.2	23.1	27.3	28.6	25.0	75.0	

Lower matrix contains per cent fixed gene difference, upper matrix contains number of loci used in comparison.

from S682 were homozygous for MDH-C^d, and lice from S679 were homozygous for MDH-A^c, while lice from S682 were homozygous for MDH-A^b (no data for S681).

Variation in lice from different host colonies

Fixed gene differences among lice from different colonies of rock-wallabies were found in six of the 11 species of lice in the *H. octoseriatus* group.

In *H. harrisoni* lice from 'Maiden Springs' were fixed for AK-A^d, while lice from the three other host colonies were fixed for AK-A^c.

In *H. insulatus*, at the ADA-A locus, lice from Cape Ferguson had a fixed gene difference when compared with lice from Alma Bay, Maud Bay and Cape Cleveland; and lice from Alma Bay and Maud Bay were fixed for EST^b and EST^a alleles, respectively (no data were available for EST from the three other localities).

In *H. insularis* lice from 'Glen Harding' had two fixed gene differences (from nine loci) when compared with *H. insularis* from the other four host colonies. These lice were fixed for IDH-C^d, an allele unique to 'Glen Harding', and MDH-A^a, an allele found in all species in the *H. octoseriatus* group. *H. insularis* was divided into two OTUs: *H. insularis*-Glenharding and *H. insularis*.

In *H. orarius*: (i) lice from Laura were fixed for the ADA-C^e and PK^b alleles, which were not found in the other *H. orarius* examined (from 10 loci); (ii) lice from 'Pinnacle' were fixed for a unique allele, AK-A^a, while the other *H. orarius* samples were fixed for AK-A^d; (iii) lice from Musgrave were fixed for ADA-A^b while the other *H. orarius* samples were fixed for ADA-A^c; and (iv) at Musgrave some lice from S865 were apparently heterozygous for the unique allele IDH-C^a—lice from S866 were fixed for IDH-C^b. *H. orarius* was divided into two OTUs: *H. orarius*-Laura and *H. orarius*.

In *H. maynesi* lice from Whitsunday Is. differed at two loci from the four samples of *H. maynesi* from *P. inornata* on the mainland (from 11 loci). Lice from Whitsunday Is. were fixed for PK^a and for ME^a, while those from the mainland had PK^b and ME^b. *H. maynesi* was subdivided into two OTUs: *H. maynesi*-Whitsunday and *H. maynesi*.

In *H. octoseriatus* lice were examined from 12 hosts (seven colonies). Eleven of 17 loci (65%) were either dimorphic (10) or trimorphic (one). (Lice from two hosts S681 (from Cooyar Ck) and S885 (from Mt Sebastopol) were excluded from the following analysis, as only one and three loci were scored.) A matrix of fixed gene differences among louse samples from these 10 hosts (Table 4) revealed surprising heterogeneity. Most samples differed from each other by more than 20% f.g.d. Some samples differed by 75% f.g.d. A cluster analysis of the proportion of f.g.d. between samples divided *H. octoseriatus* into two main groups, which were adopted as OTUs: a northern group containing lice from the four *P. p. herberti* hosts (S678, S886, S684, S685) together with lice from *P. p. penicillata* at Cooyar Ck (hereafter called *H. octoseriatus*-Northern); and a southern group containing lice from the remaining four *P. p. penicillata* hosts (S671, S688, S672, S675) (hereafter called *H. octoseriatus*-Southern, Fig. 1).

Variation between OTUs

As discussed above, four of the 11 species were subdivided into two OTUs; thus 15 OTUs are considered here. Most of the comparisons between the 15 OTUs revealed in excess of 16% f.g.d. (mean 37%, Table 5). Thirteen of the 105 comparisons, however, revealed less than 16% f.g.d. (Table 5). Seven of these involved *H. closei*. The others were *H. harrisoni* and *H. maynesi*; *H. insulatus* and *H. maynesi*; *H. octoseriatus*-Northern and *H. hughendensis*; *H. octoseriatus*-Northern and *H. insulatus*; *H. hughendensis* and *H. insulatus*; and *H. orarius* and *H. murrayi*.

DISCUSSION

According to the biological species concept a species consists of a group of individuals capable of exchanging genetic material with each other (i.e. able to produce viable and fertile offspring), but reproductively

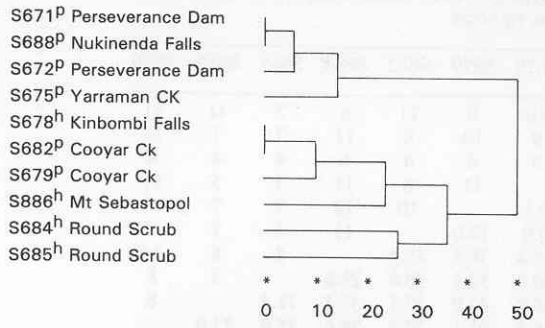

FIG. 1. Unweighted average cluster of per cent fixed gene differences (16 gene loci) among populations of *Heterodoxus octoseriatus*. S, Collection number; [p] *Petrogale p. penicillata*; [h] *Petrogale p. herberti*.

isolated from all other groups (Mayr, 1970; and updated to include the idea of ecological niches in Mayr, 1982). Thus, the essence of a 'biological species' is its genetic cohesiveness (Mayr, 1970 and discussion in Richardson *et al.*, 1986). Consequently, 'good' species can usually be differentiated by enzyme electrophoresis. As a rule of thumb, populations of the same species seldom differ at more than 10% of loci, and almost never at more than 15% of loci (Baverstock, Watts & Cole, 1977). However, 'good' species may be electrophoretically indistinguishable (e.g. Avise, Aquadro & Patton, 1982). Thus, where no electrophoretic divergence is found among populations, it cannot be concluded that those populations are necessarily conspecific. Rather, the electrophoretic data have failed to refute the null hypothesis of a single species (Baverstock, 1987). Further, electrophoretic data cannot be considered in isolation from other taxonomically informative data—in this case detailed morphological studies (Barker, in press a,b).

The first question, are the 11 species in the *H. octoseriatus* group 'good' biological species, has two parts. The first is *are some species described on morphological criteria conspecific*? Thirteen of the comparisons of gene frequencies between the 15 OTUs revealed less than 16% f.g.d.; seven of these involved *H. closei* (Table 5). The analysis, however, may have inadequately assessed the genetic variation present in these cases. In *H. closei*, for example, 11 lice only from a single host were examined. Further, data were available for comparison at a limited number of loci only—seven loci in the case of *H. closei*. Indeed, for only two of the 13 comparisons that yielded less than 16% f.g.d. (*H. octoseriatus*-Northern and *H. insulatus; H. orarius* and *H. murrayi*) were alleles at more than 11 loci compared. Moreover, there is considerable disagreement between the allozyme and morphological data for these species; a phenetic analysis of morphological characters indicates that *H. octoseriatus* and *H. insulatus*, and *H. orarius* and *H. murrayi* are well removed (Barker, in press b). Thus, the electrophoretic data did not provide strong evidence that some species described on morphological criteria are conspecific. Most species differed by at least 16% f.g.d., and thus it appears that the morphological criteria used by Clay (1981) and Barker (in press a) are valid.

The second part of the first question is *do some of the species described on morphological criteria contain cryptic species*? Based on substantial variation among samples from different localities, four species, *H. insularis, H. orarius, H. maynesi* and *H. octoseriatus*, were in each case subdivided into two OTUs. *H. insularis*-Glenharding and *H. insularis* had 20% f.g.d. (10 loci), *H. orarius*-Laura and *H. orarius* had f.g.d. at 22% (nine loci), *H. maynesi*-Whitsunday Is and *H. maynesi* had f.g.d. at 18% (11 loci), and *H. octoseriatus*-Southern and *H. octoseriatus*-Northern had f.g.d. at 36% (14 loci). Despite a thorough morphological analysis, however, no consistent differences

TABLE 5—PER CENT FIXED GENE DIFFERENCE (F.G.D.) AMONG 15 OTUS FROM THE *Heterodoxus octoseriatus* GROUP, AND *H.* SP. 14 (THE OUT-GROUP)

	harri	close	lesou	brisc	inlat	inlar	inlar-G	orari	ora-L	murra	oct-S	oct-N	mayne	may-W	hughe	*H.*14
H. harrisoni		8	16	11	21	18	12	20	10	17	14	17	11	21	6	19
H. closei	0.0		7	7	8	8	6	8	6	7	6	7	7	8	5	6
H. lesouefi	25.0	28.6		11	16	14	12	15	7	14	12	13	11	16	6	14
H. briscoei	18.2	14.3	27.3		11	11	8	10	7	10	10	11	10	11	6	9
H. insulatus	19.0	0.0	31.3	18.2		18	12	20	10	17	14	17	11	21	6	19
H. insularis	38.9	62.5	50.0	63.6	61.1		10	17	10	15	13	15	10	18	6	16
H. i.-Glenharding	50.0	66.7	58.3	62.5	58.3	20.0		12	5	12	8	9	9	11	4	11
H. orarius	30.0	25.0	46.7	40.0	35.0	23.5	16.7		9	17	13	16	11	20	6	18
H. orarius-Laura	40.0	50.0	85.7	57.1	40.0	40.0	60.0	22.2		10	8	10	7	10	5	8
H. murrayi	23.5	14.3	50.0	30.0	29.4	33.3	25.0	11.8	20.0		12	14	11	17	6	15
H. octo.-Sth*	28.6	33.3	41.7	40.0	42.9	69.2	75.0	61.5	62.5	50.0		14	10	14	6	13
H. octo.-Nth	29.4	14.3	38.5	36.4	11.8	53.3	66.7	37.5	40.0	21.4	35.7		11	17	6	15
H. maynesi	9.1	14.3	18.2	30.0	9.1	80.0	66.7	45.5	71.4	36.4	20.0	18.2		11	6	9
H. m.-Whitsunday	33.3	25.0	31.3	27.3	23.8	50.0	50.0	35.0	50.0	29.4	42.9	17.6	18.2		6	19
H. hughendensis	16.7	0.0	33.3	16.7	0.0	100.0	75.0	33.3	60.0	16.7	50.0	0.0	16.7	16.7		5
H. sp. 14	84.2	100.0	78.6	77.8	89.5	75.0	72.7	77.8	100.0	80.0	61.5	86.7	88.9	84.2	100.0	

Lower matrix is f.g.d., upper matrix is number of loci used in comparison.
**H. octoseriatus*-Southern, *H. maynesi*-Whitsunday, *H. insularis*-Glenharding, *H. octoseriatus*-Northern.

could be found between the respective OTUs. In view of the lack of morphological distinction between OTUs and the small number of loci examined, the existing species-level taxonomy is not altered. Genetic variation in *H. octoseriatus* is explored further in Barker, Close & Briscoe (1991). In summary, electrophoretic data did not provide sufficient evidence to refute the null hypothesis that the 11 species in the *H. octoseriatus* group described on morphological criteria are 'good' species.

Are populations of species in the *H. octoseriatus* genetically homogeneous (question 2)? We addressed this question using two related measures of genetic variation; the proportion of loci that were polymorphic (*P*) and the mean level of heterozygosity per locus (per louse) (*H*).

The proportion of loci that were polymorphic (*P*) in the 11 species of the *H. octoseriatus* group (0.11) was smaller than that for 93 species of invertebrates (0.40; Nevo, 1978) and 33 species of helminths (0.23; Nadler, 1990). Moreover, when data for *H. octoseriatus*, which contains extraordinary genetic variation and two OTUs, were excluded the proportion of loci polymorphic (*P*) was reduced to 0.06.

Heterozygous lice were rare in this study; none was found in eight of the 11 species of the *H. octoseriatus* group and the overall mean for *H* was 0.0017. It is possible that the use of buffers other than Tris–EDTA-maleate-$MgCl_2$ and citrate phosphate might have increased the number of heterozygotes found. The different mobilities found, however, were clear and where present, the electrophoretic patterns from heterozygous lice were unmistakable. Support media other than cellulose acetate might also have increased the number of heterozygotes found. Hillburn & Sattler (1986) considered that cellulose acetate gels did not resolve small migration differences as well as starch or acrylamide gels do. When starch support media was used for six species, however, no additional bands were detected nor were the bands more distinct.

Pooling individual lice may also have obscured heterozygotes. If in a sample of lice heterozygotes were outnumbered by homozygotes, slight understaining may have obscured the rare allele. The data presented, however, are consistent with those from the pilot study where 101 individual lice were examined, but no heterozygotes were found.

Thus, the virtual absence of heterozygotes is unlikely to be a technical artefact. Moreover, the data set is large, as 672 lice from 48 hosts were analysed for one to 21 loci.

Such low heterozygosity is unusual. The average heterozygosity (*H*) for 93 species of invertebrates was 0.11 (Nevo, 1978) and for 33 species of helminths was 0.07 (Nadler, 1990). However, some species, albeit a small proportion of the hundreds studied, are characterized by low levels or the absence of genetic variation. These species fall into four general groups:

(i) self-fertilizing species, e.g. three species of arionid slugs which are apparently invariant (Foltz, Ochman, Jones, Evalgelisti & Selander, 1982), and *Echinococcus granulosus* a cestode (McManus & Smyth; 1979; but see Lymbery & Thompson, 1989 and McManus, 1990);

(ii) haplo-diploid, eusocial Hymenoptera which may be subject to selection for reduced heterozygote advantage, and experience severe inbreeding, e.g. Australian bees from the genus *Trigona* (see Wagner & Briscoe, 1983); and *Formica* ants (Pamilo, Rosengren, Vepsalainen, Varvio-aho & Pisarski, 1978);

(iii) out-crossing species that have undergone severe demographic contractions (population bottle-necks) followed by inbreeding, either 'naturally' (e.g. *Anolis angusticeps*, a lizard, Webster, Selander & Yang, 1972), or through exploitation by man (e.g. *Mirounga angustirostris*, the elephant seal, Bonnell & Selander, 1974), and possibly *Acinonyx jubatus*, the cheetah (O'Brien, Wildt, Goldman, Merril & Bush, 1983); and

(iv) parasite species which may regularly pass through population bottle-necks and be subject to inbreeding, e.g. data in Bull, Andrews & Adams (1984) for six species of Australian reptile ticks from the genera *Aponomma* and *Amblyomma*.

Thus, low levels of heterozygosity are invariably associated with either specialized genetic structures or severe population bottle-neck and inbreeding effects.

Despite uneven sex ratios, Barker (1988, thesis cited above) found no evidence for specialized genetic structures (e.g. parthenogenesis) in the *H. octoseriatus* group—that the lice are apparently diploid (assessed by protein subunit patterns) is consistent with this conclusion. Rather, the virtual absence of heterozygotes in this study may be explained by one or more of the following demographic phenomena: (i) founder effects, that is where populations arose from small numbers of 'founder' lice; (ii) population bottle-neck effects, where population size has been regularly and severely reduced, so that subsequent generations arose from the small numbers of lice that passed through each bottle-neck; and (iii) an inbreeding pattern of reproduction.

Inbreeding may indeed occur because individual rock-wallabies were, in general, infested with small numbers of lice (median infestation = 12 adults; Barker, 1988, thesis cited above). Also, the habitat of rock-wallabies may contribute to the above phenomena. Rock-wallabies have specific habitat requirements (e.g. Short (1982) for *P. penicillata*). Consequently, patches of suitable habitat are isolated (island-like) from other suitable sites. Moreover, the social organization of rock-wallabies may limit contact between individual rock-wallabies. In at least one species, adult male and female rock-wallabies form stable relationships and the mating system is apparently controlled through a dominance hierarchy (Barker, 1990). These factors may promote inbreeding in rock-wallabies, and thus, their lice. Some populations of rock-wallabies in the *P. lateralis-penicillata* group are invariant for allozymes and apparently inbred (Briscoe, Calaby, Close, Maynes, Murtagh &

Sharman, 1982; Hopper, Campbell & Moran, 1982).

Is there much genetic variation among populations of lice in the *H. octoseriatus* group (question 3)? A population was defined above as the lice infesting a single colony of rock-wallabies.

In six species, lice from some host colonies were distinguishable at one to eight loci. All but one of these differences were fixed gene differences (Table 1). Further, in three of four colonies where electrophoretic variation in samples of lice was detected, lice from different hosts were homozygous (fixed) for different alleles.

Taken together, these allozyme data do not conform to the single panmictic unit model (*sensu* Richardson *et al.*, 1986) where random mating occurs. Rather, they are consistent with the discrete subpopulation model (*sensu* Richardson *et al.*, 1986). In this case the lice from individual hosts are the subpopulations. While mating within these subpopulations may be random, severe inbreeding may result if the subpopulations are small; as indeed they generally are in the *H. octoseriatus* group (median infestation = 12 adult lice per host; Barker, 1988, thesis cited above).

CONCLUSIONS

Except for *H. octoseriatus*, which comprises northern and southern forms (OTUs), the first prediction derived from Price's model of parasite evolution was not met: strong evidence that species in the *H. octoseriatus* group comprised many specialized races was not found.

The remaining two predictions were met, however; populations were genetically homogeneous and, where genetic markers were present, there was substantial genetic variation among populations of lice. In general, these conclusions contrast with those of Nadler (1990) for endoparasitic helminths. The differences in genetic structure at the population level may be related to fundamental differences in the biology of lice and of helminths. Lice depend on the skin and pelage of their hosts and under natural conditions probably do not survive away from their hosts for more than a day or so—all life stages are completed on the definitive host. Thus, for dispersal, lice depend on the contact their host makes with other animals. Such contact would be most frequent among conspecifics; in this case the rock-wallabies in a single colony. Thus, the habitat and social organization of hosts may have a significant effect on the genetic structure of populations of parasites.

In conclusion, data from this study support part of Price's model; populations were genetically homogeneous and there was evidence of substantial genetic variation among populations of lice. An abundance of specialized races and sibling taxa was not found, however. Rather, the 11 species in the *H. octoseriatus* group are apparently 'good' biological species.

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