Coleoptera fossils speculates that most of the North American insect fauna evolved prior to the Pleistocene. Moreover, Vickery[15] firmly believes that the Melanopline species are even phylogenetically older than that – perhaps as old as the North–South American split. This view is based on the existence of a rather close affinity between the Nearctic *Melanoplus* and the Neotropical sister genus, *Dichroplus* and the fact that the amount of radiation, speciation and distribution within each group is very extensive. Vickery also notes that the fossil record that exists for other insect groups supports the claim that most of the existing North American insect fauna evolved early. Therefore, a reasonable interpretation of the allozyme data is that commonalities of allelic distributions stem from purifying selection in the case of the Mdh and Gpdh loci and some form of balancing selection in the case of the Ldh locus. Furthermore, for species exhibiting similar patterns, it is proposed that the selection constraints, whatever their nature, are identical. It is entirely possible, of course, that the patterns stem from selection acting on closely linked loci rather than on the enzyme loci themselves. While it does seem implausible that linkage disequilibrium between the studied loci and fitness loci would be identical in separate lineages for such a long time, in the absence of map data, a hitchhiking effect cannot be ruled out. Returning to the main objective of the paper, it would have to be concluded by extrapolating from the interspecific findings that the uniform allelic distributions between populations in prairie and parkland locations (despite differences in soil, food and so on) are best explained by hypothesis 2.

The neutralist position is also untenable from another consideration. It is possible that what are called alleles in this article are, in fact, electrophoretic mobility classes comprising several alleles that correspond to polypeptides with the same electrophoretic mobilities. But as Lewontin[4] has pointed out, if a large number of alleles do comprise each mobility class, then by the law of large numbers, one would expect independently evolving populations (or species) to have the same frequency distribution of classes. An examination of the table clearly shows that this is not the case.

1 Acknowledgements. I thank B. R. Pittendrigh for his technical assistance and the Natural Sciences and Engineering Research Council (Grant A-0485) for their financial assistance.
2 Chapco, W., Can. J. genet. Cytol. *25* (1983) 88.
3 Chapco, W., and Bidochka, M. J., Heredity *56* (1986) 397.
4 Lewontin, R. C., The Genetic Basis of Evolutionary Change. Columbia, 1974.
5 Vickery, V. R., and Kevan, D. K. Mc., The Insects and Arachnids of Canada. Agric. Can. Publ. *14* (1985).
6 Chapco, W., Ann. ent. Soc. Am. *77* (1984) 11.
7 Chapco, W., Bidochka, M. J., and Hodson, F. R. H., in: Evolutionary Biology of Orthopteroid Insects. Horwood, 1987.
8 Criddle, N., Can. Ent. *55* (1933) 97.
9 Riegert, P. W., Pickford, R., and Putnam, L. G., Can. Ent. *97* (1965) 508.
10 Riegert, P. W., Can. Ent. *99* (1967) 952.
11 Beirne, B. P., Mem. ent. Soc. Can. *84* (1972) 1.
12 Gill, P., Ecol. Ent. *4* (1979) 249.
13 Futuyma, D. J., Evolutionary Biology. Sinauer, 1986.
14 Mathews, J. V., Can. Ent. *112* (1980) 1089.
15 Vickery, V. R., Personal communication, Sept. 28, 1988.

0014-4754/89/020196-03$1.50 + 0.20/0

Catalase in sulfide- and methane-dependent macrofauna from petroleum seeps[1]

E. N. Powell, A. C. Morrill and R. R. Bidigare

Department of Oceanography, Texas A & M University, College Station (Texas 77843, USA)
Received 8 August 1988; accepted 4 November 1988

Summary. Vesicomyid and lucinid clams and tubeworms from Gulf of Mexico petroleum seeps, all of which bear symbiotic sulfide-oxidizing bacteria, have much lower catalase activities than shallow-water species lacking symbionts. A petroleum seep mussel bearing methane-oxidizing bacteria is unusual in having catalase activities as high as shallow-water bivalves. Unlike sulfide-dependent meiofauna from shallow-water marine sands, catalase from all petroleum seep species was inhibited by 3-amino-1,2,4-triazole.
Key words. Catalase; sulfide; methane; oxygen; petroleum seep; bivalve; vestimentiferan.

Two types of sulfide-dependent metazoans are known. 1) Sulfide-dependent meiofauna, thiobios, are found in most shallow-water marine sediments where an oxic surface layer is separated from a sulfidic deeper layer by a chemocline[2, 3]. Thiobiotic meiofauna inhabit subsurface sediments below the oxygenated upper layer of marine sands, the sulfide system of Fenchel and Riedl[4]. The sulfide system constitutes a rigorous environment, toxic to most animals, and organisms living there have developed unusual strategies for survival. These include mechanisms for sulfide detoxification[5, 6], and the modification of some normally sulfide-sensitive pathways, aerobic respiration for example[7], to be sulfide-insensitive. 2) The second group of sulfide-dependent animals are macrofauna primarily associated with hydrothermal vents, cold water sulfide seeps and petroleum seeps. Unlike thiobiotic meiofauna, which generally lack bacterial symbionts, many of these macrofauna contain sulfur-oxidizing bacteria[8 – 10]. Their survival appears to be heavily dependent upon sulfide detoxification[11, 12] and sulfide control (e.g. sulfide-binding proteins)[13 – 15]. Consequently, those sulfide-insensitive metabolic pathways of the meiofauna have remained sulfide-sensitive in these macrofauna, as they are in most animals.

Oxygen, like sulfide, can be toxic. Oxygen toxicity is produced primarily by superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH\cdot$) which are produced by a variety of oxygen-dependent enzymatic reactions associated with electron transport systems, some immune responses, and a variety of oxidases[16 – 18]. The oxidation of inorganic sulfide[19], pathways of sulfide detoxification[20] and photochemical reactions of sunlight and water[21, 22] may also be important in oxygen radical formation.

Morrill et al.[23] examined the enzyme catalase which catalyzes the detoxification of H_2O_2:

$$2\,H_2O_2 \rightarrow 2\,H_2O + O_2$$

These investigators suspected that thiobiotic meiofauna should have little catalase because thiobios live under contin-

ual anoxia[2] and most animals living at low oxygen concentrations have low catalase activities[18,24,25]. Surprisingly, Morrill et al.[23] observed that thiobiotic meiofauna had high levels of catalase and that catalase concentrations were highest in species normally exposed to highest sulfide concentrations.

Sulfide is a potent catalase inhibitor[20,26]. Some bacteria incapable of heme synthesis possess a non-heme enzyme, pseudocatalase, that is sulfide insensitive[27]. Pseudocatalase is also insensitive to azide, cyanide and 3-amino-1,2,4-triazole[28] which inhibit all known catalases[29,30]. Morrill et al.[23] found that thiobiotic catalase was also azide and 3-amino-1,2,4-triazole insensitive. Thus, in all likelihood, this enzyme is also sulfide-insensitive and probably represents another metabolic adaptation to increase sulfide-insensitivity in thiobios.

Here, we ask the question, is the catalase of sulfide-dependent macrofauna collected at petroleum seeps sulfide-insensitive like the thiobiotic enzyme or is it sulfide-sensitive as would be expected from previous indications of retained sulfide-sensitivity in sulfide-dependent macrofauna? The recent discovery of a methane-dependent mussel[10] at these same seeps provided the opportunity to compare an animal with symbiotic methane-oxidizing bacteria to its sulfide-symbiont-containing counterparts.

Methods. Animals were collected by trawl from the Louisiana slope petroleum seeps described by Brooks et al.[31]. Each animal was dissected and frozen immediately in liquid nitrogen once aboard ship. For comparison, shallow-water molluscs were collected from Galveston Bay, Texas.

Catalase was measured using a variation of the Beers and Sizer[32] assay developed by the Sigma Chemical Co.[33] and modified for increased sensitivity by Morrill et al.[23]. Samples were homogenized in 0.1 ml seawater and 0.4 ml 0.05 M phosphate buffer using a Polytron tissue grinder and an ice bath. The entire 0.5-ml homogenate was assayed by adding it to 2.5 ml H_2O_2 in 0.05 M phosphate buffer ($A_{240} = 0.620$). Enzyme activity was measured at 25 °C by following the decrease in absorbance over a 180-s period. The initial 15 s of the reaction were occasionally anomalous, hence we recorded the change in absorbance from 15 s to 195 s, which normally was linear. The detection limit was equivalent to approximately 0.1 µg of bovine liver catalase which was used as the reference standard. Catalase was inhibited by adding 20 mM 3-amino-1,2,4-triazole and incubating the homogenate for 30 min at 25 °C prior to analysis. This concentration of 3-amino-1,2,4-triazole inhibited the liver catalase standard and natural catalase extracted from a variety of marine and freshwater invertebrates by > 90%[23]. Protein was determined by a modified Lowry technique as described in Morrill et al.[23].

Results. Catalase activity in the oyster *Crassostrea virginica*, the clam *Mercenaria mercenaria* and the mussel *Brachidontes exustus* varied from tissue to tissue but was normally above 10 units · mg protein^{-1} (table 1). Adductor muscle was generally lower than mantle or gill. Standard deviation in replicated assays obtained from different animals was usually half or less of the mean.

Five species of macrofauna from petroleum seeps were assayed: two vestimentiferans containing symbiotic sulfur-oxidizing bacteria, cf. *Escarpia* sp. and *Lamellibrachia* sp.; two clams containing symbiotic sulfur-oxidizing bacteria, *Vesicomya cordata* and *Pseudomiltha* sp.; and the mussel, cf. *Bathymodiolus* sp. bearing symbiotic methane-oxidizing bacteria (all taxonomic designations follow Brooks et al.[10]). The species harboring sulfur-oxidizing symbionts had uniformly low catalase activities (< 2 units · mg protein^{-1}) (table 2). Standard deviations of replicated assays using different animals were usually half or less of the mean. The symbiont-bearing tissue of the vestimentiferans, the trophosome, had higher activities than other vestimentiferan tissues lacking symbionts. The symbiont-bearing tissue of the clams however, the gill tissue, was not higher in catalase activity. Catalase activity was uniformly low in all clam tissues (0.5–1.2 units · mg protein^{-1}). In contrast, the mussel (with methane-oxidizing symbiotic bacteria) had catalase levels comparable to its Galveston Bay counterparts, 10–20 times the activity measured in the other petroleum seep species. The catalase inhibitor, 3-amino-1,2,4-triazole, substantially inhibited catalase activity in all species tested (table 3).

Table 1. Catalase activities of molluscan species from Galveston Bay, Texas

Taxon	Catalase activity (units · mg protein^{-1})	Number assayed
Crassostrea virginica		
Palps	50.1 ± 24.4	3
Mantle	16.6 ± 11.3	4
Gill	15.3 ± 0.5	4
Adductor muscle	13.8 ± 1.9	3
Hepatopancreas	8.1 ± 3.1	3
Mercenaria mercenaria		
Gill	41.8	1
Mantle	19.1	1
Adductor muscle	17.1	1
Foot	2.5	1
Brachidontes exustus		
Whole body	82.9 ± 50.4	3
Gill	58.7 ± 14.1	3
Adductor muscle	17.6 ± 5.4	3

Table 2. Catalase activities of molluscan and vestimentiferan species from petroleum seeps. Taxonomy follows Brooks et al.[10]

Taxon	Catalase activity (units · mg protein^{-1})	Number assayed
Pseudomiltha sp.		
Whole body	0.5	1
Gills	0.5 ± 0.2	4
Body without gills	0.7 ± 0.7	6
Vesicomya cordata		
Gills	0.9	1
Foot	1.2	1
cf. *Bathymodiolus* sp.		
Gills	22.6	1
Foot	14.3	1
Mantle	6.2	1
Hepatopancreas	10.1	1
Lamellibrachia sp.		
Trophosome	1.2 ± 0.3	4
Vestimentum	0.4 ± 0.2	2
cf. *Escarpia* sp.		
Trophosome	1.8 ± 0.2	2
Vestimentum	0.7	1

Table 3. Percent inhibition of catalase activity by 3-amino-1,2,4-triazole

Crassostrea virginica		*Lammelibrachia* sp.	
Palp	100%	Vestimentum	78%
Gill	95%	Trophosome	78%
Brachidontes exustus		cf. *Escarpia* sp.	
Gill	91%	Trophosome	72%
Whole body	94%	Vestimentum	100%
cf. *Bathymodiolus* sp.		*Vesicomya cordata*	
Gill	98%	Foot	100%
Mantle	100%	Gill	100%
Foot	65%	*Pseudomiltha* sp.	
		Gill	64%

Discussion. Catalase activities measured in shallow-water molluscs were similar to those of other shallow-water benthic biota[23]. Our values for *Mercenaria mercenaria* were somewhat higher than those measured by Blum and Fridovich[34], however we also observed that gill tissue had activities much above that of adductor muscle. In contrast, with the exception of the mussel, cf. *Bathymodiolus* sp., all petroleum seep species had low catalase activities. Blum and Fridovich[34] did not detect catalase in similar hydrothermal vent species, however our assay may have had higher sensitivity. Petroleum seep species live below the photic zone, at or near the oxygen minimum for the Gulf of Mexico[35]. Hence an important source of oxygen radicals (photochemical ionization) is absent in their habitat and ambient oxygen concentration is relatively low. Accordingly, low catalase levels in these species conform to the general trend of reduced catalase activities in animals living at lower oxygen concentrations and reduced light levels[23]. Coincidentally, perhaps, all of these species, cf. *Escarpia* sp., *Lamellibrachia* sp., *Vesicomya cordata* and *Pseudomiltha* sp., also have sulfur-oxidizing symbionts. The low catalase activities present suggest that one possible source of oxygen radicals, the reaction of sulfide and oxygen, is unimportant for these animals[19,36]. Of course, an alternative peroxidase-based system may be used rather than catalase[34,37].

The methane mussel, in contrast to all other seep species tested, had high catalase activity. One possible explanation for elevated catalase in the mussel is the use of a monooxygenase for methane oxidation by the symbiotic methanotrophic bacteria[38,39]. Methane oxidation by methane monooxygenase may involve H_2O_2[40]. Catalase activities were high in all tissues examined, not just the gills where the symbiotic bacteria are found. Hydrogen peroxide can pass through biological membranes and frequently travels relatively far from its site of production before reacting[41,42]. Perhaps high catalase activities are required in all tissues for this reason. An alternative explanation is not readily apparent. Only one individual was available for analysis, however standard deviations for replicated assays were less than half the mean in other species. Even if this animal contained more catalase by a factor of 2 than the mean for that species, catalase activities would have been 5–10 times higher than the other petroleum seep species examined.

All catalases were inhibited by 3-amino-1,2,4-triazole. We infer that they would be sulfide-sensitive as well. Thus, thiobiotic meiofauna continue to be unusual, even with respect to other sulfide-dependent animals, in having a 3-amino-1,2,4-triazole-insensitive catalase. The properties of the catalase of the petroleum seep species are consistent with the general trend of retained sulfide-sensitivity in sulfide-dependent macrofauna with a concomitant dependency on sulfide detoxification and control. The opposite adaptive strategy is present in the meiofauna[23].

1 Acknowledgments. We thank S. McDonald and the crew of R/V Gyre for assistance in animal collection. This research was funded by NSF grant OCE-8219792 to EP and NSF grant OCE-83-01538 and funds from the Offshore Operators Committee to J. Brooks. We appreciate this support.

2 Meyers, M. B., Fossing, H., and Powell, E. N., Mar. Ecol. Prog. Ser. *35* (1987) 223.
3 Meyers, M. B., Powell, E. N., and Fossing, H., Mar. Biol. (Berl.) *98* (1988) 395.
4 Fenchel, T. M., and Riedl, R. J., Mar. Biol. (Berl.) *7* (1970) 255.
5 Powell, E. N., Crenshaw, M. A., and Rieger, R. M., J. exp. mar. Biol. Ecol. *37* (1979) 57.
6 Powell, E. N., Crenshaw, M. A., and Rieger, R. M., Mar. Ecol. Prog. Ser. *2* (1980) 169.
7 Fox, C. A., and Powell, E. N., Comp. Biochem. Physiol. *86* (1987) 509.
8 Dando, P. R., Southward, A. J., Southward, E. C., and Barrett, R. L., Ophelia *26* (1986) 135.
9 Reid, R. G. B., and Brand, D. G., Veligier *29* (1986) 3.
10 Brooks, J. M., Kennicutt, M. C. II, Fisher, C. R., Macko, S. A., Cole, K., Childress, J. J., Bidigare, R. R., and Vetter, R. D., Science *238* (1987) 1138.
11 Powell, M. A., and Somero, G. N., Biol. Bull. (Woods Hole) *169* (1985) 164.
12 Powell, M. A., and Somero, G. N., Science *233* (1986) 563.
13 Arp, A. J., and Childress, J. J., Science *219* (1983) 295.
14 Powell, M. A., and Somero, G. N., Science *219* (1983) 297.
15 Arp, A. J., Childress, J. J., and Fisher, C. R. Jr, Physiol. Zool. *57* (1984) 648.
16 Machlin, L. J., and Bendich, A., FASEB J. *1* (1987) 441.
17 Frank, L., and Massaro, D., Am. J. Med. *69* (1980) 117.
18 Fridovich, I., Bioscience *27* (1977) 462.
19 Millero, F., Mar. Chem. *18* (1986) 121.
20 National Research Council, Committee on Medical and Biologic Effects of Environmental Pollutants, Subcommittee on Hydrogen Sulfide, Hydrogen Sulfide. University Park Press, Baltimore 1979.
21 Cooper, W. J., and Zika, R. G., Science *220* (1983) 711.
22 Zika, R. G., Moffett, J. W., Petasne, R. G., Cooper, W. J., and Saltzman, E. S., Geochim. cosmochim. Acta *49* (1986) 1173.
23 Morrill, A. C., Powell, E. N., Bidigare, R. R., and Shick, J. M., J. comp. Physiol. *158* (1988) 335.
24 Barrett, J., and Beis, I., Experientia *38* (1982) 536.
25 Fridovich, I., Archs Biochem. Biophys. *247* (1986) 1.
26 Hewitt, E. J., and Nicholas, D. J. D., in: Metabolic Inhibitors, a Comprehensive Treatise, vol. 2, p. 311. Eds R. M. Hochster and J. H. Quastel. Academic Press, New York 1963.
27 Kono, Y., and Fridovich, I., J. biol. Chem. *258* (1983) 6015.
28 Kono, Y., and Fridovich, I., J. biol. Chem. *258* (1983) 13646.
29 Williams, R. N., Delamere, N. A., and Paterson, C. A., Biochem. Pharmac. *34* (1985) 3386.
30 Kato, S., Alderman, J., and Lieber, C. S., Archs Biochem. Biophys. *254* (1987) 586.
31 Brooks, J. M., Kennicutt, M. C. II, Bidigare, R. R., Wade, T. L., Powell, E. N., Denoux, G. J., Fay, R. R., Childress, J. J., Fisher, C. R., Rossman, I., and Boland, G., Eos *68* (1987) 498.
32 Beers, R. F., and Sizer, I. W., J. biol. Chem. *195* (1952) 133.
33 Sigma Chemical Company Ltd., Catalog, St. Louis, Missouri (1985).
34 Blum, J., and Fridovich, I., Archs Biochem. Biophys. *228* (1984) 617.
35 Morrison, J. M., Merrell, W. J. Jr, Key, R. M., and Key, T. C., J. geophys. Res. *88* (1983) 2601.
36 Kirby, T. W., Studies of the oxygen sensitivities and defenses against oxygen toxicity in some members of the methanogenic bacteria. Ph. D. dissertation, Duke University (1983).
37 Barrett, J., J. Parasit. *66* (1980) 697.
38 Childress, J. J., Fisher, C. R., Brooks, J. M., Kennicutt, M. C. II, Bidigare, R. R., and Anderson, A. E., Science *233* (1986) 1306.
39 Higgins, I. J., Best, D. J., Hammond, R. C., and Scott, D., Microbiol. Rev. *45* (1981) 556.
40 Hutchinson, D. W., Whittenbury, R., and Dalton, H., J. theor. Biol. *58* (1976) 325.
41 Halliwell, B., and Gutteridge, J. M. C., Archs Biochem. Biophys. *246* (1986) 501.
42 Zigler, J. S. Jr, Jernigan, H. M. Jr, Garland, D., and Reddy, V. N., Archs Biochem. Biophys. *241* (1985) 163.

0014-4754/89/020198-03$1.50 + 0.20/0