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Trans-epoxide hydrolase: A key indicator enzyme for herbivory in arthropods[1]

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Summary. An epoxide hydrolase selective for a trans-epoxide substrate is more commonly associated with arthropod herbivory than is a cis-selective epoxide hydrolase. The distinct selectivities in epoxide hydrolase activities between herbivorous pests and entomophagous arthropods used in their biological control may aid design of integrated pest management systems.

The diet of an arthropod is usually controlled by perception of key chemical and physical attributes of its food. Recognition and acceptance of food often occurs prior to ingestion[2], however an arthropod must contend with the physiological consequences of its choice. Overingestion of toxins can result in decreased fitness or death, and limit host range[3,4]. Generally, enzymatic detoxification[5] is the most direct and dependable way for an arthropod to survive a chemical insult.

Plants biosynthesize trans- and higher substituted olefins including fatty acids, phenolics, alkaloids and terpenoids that are rare or absent in animals. Many of these phytochemicals are defenses against herbivory[6-8]. By contrast, cis-olefins generally have constitutive and homeostatic functions in both plants and animals[9]. Epoxidation of olefins largely by cytochrome P-450 monooxygenases (MFOs) can produce epoxides harmful to an organism[5]. The enzyme epoxide hydrolase (EH) catalyzes hydration of the epoxide, thereby detoxifying it to a more excretable 1,2-dihydroxy metabolite[10,11]. This reaction is advantageous since epoxides have high reactivities, and are common in the arthropod's environment. Exposure to epoxides can occur through extranutritional dietary chemicals[6-9], environmental pollutants (e.g. dieldrin)[10], and endogenous hormones (e.g. juvenile hormone)[12]. We recently found that a herbivorous mite had both a much higher MFO and EH activity for a trans-epoxide than a carnivorous mite. Surprisingly, cis-EH activity had the reverse tendency, favoring the predaceous mite[13]. This observation caused us to examine in greater detail the association of trans and cis EH activities with feeding specialization in arthropods.

Epoxide hydrolase activities in 30 species of macro- and microarthropods were measured. Most species were field collected; some were obtained from private or commercial sources. Actively feeding adults or last instar larvae (Aa, Cr, Da, Hc, Ma, Md, Pr, Sf only; fig.) were surveyed. The trans- and the cis-EH activities for each species were plotted in the figure, and widely differing activities (up to 350-fold) were found. Chewing herbivores had much higher trans-EH activities (greater than 11-fold on the average, $p < 0.005$ by Student's t-test and used hereafter) than

Epoxide hydrolase in adult chrysomelidae beetles relative to their host range

Subfamily Tribe Species	Gut epoxide hydrolase* trans	 cis	 trans/cis	Plant families consumed**
Galerucinae				
Luperini				
Diabrotica longicornis	38.5 ± 7.3 ad	2.59 ± 0.35 a	14.9 ± 3.7 ab	17
D. undecimpunctata howardi	28.0 ± 6.6 ade	2.46 ± 0.58 ae	11.4 ± 0.6 b	14
D. virgifera	25.7 ± 5.7 bd	1.79 ± 0.33 ae	14.4 ± 1.2 a	13
Acalymma vittata	38.0 ± 6.1 ad	4.57 ± 0.52 b	8.32 ± 0.67 c	16
Galerucini				
Trirhabda virgata	44.8 ± 5.3 a	35.0 ± 3.2 c	1.28 ± 0.05 d	1
Chrysomelinae				
Doryphorini				
Leptinotarsa decemlineata	18.2 ± 0.9 be	8.04 ± 0.11 d	2.26 ± 0.12 e	5
Chrysomelini				
Plagiodera versicolora	4.00 ± 0.96 c	7.24 ± 0.96 bd	0.55 ± 0.06 f	1
Alticinae				
Altica woodsi	3.23 ± 0.16 c	1.89 ± 0.13 ae	1.71 ± 0.20 de	1
Criocerinae				
Crioceris asparagi	2.84 ± 0.84 c	1.46 ± 0.23 e	1.94 ± 0.36 de	1

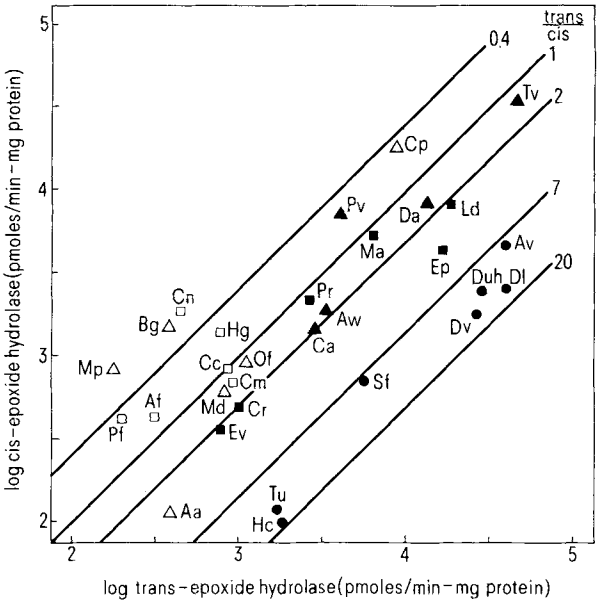
* Activity in nmoles diol formed/min/mg protein; mean ±SE for 6 determinations on 2 population samples from consecutive seasons except for Pv and Aw, where only 1 population was sampled. Pools of midgut tissue from 20 to 40 field collected adults for each species were used for activity measurements. Species with same letter are not significantly different at $p < 0.05$. ** Host range was based primarily from work cited[3,22-26].

arthropods with predaceous, parasitic, saprophagous or other specialized modes of feeding. In contrast, the cis-EH was not significantly different between herbivores vs more specialized feeders. The ratio of the trans- to cis-EH activities was also compared. We hypothesized that trans-EH may better metabolize phytochemically-derived epoxides than the cis-EH, which may play a more endogenous protective role[13]. Chewing herbivores (6.1 ± 1.4, $n = 18$; $\bar{x} \pm SE$ and used hereafter) had a 6.4-fold higher trans/cis ratio than the group containing carnivores, saprophages, sucking and more specialized feeders (0.95 ± 0.26, $n = 12$; $p < 0.005$). This conclusion was even more apparent when herbivores were grouped relative to encounter with plant allelochemicals as estimated by host plant range. Although absolute trans- or cis-EH activities were not significantly different, the specific ratios for generalist species (12.8 ± 1.4, $n = 7$) were very different ($p < 0.001$) from the oligophages (2.10 ± 0.37, $n = 6$) which in turn were different ($p < 0.05$) from the specialist herbivores (1.12 ± 0.22, $n = 8$). Thus the substrate specificity ratios appear to reflect feeding ecology and may have utility in comparing widely diverging species of arthropods.

EH levels were examined further in gut preparations from adult leaf feeding beetles of the Chrysomelidae (table). Values in EH varied widely and exceptions to increasing trans-EH activity with increasing polyphagy on plants were found. For example, *Trirhabda virgata*, a specialist on goldenrod, had the highest trans-EH of 30 species surveyed (table). Similarly, Krieger et al.[14] observed that while absolute levels of midgut aldrin epoxidase increased with host plant range for lepidopteran larvae grouped into mono-, oligo- or polyphagous species, individual species activity-host range relationships were less apparent, possibly due to 'qualitative and quantitative differences in the content of secondary substances between different plants'. Nevertheless, the ratio of trans to cis EH activity for these chrysomelids correlated well ($r > 0.92$) with number of plant families or plant genera (not shown) consumed by the individual species, and these ratios were very different ($p < 0.005$) among the specialists and generalists surveyed (table). We conclude that the ratio of one EH activity associated with herbivory relative to another EH not or only partially effected by plant components will be a more reliable index for food/arthropod relationships than comparisons based solely on absolute enzyme levels. Using ratios of enzyme activities should partly overcome problems in comparative biochemical study such as asynchronously aged animals, differing life stages and tissue sources, and unknown species difference for in vitro enzyme measurements.

Another important group difference is evident from comparison between the pest and beneficial arthropods. 17 herbivorous pests of economic importance included in this survey had EH ratios (6.2 ± 1.5) highly different ($p < 0.005$) from 6 entomophagous arthropods (0.73 ± 0.17) used in biological control. Furthermore, trans-EH was 20-fold greater in the pest over the beneficial group ($p < 0.005$), whereas cis-EH was only 3-fold greater in the pest group ($p < 0.025$). This constitutes a distinct biochemical difference between economic pests and the predaceous or parasitic arthropods used to control these pests. EH may be an appropriate biochemical lesion for development of a pest-selective toxicant, since the crop pest should be more susceptible than its natual enemies to inhibition of trans-EH. The potential applicability of such biorational pesticides in many pest-beneficial-crop systems may provide both broadspectrum pest toxicity required for industrial profitability and the relative safety to natural enemies sought for integrated pest control[15].

Presumably, trans-EH in herbivores has a detoxification role towards the widely distributed plant defensive epoxides in their diets[16]. However, little is known concerning specific allelochemical interactions with EH in herbivores. Fatty acid epoxides are components of the outer protective cuticular envelope of plants[17], and could serve as an initial chemical barrier to insect grazing. A *Fusarium* fungus retains a catabolic EH apparently to detoxify fatty acids and thus facilitate penetration through the cutin of its host[7]; perhaps trans-EH allows arthropod herbivores to 'wound' and thereby consume tissues beneath the plant cuticle. Mammals also have a trans-EH that readily cleaves fatty acid and various terpenoid epoxides[18,19]. Induction of



Enzymes were prepared at 0–4 °C. Midguts dissected as described[16] from 8 to 30 macroarthropods, or whole bodies of 20–10^3 microarthropods (Aa, Af, Mp, Pf, Tu) were thoroughly washed with water, then buffer, and homogenized (10% w/v in 150 mM potassium phosphate-50 mM sucrose, pH 7.4; buffer A) in smooth glass with Teflon® pestle. The supernatant fraction resulting from centrifugation at 15,000 × g for 10 min in an Eppendorf® microcentrifuge was used for enzyme assays. Epoxide hydrolase activities for trans-β-ethylstyrene oxide and cis-stilbene oxide were measured at 37 °C in buffer A and 0.1 M sodium pyrophosphate (pH 8.5) buffer respectively, using radiometric isooctane partitioning[19], and are plotted on a log scale with ● for generalist herbivores, ■ for oligophagous herbivores, ▲ for specialist herbivores, △ for other specialist feeders including saprophagous and sucking insects, and □ for entomophagous arthropods. Isolines of 5 trans/cis ratios of activities (0.4–20) are drawn. Each activity is the mean of 6–18 determinations except for Aw, Ma and Pv (only 3), and the SE about the mean of the activity ratios was < 25% of the mean except for Cc (94), Cm (37), Mp (29), and Bg (40). Aa, *Aedes aegypti* (Linn.); Af, *Amblyseius fallacis* (Garman); Av, *Acalymma vittata* (Fabricius); Aw, *Altica woodsi* Isely; Bg, *Blattella germanica* (Linn.); Ca, *Crioceris asparagi* (Linn.); Cc, *Chrysopa carnea* Stephens; Cm, *Coleomegilla maculata* (De Geer); Cn, *Coccinella novemnotata* (Herbst); Cp, *Chauliognathus pennsylvanicus* (De Geer); Cr, *Choristoneura rosaceana* (Harris); Da, *Delia antiqua* (Meigen); Dl, *Diabrotica longicornis* (Say); Duh, *D. undecimpunctata howardi* (Barber); Dv, *D. virgifera* Le Conte; Ep, *Epicauta pennsylvanica* (De Geer); Ev, *Epilachna varivestis* Mulsant; Hc, *Hyphantria cunea* (Drury); Hg, *Hippodamia convergens* Guerin-Meneville; Ld, *Leptinotarsa decemlineata* (Say); Ma, *Malacosoma americanum* (Fabricius); Md, *Musca domestica* (Linn.); Mp, *Myzus persicae* (Sulzer); Of, *Oncopeltus fasciatus* (Dallas); Pf, *Pediobius foveolatus* (Crawford); Pr, *Pieris rapae* (Linn.); Pv, *Plagiodera versicolora* (Laicharting); Sf, *Spodoptera frugiperda* (J.E. Smith); Tu, *Tetranychus urticae* Koch; Tv, *Trirhabda virgata* (Le Conte).

mammalian microsomal EH by an epoxide-containing but not a structurally related pyrrolizidine alkaloid toxin of rangeland weeds[20], may have important implications for grazing livestock. Tiger moths that consume *Senecio* alkaloids preferentially sequester the unsaturated analogs at the expense of the epoxide and diol bearing alkaloids[8]. Possibly an EH is used by these arctiids to egest more autotoxic pyrrolizidine alkaloids and thus retain the remainder for aposematic defenses.

Terpenoid epoxides and their precursor olefins represent another group of phytochemicals widely consumed by arthropods[6]. Many have high biological activity and are particularly abundant in the Compositae. This may explain the high EH activity of the *Solidago* specialist, *Trirhabda virgata* (table). Indeed, insects require sesquiterpene epoxides called juvenile hormones for growth regulation which are readily deactivated by insect EHs presumably similar or identical to the trans-EH characterized in mammals[12,18,19].

Plants can counter insect herbivory by synthesizing juvenile hormone mimics or antagonists of the hormone's biosynthesis or degradation within insects. Some of these defensive chemicals are terpenoid epoxides[21]. Perhaps the ubiquitous flavonoids of plants also disrupt insect endocrinology, since potent inhibitors of the trans-EH in mammals are synthetic derivatives of flavonoids[19].

Inspection of the table suggests that EH may be useful in biochemotaxonomy of arthropods. EH profiles among species of chrysomelids in the same genus are more similar than species from other genera in the same tribe, and the relatedness decreases as expected from the tribe to subfamily levels. Predaceous coccinellids from the same tribe (Cn, Hg, Cm) had a significantly different enzyme profile from that of a herbivorous coccinellid in another tribe (Ev, fig.). More species need to be examined with additional epoxide substrates to clarify the utility of this enzyme for distinguishing species with similar morphological characters.

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Identification of raffinose in honeydew[1]

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Summary. The presence of small amounts of raffinose in the honeydew of 6 aphid species was demonstrated by means of TLC and GLC after invertase hydrolysis. The method allows the detection of this sugar even in the presence of a high percentage of melezitose.

The honeydew produced by many aphids and the corresponding honeydew-honey are complex mixtures of mono-, di-, and oligosaccharides, often characterized by a very high melezitose content. The presence of raffinose in the trisaccharide fraction is usually accepted[2,3], but recently doubts have been raised as to whether it has been adequately demonstrated[4].

The aim of our work was to demonstrate the presence of