Odonatologica 23(4): 421-429 *December 1, 1994*

AN ELECTROPHORETIC COMPARISON OF ENZYMES FROM *ANAX JUNIUS* (DRURY) AND *ERYTHEMIS SIMPLICICOLLIS* (SAY) (ANISOPTERA: AESHNIDAE, LIBELLULIDAE)

S.J. HARRISON[1*], A.P. PLATT[2] and S.P. SANDERS[3]

[1] Biology Department, Towson State University, 320 Smith Hall, Towson, MD 21204-7097, United States

[2] Department of Biological Sciences, University of Maryland Baltimore County (UMBC), Room BS 431, 5401 Wilkens Avenue, Catonsville, MD 21228-5398, United States

[3] Division of Pulmonary and Critical Care Medicine, Johns Hopkins Asthma & Allergy Center (JHAAC), Room 4B.72, 5501 Hopkins Bayview Circle, Baltimore, MD 21224-6821, United States

Received June 8, 1994 / Revised and accepted July 22, 1994

Using starch-gel electrophoresis, 21 different enzymes were examined. Both spp. were collected from unpolluted lentic environments. Different gel systems which were appropriate for the specific enzymes tested for were utilized. Enzymatic polymorphisms between and within species have been documented. Several enzymes, such as alcohol dehydrogenase and superoxide dismutase, were unexpectedly absent in one or both of the spp. Numbers and frequencies of alleles detected for both spp. are given.

INTRODUCTION

The use of aquatic organisms as environmental indicators is common practice (HIGLER, 1974; HILSENHOFF, 1982; MARTIN et al., 1986). Generally, this has involved population studies of whole organisms, using their presence or absence as indicative of water quality. The health of the organisms however, is frequently not taken into consideration. It is therefore possible, that apparently healthy animals may actually be metabolically impaired. Since several studies have demonstrated that enzymes can be affected by contact with heavy metals (DIXON & WEBB, 1964; EISENBERG, 1970; HARRISON et al., 1994), the

* **Current address**: Division of Pulmonary and Critical Care Medicine, Johns Hopkins Asthma & Allergy Center (JHAAC), Room 4B.72, 5501 Hopkins Bayview Circle, Baltimore, MD 21224-6821, United States

measurement of enzyme activity may be a more sensitive measurement of water quality.

Few studies have characterized enzymes in the odonates. An electrophoretic analysis by SCHOTT & BRUSVEN (1980) showed that enzymatic activities varied relative to a temperature gradient in Zygoptera. They found differences in four enzymes: glucose-6-phosphate dehydrogenase, lactate dehydrogenase, leucine aminopeptidase, and tetrazolium oxidase. Malate dehydrogenase and esterase showed no differences. DIERICKX (1984) studied the activity of glutathione S-transferase in aquatic macroinvertebrates subjected to various pollutants. He found an activity level in odonates six times higher than that of other aquatic insects. Since glutathione S-transferase is a detoxification enzyme, his data may explain why dragonflies are more resistant to pollution than are many other aquatic insects. TEMBHARE & ANDREW (1991) reported that protease activity in dragonflies was regulated by medial neurosecretory cell hormone, and TEMBHARE & MUTHAL (1992) determined optimal activity conditions for several of the midgut digestive enzymes.

Other studies have used enzymatic analysis as a method to determine genetic distances in Odonata (CARIUS, 1988; 1993; COBOLLI et al. 1992). KNOPF (1977) carried out an electrophoretic study of gomphids, (and members of four other anisopteran families) of unspecified life-stage, collected mainly from Florida. He analyzed 22 loci from the Gomphidae. In addition to *Gomphus*, and *Progomphus*, Libellulidae, Aeshnidae (including *Anax*), Macromiidae, and Corduliidae were studied by Knopf. However, in his study, many species were represented by very few (sometimes single) specimens. In related studies of odonates, ANDERSON et al. (1969-1970) using gel disc electrophoresis, noted variations in unspecified proteins that were related to instar or life stage in libellulids and aeshnids, although ZLOTY et al. (1993a) were able to identify larvae of several species of *Hetaerina* by comparing larval and adult enzymes using cellulose acetate gel electrophoresis. ZLOTY et al. (1993b) were also able to identify early instar larvae by comparing their enzymes with later instar larvae.

Since there are limited studies of enzymatic functions in Odonata, it was necessary to develop a baseline establishing which enzymes were present in organisms from an unpolluted area and how the enzymes migrated on gels prior to studying environmental effects on enzymatic function. This paper reports such a study.

MATERIAL AND METHODS

Two types of odonate larvae were collected and the species were determined microscopically using appropriate dichothomous keys (NEEDHAM & NEEDHAM, 1962; PENNAK, 1978; BORROR et al., 1981; MERRITT & CUMMINS, 1984). Eighteen larval *Erythemis simplicicollis* (Say) were collected from Pig Pen Pond on the University of Maryland Baltimore Co. (UMBC) campus during June. Five *Anax junius* (Drury) larvae were taken in November from among cattails *Typha latifolia*

L. in a temporary pond, also on the UMBC campus. Larvae of similar sizes in each group were selected. The animals were starved for five days, an arbitrary time chosen to prevent possible gut-content interference in the assays (see TERRA, et al., 1990). After the fifth day, all remaining animals were frozen at -70° C and stored until the gels could be run.

Individual animals were homogenized in a neutral pH extraction buffer solution. Although whole animal preparations were used in this study, only a tiny amount of material from each animal was necessary for each gel run due to the catalytic nature of enzymes. In a study of the copepod *Eurytemora affinis* (Poppe), NIXON et al. (1983) successfully used as few as two individuals with volumes ranging from > 1 µl to <100 µl. ANDERSON et al. (1969-1970) were able to resolve bands on disc gels using just 8-10 µl of haemolymph drawn from living aeshnid and libellulid larvae.

Enzymatic mobilities were determined by horizontal, starch-gel electrophoresis. The procedures used (HARRISON, 1993) were based on those of McCRACKEN (1976) and MAY et al. (1979). Since different enzymes run, and separate more clearly, under different chemical conditions, three different buffer systems: C (CLAYTON & TRETIAK, 1972), R. (RIDGEWAY et al., 1970), and 4 (SELANDER et al., 1971) were used. The selection of the gel depended upon the list from BRUSSARD et al. (1985). The liquid gels were prepared 12 h before they were to be run and immediately poured onto glass plates on which plexiglass frames of 21.5 cm × 12.5 cm × 1.5 cm had been clamped. They were allowed to set, were then covered with plastic wrap and left to age 12 h at room temperature. Prior to use, the gels were chilled at 4°C for 1 hr, then removed from the refrigerator and a lateral perpendicular slice made 4 cm from the bottom – this is referred to as the origin.

Dits soaked in homogenized insect samples or red dye were placed in the origin. A Heath Zenith SP-2717A power supply was attached to platinum wire electrodes in the tray buffers. The setting was maintained at 75 ma or 225 V, whichever was greater. Timing for the gel runs were: C 5 hr, R 5-6 hr, and 4 6-7 hr. Gels were maintained at 4°C while running to slow degradation of the enzymes.

Gels were run for 21 different enzymes. During the initial run, nine Lepidoptera (*Limenitis* spp.) were run along with the odonates as they had been run before (A.P. Platt, unpubl. data), and served as known controls. The enzymes assayed were: Adenylate kinase (AK); Alcohol dehydrogenase (ADH); Aldolase (ALD); Diaphorase (DIA); Esterase (EST); Galactosaminidase (GAM); Glucosekinase (GK); Glucosephosphate isomerase (GPI); α-Glycerophosphate dehydrogenase (αGPD); Hydroxybutyryl dehydrogenase (HBDH); Isocitrate dehydrogenase (IDH); Leucine aminopeptidase (LAP); Malate dehydrogenase^{+} (MDH^{+}); Malate dehydrogenase^{-} (MDH^{-}); Malic enzyme (ME); Peptidase (PEP); Phosphoglucomutase (PGM); Phosphogluconate dehydrogenase (PGD); Octanol dehydrogenase (ODH); Superoxide dismutase (SOD); and Xanthine dehydrogenase (XDH).

Most enzymes have different allozymes and isozymes, however, if the activities were maintained, the bands developed and stained when present. The assay for esterase, however, was non-specific and used synthetic compounds as substrates. The natural substrates upon which various esterases act are not always known (SHAW, 1965). The PEP assay was also somewhat non-specific. The assay used was for peptidase – leucyl-leucyl-leucine (PEP – III), however, it had been previously determined in *Limenitis* spp. (A.P. Platt, unpubl. data) that PEP – III cross-reacted with: PEP – glycyl-leucine, PEP – leucyl-alanine, PEP – leucyl-glycyl-glycine, and PEP – phenyl-alanyl-proline; therefore, the specific form of peptidase detected on these gels was indeterminate.

After running, the gels were sliced, placed in the appropriate substrate, and incubated at 37°C until bands appeared. The substrates were aqueous solutions of critical co-factors which were necessary components of secondary reactions required for development and staining of the product to be detected. Therefore, these assays detected the reaction products of the enzymes and their substrates, not the enzymes themselves. Each of the solutions contained, in addition to the substrate, a number of additional chemicals necessary for the bands to stain. All of the cofactors were added in excess as the enzymes could not perform their catalytic functions unless the cofactors were present in equal or greater amounts than were the enzymes (WOLFE, 1986). A few gels (especially transferases and isomerases) required the stains to be prepared in agar overlays. The coupling enzymes which were

needed to convert the enzyme products into substrates for the dehydrogenases in the mixture, penetrated the gels poorly. As a result, the coloured products appeared only on the surfaces, and the agar was added to prevent zone spreading (HAMES, 1990). The recipes for development and staining of bands were followed exactly to avoid artifacts due to viscosity, ionic strength, or chemical properties (WILKINSON, 1966). Also the addition of certain compounds, such as sucrose, may cause additional bands to appear (ANDERSON et al., 1969-1970). After staining, the slices were removed from the substrate and photographed using black and white film. As the bands were sometimes pale, and did not contrast well enough for photographs of the results to be useful, acetate sheets were laid over the gel slices and ink tracings were made and xeroxed to provide permanent records of the runs.

Table I

The number and types of alleles found for each isozyme, followed by the number of animals in each group having that enzyme. The fastest allele i.e. the one that moves farthest from the origin, whether anodally or cathodally, is designated A, second fastest is B, third fastest is C, etc. – [X = enzyme was not present, O = gel did not develop]

Gel	Enzyme	*A. junius* (N=5)	*E. simplicicollis* (N=18)
C	ALD	A, 5	O
C	DIA	A, B, 5; C, 3; D, 2	A, 18
C	GAM	A, 5	A, 5; B, 13
C	HBDH	X	X
C	MDH^-	A, 5	A, B, 4; C, D, 1
C	MDH^+	A, 5; B, 1	A, B, 18
C	PGM	A, 5	A, B, 18
R	ADH	X	X
R	AK	X	A, 1; B, 2
R	EST	A, 3; B, 3; C, 5; D, 1	A, B, 1; C, D, E, F, 18; G, 1
R	GPI	A, 5; B, 5; C, 5	A, 15
R	LAP	A, 4; B, 1; C, D, E, 5	A, 2; B, 1; C, 16; D, 15; E, 2
R	PEP	A, 3; B, 2; C, D, 5	A, B, 18
R	ODH	X	X
4	GK	X	X
4	αGPD	A, 5	X
4	IDH	A, B, C, 1	X
4	ME	X	X
4	PGD	X	X
4	XDH	A, 5	A, 18
4	SOD	A, 5	X

RESULTS

Table I shows the results of the runs, and describes how the bands were scored. Ten enzymes were present in both libellulids and aeshnids, although often as different isomers having different mobilities. Of the eleven enzymes that appeared on the libellulid gel, eight were polymorphic; the aeshnid gel had seven poly-

morphic loci but from a total of 14. EST had the most alleles in the libellulids with seven, however three of these, A, B, and G were found in only one animal. The others, C, D, E, and F were found in all 18 individuals. LAP had a somewhat similar pattern with C, which was found in 16 animals, and D in 15, but A, B, and E were found in only one or two animals. Of the others, only MDH$^-$ (4 alleles) had more than two alleles for any enzyme. In the aeshnids, LAP had the most alleles with five; DIA, EST, and PEP each had four. The other enzymes had from one to three alleles.

No bands developed for six of the 21 enzymes: ADH, GK, HBDH, ME, PGD, and ODH, on any gels for odonates but these did appear for the Lepidoptera. ALD on the libellulid gel developed bands neither for Odonata nor Lepidoptera.

On four of the libellulid gels, odd bands that did not match those on the rest of the gel developed in a few of the lanes. Only three (polymorphic) bands, from three different animals, developed on the AK gel. MDH$^-$, EST, and LAP each have a small number of bands with patterns that varied inconsistently from the others on their respective gels.

DISCUSSION

Using starch-gel electrophoresis, this study has examined 21 different enzymes in two species of larval odonates. Many differences within and between the species were observed.

Polymorphisms have been reported in enzymatic studies (GOTTLIEB, 1971; HARRISON, 1977; JOHNSON, 1974; SHAW, 1965). The gels in this study demonstrated several polymorphic enzymes in the two species of odonates (Tab. I). No clear-cut trends involving monomorphic vs. polymorphic loci, either within or between species were evident from our data. However, if an enzyme was present or absent in one species it often followed a similar pattern in the other. Considering that both species are from the same insect order, both live in lentic environments, and both are predatory in nature, it was not unexpected to find many such similarities.

As in this study, KNOPF (1977) found EST $^-$ 2 to have the greatest number of alleles (18) although most of his were found in different species. He also found high numbers for GPI (11 alleles), ME and PGM (ten each), ADH (nine), IDH$^-$ 1 & 2 (eight and five, respectively), and HBDH (six), also from different species. All of the remaining enzymes he assayed for had either two or three alleles. Even so, the overall variation among dragonflies was lower than in other insects, such as *Drosophila* spp., which have been more intensively studied.

Some enzymes are puzzling by their absence. SOD is an ubiquitous enzyme that dismutases O_2^- (GOODSELL, 1992; RUSTING, 1992; SANDERS et al., 1993). Hence it should be found in most animals but it was absent from the libellulid gel. AK, sometimes called myokinase, catalyzes the rephosphorylation

of AMP to ADP (LEHNINGER, 1975) and its presence would also be expected. Likewise, ADH and ODH should have been present. Since these enzymes may carry out more than one function, eg. lipid metabolism and breakdown of dietary alcohols (GOODSELL, 1992; JOHNSON, 1974), starvation has been proposed as an explanation for its absence. The relative sizes and seasonality of the animals might help explain such differences as GK's absence in libellulids but its presence in aeshnids. This enzyme is produced in response to an excess of sugar in humans (LEHNINGER, 1975); if the same is true in insects, its absence in starved animals is expected. The aeshnids being larger, and also collected later in the year, might have reserves stored in their bodies that could account for the presence of the enzyme.

The odd bands that develop on the gels may result from rare alleles; but they may also result from the fact that odonate larvae are host to a number of internal and external parasites such as mites, and horsehair worms (ÅBRO, 1990; CAMPBELL, 1985; FRYE & ROBINSON, 1987; HERMES, 1943; PARSONS et al., 1966; ROBINSON, 1983; WILLEY, 1972). Different species, even when totally unrelated, often possess many of the same enzymes, or very similar isozymes. If parasites were present and contained sufficient quantities of the type of enzymes being assayed (and very little is required), they might have influenced the gels by providing bands indistinguishable from the odonate bands unless they had different mobilities. That potential parasitic infestation might appear in the libellulids and not the aeshnids may result from the former being collected in an old pond where a wide range of organisms (including parasites) could have been well established, while the aeshnids were taken from a recently formed, temporary pond. Whether the odd bands expressed represented genetic factors inherent in animals at different instars, or whether there are external agents such as environmental causes or parasites, needs to be determined before far-reaching conclusions can be drawn based on such gel-mobility patterns. Such difficulties of interpretation often occur when assaying wild-collected animals which have unknown developmental exposure, and environmental histories.

CONCLUSION

Two genera of larval odonates belonging to separate families have been examined and the data showed that enzyme variants of a number of basic metabolic proteins could be detected in both groups. Different allelic mobilities within species were often found on the gels. There were differences both between and within species. Although some enzymes, such as the non-specific EST, tended to exhibit polymorphisms, there was not enough evidence to conclude that any given enzyme would always exhibit polymorphisms. The presence of certain enzymes in a given species, however, may increase this likelihood. Further work should focus on whether such enzymes exhibit similar mobilities in both larval

and imaginal odonate populations, and whether external factors influence mobilities.

ACKNOWLEDGEMENTS

The authors thank Dr DAVID FLAIM of UMBC for assistance with collecting and identifying the dragonflies; and Drs DONALD C. FORESTER and AUBREY G. SCARBROUGH of Towson State University for comments on an earlier form of the manuscript. This work was supported by graduate student grants from TSU and UMBC.

REFERENCES

ÅBRO, A., 1990. The impact of parasites in adult populations of Zygoptera. *Odonatologica* 19(3): 223-233.

ANDERSON, M., L. HALGREN & L. NUTI, 1969-1970. Protein patterns of dragonfly hemolymph as shown by gel disc electrophoresis. *J. Minn. Acad. Sci.* 36(2-3): 75-76.

BORROR, D.J., D.M. DeLONG & C.A. TRIPLEHORN, 1981. An introduction to the study of insects [5th ed.]. Saunders Coll., Philadelphia.

BRUSSARD, P.F., P.R. EHRLICH, D.D. MURPHY, B.A. WILCOX & J. WRIGHT, 1985. Genetic distance and the taxonomy of checkerspot butterflies (Nymphalidae: Nymphalinae). *J. Kans. ent. Soc.* 58(3): 403-412.

CAMPBELL, A., 1985. Worm-like invertebrates. *In*: G. Bateman, [Ed.], All the world's animals – aquatic invertebrates. Torstar Books, New York.

CLAYTON, J.W. & D.N. TRETIAK, 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. *J. Fish. Res. Bd Can.* 29(8): 1169-1172.

CARIUS, W., 1988. *Verwandschaftsanalyse der europäischen Vertreter der Gattung Sympetrum (Newman, 1833) (Libellulidae, Odonata).* DiplArb. Univ. Bremen.

CARIUS, W., 1993. *Zur Verwendbarkeit biochemischer Daten in der Verwandschaftsanalyse von Insekten. Elektrophoretische Untersuchungen an Archeocarabus (Bengtsson, 1927) (Carabus, Carabidae, Coleoptera) und der Gattung Aeshna Fabricius, 1775 (Aeshnidae, Odonata).* Diss. Univ. Bremen.

COBOLLI, M., E. DE MATTHAEIS & G. CARCHINI, 1992. Biochemical systematics in Odonata: the mediterranean Coenagrionidae. *Proc. 19th Int. Congr. Ent., Beijing*, p. 27. – [Abstract only].

DIERICKX, P.J., 1984. Glutathione S-transferase in aquatic macro-organisms and its interaction with different organic micropollutants. *Sci. total Env.* 40: 93-102.

DIXON, M. & E.C. WEBB, 1964. *Enzymes.* [2nd ed.]. Academic Press, New York.

EISENBERG, D., 1970. X-ray crystallography and enzyme structure. *In*: P.D. Boyer, [Ed.], The enzymes: structure and control, Vol. 1. [3rd ed.]. Acad. Press, New York.

FREY, B.L. & J.V. ROBINSON, 1987. Ischnura posita (Hagen) and Telebasis salva (Hagen) (Odonata: Coenagrionidae) as potential fungal dispersal agents. *SWest. Nat.* 32(1): 131-134.

GOTTLIEB, L.D., 1971. Gel electrophoresis: new approach to the study of evolution. *BioScience* 21(18): 939-944.

GOODSELL, D.S., 1992. A look inside the living cell. *Am. Scient.* 80(5): 457-465.

HARRISON, R.G., 1977. Parallel variation at an enzyme locus in sibling species of field crickets. *Nature, Lond.* 266: 168-170.

HARRISON, S.J., 1993. *Variation in enzymatic mobility of anisopteran naiads exposed to sub-lethal concentrations of Cu^{2+}.* M.Sc. thesis, Towson St. Univ.

HARRISON, S.J. & A.P. PLATT, 1994. Environmental exposure to $Cu2^{+}$ in Anax junius (Drury) (Anisoptera: Aeshnidae) naiads causes disruption in enzymatic mobilities. *Md. Entomol.* – [In press].

HAMES, B.D., 1990. One-dimensional polyacrylamide gel electrophoresis. *In*: B.D. Hames & D. Rickwood, [Eds], Gel electrophoresis of proteins. IRL Press, New York.

HERMES, W.B., 1943. *Medical entomology.* [3rd ed.]. MacMillan, New York.

HIGLER, L.W.G., 1974. The biological assessment of water by means of macrofauna. *Hydrobiol. Bull.* 8(3): 285-287.

HILSENHOFF, W.H., 1982. Using a biotic index to evaluate water quality in streams. *Tech. Bull. Dept nat. Res. Madison Wisc.* 132: 1-24.

JOHNSON, G.B., 1974. Enzyme polymorphism and metabolism. *Science* 184: 28-37.

KNOPF, K.W., 1977. *Protein variation in Gomphus (Odonata: Gomphidae).* Ph.D. thesis Univ. Fla, Gainesville.

LEHNINGER, A.L., 1975. *Biochemistry.* Worth, New York.

MARTIN, S.R. & P.S. CLAYTON, 1986. *Technical summary, Jones Falls watershed urban stormwater runoff project.* Regional Planning Council, Baltimore/MD.

MAY, B., J.E. WRIGHT & M. STONEKING, 1979. Joint segregation of the biochemical loci in Salmonidae: results from experiments with Salvelinus and review of the literature on other species. *J. Fish. Res. Bd Can.* 36: 1114-1128.

McCAFFERTY, W.P., 1981. *Aquatic entomology.* [2nd ed.]. Jones & Bartlett, Boston.

McCRACKEN, G.F., 1976. *The population biology of the white-lipped land snail, Triodopsis albolabris.* Ph.D. thesis, Cornell Univ., Ithaca/NY.

MERRITT, R.W. & K.W. CUMMINS, 1984. *An introduction to the aquatic insects of North America.* [2nd ed.]. Kendall/Hunt, Dubuque.

NEEDHAM, J.G. & P.R. NEEDHAM, 1962. *A guide to the study of fresh water biology.* Holden--Day, San Fransisco.

NIXON, B.F., T.J. COLE & B.P. BRADLEY, 1983. Biochemical analysis of the copepod Eurytemora affinis: a modification of standard starch gel techniques. *Comp. Biochem. Physiol.* (B)75(4): 659-663.

PARSONS, W.M., H.E. SCHLICTING & K.W. STEWART, 1966. In-flight transport of algae and protozoa by selected Odonata. *Trans. Am. microsc. Soc.* 85(4): 520-527.

PENNAK, R.W., 1978. *Fresh water invertebrates of the United States.* Wiley, New York.

RIDGEWAY, G.J., S.W. SHERBURNE & R.D. LEWIS, 1970. Polymorphism in the esterases of Atlantic herring. *Trans. Am. Fish. Soc.* 99(1): 147-151.

ROBINSON, J.V., 1983. Effects of water mite parasitism on the demographics of an adult population of Ischnura posita (Hagen) (Odonata: Coenagrionidae). *Am. Midl. Nat.* 109(1): 169-174.

RUSTING, R.L., 1992. Why do we age? *Scient. Am.* 267(6): 130-141.

SANDERS, S.P., J.L. ZWEIER, P. KUPPUSAMY, S.J. HARRISON, D.J.P. BASSETT, E.W. GABRIELSON & J.T. SYLVESTER, 1993. Hyperoxic sheep pulmonary microvascular endothelial cells generate free radicals via mitochondrial electron transport. *J. clin. Invest.* 91(1): 46-52.

SELANDER, R.K., M.H. SMITH, S.Y. YANG, W.E. JOHNSON & J.B. GENTRY, 1971. Biochemical polymorphism and systematics in the genus Peromyscus. 1. Variation in the old-field mouse (Peromyscus polionotus). *Univ. Tex. Publs* 7103: 49-90.

SHAW, C.R., 1965. Electrophoretic variation in enzymes. *Science* 149: 936-943.

SCHOTT, R.J. & M.A. BRUSVEN, 1980. The ecology and electrophoretic analysis of the damselfly, Argia vivida Hagen, living in a geothermal gradient. *Hydrobiologia* 69(3): 261-265.

TEMBHARE, D.B. & R.J. ANDREW, 1991. Neuroendocrine influence on haemolymph protein concentration and midgut protease activity in the final instar larva of Tramea virginia (Rambur) (Anisoptera: Libellulidae). *Odonatologica* 20(1): 91-95.

TEMBHARE, D.B. & A. MUTHAL, 1992. Midgut digestive enzyme activity in the dragonfly, Tramea virginia (Rambur) (Anisoptera: Libellulidae). *Odonatologica* 21(1): 111-117.

TERRA, W.R., F.J.A. LEMOS & B.P. JORDÃO, 1990. Enzyme depletion in insect feeding experiments: fact or artifact? *Comp. Biochem. Physiol.* (B)96(1): 59-61.

WILLEY, R.L., 1972. The damselfly (Odonata) hindgut as host organ for the euglenoid flagellate Colacium. *Trans. Am. microsc. Soc.* 91(4): 585-593.

WILKINSON, J.H., 1966. *Isoenzymes*. Lippincott, Phildelphia.

WOLFE, D.H., 1986. *Essentials of general, organic and biological chemistry*. McGraw-Hill, New York.

ZLOTY, J., G. PRITCHARD & C. ESQUIVEL, 1993a. Larvae of Costa Rican Hetaerina (Odonata: Calopterygidae) with comments on distribution. *Syst. Ent.* 18(3): 235-265.

ZLOTY, J., G. PRITCHARD & R. KRISHNARAJ, 1993b. Larval insect identification by cellulose acetate gel electrophoresis and its application to life history evaluation and cohort analysis. *Jl N. Am. benthol. Soc.* 12(3): 270-278.