Entomol. exp. appl. **52**: 167–172, 1989.

Isozyme analysis of *Hemisarcoptes* and its beetle associate *Chilocorus*

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Accepted: May 25, 1989

Key words: *Chilocorus*, coevolution, *Hemisarcoptes*, phoresy, electrophoresis

Abstract

Mites of the genus *Hemisarcoptes* (Acari: Hemisarcoptidae) are cosmopolitan predators of diaspidid scale insects (Coccoidea: Diaspididae). Heteromorphic deutonymphs (= hypopi) are phoretic on beetles of the genus *Chilocorus* (Coleoptera: Coccinellidae). Isozyme analysis was performed on *H. malus* Shimer and *H. cooremani* Thomas and two species of *Chilocorus* (*C. cacti* (L.) and *C. nigritus* (F.) using starch gel electrophoresis. Of the 28 enzymes screened, four banding patterns were completely resolvable for differences in the two mite species. Thirteen were resolvable for the beetles species tested. *Hemisarcoptes cooremani* adults and deutonymphs have the same banding pattern for resolved systems. In analysis of combined *H. cooremani* and *C. cacti* extracts, two enzyme systems produced unambiguous bands for the mites which were distinguishable from banding patterns of the beetles. Results are discussed in reference to a research program emphasizing the species radiations and coevolution of phoretic interactions of this mite-beetle complex.

Introduction

Hemisarcoptes (Acari: Hemisarcoptidae) is a genus of small ($\sim 300\ \mu m$) astigmatid mites that is global in distribution. The ontogeny of *Hemisarcoptes* usually consists of four developmental stages: larva, protonymph, tritonymph, and adult. The ontogenetic sequence of some individuals, however, includes a facultative insertion stage. This stage is the deutonymph, or hypopus, which is heteromorphic.

Larvae, protonymphs, tritonymphs, and adults of *Hemisarcoptes* are all obligate predators of armored scale insects (Coccoidea: Diaspididae) (Gerson *et al.*, 1989). They are, however, polyphagous within the context of the 340 scale-insect genera included in this family. For this reason there is interest in the mite as a biological control agent.

Hypopi are non-feeding stages. They are specifically adapted for dispersal (phoresy), with elaborate ventral suckers for host attachment. Hypopi of *Hemisarcoptes* are phoretic only on beetles of the genus *Chilocorus* (twice-stabbed ladybird beetles).

In North America two species of *Hemisarcoptes* have been described, *H. malus* Shimer and *H. cooremani* Thomas. *Hemisarcoptes malus* inhabits most of the USA and Canada and *H. cooremani* is restricted to the southern US tier states. Species-level differences in morphology are subtle, and studies have yielded few taxonomically discriminating characters to distinguish these morphologically conservative species.

There are approximately 70 extant species of *Chilocorus* worldwide (Gordon, 1985). In the United States *Hemisarcoptes* is associated with two species of *Chilocorus*, *C. cacti* (L.) and *C. stigma* (Say). The exception is in California, where *C. stigma* has been replaced by a species complex (*C. fraternus* LeConte, *C. orbus orbus* Casey, and *C. orbus monticolus* Drea) (Drea, 1956). The regions of dominance of the mites correspond roughly to the geographic distributions of their hosts, *Chilocorus stigma* to the north and *C. cacti* to the south.

Chilocorus is itself an obligate scale-insect predator of most species of diaspidids. This assures that the phoretic hypopi have a high probability of being transported to a new habitat containing required resources. Once in this new habitat, the hypopi disembark and molt and the developmental sequence is completed.

Research on the systematics of *Hemisarcoptes* has clarified some very real impediments to the interpretation of ontogenetic and phylogenetic relationships within the genus *Hemisarcoptes*. Interpretation of ontogenetic and phylogenetic relationships is fundamental to understanding the interspecific interactions of phoretic coevolution.

Due to the vagaries of field work, one is almost never fortunate enough to find beetles, hypopi, and predatory mite stages in any particular field survey. Mites recovered from museum specimens of *Chilocorus* are always hypopi. The coupling of the extensive museum data with field records is currently imperfect but most convincing where rearing of field material has occurred. Determination of morphospecies of *Hemisarcoptes* is currently tedious and labor intensive. The challenge, then, was to find enzyme systems resulting in electrophoretic banding patterns shared by hypopi and predatory stages which would anchor them in an unquestionable ontogenetic context. And, to determine potential enzymes which would discriminate among species of beetles.

Techniques of electrophoresis were applied to the following questions:

1) Do genetic data corroborate preliminary conclusions, based on morphology, that *H. malus* and *H. cooremani* represent distinct species?
2) Do isozyme analyses of deutonyphal and adult tissues of *Hemisarcoptes* yield identical banding patterns so that an evaluation of either stage will be useful in determining geographic boundaries of species?
3) What are the potential enzyme systems for examining species relationships among *Chilocorus* beetles?
4) Can we establish enzyme systems that would allow us to process beetle elytra containing hypopi of mites, and to recognize discrete banding patterns specific to beetle proteins separate from those attributable to the mites?

Methods and materials

Hemisarcoptes malus and *H. cooremani* were harvested from laboratory strains originally collected in 1984. Cultures contained thousands of individuals, were enhanced with wild inoculates in December, 1986, and were thus assumed to represent genetic correlates to natural populations.

Hemisarcoptes malus were field collected from *Lepidosaphes ulmi* (L.) (oystershell scale) on apple trees in Danby, New York. *Hemisarcoptes cooremani* were obtained from *Lepidosaphes beckii* (Newman) (purple scale) on orange trees in Donna, Texas. Mites were cultured in the laboratory using the scale insect *Aspidiotus nerii* Bouché (oleander scale) as prey.

Because of the small size of the mites and because I was seeking discrete rather than quantitative differences, three hundred mites represented a single sample. This number was chosen because it was previously shown to yield a volume of material sufficient for starch-gel electrophoresis, and because it is a realistic expectation of numbers available in a field population. As many as 800 mites per elytra have been found on a single beetle (personal observation).

Mites were collected individually, using a single-haired sable brush, into dry vessels (on ice) until the collection of each sample was completed, after which it was stored briefly at $-70\ ^{\circ}C$ until all samples could be assayed.

Chilocorus cacti were sampled from laboratory

cultures grown on *A. nerii*. *Chilocorus cacti* was originally collected from the same site in Texas as the mite *H. cooremani* and was also inoculated with recruits in December, 1986. One beetle represented an electrophoretic sample. A second species, *C. nigritus* (F.), was obtained from the cultures of Dr. Robert F. Luck (The Univerity of California, Riverside), where it was grown on the scale *Aonidiella aurantii* (Maskell) (California red scale). *Chilocorus nigritus* is native to India, but this particular culture represents a collection of beetles successfully introduced into South Africa and exported from there to California in 1984. The beetles were transferred to cultures of *A. nerii* several months prior to sampling.

Eight independent samples were included for evaluation of electromorphs: 1) adult females of *H. malus*; 2) all stages of *H. cooremani* combined; 3) hypopi of *H. cooremani*; 4) adult females of *H. cooremani*; 5) *C. cacti* elytra bearing *H. cooremani* hypopi; 6) intact specimens of *C. cacti* bearing *H. cooremani* hypopi; 7) *C. cacti* bodies with the elytra removed (no hypopi); 8) intact specimens of *C. nigritus* (no hypopi). No hypopi of *H. malus* were included in this survey because the

Table 1. The names, abbreviations, and E.C. numbers for enzymes used in the initial screening; * = unscorable enzymes, ** = enzymes with limited activity

Enzyme name	Abbreviation	E.C. number	Buffer
Acid phosphatase	ACP	3.1.3.2	C[1]
Adenosine deaminase	ADA**	3.5.4.4	M[2]
Adenylate kinase	AK*	2.7.4.3	M
Alcohol dehydrogenase	ADH**	1.1.1.1	S-4[3]
Aldolase	ALD	4.1.2.13	M
Aspartate aminotransferase	AAT**	2.6.1.1	R[4]
Diaphorase	DIA	1.8.1.4	C
Esterase	EST-F-A	–	M
Fumerase	FUM	4.2.1.2	C
Galactosaminidase	GAM	–	R
Glucosephosphate isomerase	GPI	5.3.1.9	S-4
Glutathione reductase	GR	1.6.4.2	R
Glycerol-3-phosphate dehydrogenase	G3P	1.1.1.8	C
Hydroxybutyric dehydrogenase	HBDH**	1.1.1.30	R
Isocitrate dehydrogenase	IDH	1.1.1.42	S-4
Lactate dehydrogenase	LDH	1.1.1.27	S-4
Malate dehydrogenase	MDH	1.1.1.37	C,S-4
Malic enzyme	ME*	1.1.1.40	R
Mannosephosphate isomerase	MPI**	5.3.1.8	C
Methylumbelliferyl phosphate	MUP*	–	C
Peptidase with glycyl-leucine	PEP-GL	3.4.11-13	R
leucyl-glycyl-glycine	-LGG*	–	R
phenyl-alanyl-proline	-PAP*	–	R
Phosphoglucomutase	PGM*	5.4.2.2	S-4
Phosphogluconate dehydrogenase	PGD	1.1.1.43	C
Superoxide dismutase	SOD	1.15.1.1	S-4
Triosephosphate isomerase	TPI	5.3.1.1	S-4

[1] Clayton & Tretiak, 1972.
[2] Markert & Farlhaber, 1965.
[3] Selander *et al.*, 1971.
[4] Ridgway *et al.*, 1970.

particular population from which the original stocks were obtained has apparently lost the insertion stage.

Isozyme analysis was performed by horizontal starch-gel electrophoresis after the protocol of Utter *et al.* (1974) as modified by May (1980). The screening included a total of 28 enzyme-buffer combinations. Due to the use of pooled samples, no attempt was made to establish the identity of specific loci where electromorphs were determined.

Results

Of the 28 enzyme systems screened (Table 1), 17 were scorable and provided sufficient resolution. Eleven systems could not be scored due to insufficient activity on the gels or uninterpretable banding patterns. Eight of the 17 systems were resolvable for mites and 15 of 17 were resolvable for beetles (Table 2). Of these, 4 provided discrimination among samples of mites and 13 provided discrimination among samples of beetles.

In general, more between-species variation and within-locus polymorphism was found in the host, *Chilocorus*, than was found in the phoretic mite, *Hemisarcoptes*. And there is significant differentiation between the geographically disjunct populations of *Chilocorus* examined.

Hypopi and predatory stages of *H. cooremani* had the same banding pattern for all resolved enzyme systems (TPI, SOD, PEP-GL, GPI, ALD, and MDH/C, S-4); GPI and MDH/S-4 were polymorphic within samples of *H. cooremani*.

The two morphospecies of the mite (*H. malus* and *H. cooremani*) were completely discriminated by four enzymes (PEP-GL, GPI, MDH/C, and MDH/S-4). Females of *H. malus* and *H. coore-*

Table 2. Qualitative mobility of isozymes which discriminate *Hemisarcoptes* and *Chilocorus*. Numbers represent electromorphic designations; lower scores represent higher mobility. Dashes indicate no activity

Enzymes	Mites				Beetles				Resolved Among:
	H. malus females	*H. cooremani*			*C. cacti*			*C. nigritus* whole beetle (no hypopi)	
		all stages	hypopi	females	elytra (hypopi)	whole beetle (hypopi)	body (no hypopi)		
	Electromorphs				Electromorphs				
EST-F-A	–	1	–	1	–	–	–	–	–
GAM	–	–	–	–	1	1	1	2	Beetles
TPI	3	3	3	3	2	2	2	1	Beetles
SOD	2	2	2	2	3	3	3	1	Beetles
FUM	–	–	–	–	2	2	2	1	Beetles
PEP-GL	1	3	3	3	2, 3	2, 3	2	4	Mites & Beetles
GPI	2	2, 3	2, 3	2, 3	1	1	1	1	Mites
ALD	2	2	2	2	1	1	1	1	–
MDH/C	2	1	1	1	–	–	–	–	Mites
MDH/S-4	3	2, 6	2, 6	2, 6	4, 5, 6	4, 5, 6	5	1, 2	Mites & Beetles
IDH	–	–	–	–	2, 4	2, 4	1, 2, 4	2, 3	Beetles
GR	–	–	–	–	1, 2	1, 2	1, 2	1, 3	Beetles
PGD	–	–	–	–	2	2	2	1	Beetles
G3P	–	–	–	–	2, 4	2, 4	2, 4	1, 3, 5	Beetles
DIA	–	–	–	–	1, 4	1, 4	1, 4	2, 3	Beetles
ACP	–	–	–	–	1	1	1	2	Beetles
LDH	–	–	–	–	2, 4	2, 4	2, 4	1, 3	Beetles

mani had identical banding patterns for enzymes TPI, SOD, and ALD, but had alternate banding patterns for PEP-GL and MDH/C. *Hemisarcoptes malus* shared one of these alleles with *H. cooremani* (*Gpi*) but the other (*Mdh/C*) was unique.

The beetles *C. cacti* and *C. nigritus* shared two banding patterns (GPI and ALD). However, they differed with enzymes GAM, TPI, SOD, FUM, PGD and ACP, which provided clear discrimination between the beetle species. Beetles were polymorphic for alternate alleles at *Pep-gl*, *Mdh/S-4*, *Idh*, *Gr*, *G3p*, *Dia*, and *Ldh*.

In the effort to produce interpretable banding patterns of combined *H. cooremani* and *C. cacti* extracts, many enzymes produced patterns for the mites that were uninterpretable due to overlap with banding patterns of the beetles. Two enzymes, however, gave good results: PEP-GL which produces unambiguous monomorphic bands, and MDH/S-4 for which the mite and beetle banding patterns are less distinctive due to significant polymorphism. All but one of the enzymes (IDH) present in the whole body extracts of *C. cacti* were also present in the elytra. And, one enzyme (MDH/S-4) was present in the elytra but not scorable in the body extracts of *C. cacti*.

Discussion

Electrophoretic data from female mites indicate that isozyme analyses corroborate morphometric evidence that *Hemisarcoptes malus* and *H. cooremani* represent distinct allopatric species. In addition, hypopi and adults of *H. cooremani* are consistent in their patterns of isozymes irrespective of the heteromorphy in morphology. Results of this genetic survey of variation within and among populations of *Hemisarcoptes* and *Chilocorus* offer encouraging evidence of the importance and applicability of the methods for studies of coevolution of *Hemisarcoptes* and *Chilocorus*. The realistic option of using either adult or hypopal mites to diagnose species boundaries will allow more rapid progress in systematic studies. Also, where beetles are collected carrying phoretic mites, elytral samples are the appropriate target of analysis for efficiently recovering host and mite identifications in parallel and for the determination of geographic boundaries of the phoretic association.

Electrophoretic techniques also hold promise in a practical way in bio-control studies investigating native and exotic introductions of *Hemisarcoptes*. Once electrophoretic profiles are established by species, rapid monitoring of the spread, assimilation, or displacement of individual species will be possible without tedious preparation and examination of permanent slide mounts as has previously been required.

The identification of these discriminatory isozymes will be applied to future systematic and coevolutionary questions concerning *Hemisarcoptes* and *Chilocorus*. The techniques are particularly appropriate in studies such as this which examine morphologically cryptic species, with heteromorphic life histories, especially where populations range over large geographic areas (Berlocher, 1984).

Acknowledgments

I thank Dr. Kenneth R. Summy of Weslaco, Texas for cooperation in helping locate infestations of *Lepidosaphes beckii* from which *Chilocorus cacti* and *Hemisarcoptes cooremani* were collected; Dr. Robert F. Luck, Riverside, California for providing stocks of *Chilocorus nigritus*; Dr. Richard E. Strauss provided constructive comments on the manuscript. The isozyme analysis was performed under the direction of Dr. Bernie May, Cornell University. Supported by NSF (#BSR-83-07711) and BARD (#IS-1397-87).

Résumé

Analyse des isozymes de l'acarien, Hemisarcoptes *et de la coccinelle*, Chilocorus, *qui est son hôte phorétique*

Hemisarcoptes est un petit acarien astigmate. Son ontogenèse comprend une deutonymphe (hypopus) phorétique des coccinelles du genre

Chilocorus. Aux USA, il y a deux espèces d'*Hemisarcoptes*: *H. malus* Shimer et *H. cooremani* Thomas aux différences peu apparentes, mais une analyse discriminante des caractéristiques de la femelle permet une distinction complète. Des techniques d'électrophorèse horizontale sur gel d'amidon ont permis de placer les adultes et les hypopi dans un contexte ontogénique indiscutable et de relier les espèces d'acariens et de coccinelles en termes de relations coévolutives. 28 combinaisons enzyme-tampon ont été examinées. Les enzymes PEP-GL, GPI, MDH/C et MDH/S-4 séparent totalement les deux espèces d'acariens. Les enzymes GAM, TPI, SOD, FUM, PEP-GL, MDH/S-4, IDH, GR, PGD, G3P, DIA, ACP et LDH séparent les coccinelles *C. cacti* et *C. nigritus*. En combinant les extraits de *H. cooremani* et de *C. cacti*, PEP-GL et MDH/S-4 donnent de bandes séparant nettement les acariens des coccinelles.

Les données élecrophorétiques montrent: 1) une confirmation par analyse des isozymes des arguments morphologiques de séparation en espèces différentes de *H. malus* et *H. cooremani*; 2) la présence des mêmes bandes quelles que soient les différences morphologiques entre les hypopi et les stades prédateurs de *H. cooremani*; 3) que pour les coccinelles récoltées avec leurs acariens phorétiques, les échantillons d'élytres sont les meilleurs éléments pour identifier efficacement en parallèle l'hôte et l'acarien et pour déterminer les limites géographiques de l'association phorétique; 4) la présence de différences pour de nombreux systèmes enzymatiques chez la coccinelle.

Les résultats de cet examen génétique de la variabilité intra et inter populations de *Hemisarcoptes* et de *Chilocorus* apportent des éléments encourageant l'utilisation de ces méthodes dans l'étude de leur coévolution.

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