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THE EVOLUTION OF THERMOPHILY IN HOT SPRINGS

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ABSTRACT

The origins of the blue-green algae of hot springs are uncertain, but the snails and flies that eat the algae, and the mites parasitic on the flies, seem to have evolved from species that lived in water-margin habitats. The wet shores of water margins are regularly warmed up to 40° C by the sun. Although the origins of many thermophiles are similar, each taxonomic group evolves to a characteristic temperature limit: bacteria > 95° C, blue-green algae < 74° C, fungi and eucaryotic algae < 60° C, and metazoans < 50° C. The selective pressures operating on each population can be defined from evolutionary models, and various explanations tested. Procaryotes might be driven to the highest temperatures by intraclonal competition for resources in the water. Heterotrophs and chemoautotrophs tolerate up to the boiling point of water, but autotrophs have evidently reached some physicochemical limit at 74° C. When the fitness of individuals in a population of sexual organisms is averaged out by means of out-breeding, their evolutionary equilibrium may be lower because it is determined in part by the laws of cooling that determine area-temperature relationships. In eucaryotic autotrophs the fixed supply of chemical resources, as well as area, is a determining factor. The maximum temperature tolerated by eucaryotic autotrophs is 10° C higher than that tolerated by herbivores, which evolve thermophily only by trading larger, cool, grazing areas of algal mat for smaller hot areas.

INTRODUCTION

THERMOPHILY is best defined as the adaptation to waters that are warmer than the mean annual temperature of the general region (see the review by Tuxen, 1944). Thermophiles include the symbionts of homoiotherms, the complexes of molds and bacteria that are responsible for self-heating decay, and, finally, the organisms of hot springs. Each system has unique properties. The symbionts of homoiotherms live in a virtually constant environment with a rich supply of nutrients. The self-heating flora is a set of forms that exploit accumulations of dead plant material (Cooney and Emerson, 1964; Tansey, 1973). Neither of these systems of thermophiles is independent, because some outside action—the feeding of vertebrates or a heaping up of vegetable material—must concentrate food material so that some of the energy is released as heat. Hot springs are independent geothermally heated systems with an intrinsic supply of chemical nutrients; hence, they are closed stable systems within which a set of organisms evolved and formed reasonably stable ecosystems that are isolated by temperature regimens that are lethal to the adjacent flora and fauna.

Certain common characteristics of hot springs are determined by the properties of thermal outflows. Thermal waters generally come from surface water that percolates down to the vicinity of hot magma, is heated, and then is driven to the surface. The hot water dissolves minerals as it moves upwards, and therefore emerges with a mineral content that is characteristically quite high (Castenholz, 1969b).

Local thermal outflows tend to be constant over long periods (tens to hundreds of years), and consistent activity in a small thermal region will persist for thousands of years. Such areas of thermal activity can be the locus for several independent evolutionary events that result in a spring acquiring a complete endemic ecosystem consisting of green plants, herbivores, carnivores, and decomposers (Brock, 1967b). These systems are the best known, as a result of the studies of Brues (1938), Brock (1970a), and Wiegert and Mitchell (1973) in Yellowstone National Park (U.S.A.) where bacterial, algal, and herbivore components have been studied in detail.

Each hot spring is a sequence of communities that decrease in complexity toward the source. In the hottest water there are only heterotrophic bacteria, and these grow all the way up to the boiling point (Bott and Brock, 1969; Brock and Darland, 1970; Castenholz, 1969b). Algae do not grow above 73° C, but wherever algae occur there is a very close physical, and probably chemical, association with the bacteria; this association results in a well-defined "algal mat." These organisms have been proven to be acceptable and adequate food for herbivorous thermophilic metazoans (Brock, Wiegert, and Brock, 1969) which are generally completely absent at temperatures above about 45° C. That temperature is well below the range of optimal growth for the algae and bacteria they eat (Wiegert and Mitchell, 1972). Until recently such relations have been ignored because the upper temperature limit was assumed to be determined by the physicochemical stability of cells, and no one has yet attempted to analyze the enigmatic failure of thermophilic herbivores to evolve a temperature range overlapping that of their food resource (Castenholz, 1969b; Wiegert and Mitchell, 1972).

The evolution of thermophily has been treated as a physiological question, not an ecological question. A number of authors have argued that the sequence of temperature limits

$$\text{metazoans} < \text{green algae} < \text{blue-green algae, fungi} < \text{bacteria}$$

corresponds with decreasing cellular complexity (Brues, 1938; Brock, 1970a). These authors infer that the primary limits to the evolution of tolerance of high temperatures are related to cellular complexity (Tansey and Brock, 1972).

Arguments based on the assumption that some cellular function limits evolution have not progressed beyond such statements as the following: "I postulate that there is an inherent fundamental limitation in eucaryotic membrane structure which cannot be overcome by further evolutionary changes . . ." (Brock, 1969). This is really two postulates in one. The first postulate is that each major eucaryotic taxon has a characteristic maximum temperature sensitivity that is presumably based on physiochemical properties that are impossible to alter through mutation. Such a postulate cannot stand alone. It must be accompanied by the postulate, or preferably by evidence, that there are positive selective pressures favoring the evolution of temperature tolerance in excess of the observed levels.

Despite the preoccupation of many authors with temperature limits, there has been no attempt to identify the locus of cellular failure. Recent reviews of cytoecology (Troshin, 1967) clearly indicate that temperature tolerance is not a simple function of cellular complexity (Poljansky and Sukhanova, 1967; Precht, 1967), and the work these authors have reviewed casts doubt on the statement that "the key point is that the internal membranes of eucaryotes *must* [sic] be loose (and hence thermolabile) if the necessary functions of the organelles they surround are to be maintained" (Brock, 1969). Tansey and Brock (1972) persist in using this postulate to account for the 62° C limit for temperature tolerance in eucaryotic cells.

The simple argument that there are absolute limits to the evolution of membrane function is impossible to test unless, in order to deal with evolutionary matters, it is expanded into two questions: (1) is there an evolutionary advantage to be gained above 62° C?; and (2) does the genome have the capacity to respond to such selective pressures?

The hitherto neglected analysis of the evolution of thermophily has left it difficult to consider alternatives to either simple rejection or acceptance of the postulates of Brues (1938) and of Brock (1970a). Data on the elements of evolutionary pressures will allow one to estimate selective pressures and to model their effect under a variety of circumstances. The purpose of this paper is to show that it is of crucial importance to broaden the study of thermophily to consider its evolutionary aspects. If the selective pressures driving the evolutionary process can be understood and defined, it will necessarily clarify our views of the question of why the evolution of thermophiles follows such a regular pattern.

THE EVOLUTIONARY MODEL

Evolution moves in a given direction only so long as there is a gain in fitness. Darwinian fitness (w) is expressed as the number of young (n) produced by a female, hence $w = n$ and this can be expanded to express the mean fitness of a homogeneous population as follows:

$$\bar{w} = n = \frac{(\text{habitat} \times \text{resource density}) \times (\text{cellular functions})}{m}$$

m being defined as the total calories of energy expended by the parents divided by the number of young. From this value can be derived the number of young produced per calorie taken in. The potential calories of resource are defined by area × calories per unit area. Cellular functions include all the processes, responses, and work expended in collecting and capturing food, as well as the ultimate budgeting of calories to activity and growth. Such a function can be unambiguously defined in theory and can be treated as *efficiency,* the fraction of the energy collected and processed that is put into successful offspring. This method usefully separates area × density from biological functions. In fact, the biology of capturing resources and converting them into biomass is a complex set of functions extending from the cellular to the population level (Kitizawa, 1959; Nakano and Monsi, 1968). These complexities, as yet unknown, need not be considered in testing the reasonableness or necessity of postulates based on cellular disasters such as the loss of membrane function.

It is obvious from the above explanation that the evolution of thermophily may be limited by either of two things: cellular functions or resources. Hence, an evolutionary explanation that considers only one function may be correct, but it will remain incomplete and unconvincing until the roles of both resources and physicochemical functions are considered.

Since resources are the energy base and cellular functions are a dimensionless conversion coefficient, the first step in dealing with the problem is through models that define the dimensions of resource availability. Two models that represent the extremes of the varied patterns described by Precht (1967) will adequately illustrate the general evolutionary problems and show how the interpretation of the significance of temperature limits must differ as a function of whatever adaptive tactics are thought to be operating. Under the first tactic, the range of temperature tolerance could be expanded by extending the upper limit independently of the lower limit. Under the second tactic the temperature range may be a fixed dimension that cannot be expanded; thus, thermophily can evolve only if the range is shifted upward.

The changes in resources can be rather simply defined. Consider the temperature range of 15 to 30° C to be broken up into 5° ranges with $A = 15\text{–}20°$, $B = 20\text{–}25°$, and $C = 25\text{–}30°$ C. If a species living at the 15–25° C temperature range ($A + B$) evolved a capacity to live at 25–30° C in addition to its former range, then the difference could be measured as a quotient for an expanding range tactic (Q_e).

$$Q_e = \frac{A + B + C}{A + B} > 1.0 \qquad \text{(Tactic 1)}$$

and Q_e must always be greater than one. The quotient for a shifting range strategy (Q_s), in which the low temperature tolerance is given up in order to gain high temperature tolerance, will be

$$Q_s = \frac{B + C}{A + B} \qquad \text{(Tactic 2)}$$

The value for Q_s can be 1.0 only if $A = C$. It must be less than 1.0 when $A > C$ and greater than 1.0 when $A < C$.

If higher temperature tolerances evolve by shifting the range (Tactic 2), and the efficiencies at the two ends of the distribution do not change, then the resources lost at the lower end of the range must be less than the resources gained at the upper end. Greater temperature tolerances will evolve if there is a net gain of resources and an inherent physicochemical limit is not reached.

The total primary production at a specified temperature range is fixed by the area at that temperature, and the relative area of temperature ranges can be estimated because water loses heat as a function of the difference between the air temperature (T_A) and water temperature (T_W). The loss of heat varies from spring to spring because each channel configuration uniquely affects exposure and turbulence, yet the controlling function for the rate of heat loss, and consequently, the area of a temperature zone, is $T_W - T_A$. Area limits the primary production of a system; thus the earlier formulation for fitness can be rearranged for hot springs to separate out the area function, which presumably determines primary productivity and is determined by physical laws that apply to all springs. In this context the physical factors define the total potential yield from a zone in the same terms, and mean Darwinian fitness for a population is defined as

$$\bar{w} = \text{area (density of resources} \times \text{cellular functions)}/\text{m}$$

Area relations, as defined in Table 1, are the unique variable and can be simulated by a beaker of hot water representing a mass of hot water floating down a non-turbulent stream of constant geometry. The time it takes to cool 5° C will then be proportional to the area of that temperature zone.

This simulation can be used to estimate the changes in area and, presumably, in resources under an expanding (Q_e) or a shifting (Q_s) evolutionary tactic (Table 1). There is always a gain of resources if thermophily evolves through range extension, but the gain for adding 5° range extensions above 50° C is very slight (<0.05). Hence, an inherent limit above 50° C need not imply a catastrophic cellular failure, such as a loss of membrane function; and the physicochemical limit need be no more than small losses of efficiency in some cellular function even if assumptions more realistic than those of Table 1 are used. Within a given population the Q_{10} effects will profoundly affect genetically similar individuals at the extremes in a way that increases the disadvantages of the tactic of expanding the temperature range.

Under the alternative of shifting range (Tactic 2, Table 1), there will never be an advantage for thermophily because organisms must give up resource area if their temperature range is to be shifted upward. There will be selective pressure for thermophily only if secondary factors, such as predation, competition, or low standing crop reduce the survivorship or resource availability at the low temperature range so that the total yield from the larger low-temperature areas is less than that from the smaller areas at high temperatures. The differential in availability that would be necessary to compensate for area differences and to allow thermophily to evolve under Tactic 2 (shifting range) can be estimated.

An examination of these elemental area relations makes it clear that general explanations for the evolution of temperature tolerances among thermophiles must consider how three

TABLE 1

Cooling water and models for the evolution of thermophily

The times are given for 500 cc of water in a one-liter beaker to cool in 5° C steps (at T_A = 22° C). These times are the primary determinants of the relative area at that temperature and are used to calculate quotients for area changes (as explained in the text) under the tactic of range expansion (Q_e) and the alternative of shifting the range (Q_s).

		TACTIC 1			TACTIC 2		
TEMP. °C	TIME TO COOL (MIN.)	OLD RANGE	NEW RANGE	Q_e	OLD RANGE	NEW RANGE	Q_s
30-25	107						
35-30	50	35-25	35-25	1.0	35-25	35-25	1.0
40-35	35	35-25	40-25	1.22	35-25	40-30	.54
45-40	25.5	40-25	45-25	1.32	40-30	45-35	.71
50-45	18.5	45-25	50-25	1.08	45-35	50-40	.73
55-50	13	50-25	55-25	1.06	50-40	55-45	.72
60-55	10.5	55-25	60-25	1.04	55-45	60-50	.75
65-60	8.5	60-25	65-25	1.03	60-50	65-55	.81
70-65	8	65-25	70-25	1.03	65-55	70-60	.87
75-70	7.5	70-25	75-25	1.03	70-60	75-65	.84

different parameters (fundamental physicochemical limits; the presence or absence of resources; and the relative amounts of resources—i.e., area times standing crop—available at various temperature zones) interact with the biology of the organism (by way of competitive response, predation, and the like) so as to determine the fitness equilibrium with respect to temperature. The first parameter has been the major point of speculation in all recent reviews of thermophily. There is ample evidence (Brock, Wiegert, and Brock, 1969; Castenholz, 1969b; Wiegert and Mitchell, 1972) for rejection of the second parameter as a general explanation. The interactions involving the third parameter (essentially, resources) have been neither defined nor critically examined in previous work. They must be understood in order to determine whether fundamental physicochemical limits are relevant to the evolution of thermophily.

RESOURCE LIMITS

The following examination of the availability of resources uses the tactical models defined above and specified in Table 1 to consider the evolution of thermophily. Only populations that are genetically homozygous or have a random dispersion of genotypes fit these traditional evolutionary models. Procaryotic thermophiles obviously do not fit because the major changes in the abundance of genotypes in a procaryote population seem to be the result of differential rates of growth and death. New genotypes appear as the result of mutation rather than through sexual processes. Genetic material can be exchanged, and additions to it do occur (Grant and Howe, 1971), but the significance of such events in natural populations is not yet known (Brock, 1970b). These differences in evolutionary mechanisms make it necessary to treat the procaryotes and eucaryotes separately.

Resources and Thermophily in Procaryotes

The evolution of thermophily among the procaryotes of hot springs should be particularly easy to study because the species diversity of both bacteria and algae is low over a wide range of temperatures (Brock, 1969; Castenholz, 1973). The flow and quality of water of each spring are quite constant and, in high-temperature zones, a virtually uniform mineral substrate is precipitated from the water. Temperature is the only major edaphic variable in the run of a spring, and it is a daily and seasonal function of $T_W - T_A$.

Above the upper limit for photoautotrophs, at about 75° C, almost monotypic bacterial colonies occupy a uniform habitat that is immense relative to bacterial dimensions (Brock, 1969). Such colonies are generally attached rods or filaments (Brock and Darland, 1970), and these colonies experience a significant decline in temperature from the upstream to the downstream end of the colony. The genotypes reproducing most rapidly at the upstream (high-temperature) end of the colony may differ from the successful genotypes at the cooler, downstream end of the colony. If temperature-sensitive growth characteristics did differ among the cells of an attached procaryote, the species might appear to have broadened its range (Tactic 1) when in fact the colony was a cline of genotypes differing with respect to temperature tolerance. The utter intractability of extreme thermophiles in culture (Brock, Brock, Bott, and Edwards, 1971) may mean that the presence or absence of clonal differences will remain a matter of conjecture.

Such a cline would consist of a set of cell populations with genetically determined differences in temperature optima, in contradistinction to a population possessing a common temperature optimum. This situation has been demonstrated to obtain for high-temperature bacteria (Brock and Brock, 1968) and for the blue-green alga *Synechococcus* (Brock, 1967c). Temperature strains are presumably genetically different in the blue-green algae *Mastigocladius* (Castenholz, 1969a) and *Synechococcus* (Peary and Castenholz, 1964). There is no direct information on the genetics of the high-temperature bacteria, because they have not yet been cultured (Brock, Brock, Bott, and Edwards, 1971).

The achievement of high-temperature tolerance by bacteria requires an inherent capacity to withstand heat, as well as selective pressures that make it advantageous for bacteria to evolve tolerances up to the boiling point of water. This selective pressure might result from competition for the fixed quantity of solutes emerging in the constant outflow of thermal water. If competition affects the downstream populations by reducing resources, then the model for evolution by range extension (Tactic 1) is inappropriate; Tactic 2, however, could be modified to define fitness as a function of the differential availability of resources relative to each of the genotypes which differ in their temperature ranges.

There should be a gain in fitness with higher temperature tolerances only so long as cells with higher temperature tolerances are able to take up resources before these resources reach the remainder of the population. If the upstream cells do not affect the downstream cells by reducing the concentration of resources, then there will be no advantage for thermophily, with respect to resources at least. Geophysical functions may make absorption an exceptionally important factor. Thermal streams generally deposit rather than erode their substrate; thus, outflows evolve into deltoid or terraced systems, over which the water flows in shallow sheets. A thin film of bacteria or algae could easily extract nutrients from the entire mass of water. Phosphate and nitrate do not appear to be limiting for autotrophs (Brock, 1967a; Castenholz, 1969b), but Wiegert and Fraleigh (1972) and Castenholz (1973) have suggested that carbon dioxide may be the limiting factor for autotrophy in some springs. The bacteria living at temperatures above the limit for autotrophs evidently depend on the low levels of organic carbon leached from rocks (2 ppm—see Brock, 1967a, 1969), but whether absorption of this resource does in fact limit the growth of downstream populations is not known.

The role of nutrient levels is still being debated, but an undisputed mid-range peak for primary production lies around 55° C (Brock, 1967a, 1967c; Wiegert and Fraleigh, 1972). The decline in production below 55° C is not due to the removal of algae by grazing herbivores because the principal herbivores are not found and cannot tolerate temperatures over 40° C (Brock, 1967a; Wiegert and Mitchell, 1972). It has been argued that production declines because resources are bound by the mat and may become available only when there is herbivory (Brock, 1967a). Thus, the binding of nutrients may result in a decline in productivity over the range from 55° C to about 40° C, at which point herbivory becomes significant in releasing nutrients bound by the plants. There is no direct evidence on this point, however.

Competition for nutrients would generate a selective advantage for high-temperature tolerance all the way up to the source (where the water is at the boiling point). Castenholz's (1968) description of the extraordinary motility and behavior of *Oscillatoria* along the isotherm of its tolerance shows how mutants might compete for upstream nutrients when such nutrients are limiting and are rapidly absorbed. Clonal differences are not known for *Oscillatoria*, but there are two morphologically distinct temperature clones of *Mastigocladius* (Castenholz, 1973), and a sequence of four temperature clones is known for *Synechococcus* (Castenholz, 1969a; Peary and Castenholz, 1964). There are obvious differences among these well-known thermophiles, as would be expected from the fact that no two evolutionary regimens are the same.

The most powerful evidence for an inherent physicochemical limit to temperature tolerance would be derived from evidence that there is competition for nutrients over relatively short distances of flow and that there is a series of clones of declining efficiency. That is precisely the case for *Synechococcus*, in which each increment of temperature tolerance results in a decrement in growth rate (Peary and Castenholz, 1964; Meeks and Castenholz, 1971) until the doubling time of the clone at the highest temperature (70° C) is five times that of the clone at 40° C (2 doublings at 70° C for every 10 at 40° C—see Peary and Castenholz, 1964).

If resources were limited, then the very slow-growing cells of blue-green algae nearest the spring source would be at an advantage, and the failure of blue-green algae to evolve tolerances to temperatures above about 75° C under such circumstances would have to be the result of a temperature-dependent reduction in growth rate or a physicochemical failure of the algal cell at that temperature (Brues, 1938; Brock, 1969; Meeks and Castenholz, 1971).

There is no information on resource limitation for *Synechococcus*, and there is disagreement regarding the role of resources as limiting factors for the growth of procaryotes at high temperatures. Any test of evolutionary hypotheses must await more information about the available resources. Once the temperature characteristics and resource relations of the cells from various temperatures are well known, one may be able to use the tactical models to estimate the minimum loss of efficiency needed to account for a limit. The loss of efficiency need only be greater than the gain in resources in order that an equilibrium be reached. If there is no competition, resource gains would be zero for all resources, except that CO_2 would be lost at high-temperature zones, because all essential resources are fixed in quantity per cubic centimeter of emerging water. Thus, small losses of enzyme efficiency may easily account for limits that lie in the 70° C range.

In acid waters, the temperature tolerances of all procaryotes are much lower than at neutral or basic *p*H ranges (Brock and Darland, 1970) and may fall below those of certain eucaryotes—e.g., *Cyanidium* (Doemel and Brock, 1971). This seems to be clear evidence of a chemical susceptibility that involves complex interactions, and that is likely to indicate a loss of cellular efficiency. The minimum loss of efficiency needed to explain the temperature limit can be estimated from data such as those given in Table 1, once the temperature-tolerance characteristics have been well defined.

Thermophily among Eucaryotes

Because eucaryotes, especially metazoans, are easily classified on the basis of biological characteristics, comparisons of thermal species with non-thermal relatives can provide a basis for evolutionary speculation. It may be an idle exercise to attempt to search out the phylogenetic history of individual species of thermophiles, but the similarities and differences between sets of thermal and non-thermal species to be presented here may reveal common patterns which can be the basis for a plausible general picture for the evolution of thermophily.

Algae and Fungi

Thermophily may evolve by the exploitation of preadaptations for heat tolerance that occur purely by chance. Alternatively, the preadaptations may be associated with some taxonomic group or set of species that has some common ecological trait. Differences between the procaryotic and eucaryotic algae are clearly shown in the data of Kullberg (1971) plotted in Fig. 1 and in Stockner's (1967) studies in Washington. At 35° C, about 40 per cent of the algal species are eucaryotes. In contrast, between 50° and 60° C there is an average of six species of procaryotic algae, but never more than a single species of eucaryote. The shift in relative abundance, of course, is presumed to be due to a greater temperature sensitivity among eucaryotic algae than among procaryotic algae.

In acid waters blue-green algae may be absent. Under such conditions the eucaryotic alga, *Cyanidium calderum,* exists at much higher temperatures than is its limit in alkaline waters (Doemel and Brock, 1971).

C. calderum is evidently an asexual alga (Brock, pers. commun.), hence temperature strains might be expected; yet a very careful study (Doemel and Brock, 1971) has failed to reveal temperature strains. *C. calderum* occupies a variety of adjacent habitats where the temperature regime is not constant and the acid springs have less constant temperatures than alkaline springs (Doemel and Brock, 1971); hence this population may experience rather different kinds of selection pressures than most thermophiles. Could it be that there is less competition in acid habitats and that there the expanding range tactic (Q_e of Table 1), which may be biologically inefficient (as defined by the fitness model above), can nevertheless evolve? It is tempting but presumptuous to use the range of *C. calderum* and Tansey and Brock's (1973) definition of the astonishing range of the fungus *Dactylaria gallopava* to argue that some general differences exist between the acidophiles and the inhabitants of alkaline habitats. One obvious difference is the lack of competition in the former situation.

The species' mode of reproduction (*C. calderum* is evidently asexual) and the nature of its chemistry and competition must be considered in any explanation of the differences between the eucaryotes of alkaline and acid springs. Blue-green algae are often more numerous on wet shores than elsewhere (Castenholz, 1969b). These habitats regularly reach high temperatures because of solar heating (Mitchell, 1960) and it is possible to argue that this could preadapt blue-green algae and fungi to live at continuous high temperatures. Two important thermophiles, *Synechococcus* and *Oscillatoria,* may not have evolved from water-margin species (Castenholz, pers. commun.); thus, the different slopes for the two algal groups in Fig. 1 suggest either that independent

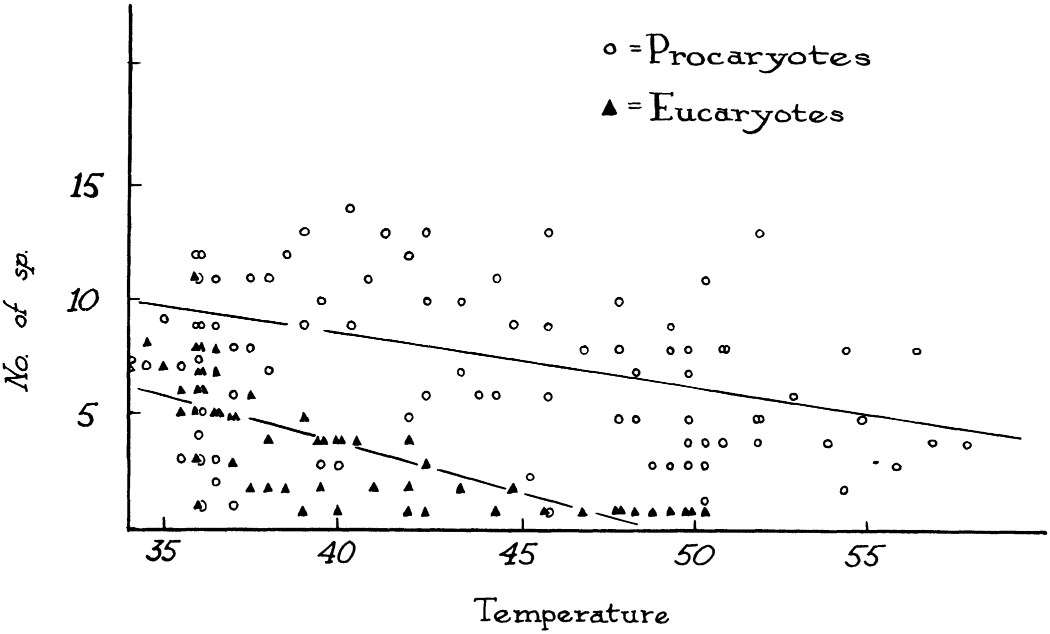

FIG. 1. THE NUMBERS OF SPECIES OF PROCARYOTES AND EUCARYOTES IN 94 COLLECTIONS FROM 5 SPRINGS IN MONTANA
Based on data of Kullberg, 1971.

preadaptation to high temperature is characteristic of blue-green algae, or else that there is a tendency for blue-green algae to dominate in water-margin habitats where tolerance to high temperatures must be secondarily evolved. The pattern exists, but a simple correlation provides no evidence in favor of either explanation.

If procaryotic and eucaryotic algae compete for a shared pool of dissolved solutes, the competition should result in all autotrophs gaining a selective advantage through thermophily; yet, the upper limit for most eucaryotic algae, namely, 56° C (Brock, 1967b), is 20° C less than the usual upper limit for procaryotic algae. This difference suggests a physicochemical limit. Such a conclusion is premature, however, because great differences in population structure and adaptive potential could result from sexuality. Sexuality is common among the genera of eucaryotic algae listed by Kullberg (1971), but there are no data on sexuality among the thermophilic species of algae. Even a small amount of genetic exchange will distribute traits uniformly through the population and prevent the differentiation of temperature strains within the population of a spring. Selection operating on such populations would optimize the fitness of each individual for occupying any temperature in the entire range of temperatures experienced by the interbreeding population. The optimum for such a population relative to temperature will be determined by the contributions of gametes from the lower part of the temperature range versus those from the upper part. The relationships between area and temperature always make the areas at lower temperatures larger and may necessarily result in a selective optimum that is lower for sexual organisms than it is for sets of competing clones. The lower temperature tolerance of eucaryotic algae could be due to sexual reproduction or to an inherent physicochemical limit. Studies of the genetics of thermophilic algae should allow one to consider the significance of these two possible explanations for the evolution of algae in thermal waters.

The Metazoans

There is much more information about thermophily in metazoans which are definitely sexual. Brues (1924, 1928, 1932, 1938) made extensive collections of thermophiles from hot springs throughout the western United States, but recorded the phenomenon in only 17 families of insects from two or more collections of at least two specimens of a species. In contrast, Usinger (1963) has recorded thermophily in 101 families of aquatic insects from North America.

The hot springs fauna is obviously a non-random sample, because the orders of insects that are dominant in western streams (Plecoptera, Neuroptera, Odonata, Trichoptera, and Ephemeroptera) are virtually absent from thermal waters. None of the orders of insects dominant in Yellowstone streams (Vincent, 1967) is ever found in thermal waters. Beetles of the family Hydrophilidae and three families of flies (Chironomidae, Stratiomyidae, and Ephydridae) dominate in hot springs and, except for the chironomids, these families of insects are never common in running water.

The highly evolved thermal communities are quite unlike other rheophilic communities. Flies are the main herbivores in them and eat the algal mats formed by blue-green algae. Ephydrid and chironomid flies are the dominant herbivores in communities known from Yellowstone (Brues, 1924, 1928, 1932, 1938; Brock, Wiegert, and Brock, 1969; Lynn and Brock, 1969; Wiegert and Mitchell, 1972), New Zealand (Winterbourne, 1969), and Iceland (Tuxen, 1944); and a stratiomyid community was described from the state of Washington (Stockner, 1968).

The random invasion hypothesis diagrammed in Fig. 2A requires upstream invasion of a few species; the thermophilic insects, however, belong to different families than those found in downstream habitats. A close examination of thermophiles shows them to be the kinds of insects normally found in the wet margins of streams and ponds. Such margins are heated by the sun, and the temperatures experienced by mites from these habitats have been characterized (Mitchell, 1960). The same temperatures are experienced by the insects of water margins, such as the ephydrids (Deonier, 1964, 1972). The maximum temperatures experienced along a gradient up the shore of a stream increases, while adaptations to resist the current become less important in zones of high temperature (Fig. 2).

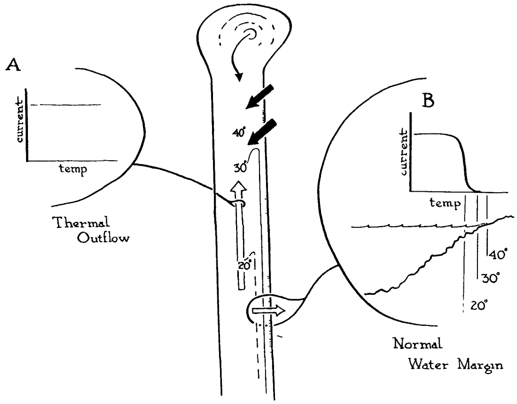

Fig. 2. Two Alternative Hypotheses for the Origin of Thermophiles and the Relations of Various Stream Faunas to Current and to Temperature.

A, mid-stream species moving up a temperature gradient must adapt to higher temperatures, but will not need to adapt to differences in current.

B, species in water margins experience high temperatures on wet shores due to insolation but do not experience currents. Thus, invasions of thermal waters from water margins (the black arrows) do not necessarily require alterations in the range of temperature tolerances, but may require adaptations to resist current.

The mites and insects of thermal waters clearly evolved from water-margin habitats; this is also true for the Mollusca, because the genera of mollusks found in hot springs are not characteristic of running water. Instead, they are the pulmonate snails that are typical inhabitants of shallow or still water (*Succinea, Physa,* and *Limnaea*—Brues, 1924, 1928, 1932, 1938; Tuxen, 1944). These snails normally live in very shallow waters or on wet surfaces where they must experience very high temperatures on sunny days. Even the peculiar snails of warm springs in Cuatro Cienegas, Mexico, and in Death Valley, U.S.A., are not typical stream species (Taylor, 1966, pers. commun.). Thus, the three major groups of hot spring endemics, the arachnids, the insects, and the mollusks, all seem to have evolved from water-margin habitats.

Two problems face those metazoans that evolve into thermal waters from water margins: (1) continuous exposure to high temperatures, and (2) life in swiftly flowing currents. The first problem is physiological. At present, the only related physiological studies of a metazoan involve the fish *Cyprinodon* in Death Valley (U.S.A.). The maximum water temperatures in the non-thermal waters of Death Valley reach or approach the low forties and the highest temperature tolerated by a hot spring species is 43.5° C (Brown and Feldmeth, 1971; Sumner and Lanham, 1942). The species of *Cyprinodon* are not typical stream species but are most common in shallow waters that regularly reach high temperatures.

Death Valley is one of the few areas in which ambient stream temperatures are likely to be well above the 20–30° C range. Only sketchy information is available for the three known thermal communities with fish populations at temperatures over 30° C: Death Valley, Nevada (Brown and Feldmeth, 1971); Cuatro Cienegas, Mexico (Taylor, 1966); and Hamman Meskoutine, Algeria (Mason, 1938). All of these, however, are areas in which the adjacent nonthermal waters are likely to have transient daytime temperatures that equal the maxima tolerated by the fish in thermal waters (Brown and Feldmeth, 1971).

No metazoan seems to have evolved a maximum temperature tolerance much above that experienced by related non-thermal populations living in adjacent non-thermal habitats. Numerous examples of high temperature tolerance associated with non-thermal habitats are given in the volume edited by Troshin (1967). The evolution of high temperature tolerance cannot be limited by the lack of resources at higher temperatures (Brock, Wiegert, and Brock, 1969; Castenholz, 1969b). Indeed, the ephydrid fly *Paracoenia* cannot live at temperatures that are optimal for the growth of its principal food (Wiegert and Mitchell, 1972); yet the flies have not moved upstream. The algae that grow at high temperature become available at low temperatures because of erosion or the diversion of the original flow of water Weigert and Mitchell, 1972). Thus, there might be an advantage in feeding at higher temperatures only if intraspecific or interspecific competition made it advantageous to eat the food just where it is produced.

Unless there is assortative mating, the direction of evolution with respect to temperature will be determined by the relative number of individuals produced at the upper half of the temperature range relative to the number from the lower half. Since the area of the upper half is inevitably smaller (Table 1), there will never be evolution of thermophily under Tactic 2 (shifting range) unless there is assortative mating or unless the reproduction or the survivorship of young per female is reduced at low temperatures or both. The average differential in production that is needed for evolution to favor individuals at above average temperatures can be easily calculated from temperature-area estimates such as those of Table 1.

The randomness of genetic exchange among the sexual metazoans will vary with their habits. Among the Diptera, mating occurs after flies emerge from the water and is surely haphazard with respect to the temperatures at which the flies have developed. The dispersion of water mites will be randomized when they are aerial larvae and become attached to adult flies, but the water mites will be subject to differing temperature regimens during the month or more of their development in the hot spring prior to mating. Mating may then be non-random with respect to temperature tolerance.

There is no dispersal phase among ostracods; hence their mating may be assortative. In addition, they appear to be food-limited (Castenholz, 1973). The individuals able to tolerate the highest temperatures, where there is less competition for algae, presumably gain more food and may enjoy higher fecundity. If they have greater fitness it may explain why their equilibrium is higher than in other metazoans (Wickstrom and Castenholz, 1973).

The overall pattern of temperature limits for metazoans lies in the range to be expected if they have evolved from water-margin habitats. In addition, the small differences between the three well-known metazoan thermophiles are as expected if randomness of mating was a factor determining the selective equilibrium and upper temperature limit:

ephydrids <	water mites <	ostracods
40° C	45° C	50° C

The general correspondence of the upper temperature limit for metazoans with the maximum temperatures in adjacent non-thermal habitats suggests that there is no selective advantage for thermophily. Since resources are present above the limit, it seems likely that thermophily must have evolved under a shifting range tactic, and that fact would account for the low temperature limits characteristic of metazoans.

If this hypothesis is rejected, and it is argued instead that the upper thermal limit is determined by physicochemical functions, then the congruence of the maxima in non-thermal habitats with that of thermal species must be dismissed as a meaningless correlation.

The hypothesis that water-margin species evolved from warm water-margin habitats into midstream thermal habitats accounts for temperature tolerance as a preadaptation. A water-margin form would seem to face serious problems in adapting to flowing water habitats, but two factors probably make this transition unusually easy in hot springs. First, most thermal waters deposit minerals to form deltoid, terraced flows. Second, the blue-green algae grow entangled with flexibacteria to form firm algal mats that often divert and spread the water out into deltoid flows, with many islands and shallow channels (Wiegert and Mitchell, 1972). Currents are usually gentle in thermal outflows, and thus water-margin forms have been able to invade such habitats without any great change in structure or habits.

SUMMARY

The autotrophs of thermal waters may have evolved from heat-tolerant species of water-margin or pond habitats, or else from cool downstream habitats. There is no firm evidence as to which source is of the most importance. The dominant metazoans in virtually all, if not all, thermal ecosystems evolved from water-margin habitats, where solar radiation may warm the surface to around 40° C. Thermophilic flies, mites, and snails come from such habitats, and their adaptations to transient high temperatures in water margins was evidently the critical preadaptation for thermophily.

Although thermophiles may in general have evolved from similar habitats, each group has evolved to a maximum that is characteristic for itself. That maximum is an independently determined evolutionary end-point in each thermophilic area. The close similarities of the evolutionary end-points of these replicated natural experiments suggest that some shared characteristic of the several taxonomic groups or an interaction with some common features of all thermal waters, or both, will account for the characteristic patterns of thermophily. Explanations for differences in temperature tolerances must consider three factors: (1) inherent physicochemical limits of cell function associated with phylogenetic position (N.B.: this refers to the maximum limit that could evolve, not the observed limit); (2) the availability of resources; and (3) the interactions between evolutionary mechanisms and resource abundance patterns that determine fitness. At present the maximum temperature limits are said to be a function of cell complexity, and the possible role of the other two factors has not been considered.

Bacteria extend to the boiling point of water, and neither the resource limits nor physicochemical limits have been reached. Since hot springs are depositional systems with shallow water flows, cells upstream will be at an advantage in absorbing nutrients from the water. If there were interclonal competition, it would drive bacteria to adapt to higher and higher temperatures and would account for their evolution of tolerance up to the boiling point. The only direct evidence for this hypothesis lies in the clonal differences along a thermal gradient of one bacterium and two species of blue-green algae. The role of resources and their supply is still not understood, but in the case of *Synechococcus* there is a trade-off between growth rate and temperature tolerance such that the limit may fall at a point at which the population fails to replace itself.

There is ample evidence of poor growth in the downstream part of the blue-green algae colonies, but there is much dispute as to whether this is due to a removal of nutrients by the cells upstream. If nutrients were not limiting, the situation would constitute the strongest possible evidence that the temperature limit of 73° C is a physicochemical limit for the cells of blue-green algae.

Green algae and fungi have a maximum temperature tolerance almost 15° C lower than that of blue-green algae, and there is evidence of competition for resources at these lower temperatures. The idea that their more complex cells are inherently more heat-sensitive than those of blue-green algae is a tempting postulate, but is unsupported by direct evidence. The majority of the green algae in thermal waters are from genera that are usually sexual. If the thermal species are also sexual, then the clonal differences that occur among procaryote populations would be absent in them, and fitness would be averaged to the extent that genetic exchange is random.

With genetic exchange, a fitness equilibrium with respect to temperature will be reached when the individuals at the upper (warmer) half of the range produce as many successful gametes as the individuals at the lower half of the range. The single factor common to all springs is that cooling is proportional to the difference between the water and ambient temperature; thus the area within a specified number of degrees will be smaller at high temperatures than at low temperatures. Such area differences may well determine the fitness equilibrium for a sexual organism, and that equilibrium may be far below the inherent physicochemical limit of the cell.

Sexual eucaryotic algae compete for nutrients, and there is probably a degree of nonrandomness in their exchange of gametes in the flowing stream of a hot spring. These two factors may account for their upper limit (<60° C) being ten degrees higher than that of metazoans (<50° C). There are small differences in the maxima for metazoans. These differences correlate well with the randomness of their genetic exchange: the more random, the lower the temperature limit. As a rule, metazoans are limited to temperature maxima equal to the warm season maxima of wet water margins rather than those of deeper waters, ponds, and streams.

The fauna of hot springs is almost never drawn from the adjacent ponds and streams. Most thermophilic animals are a sampling of the fauna living on wet, muddy margins of streams and ponds. Such organisms are regularly exposed to temperatures in the low forties, and the evolution of thermophily may be no more than acquisition of tolerance to continuous exposure to temperatures in that range. The evidence will not support the argument that there is some absolute thermal limit to the evolutionary potential of the metazoan cell.

Careful studies of the endemic thermophilic ephydrid flies suggest that they would lose fitness be evolving greater temperature tolerance unless they broadened their range. Since these conclusions depend on relations definable by the general properties of water-cooling and algal growth, rather than the biological peculiarities of ephydrid flies, it is possible that the explanation may account for the thermal limits of other herbivorous metazoans.

In every known case of thermophily there is one species, and only one species, that occupies the highest temperature zone. That zone is likely to be a rather large one. If the upper temperature limit is strictly a physicochemical limit, why should we never find more than one species of a group sharing the high-temperature zone? This may be a telling question because physicochemical functions cannot explain why only one species ever reaches the limit in any single spring. The ecologist would expect this outcome because hot springs are simple stable habitats in which competitive exclusion would reduce diversity. Explanations for thermophily should not be either physicochemical or ecological. They must be an amalgam of both. As this synthesis progresses, it is likely that hot springs will be found to be sites of many extraordinarily interesting natural evolutionary experiments.

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