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Enzymatic characterization of nine endoparasite species of small ermine moths (Yponomeutidae)

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Summary. Eight hymenopterous and 1 dipterous species, all endoparasitic in eggs, larvae, or pupae of small ermine moths *(Yponomeuta)* were investigated for their allozyme variation at 3–29 loci. The mean heterozygosity level of the hymenopterous species is one-third of that of the dipterous species. Zymogram patterns of the parasite larvae do not interfere with those of the host.

Electrophoresis is at present the major technique of biochemical systematics[1]. In field samples of insects, one of its possible sources of error may arise from endoparasite proteins, which can be erroneously interpreted as allelic variations of the host. The general zymogram picture of a developing endoparasitisation is an increase in activity of the parasite-specific bands with a concomitant reduction in activity of those of the host[2,3].

Eight parasites of small ermine moths were investigated allozymically as adults and another as larva; they are briefly discussed below (see also data in Dijkerman[4]). The *Yponomeuta* hosts on which they occur are abbreviated as follows and given in parentheses, with those from which they were analyzed in italics: *Y. cagnagellus,* C; *Y. evonymellus,* E; *Y. mahalebellus,* M; *Y. malinellus,* Ma; *Y. padellus,* P; *Y. plumbellus,* Pl; *Y. rorellus,* R; *Y. vigintipunctatus,* V.

The list of parasites comprises the following species (see the table for generic names and families; – note that L2, L3, etc. below denotes the 2nd, 3rd etc. larval stages). *A. fuscicollis:* attacks eggs (*C,* E, M, Ma, P, *R*). Polyembryonic cleavage from L4 on. On the average 80–90 individuals from 1 egg. *D. armillata:* attacks L2–L3 *(V)* or L3–L4 (C, E, M, Ma, P, R). Larva leaves host after it has spun its cocoon. *I. maculator:* attacks pupae, even just before emergence of the adult moth, sometimes L5 (C, E, *Ma, P*). *M. vittator:* attacks presumably late L4 larvae (C, E, M, Ma, P, Pl, *V*). Hyperparasite, parasitises *Diadegma* species. *P. turionellae:* attacks pupae (C, E, P). Reared on *Galleria mellonella* (Pyralidae) in the laboratory. *T. yponomeutae:* attacks L3–L4 *(V).* Presumably thelytokous, only ♀♀ have been reared in the department during the last 10 years (some ♂♂ known from museum collections). *T. tricarinatus:* as *T. yponomeutae* but bisexual. *T. evonymellae:* gregarious, attacks L4, L5 and sometimes pupae (C, E, M, *Ma,* P, R). *D. hyponomeutae:* attacks L4–L5 (C, *Ma, P*).

Electrophoretic techniques and staining methods are the same as described for *Yponomeuta*[3]. Genetic interpretation of the observed variation is inferential. Available data on levels of enzyme polymorphism in Hymenoptera indicate a reduced intrapopulation variability relative to diploid insects[5–7]. Values presented here fall in the limited range of heterozygosity *(H)* levels of the haplodiploid species known[7] ($\bar{H} = 0.029 \pm 0.023$; range 0.000–0.056). When we disregard those species in which less than 10 genomes were investigated (i.e. *I. maculator* and *M. vittator*) the outcome remains the same ($\bar{H} = 0.030 \pm 0.021$; range 0.000–0.055). This average H level for Hymenoptera is less than one-third that of the only dipteran studied ($H = 0.96$). Evidence for the hypothesis that haplodiploidy reduces variation is, however, very weak[9].

Except for *A. fusciollis,* only electrophoresed parasites were studied, whereas larval patterns may interfere with those of their host. The 4 enzymes (namely α-glycerophosphate, lactate and malate dehydrogenase and phosphoglucose isomerase [*Pgi*]) that occur in gels where *Yponomeuta* larvae, parasitized by *A. fuscicollis,* are electrophoresed are all coded for by the same locus in the larval and adult stage, as samples of adults give bands at the same distance of migration as those of the larvae. This also holds for lactate

Nine species of endoparasitic insects, the number of individuals and loci analyzed electrophoretically per species together with the enzymes that show variation, the mean number of alleles per locus ($\bar{A}$), mean proportion of loci polymorphic per species ($\bar{P}$) and heterozygous per individual (H)

Species	Family	Number of individuals ♂	♀	Loci	Polymorphic enzymes**	$\bar{A}$	$\bar{P}$	H***
Hymenoptera								
Ageniaspis fuscicollis	Encyrtidae	13*		25	k	1.080	0.040	0.019****
Diadegma armillata	Ichneumonidae	9	8	19	g, h, k	1.158	0.158	0.048
Itoplectis maculator	Ichneumonidae	5	-	20	g, h, i	1.150	0.150	0.056
Mesochorus vittator	Ichneumonidae	2	1	3	-	1.000	0.000	0.000
Pimpla turionellae	Ichneumonidae	-	12	29	d, e, g, i	1.138	0.138	0.038
Triclistus yponomeutae	Ichneumonidae	-	11	20	a, g	1.100	0.100	0.020
Trieces tricarinatus	Ichneumonidae	14	-	14	-	1.000	0.000	0.000
Tetrastichus evonymellae	Eulophidae	-	10	20	c, h, j	1.150	0.150	0.055
Diptera								
Discochaeta hyponomeutae	Tachinidae	-	5	16	b, f, g, h	1.250	0.250	0.096

*All larvae; 13 clones of 20–30 individuals each. **A, malate dehydrogenase; b, malic enzyme; c, NADH dehydrogenase; d, aldehyde oxidase; e, glucose oxidase; f, hexokinase; g, phosphoglucomutase; h, esterase; i, alkaline phosphatase; j, leucine aminopeptidase; k, phosphoglucose isomerase. ***Values computed from field populations except for *P. turionellae* (see text). ****Computed assuming a 1:1 sex ratio and no differentiation in allozyme frequencies between the sexes[8].

dehydrogenase in *D. armillata*. Assuming identical larva-adult patterns, all other parasites were not detected in *Yponomeuta* larvae (routinely electrophoresed as L4 or L5) due to their rare occurrence and probably to the fact that they parasitize in general later stages of *Yponomeuta* than do *D. armillata* and especially *A. fuscicollis*. As larvae were opened and investigated prior to electrophoresis, at least those parasite larvae that have reached a size at which parasite-specific bands may be intense enough to interfere with the host bands were detected. In *A. fuscicollis Pgi* occurs with 3 alleles, whereas all other polymorphic enzyme systems listed in the table have 2 alleles (so $\bar{A} = 1 + \bar{P}$). Moreover, *Pgi* appears to be a monomer in this species as only 1 or 2 banded patterns occcur; normally this enzyme is a dimer (e.g. in *Diadegma* and *Yponomeuta*[3,10–12]).

It is possible to compute percentages of parasitization in a population from analysis of the zymograms of a sample of individual host-larvae. A total of 456 specimens were analyzed from a population of *Y. cagnagellus* in a dune area near The Hague[11]. *A. fuscicollis* was found to parasitize 10.5%. In a restricted part of this area intensive sampling and subsequent rearing resulted in 7.8 and 13.1% by direct count of the emerging parasites (N = 795 and 703 respectively), whereas based on interpretation of the zymograms of hosts from this area this value was 12.9% (N = 54).

Parasites, analyzed from more than 1 sympatric host (e.g. *A. fuscicollis* from *Y. cagnagellus* and *Y. rorellus*) showed similar variation patterns, probably indicating that they belong to interbreeding populations. The *T. yponomeutae* population is apparently composed of genetically different clones as all 11 ♀♀ originated from 1 single population of *Y. vigintipunctatus*.

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Adrenergic nerves and mast cells after skin freezing. A hypothesis based on fluorescence microscope observations in the rat*

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Summary. After freezing and thawing of rat skin, degranulation and disappearance of mast cell fluorescence became apparent in the skin up to 1 h after thawing. Gradual disappearance of catecholamines from the adrenergic nerves of the injured area occurred during the 1st 24 h. Both mast cells and adrenergic nerves may play a role in tissue destruction after freezing injury.

The pathogenesis of tissue injury caused by freezing and cold is still not fully understood. Many factors such as direct cell injury and resultant biochemical changes and the development of microthrombi are likely to be involved[1,2]. Sympathectomy has been used in the treatment of frostbite[3–5] and intra-arterial reserpine, which pharmacologically depletes catecholamines, has been put forward as possible treatment of frostbite[6,7]. However, there is no direct