

## GENETIC DIFFERENCES AMONG HOST-ASSOCIATED POPULATIONS OF WATER MITES (ACARI: UNIONICOLIDAE: *UNIONICOLA*): ALLOZYME VARIATION SUPPORTS MORPHOLOGICAL DIFFERENTIATION

Dale D. Edwards and Mark Labhart

Department of Biology, University of Evansville, Evansville, Indiana 47722

**ABSTRACT:** *Unionicola poundsi* and *U. lasallei* are recognized as closely related, morphologically distinct species of water mites living in symbiotic association with the mussels *Villosa villosa* and *Uniomereus declivus*, respectively. However, results of a transplant experiment suggested that the morphological characters used to separate these species are plastic and are influenced by the host species in which these mites metamorphose. These results indicate that *U. poundsi* and *U. lasallei* are variants of the same species. To test the validity of these contrasting notions, the genetic structure of mite populations from *Uniomereus declivus* and *V. villosa* was compared. An examination of allozyme variation at 9 enzyme loci revealed a high degree of genetic differentiation between these host-associated populations, with mites from *U. declivus* and *V. villosa* being fixed for different alleles at 3 loci and exhibiting significant allele heterogeneity at 71% of their polymorphic loci. Coefficients of genetic similarity and genetic distance for mites from *U. declivus* and *V. villosa* were 0.36 and 0.95, respectively. The results of this study suggest that mite populations from *U. declivus* and *V. villosa* are genetically distinct and complement morphological data recognizing them as valid species.

Parasitologists have traditionally viewed host–parasite phylogenies as being highly congruent, with the historical development and splitting of parasite lineages paralleling those of their hosts (Fahrenholz, 1913). Although comparisons of several host–parasite genealogical trees lend some support to this commonly held view (Hafner and Nadler, 1988, 1990; Hafner et al., 1994), other studies of parasite evolution have failed to uncover this or any other pattern in the evolutionary diversification of parasites (see Brooks and McLennan, 1993, for review). The former pattern would be predicted if there is a causal relationship between host specificity and the diversification of parasites (Humphrey-Smith, 1989); the latter outcome would be expected if host specificity is not directly involved in parasite speciation (Brooks and McLennan, 1993).

Despite these conflicting views regarding patterns and processes of parasite diversification, there is general agreement that our understanding of parasite evolution must be examined within a phylogenetic framework (Brooks and McLennan, 1993; Thompson, 1994). Furthermore, estimates of host preferences of parasites under natural conditions are necessary prerequisites to reconstructing the evolutionary histories of parasites. Unfortunately, information pertaining to patterns of host utilization for many species of parasites is often not well documented, owing in part to the problems of delineating species populations in nature. For example, morphologically indistinguishable host-associated populations that utilize 2 or more host species may, based on other criteria, represent genetically differentiated populations, each utilizing a distinct host taxon (Waring et al., 1990). Conversely, groups of closely related yet morphologically distinct parasites occurring in association with separate host species may be incorrectly labeled as host specialists if the differences in morphology are due to the hosts (Downes, 1990).

Electrophoretic studies have been useful in elucidating patterns of host utilization among parasitic organisms and in many cases have uncovered considerable amounts of hidden specialization. Parasites previously regarded as host generalists over the entire range of the species are, at the local level, either

genetically distinct host-associated populations that are capable of interbreeding (Waring et al., 1990; Roininen et al., 1993) or complexes of sibling species (Perring et al., 1993; Edwards and Dimock, 1997). In the present study, we used an electrophoretic analysis to address patterns of association between water mites and their host mussels.

Water mites of the genus *Unionicola* are common symbionts of freshwater mussels of the family Unionidae. Their life cycle is complex and includes larvae that leave a host mussel and undergo a brief parasitic phase with chironomid dipterans. Following this association, larvae reinvade a host mussel and undergo developmental transformation that is typical of acariforms, eventually becoming sexually mature adults. The extent to which members of the genus depend on host mussels is variable. Some species are free living as nymphs and adults, using mussels only as a site for oviposition and for postlarval developmental transformations. Other species are obligate symbionts of their bivalve hosts.

The subgenus *Unionicoloides* contains the most diverse assemblage of mussel parasites in North America. Distributional data suggest that members of this subgenus are host specific in 1 or a few related species of freshwater mussels (Vidrine, 1987). Many species within the subgenus are morphologically very similar and are distinguished based on slight differences in a few characters. For example, *Unionicola poundsi*, which occurs in association with the mussel *Villosa villosa*, can be separated from *U. lasallei*, which is specific for the mussel *Uniomereus declivus*, based on subtle differences in the shape of the tarsal claws of legs II–IV and by the shape of the setae of leg I (Vidrine, 1986, 1996). Downes (1986) examined the host recognition behavior of *U. poundsi* from *Villosa villosa* and *U. lasallei* from *Uniomereus declivus* and suggested that these mites were not host specific because they failed to discriminate among these hosts when presented with a choice. Furthermore, Downes (1990) suggested that the morphological characters that were used to distinguish *U. poundsi* and *U. lasallei* were plastic and influenced by the host species in which these mites metamorphosed. Based on these findings, Downes (1990) suggested that mite populations from *Uniomereus declivus* and *V. villosa* comprised a single species of *Unionicola*.

TABLE I. Allele frequencies of polymorphic loci in populations of *Unionicola lasallei* and *U. poundsi*.

Locus	Alleles	<i>U. lasallei</i>	<i>U. poundsi</i>
FUM	100	—	0.04
	80	—	0.96
	60	1.00	—
	n*	26	27
MDH	100	0.26	0.58
	90	0.74	0.37
	77	—	0.05
	n	19	19
MPI	100	0.50	0.40
	86	0.46	0.22
	71	0.02	0.31
	57	0.02	0.07
	n	25	21
PEP	100	—	0.32
	88	—	0.15
	75	0.52	—
	63	0.02	0.52
	25	0.46	—
	n	25	20
PGI	100	1.00	—
	71	—	0.65
	14	—	0.35
	n	20	20
PGM	100	0.48	—
	89	0.50	0.39
	79	0.02	0.50
	68	—	0.11
	n	20	18
6PGDH	100	0.42	0.92
	80	0.04	—
	66	0.54	0.08
	n	22	25

\* n = number of individuals that were sampled/locus/species population.

To test the validity of these contrasting notions regarding the species status of mites from *Unioemerus declivus* and *V. villosa*, the genetic structure of these host-associated mite populations was compared. A preliminary study by Dimock et al. (1995) revealed differences in allozyme banding patterns between *U. poundsi* and *U. lasallei*, lending credence to the original species descriptions of Vidrine (1986).

## MATERIALS AND METHODS

The host mussels, *Unioemerus declivus* (harboring *Unionicola lasallei*) and *Villosa villosa* (harboring *U. poundsi*), were collected from the St. Mark's River, Leon County, Florida (30°24'W, 84°17'N). This study site was used by Downes (1986, 1990) when she examined host specificity and host-induced changes in morphology for *U. lasallei* and *U. poundsi*. Mussels were collected in June 1998 and were transported to the University of Evansville in insulated coolers packed with ice.

In the laboratory, *U. lasallei* and *U. poundsi* were removed from their respective host mussels and washed several times in deionized water. In some instances, mites were electrophoresed within 24 hr of being removed from a host mussel. However, most mites were not used immediately and were stored at -70 C until processed.

Cellulose acetate electrophoresis was used to examine the degree of genetic divergence between *U. lasallei* and *U. poundsi* following the

TABLE II. Results of *G*-contingency tests for allele frequency heterogeneity for the polymorphic loci among the populations of *Unionicola lasallei* and *U. poundsi*.

Locus	<i>G</i>	df	<i>P</i>
FUM	36.5	2	‡
MDH	3.0	2	NS
MPI	5.3	3	NS
PEP	29.0	4	‡
PGI	27.6	2	‡
PGM	13.1	3	†
6PGDH	7.5	2	*

\* *P* < 0.05.

† *P* < 0.01.

‡ *P* < 0.001.

methods of Hebert and Beaton (1989). From each population of mites, 9 enzyme loci were examined, and approximately 20 (range, 18–27) mites were tested for each enzyme locus. The buffers used and the enzymes surveyed were as follows: tris glycine buffer for alcohol dehydrogenase (ADH, EC 1.1.1.1), arginine kinase (ARK, EC 2.7.3.3), fumerate hydratase (FUM, EC 4.2.1.2), mannose phosphate isomerase (MPI, EC 5.3.1.8), peptidase (PEP, EC 3.4.11), phosphoglucomutase (PGM, EC 5.4.2.2), 6-phosphogluconate dehydrogenase (6PGDH, EC 1.1.1.44), and phosphoglucose isomerase (PGI, EC 5.3.1.9) and tris citrate buffer for malate dehydrogenase (MDH, EC 1.1.1.37). Enzymes were resolved following the staining recipes of Hebert and Beaton (1989).

Gels were scored by comparing the mobility of enzymes for *U. lasallei* and *U. poundsi*. Enzymes that migrated the greatest distance from the point of origin were assigned a value of 100, and the mobilities of all other allozymes were reported as a ratio of their migration distance relative to that of the most mobile form. Allozyme phenotypes were classified as genetic polymorphisms when the phenotypes of putative heterozygotes were congruent with those expected on the basis of the quaternary structure of the enzyme in question.

The percentage of polymorphic loci (*P*) was determined by dividing the number of polymorphic loci by the total number of loci examined. A locus was considered polymorphic if the most common allele had a frequency  $\leq 0.95$ . Heterozygote estimates were based on actual counts of detectable heterozygotic genotypes. Mean heterozygosity ( $\bar{H}$ ) was calculated by dividing the number of heterozygotic genotypes in a sample by the product of the number of individuals and the number of allozymes surveyed. *G*-contingency tests were used to test for allele frequency heterogeneity among *U. lasallei* and *U. poundsi*. Genetic divergence between *U. lasallei* and *U. poundsi* was determined by calculating Nei's (1978) coefficients of genetic similarity (*I*) and genetic distance (*D*) for the 2 populations.

## RESULTS

Of the 9 loci that were scored, 2 (ADH and ARK) were monomorphic for both species. The allele frequencies of the polymorphic loci for *U. lasallei* and *U. poundsi* are presented in Table I. Significant differences in the frequency of alleles were observed at 5 of 7 (71%) polymorphic loci (Table II). The proportion of polymorphic loci was identical (*P* = 0.55) for *U. lasallei* and *U. poundsi*, and there was very little difference in average individual heterozygosity between these 2 species ( $\bar{x} \pm SE = 0.33 \pm 0.15$  for *U. poundsi*,  $0.22 \pm 0.14$  for *U. lasallei*).

*Unionicola lasallei* and *U. poundsi* exhibited fixed allele differences for 3 enzyme loci, ADH, FUM, and PGI. In addition, Nei's coefficients of genetic similarity (*I* = 0.36) and genetic distance (*D* = 0.95) revealed a substantial degree of genetic differentiation between these 2 species.

## DISCUSSION

Results of the electrophoretic analysis indicate a high degree of genetic differentiation between *U. poundsi* from *V. villosa* and *U. lasallei* from *Uniomereus declivus*. The *D* value for *U. poundsi* and *U. lasallei* in the present study is substantially greater than that reported for sibling species of *Unionicola* occurring in association with different host mussels. Comparisons between populations of *U. formosa* from *Pyganodon cataracta* and *U. foili* from *Utterbackia imbecillis* revealed a mean *D* value of 0.186 (SE = 0.002) (Edwards and Dimock, 1997). A comparison of the genetic similarity value for *U. poundsi* and *U. lasallei* with values that have been reported from electrophoretic studies used to distinguish among closely related species of insects reveals that the value of *I* for the 2 mite species is substantially lower than mean values of *I* (0.61) reported for nonsibling species of insects but is in accord with the average values of genetic identity (0.30) observed among insect genera within subfamilies (Brussard et al., 1985). The genetic similarity and distance values reported for *U. poundsi* and *U. lasallei* in the present study are consistent with those expected among closely related yet morphologically distinct species of *Drosophila* (Ayala, 1975). The fixed allele differences at 3 enzyme loci (ADH, FUM, and PGI) provide strong evidence that *U. poundsi* from *V. villosa* and *U. lasallei* from *Uniomereus declivus* are reproductively isolated. These data suggest that *U. poundsi* and *U. lasallei* are good biological species, and these data complement the morphological data that distinguish mites from *V. villosa* and *Uniomereus declivus* as separate species (Vidrine, 1986, 1996).

Downes (1990) argued that morphological differences between mites from *V. villosa* and *Uniomereus declivus* were influenced by the host species in which these mites metamorphosed and concluded that these host-associated populations were variants of the same species. It is uncertain whether Downes's study conclusively supports this contention. Downes (1990) claimed that whereas adult *U. poundsi* and *U. lasallei* were consistently found in association with *V. villosa* and *Uniomereus declivus*, respectively, their nymphs occurred with both species of hosts. The data that support this latter assertion were, however, based on extremely small sample sizes. For example, Downes (1990) concluded that nymphs of *U. poundsi* occurred in association with *Uniomereus declivus* when only 1 of the 40 nymphs recovered from field populations of this host species morphologically resembled *U. poundsi*. Downes (1990) also claimed that nymphs of *U. lasallei* metamorphosed into adults that morphologically resembled *U. poundsi* (5 adult mites resembling *U. poundsi* were recovered from 12 mussels) when they were experimentally introduced into *V. villosa*. Whether these findings support host-induced changes in morphology of these mites is questionable for at least 2 reasons. First, Downes did not report how many nymphs were introduced into these mussels at the beginning of the experiment. Second, she did not perform a reciprocal transplant experiment in which nymphs of *U. poundsi* were introduced into *Uniomereus declivus* to determine whether they would metamorphose into adults that morphologically resembled *U. lasallei*.

These criticisms coupled with the genetic data reported in the present study cast seriously doubt on Downes's (1990) conclusion of host-induced morphology among water mites. Conse-

quently, the concerns that she raised regarding the implications of this phenomenon for water mites systematics and patterns of association between water mites and host mussels presently are unfounded. It is possible that the allele frequency heterogeneity and fixed allele differences observed for *U. lasallei* and *U. poundsi* are host induced. If the allozyme data reported for this study are to unequivocally support the contention that *U. lasallei* and *U. poundsi* are distinct species, then it will be important to determine whether and how the host associations established by these species selectively maintain allozyme differences as is true for some phytophagous insects (Feder et al., 1997).

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