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Temperature Effects on Mitochondria from Hydrothermal Vent Invertebrates: Evidence for Adaptation to Elevated and Variable Habitat Temperatures

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Abstract

The effects of elevated temperatures on mitochondrial respiration and the activities of selected mitochondrial enzymes were measured for several invertebrates endemic to the deep-sea hydrothermal vents to determine whether the differences in distribution patterns noted among these species are related to differences in temperature adaptation. Parallel studies were done with related (congeneric or confamilial) species from shallow marine habitats. Mitochondrial respiration of hydrothermal vent species living in zones with high water flux (high, variable temperatures), including the large tube worm Riftia pachyptila, *the Pompeii worm* Alvinella pompejana, *and the brachyuran crab* Bythograea thermydron, *was more resistant to high temperatures than was mitochondrial respiration of hydrothermal vent species or shallow-living species in cooler waters, for example, the vent clam* Calyptogena magnifica *and mussel* Bathymodiolus thermophilus. *The temperatures at which Arrhenius plots of respiration rate exhibited sharp breaks were correlated with the apparent maximal habitat temperature of the species. The apparent Arrhenius activation energies of respiration also correlated with maximal habitat temperature, although not as closely as the Arrhenius break temperatures. Temperature inactivation of mitochondrial enzymes generally adhered to the pattern noted in the interspecific comparisons of mitochondrial respiration. These findings suggest that differences in thermal adaptation*

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exist among endemic vent species and that these differences are correlated with the microhabitat conditions the different species encounter at the vent sites.

Introduction

Hydrothermal vents are unique deep-sea habitats. The density of animal life is extremely high, the food chain is based on chemosynthesis rather than photosynthesis, and the water temperatures are much higher and more variable than those typical of the deep sea (2°–3°C) (Hessler and Smithey 1984; Grassle 1985). Research on vent animals has focused on the symbioses between invertebrates and chemolithoautotrophic sulfur bacteria and has explored the exploitation of hydrogen sulfide by these symbioses and the mechanisms enabling these organisms to resist poisoning by sulfide (Grassle 1985; Vetter et al. 1987; Somero, Childress, and Anderson 1989). Although some studies have been conducted on the temperature relationships of endemic vent species, such as those of the brachyuran crab *Bythograea thermydron* (Mickel and Childress 1982*a*, 1982*b*) and the zoarcid and bythitid fishes of the vents (Dahlhoff, Schneidemann, and Somero 1990), no systematic investigation has been made of possible temperature-adaptive differences among the vent invertebrates in relation to their different patterns of distribution in the vent environment. Vent invertebrates and fishes are not uniformly distributed in the vent environment, and this phenomenon appears to be based, in part, on the ability of some species to detoxify and/or exploit sulfide (reviewed by Somero et al. [1989]). Temperature is positively correlated with sulfide concentrations in vent water (Johnson, Childress, and Beehler 1988), which suggests that some vent species may be exposed to elevated temperatures as well as high sulfide concentrations.

To determine whether several of the dominant invertebrates endemic to the vents differed in thermal adaptation, we examined the effects of temperature on mitochondrial respiration and on the activities of selected mitochondrial enzymes. Our interest in this initial study was in establishing correlations between the thermal sensitivities of homologous processes in these species and their habitat temperatures; in the companion article (O'Brien, Dahlhoff, and Somero 1991), we propose a mechanistic basis for the thermal perturbation of mitochondrial function. We examined species from several different sites in the eastern Pacific: the Galápagos spreading center and sites at 11°, 13°, and 21°N along the East Pacific Rise (EPR). The Galápagos vent site is characterized by waters whose temperatures probably

do not exceed 30°C (Johnson et al. 1988). The EPR sites have much higher and more variable temperatures. In addition to low-temperature vents with temperatures no higher than ~17°C (Hessler, Smithey, and Keller 1985), "smoker" chimneys may emit waters with temperatures up to ~20°C ("white smokers") or 380°C ("black smokers") (Hekinian et al. 1983). The species that are likely to encounter the highest temperatures are those living on or very near these smoker chimneys, for example, the polychaete worms *Alvinella pompejana* and *Alvinella caudata,* the vestimentiferan tube worm *Riftia pachyptila,* and the brachyuran crab *B. thermydron* (Fustec, Desbruyeres, and Juniper 1987). The vent bivalves, the mussel *Bathymodiolus thermophilus* and the clam *Calyptogena magnifica,* are abundant at cooler vents and at the periphery of vent fields, where water temperatures are only a few degrees above ambient deep-sea temperatures. On the basis of these distribution patterns, it is likely that *B. thermophilus* and *C. magnifica* rarely encounter temperatures greater than ~15°C at either the Galápagos or EPR sites (Hessler et al. 1985; Fustec et al. 1987; Johnson et al. 1988).

In addition to these six endemic vent species, we studied mitochondrial function in four shallow-living invertebrates: a congener of the vent clam, *Calyptogena elongata,* the mytilid *Mytilus galloprovincialis,* a protobranch clam *Solemya reidi,* and the crab *Cancer antennarius.* These species occur in a variety of well-defined thermal conditions, and their taxonomic affinities to the vent species (in most cases) suited them for use as comparison species.

For each of the 10 species we examined the effects of elevated temperature on mitochondrial O_2 consumption and enzyme activity, using mitochondria isolated from freshly collected animals. By determining the temperatures at which mitochondrial respiration and enzyme activities for each species were inactivated, we sought to establish the existence of interspecific differences in thermal adaptation that could explain, in part, the distribution patterns of vent species within the vent environment.

Material and Methods

Animal Collections

Vent animals were collected during three expeditions to eastern tropical Pacific hydrothermal vents in 1987, 1988, and 1990. Animals were collected by submersible at a depth of approximately 2,500–2,600 m and were transported from the collection site to the surface in an insulated box. Mito-

chondria were isolated within several hours of the submersible's return to the surface. All respiration measurements were made with mitochondria isolated from freshly collected individuals. Enzyme activities were measured in both freshly isolated mitochondria and in preparations that had been frozen in liquid N_2 after preparation and defrosted immediately before use. *Solemya reidi,* a gutless bivalve found in sulfide-rich habitats, were collected by modified Van Veen grab at depths of approximately 100 m near the Hyperion sewage outfall in Santa Monica Bay, California, from the research vessel *Sproul* (November 1987). *Calyptogena elongata* were collected by otter trawl at 500 m from the Santa Barbara basin using the *Sproul* (July 1990). Specimens of *S. reidi* and *C. elongata* were maintained in mud from the site in a dark aquarium (8°C for *S. reidi,* 5°C for *C. elongata*). *Cancer antennarius* were collected near San Diego, California, and purchased live from a local seafood supplier. *Mytilus galloprovincialis* were collected by skin diving from the Scripps Institution of Oceanography pier. The coastal crabs and mussels were kept in ambient seawater aquaria (~15°C) until analyzed.

Mitochondria Isolation

For all species except *M. galloprovincialis,* mitochondria were isolated by differential centrifugation in a medium consisting of 0.4 M sucrose, 0.4 M mannitol, 0.5 mM ethylene glycol bis-(1-aminoethyl ether) *N,N,N',N'*-tetraacetic acid (EGTA), 10 mM *N*-2-hydroxyethylpiperazine *N'*-2-ethanesulfonic acid (HEPES) pH 7.4 at 20°C, and 0.4% (wt/vol) bovine serum albumin (BSA). Mitochondria from *M. galloprovincialis* gonad were isolated in 0.5 M sucrose, 100 mM KCl, 50 mM NaCl, 5 mM EGTA, 1 mM ethylene diamine tetraacetic acid (EDTA), and 50 mM HEPES, pH 7.5 at 20°C. One to 10 g of tissue were homogenized in approximately 5 vol of ice-cold isolation medium with a Potter-Elvhjem homogenizer (Wheaton Scientific, Millville, N.J.) driven by a drill press. A gimbaled Sorvall centrifuge was used for all isolations performed at sea. The homogenate was first centrifuged at a low speed for 10 min to remove large debris and the large subcellular fraction. Mitochondria were subsequently pelleted from the supernatant by centrifugation for 10 min at a higher speed. This pellet was resuspended in buffer and washed in an identical high-speed spin (centrifugation speeds were modified for each species and are described in detail in the paragraph below). The final pellet was resuspended in a minimal volume of buffer and used immediately for respiration measurements and some enzyme assays. A number of preparations were frozen immediately in liquid N_2 and thawed

immediately prior to enzyme assays. All buffers and mitochondrial isolates were kept ice-cold between experiments.

Mitochondria were isolated from *Riftia pachyptila* plume by the method described by O'Brien et al. (1991). For the *Alvinella* species, gill tissue was homogenized as described above and centrifuged for 10 min at 600 *g*. During initial studies, the first spin was at 400 *g* (*Alvinella pompejana* only). The two high-speed spins were at 8,400 *g*. For *Bythograea thermydron* and *C. antennarius,* hepatopancreas was used. The first centrifugation was at 800 *g*. The high-speed spins were at 8,800 *g*. For some preparations of *B. thermydron,* and all *C. antennarius,* an additional first spin at 8,800 *g* was performed to remove (soluble) digestive enzymes as quickly as possible. The pellet was then resuspended and subjected to the normal procedure. Gills from *Bathymodiolus thermophilus* were homogenized and centrifuged for 10 min at 1,300 *g* followed by two spins at 10,800 *g*. For the *Calyptogena* congeners and the mussel *M. galloprovincialis,* soft gonadal tissue was homogenized and centrifuged for 10 min at 700 *g*. High-speed spins were 8,800 *g* for these species. For *Calyptogena magnifica,* mitochondria were also isolated from soft mantle. Centrifugation speeds were identical. Mitochondria were isolated from *S. reidi* as described by O'Brien and Vetter (1990). For the alvinellids, the crabs, *C. elongata,* and *S. reidi,* it was necessary to pool tissue from several animals to obtain an adequate sample.

Experimental Design

Isolated mitochondria were exposed to elevated temperatures as follows. Mitochondria were left on ice until the beginning of each assay. Fresh aliquots of mitochondria (i.e., those previously unexposed to elevated temperatures) were used for each measurement temperature. Initial rates of mitochondrial respiration and enzyme activities were measured at 5°C intervals from 5° to 70°C. Duplicate measurements were made at each temperature.

From these data, the Arrhenius break point (the temperature above which respiration or enzymatic activity drops off dramatically) was determined through a continuous two-phase regression by the method of Nickerson, Facey, and Grossman (1989). This method compares sequential linear regressions and selects two intersecting lines that best fit the data. The break temperature was calculated by determining the intersection of these two lines (fig. 1*A*). The break temperature was calculated in degrees Kelvin. The data reported in table 1 and elsewhere have been converted to degrees Celsius for clarity. The SE of determination of each Arrhenius break temperature was estimated

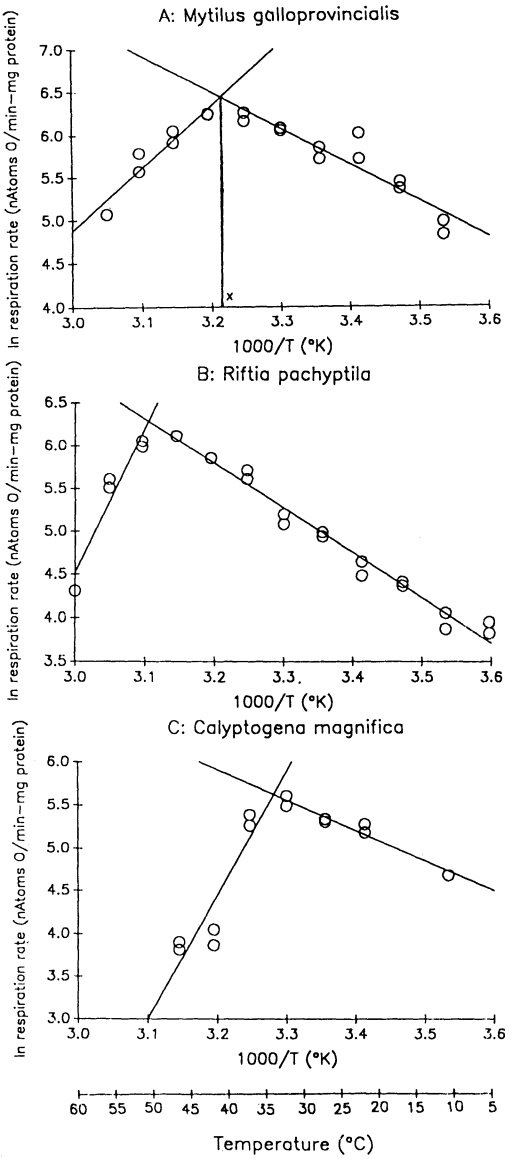

Fig. 1. Examples of Arrhenius plots used in this study. Assay temperature increases to the left. The Arrhenius break temperature (x)*, the temperature above which respiration rates decrease sharply, was calculated by the method of Nickerson et al. (1989). Different species showed characteristic break temperatures that appeared to correlate with the species' maximal habitat temperature. For example, the break temperature of mitochondria from the tube worm* Riftia pachyptila *(B) was much higher than that of* Mytilus galloprovincialis *(A); the break temperature of mitochondria from* Calyptogena magnifica *(C) was much lower than either the vent worm or the coastal mussel. These plots represent one determination* (n)*, with each point representing one assay; multiple determinations were made for most species (table 1).*

TABLE 1

Species studied, their habitats and envirionmental temperature ranges, and Arrhenius break temperatures of mitochondrial respiration

Species	Habitat Temperature (°C)	Arrhenius Break Temperature (°C)
Deep-sea, warm (hydrothermal vents):		
Alvinella pompejana (n = 3)	2–40[a]	48.6 ± 2.4
Alvinella caudata (n = 2)	2–40[a]	48.0 ± 2.0
Riftia pachyptila (n = 4)	2–28[b]	47.0 ± 2.7
Bythograea thermydron (n = 2)	2–35[a]	46.3 ± 3.4
Deep-sea, cool (hydrothermal vents):		
Calyptogena magnifica (n = 2)	2–7[c]	33.0 ± 3.0
Bathymodiolus thermophilus (n = 5)	2–14[d]	32.7 ± 2.0
Shallow, warm:		
Mytilus galloprovincialis (n = 2)	12–20[e]	37.4 ± 2.7
Cancer antennarius (n = 1)	14–17[e]	31.0 ± 2 6
Shallow, cool:		
Solemya reidi (n = 1)	5–8[f]	31.3 ± 1.8
Calyptogena elongata (n = 2)	6–8[f]	32.6 ± 3.6

Note. Number of preparations in parentheses. For some species (*C. antennarius, S. reidi, C. elongata,* and the alvinellids), multiple individuals were used for each preparation. Break temperature values and the SEs of determination were calculated as described in Material and Methods.

[a] D. Desbruyeres (Institut français de le recherche pour l'exploration de la mer–Centre de Brest), personal communication.

[b] K. Johnson (Moss Landing Marine Laboratory), personal communication.

[c] Hessler et al. 1985.

[d] Fisher et al. 1988.

[e] Ricketts, Calvin, and Hedgpeth 1968.

[f] Our unpublished observations.

with a method of linear calibration (Snedecor and Cochran 1980). All respiration rates reported were normalized to total mitochondrial protein concentration as determined by the bicinchoninic acid (BCA) procedure (Pierce, Rockford, Ill.). When two or more preparations for a given species were analyzed, data were normalized to the 20°C rate to account for slight differences in total protein concentration between preparations.

Respiration Measurements

Oxygen-consumption measurements were made with a Clark-type O_2 electrode (Strathkelvin Instruments 1302 microcathode electrode) in a glass water-jacketed chamber. Mitochondrial respiration was assayed in a medium of 0.5 M glycine, 0.15 M KCl, 5 mM $MgCl_2$, 10 mM potassium phosphate, and 50 mM imidazole-Cl (pH 7.2 at 20°C). One minute after addition of mitochondria, 5 mM pyruvate/5 mM malate (for *C. magnifica, C. elongata,* and *M. galloprovincialis*) or 4 mM succinate (for all other species studied) was added to stimulate respiration. At the beginning of this study, succinate was used to stimulate respiration in isolated mitochondria. We later discovered that the pyruvate-malate mixture was more effective for stimulating respiration in mitochondria from bivalves, and it was therefore used for all subsequent bivalve species. Unfortunately, we were unable to obtain live *B. thermophilus* at this point to repeat measurements using pyruvate-malate to stimulate respiration. However, we did find that for at least one species (*B. thermydron*), the break temperature was the same with either substrate.

The respiration measurements were made in the absence of a respiratory uncoupler. However, control experiments with *S. reidi, C. magnifica* and *Riftia pachyptila* showed that neither ADP addition nor addition of the uncoupler carbonylcyanide-*p*-(trifluoromethyl)phenylhydrazone stimulated respiration. Measurements of ATP production by mitochondria of *R. pachyptila* and *A. pompejana* also indicate that these mitochondria were completely uncoupled (J. O'Brien, unpublished data). We therefore assert that the rates we measured for all species were uncoupled (i.e., state 3) rates. For hydrothermal vent animal mitochondria, this may be in large part due to the difficulty in obtaining coupled mitochondria from deep-sea animals. However, coupled mitochondria have routinely been isolated from *S. reidi* under different conditions (O'Brien and Vetter 1990), and we hypothesize that the mitochondria used in this study were probably uncoupled by the imidazole buffer in the assay medium.

Oxygen Electrode Calibration

The temperature dependence of the concentration of O_2 in fully air-saturated water necessitated the generation of a standard curve to describe this function. The concentration of O_2 in fully air-saturated media was assayed by the method of O'Brien and Vetter (1990). A standard curve was generated by measuring the O_2 concentration of mitochondrial assay medium at 5°C increments from 15°C to 40°C. The assay medium was equilibrated for at

least 10 min, with frequent shaking, at each assay temperature. A minimum of two O_2 determinations, consisting of at least two assays each, was made for each 5°C increment and the electrode was zeroed at each temperature. The resulting standard curve for the mitochondrial assay medium was approximated by a line with the equation

$$y(n \text{ atoms O/mL}) = 496.9 - 4.4x. \quad (1)$$

Equation (1) was used to calculate the oxygen content of the medium at each assay temperature (x = temperature, °C).

Enzymatic Activity Determinations

The following enzymes were studied in two vent species (*R. pachyptila* and *B. thermophilus*) and one coastal species (*S. reidi*): L-malate dehydrogenase (MDH, EC 1.1.1.37; L-malate: NAD^+oxidoreductase); cytochrome c oxidase (Cyt. ox., EC 1.9.3.1; ferrocytochrome c: O_2 oxidoreductase); and succinic dehydrogenase (SDH, EC 1.3.99.1; succinic dehydrogenase). Freshly isolated mitochondria were diluted 10× by the addition of 10 mM HEPES (pH 7.4 at 20°C) and assayed for either Cyt. ox. or SDH activity. Frozen mitochondria were defrosted, diluted with 10 mM HEPES, and immediately assayed for either Cyt. ox., SDH, or MDH activity. All enzymes studied, including Cyt. ox., were found to be stable to freezing when stored as frozen mitochondrial preparations. A fresh aliquot was used for each determination. Assays were started by the addition of 25 μL of the diluted mitochondrial preparation, and initial rates of enzymatic activity were measured. Rates reported were normalized to total mitochondrial protein concentration, as determined by the BCA method.

The MDH activity was measured using a modification of the protocol of Childress and Somero (1979). The assay medium contained 100 mM imidazole-Cl (pH 7.0 at 20°C), 100 mM KCl, 0.4 mM oxaloacetic acid, and 0.15 mM NADH (nicotinamide adenine dinucleotide, reduced). The concentrations of substrate and cofactor used were determined to be saturating and noninhibitory. The oxidation of NADH was followed at 340 nm.

The SDH activity was assayed by a modification of the method of Ackrell, Kearney, and Singer (1978). To minimize inactivation of the enzyme by endogenously formed oxaloacetate, a high concentration of succinate was added to the enzyme preparation prior to conducting experiments. One molar sodium succinate (pH 7.0 at 20°C) was added to the diluted mitochondrial preparation to bring the concentration to 10 mM. The preparation

was incubated at 20°C for 30 min. The assay medium contained 50 mM imidazole-Cl (pH 7.2 at 23°C), 20 mM sodium succinate, 50 μM oxidized cytochrome c, 50 nM antimycin A, 0.5 mM KCN, and 0.033% (wt/vol) phenazine methosulfate.

Cytochrome c oxidase activities were measured with the protocol of Yonetani and Ray (1965). The assay medium contained 0.1 M 2(*N*-morpholino) ethanesulfonic acid (MES; pH 6.0 at 20°C), 1 mM EDTA, and 50 μM reduced cytochrome c (equine heart, type VI, Sigma Chemical, Saint Louis). Reduced cytochrome c was prepared according to the method of Hand and Somero (1983). The oxidation of cytochrome c was followed at 550 nm.

Results

The effects of measurement temperature on O_2 consumption by mitochondria exhibited the same general pattern in all species: the rate of respiration increased up to a certain temperature, beyond which further increases in temperature led to a sharp reduction in respiration. These patterns are shown most clearly when the data are presented on Arrhenius plots (fig. 1), which exhibit discontinuities in slope (break temperatures) that differ among species (table 1). The Arrhenius break temperatures correlate well with the known, or conjectured (for some vent species), habitat temperatures of the species. That is, the break temperatures of species from warm vent microhabitats (*Alvinella pompejana, Alvinella caudata, Riftia pachyptila,* and *Bythograea thermydron*) were significantly different from those of species from cool microhabitats (the vent bivalves *Calyptogena magnifica* and *Bathymodiolus thermophilus,* as well as *Calyptogena elongata,* and *Solemya reidi*) ($P > 0.980$; Wilcoxon signed-ranks test).

Because these species differ in maximal habitat temperature, mean habitat temperature, and habitat temperature range (the difference between the high and low temperature, in degrees Celsius, that an individual of a given species may experience), we sought to determine which of these three aspects of the thermal environment was most closely correlated with mitochondrial thermal tolerance. The regression analyses given in figure 2 show that maximal habitat temperature and variation in habitat temperature both can explain most of the interspecific variation in break temperature. Mean habitat temperature is less closely linked to the differences in break temperature.

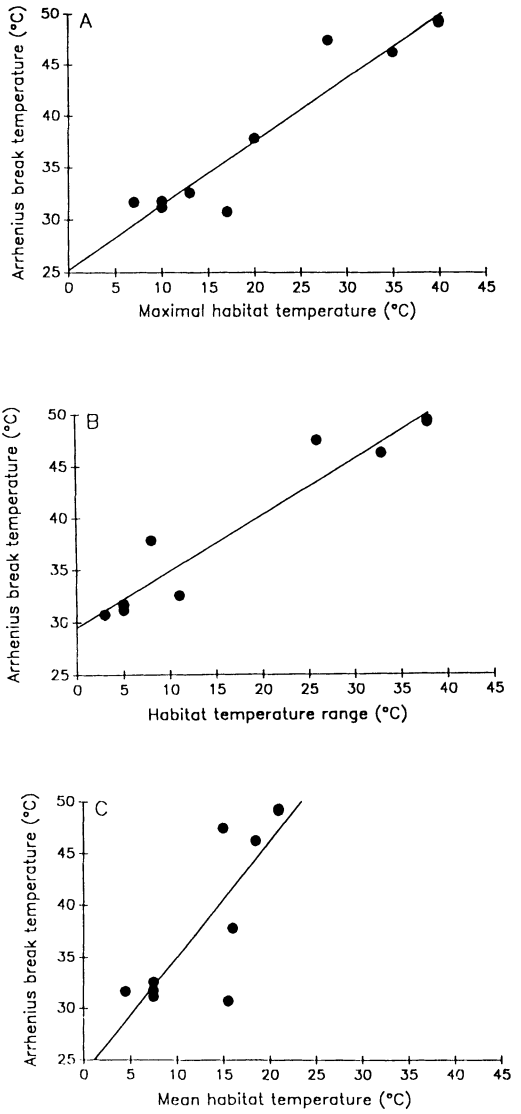

Fig. 2. Correlation between Arrhenius break temperature of mitochondrial respiration and thermal aspect of habitat. A high degree of correlation was found between Arrhenius break temperature and both maximal habitat temperature (A, $y = 25.221 + 0.615x$; $R^2 = 0.909$) *and habitat temperature range* (B, $y = 29.482 + 0.538x$; $R^2 = 0.928$; *habitat temperature range is defined as the number of degrees Celsius between the highest and lowest habitat temperatures a species experiences). Arrhenius break temperature appears to be less closely correlated with mean habitat temperature* (C, $y = 23.773 + 1.112x$; $R^2 = 0.690$). *Break temperatures used for these regressions are those reported in table 1.*

The slope of an Arrhenius plot equals $-E_a/R$, where E_a ($= \Delta H‡ + RT$) is the apparent activation energy of the process and R is the gas constant. The apparent activation energy of mitochondrial respiration was calculated from the slope of the regression line at temperatures below the break temperature and plotted against maximal habitat temperature (fig. 3). The E_a of mitochondrial respiration was higher for species living at high temperatures than for those living in cool habitats ($R^2 = 0.630$).

Temperature effects on two membrane-associated mitochondrial enzymes, Cyt. ox. and SDH, differed among the three species studied, *R. pachyptila, B. thermophilus,* and *S. reidi,* in a manner consistent with the trend observed for mitochondrial respiration. Figure 4 illustrates Arrhenius plots (with break temperatures in °C), for the activity of Cyt. ox. from these three species. Identical treatments of SDH from *R. pachyptila, B. thermophilus,* and *S. reidi* showed break temperatures at 50°, 46°, and 40°C, respectively. The soluble enzyme MDH exhibited only minor differences in thermal stability (<3°C), and these differences were not correlated with adaptation temperature (data not shown).

Discussion

In this study the effects of temperature on mitochondrial respiration and enzyme function are used as an index of thermal adaptation. These data suggest that hydrothermal vent invertebrates differ significantly in their ability to tolerate elevated temperatures. Before discussing the ecological implications of these results, several potential problems with these data should be addressed.

Because respiration was measured for mitochondria from very different species, differences in the Arrhenius break temperature of respiration between these species may be due to differences in mitochondrial preparation quality; that is, some mitochondria may be partially uncoupled, while others are fully uncoupled. However, there are several reasons for concluding that the process being measured is similar for mitochondria from each species. Neither respiratory uncouplers nor ADP appeared to affect respiratory rate for any species in which it was measured (see Methods), which suggests that the mitochondria were completely uncoupled. This assessment is further supported by the fact that no ATP production could be measured in mitochondria from several vent species. Additionally, the imidazole assay buffer appeared to uncouple mitochondria (our unpublished observations).

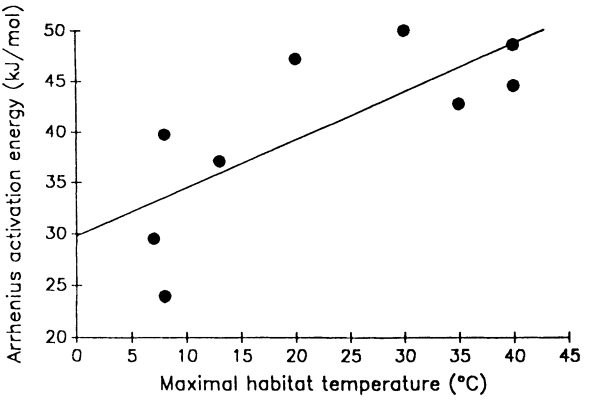

Fig. 3. Correlation between apparent activation energy of mitochondrial respiration and maximal habitat temperature. Mitochondria of species from warm-temperature habitats have higher apparent activation energies of mitochondrial respiration than those of species from cooler habitats ($y = 30.301 + 0.438x$; $R^2 = 0.630$). *Activation energies were calculated from the break temperatures reported in table 1.*

Therefore, we believe that the interspecific differences found in these comparisons were not a consequence of differences in coupling state.

It is also pertinent to consider whether any of the interspecific differences we observed reflect adaptation to pressure, rather than to temperature, and whether the use of 1 atm as the experimental pressure introduced artifacts. There are two bases for concluding that effects due to pressure adaptation would have had a minimal effect on the observed trends. First, all of the vent species live at the same pressure, approximately 250–260 atm. Differences between the low- and high-temperature vent species should therefore not be the result of differential adaptation to pressure. Furthermore, if pressure-adaptive differences in the mitochondria from shallow- and deep-living species affected the Arrhenius break temperatures to a great extent, then shallow- and deep-living species that experience the same habitat temperatures should have exhibited significant differences in Arrhenius break temperatures. This was not the case (table 1). While it is conceivable that assays done at the in situ pressures of the different species could have yielded different results, we conclude that neither differences in adaptation pressure nor the use of 1 atm as the experimental pressure significantly affected the conclusions reached in this study.

Even with the problems inherent in its measurement (Silvius and McElhaney 1981), the Arrhenius break temperature of mitochondrial respiration

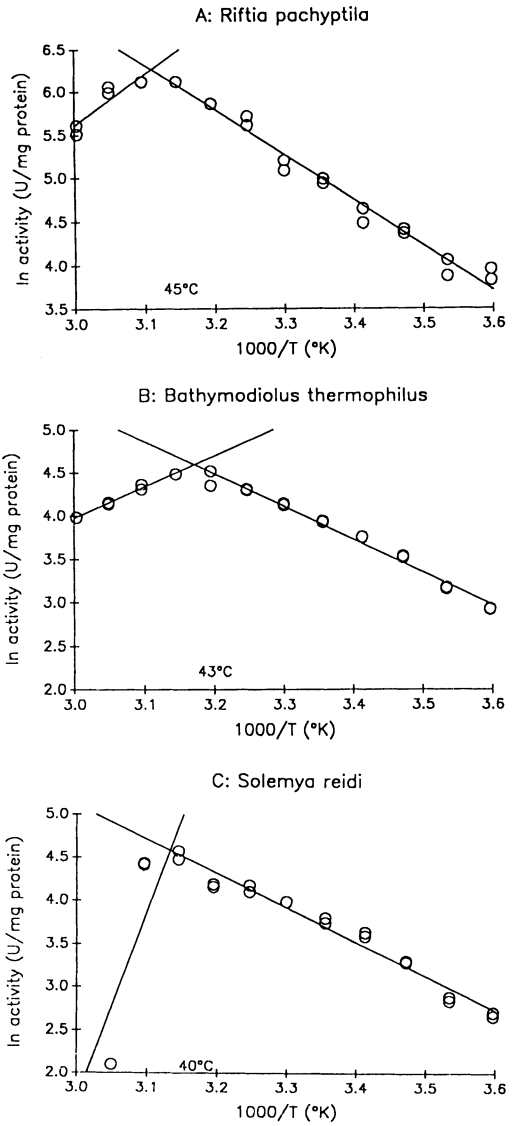

Fig. 4. Arrhenius plots of Cyt. ox. from three invertebrates living in different thermal habitats. Cytochrome c oxidase from the warmest living species, Riftia pachyptila *(A), had the highest break temperature; Cyt. ox. from* Solemya reidi *(C), the coolest living species, had the lowest break temperature. The break temperature of Cyt. ox. from the vent mussel* Bathymodiolus thermophilus *(B) was between that of* R. pachyptila *and* S. reidi, *which is consistent with the trends observed for mitochondrial respiration. Data shown are for one individual of each species; each point represents one assay. Break temperatures were identical when measured for mitochondrial enzymes from at least three individuals of each species.*

appears to be an excellent indicator of adaptation temperature. We therefore used this index of thermal adaptation to further examine fine-scale adaptation of vent species. The Arrhenius break temperature was used to examine which aspect of the thermal habitat is most important to an organism. Figure 2 illustrates this analysis. There is an extremely high correlation between maximal habitat temperature and interspecific variation in break temperature. The strong correlation between habitat temperature range and break temperature (fig. 2*B*) is, we suggest, also a reflection of selection that is driven primarily by the maximal habitat temperature. All of the hydrothermal vent species have the same lower habitat temperature (near 2°C), although they spend very different fractions of their lives at this lowest temperature. The temperature range, the number of degrees Celsius difference between the high and low habitat temperature a given species experiences, is therefore essentially defined by the maximal temperature for vent species. In examining adaptation of enzymatic kinetic properties to temperature, it was similarly found that strong correlations existed between thermal resistance of enzyme function and both maximal temperature and temperature range in Sciaenid fishes (Coppes and Somero 1990). The conclusion reached in that study, too, was that selection for resistance to the highest habitat temperature was most critical and that the correlation between resistance and habitat temperature range was again a consequence of the fact that the species experiencing the highest habitat temperatures also had the widest range of temperatures. One difference between Coppes and Somero's study and the present one is that the fishes they studied experience their maximal habitat temperature for somewhat extended periods of time. The maximal habitat temperature of many hydrothermal vent species (reported in table 1) is the highest temperature ever recorded for water bathing the species in question and is therefore probably much higher than the temperature a given species is exposed to most of the time. The water temperature at vent sites is directly correlated with the flow of water from vents. This flow, and therefore the temperature of water surrounding the vent openings, fluctuates greatly and reaches the hottest temperatures for the shortest intervals (<1 s; Johnson et al. 1988). The maximal sustainable temperature for vent species is therefore probably much lower than the values reported in table 1, especially for *Riftia pachyptila*. This problem is discussed in detail in Dahlhoff and Somero (in press).

The Arrhenius analysis of respiration data allows further examination of temperature adaptation by the calculation of an apparent E_a. This apparent E_a was also correlated with maximal habitat temperature, although not as closely as was the Arrhenius break temperature (fig. 3). This weaker cor-

relation may reflect some of the problems associated with assigning a single E_a to a complex process like mitochondrial respiration. The Arrhenius analysis assumes that a single rate-limiting step governs respiration at all temperatures below the break and that this rate-limiting step is the same at all temperatures. This assumption may not be valid. For example, the data in figure 1 show that the Arrhenius plot of respiration below the break temperature for *R. pachyptila* is virtually linear while that for *M. galloprovincialis* is more curvilinear, which suggests that the rate-limiting step in respiration may shift for some species and not for others. However, it must be emphasized that the correlation between activation energy and habitat temperature does exist ($R^2 = 0.630$), which suggests specific adaptations to temperature that involve shifts in activation energy. Gibbs and Somero (1989) observed a similar correlation for the activation energies of Na^+-K^+-ATPase activity from deep- and shallow-living fishes and suggested that this phenomenon was due, in part, to differences in fluidity of the membrane associated with the ATPase (Gibbs and Somero 1990).

Thus far, this discussion has assumed that interspecific differences in the Arrhenius break temperature of mitochondrial respiration are due to genetically fixed adaptations. However, some differences observed may be due to thermal acclimation, as has been observed in other systems. Newell and Pye (1971) examined mitochondria from the snail *Littorina littorea* and showed that the effect of temperature on mitochondrial function was altered by acclimation to different temperatures. Additionally, Cossins and Bowler (1987) have reported bulk fluidity changes of plasma membranes isolated from goldfish acclimated to different temperatures. These studies suggest that mitochondrial function can be acclimated to variations in habitat temperature. While this may be the case for vent species as well, our inability to maintain healthy specimens in the laboratory for longer than a few days precluded further experimentation with these organisms.

The two membrane-associated mitochondrial enzymes, SDH and Cyt. ox., differed among species according to the pattern noted for respiration, but the interspecific differences in Arrhenius break temperature were smaller for enzymatic function. The observation that the soluble enzyme MDH did not show differences in thermal stability related to body temperature suggests that it is the integrity of the mitochondrial membrane and membrane-protein interactions that generate the high temperature sensitivity of intact mitochondrial function rather than protein stability per se. This hypothesis is tested in the following article (O'Brien et al. 1991).

In conclusion, these studies using thermal effects on mitochondrial respiration and enzyme function as indices of species' habitat temperatures

suggest that hydrothermal vent invertebrates differ significantly in their tolerances of elevated temperature. The distribution patterns of hydrothermal vent invertebrates within the vent environment therefore may be established in part by adaptive differences in the thermal optima or tolerance limits of their physiological and biochemical systems. The high thermal resistance of mitochondrial respiration of the four species observed to occur on smoker chimneys, *Alvinella pompejana, Alvinella caudata, R. pachyptila,* and *Bythograea thermydron,* may be a reflection of pervasive physiological and biochemical adaptations to elevated temperatures by these deep-sea species, a conclusion supported by recent studies of temperature and pressure effects on enzyme kinetics of vent fishes (Dahlhoff et al. 1990) and invertebrates (Dahlhoff and Somero 1991). The results reported in this study suggest that high temperatures, like high sulfide concentrations, preclude many deep-sea organisms from exploiting food-rich vent habitats and that temperature was an important selective factor for the initial colonization of these habitats by the ancestors of modern vent species.

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