

Evolutionary Challenges of Extreme Environments (Part 2)

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Despite a plethora of theories, basic laws of nature seem elusive in biology even though they have usually been considered the ultimate goal of physics and chemistry (Waterman, '68). Perhaps their evasiveness in biology stems from the quite special entities with which it deals. Living beings have many complex components, dynamically interconnected in multiple ways. These are clearly rather different from those of a falling apple, radiation propagating through space, or the combustion of glucose in a flask filled with oxygen gas. Some scientists suggest that biology is more like engineering, because it often uses the laws of physics and chemistry to explain living material and its information systems (Hengeveld and Walter, '99). Whether this is true or not, biology and engineering often have mutually rewarding roles as in cybernetics and robotics (Ritzmann et al., 2000).

Even so, quite a few broad rules or laws about life have been proposed. Arguably, the broadest and most persistent of such laws (Kleiber's Law)

deals with relative growth and *quantitatively predicts* the relation between metabolic rate and size in major groups from microbes to elephants (Smil, 2000). Despite much data and thought, a widely acceptable explanation of this impressive generalization remains to be agreed upon (Dodds, et al., 2001). Quite often, such biological rules are soon forgotten, or frequently rejected, sometimes with jeers, by second thoughts of others. Yet some of them have considerable staying power.

For instance, life's vigorous persistence in an unstable and often highly stressful world may depend quite typically on two pairs of remarkable, seemingly contradictory, traits:

- *diversity* and *unity*
- *flexibility* and *stability*

On the one hand, the exuberant diversity of the millions of different species and kinds of living things, plus their innumerable component organs, cells, genes and special protein molecules, would seem to contradict any notion of underlying uniformity.

On the other hand, all living organisms are built of the same chemical elements and do function basically in the same way, subject to the classic laws of thermodynamics. In other words, they all share a remarkable *unity*, particularly in the nature of cytoplasm, the anaerobic core of energy metabolism, the basic genetic code, and the drives to survival and self-replication. This oneness of life imposes limits and constraints on evolution that often seem to be overlooked by biologists.

In addition, flexibility and stability also appear quite opposite. Yet they are complementary and necessary aspects of life in a world with many kinds of habitats, constantly changing on shorter

This essay is a more technical and detailed version of the last chapter of the author's book about extremophile animals, *Animal Frontiers*, to be published by the Yale University Press. Some additional material has been drawn from earlier chapters to make this part of the book stand on its own. Part one of three (Waterman, 1999) focused on currently productive ways to study the evolution of animals living on the environmental frontiers. This second part concentrates on relevant long-term evolutionary trends and their relation to natural selection in extremophiles. The last part will discuss evolution and the environment, including the frontiers, as well as sources of phenotypic variation, evolutionary rates, and extinction as potential components of extremophile evolution.

Part 1 of this discussion already showed that the challenges of the essay's title are twofold. Biologically, potential animal extremophiles have been frequently challenged for at least 500 million to 600 million years to maintain their fitness in environments that demanded greater hardiness and more stress avoidance than they had previously experienced. Failure to meet such environmental challenges obviously would block a species or its group from becoming more extremophilic despite the currently steep deterioration of global habitats. Professionally motivated biologists are also challenged to extend and integrate the rather scattered and sparse existent data on extremophile evolution, as well as to analyze the mechanisms responsible and their ultimate relevance to the rapidly changing biosphere and its future.

Part 3 will discuss sources of phenotypic variation, rates of evolution extinction as a component of evolution, and extremophiles' future.

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or longer scales of time and space. Diversity and flexibility (plasticity, evolvability) are obviously central to our preoccupation with evolution. Yet they are critically interrelated with the unity and stability that have been responsible for life's *persistence* for billions of years.

Another more controversial aspect of life relates to its *adaptedness*. Observers of nature have for millennia noticed that animals and their environments seem to match each other, often to an extraordinary degree. Such correlations are often particularly dramatic in extremophiles. Desert animals, polar animals, deepsea animals, and high mountain animals, for instance, are usually notable for a variety of structural, functional, and behavioral features closely correlated with the stressful aspects of their extreme habitats. These correlations with the environment have long been called *adaptations* and have often been considered as basic characteristics of life.

In the 1960s and 1970s, the Darwinian belief that this pervasive match between organisms and their environment arose mainly if not exclusively, by natural selection, was widely accepted by biologists (Amundson, '96). Even so, the interdisciplinary research necessary to prove such a causal link was scarce, difficult to carry out, and, in fact, not widely pursued despite considerable speculation that fine-tuned adaptation was indeed the rule. This state of affairs was vigorously denounced by Gould and Lewontin in 1979 (see also Pigliucci and Kaplan, 2000) amid architectural and literary flourishes, and for some readers, potential links with the "sociobiology wars" (Brown, '99; Sterelny and Griffiths, '99; Segerstråle, 2000).

This outspoken indictment criticized weakly documented evolutionary "adaptationism" and the irresponsible "adaptationists" who practiced or accepted it. Whether from guilt as charged, or sheer vulnerability, a topic of central interest to biology, seemingly at one blow became a pejorative term not to be mentioned in respectable company. Some 20 years later adaptation and its degree of precision are still matters of controversy (Weibel et al., '98). Yet there are sustained signs that the subject may be making a substantial comeback (Rose and Lauder, '96; Bijlsma and Loeschke, '97; Givnish and Sytsma, '97; Van de Vijver et al., '98; Koslowski, '99).

Part of the problem, as is so often the case, was rooted in terminology. For instance, following Darwin strictly, any of an animal's features that increase its fitness, but have not evolved

through natural selection, should not to be considered adaptive (Rose, '96). But more general definitions are commonly used by scientists and engineers referring to the adaptation, Darwinian or not, of many complex systems, both biotic and nonbiotic: for instance, sophisticated robots (Ritzmann et al., 2000; Frank, '96; Givnish and Sytsma, '97; Auyang, '98).

As is evident from this essay, the author, as an emeritus comparative physiologist who worked mainly on underwater vision and orientation (Waterman, '81; Waterman, '82; Waterman, '97), was used to a far broader definition of adaptation (Slobodkin and Rapoport, '74) than one based strictly on its evolutionary dependence on natural selection. Adaptation and accommodation in eyes, as well as many other kinds of physiological regulation, acclimatization, behavior, learning, reproductive and developmental patterns, phenotypic plasticity, symbiosis, human culture and so on, are surely parts of the usual match between organisms and their environments (Frank, '96), not to mention the fitness of the environment itself (Henderson, '13). Clearly, adaptation as Darwin conceived it is not the only factor in the whole evolutionary process.

Another broad biological rule of some interest here is Malthus's Law. It was suggested in part I of this essay to be an emergent universal property of life arising from its complex system properties and providing a critical internal driving force relevant to animal evolution (Koslowski, '99). If so, this "law" might explain how animals are impelled into becoming extremophiles. Malthus's Law states that living things tend to reproduce themselves indefinitely until their *numbers* reach or exceed the limits of the ecological resources they require, including living space.

The resulting Malthusian population pressure to expand any given animal's range was suggested as a likely mechanism bringing animals to the frontiers of extreme environments and constantly challenging their capacity to survive greater stresses. In addition to this increase in biological numbers that creates crowding pressure for expansion, several other possible evolutionary trends were mentioned briefly in part 1. Despite some risk of resurrecting old controversies, these deserve further discussion here as probable trends followed by innovative forms of life beyond the pioneering prokaryote extremophiles, such as deepsea hyperthermophilic chemoautotrophs, which still flourish two billion or more years after their origin.

EVOLUTIONARY TRENDS

Since life began, fossil and geological evidence demonstrates that organisms' individual *size*, *complexity*, and taxonomic *diversity* have all increased dramatically overall. Along with numbers of individual creatures that have ever lived, these evolutionary "growth factors" may seem somewhat redundant. Life's defining properties no doubt include *reproduction*, which increases number; *growth*, which increases size; and *evolution*, which often increases various kinds of diversity. Both growth and *evolution* also typically include increased *complexity* that is often positively correlated with *size* (Bell and Mooers, '97).

All four trends would seem to contribute to crowding and a need for expansion because they usually require more space and additional resources. The obvious circularity of this argument suggests that evolution has had a net *direction*, even though it is usually considered to be unprogrammed. Moreover, these predominant, mainly phenotypic, increases were, in detail, irregular and often locally or temporarily reversed. Evolutionary regressions, both structural and functional, were sometimes drastic, as in many parasites; in addition, extinctions were sometimes massive and perhaps inevitable (part 3).

In so far as they may seem nonDarwinian, the four positive trends cited remain largely problematical (Futuyma, '98). Theoretically, such changes may be *emergent* properties of complex self-organizing living systems. Probably the most spectacular and familiar emergent feature of animals is the basically self-programmed development of the single-celled "simple" zygote into its specific kind of many-celled complicated adult (Wolpert et al., '98; Peterson and Davidson, 2000).

Although considered by some biologists to be suspiciously metaphysical, intrinsic system properties may provide biological evolution, including that of extremophiles, with an internally driven direction and/or a terminal state (Auyang, '98; Csányi, '98). These could act in addition to genetic drift and the usually accepted Darwinian *external* selective forces acting on variation. They must also be taken into account when analyzing evolvability as well as the role of chance in evolution discussed later in this article.

Overall evolutionary gains in number, size, complexity, and diversity of life seem to have prevailed from the earliest microorganisms to the much later origins and subsequent evolution of the many celled plants, fungi, and animals (Szathmary and

Maynard Smith, '95; Baldauf, '99). This appearance of multicellular organisms bypassed limitations to the usual minute size of single cells. Interestingly, the typical size increase in many-celled animals took place mostly through increases in the *number* of cells per individual rather than through further increases in cell *size*, with some exceptions such as nerve cell processes that have to reach the body's periphery from the central nervous system.

Dendrites and axons may be one or two meters in length in a large dinosaur or a giraffe. However, cell numbers and sizes have themselves been components of local evolution by particular groups of animals as diverse as nematodes and amphibians. Also, the genetic and hormonal controls of cell size and cell number differ so that, at least in *Drosophila* and mammals, organ or animal size can depend either on cell growth or cell division (Montagne et al., '99). However, among invertebrates of many kinds increased cell size, along with cell numbers, may be largely responsible for the sometimes major increases in body size correlated with larger genome sizes, discussed later in part 3 (Gregory et al., 2000).

More broadly, the reality of a tendency of increasing animal size among the outer branches of higher phyla (McMahan and Bonner, '83; Jablonski, '96; Gould, '97) has been a topic for considerable discussion and controversy. A number of biologists have even suggested that the abrupt appearance of most metazoan phyla as fossils around 550 million years ago resulted from a massive size increase plus a sudden burst of body plan innovation (Davidson et al., '95; Fortey et al., '97; also discussed later in part 3). Size increase and the other trends cited have been considered by some other biologists as largely intuitive (McShea, '96) and unsupported or even confounded by the facts. Others consider them too biased by human-centered notions of cultural "progress" to be scientifically acceptable (Ruse, '96).

With regard to *size*, the earliest extremophilic bacteria were unquestionably far smaller than the first single-celled eukaryotes, including protozoans. The pioneer many-celled animals in turn were clearly smaller than many of those that evolved later, even though many kinds of the smallest early creatures, such as innumerable bacteria and archaea, obviously continue to flourish in the modern world. Conversely simpler wormlike invertebrates such as flatworms and nematodes are notably smaller than cephalopod giant squids and crustacean spider crabs although some marine jellyfish and the

human tapeworm achieve one sizable dimension. The longest tapeworm, for instance, can match the blue whale's length at about 30 m.

Yet the biggest invertebrates in turn are clearly outsized by vertebrate whale sharks and many fossil reptiles. In any case, the largest animal known ever to have evolved on earth is the blue whale, still living in the world ocean. The most massive individuals of this whale weigh in at over 150 tons, compared with 50 to 60 tons estimated for the largest dinosaurs known so far. The whole whale family to which the blue whale belongs is only about 12 million years old (Carroll, '97). If so, that record for largest animal size has been set rather recently, geologically speaking. Size *increases* in smaller branches of invertebrate and vertebrate evolutionary trees, are quite common, but comparable evolutionary size *decreases* may be significantly less so (McMahon and Bonner, '83). Generation time is usually positively correlated with size so that size increases are often inversely correlated with the rate of evolution (Mackenzie '99).

However, an exceptional evolutionary trend toward notably smaller size has occurred repeatedly in a variety of different kinds of marine invertebrates among the *meiofauna*. These tiny creatures, less than a millimeter in diameter, live between sand grains. Also miniaturization of vertebrates is a striking local trend in newts (Hanken, '99), frogs, hummingbirds, and others (Miller, '96). Yet minimum vertebrate adult sizes are far larger than the invertebrate ones. The smallest vertebrates (certain bony fish) are clearly much larger than the smallest nematodes, insects, or crustaceans.

As a result, the tiniest mature fish is much too big to live between sand grains. In turn the smallest mammals and birds are larger than the smallest fish. The lightest adult simian primate, the pigmy marmoset, is a lot larger than that littlest fish (Genoud et al., '97) or even the smallest shrew. The smallest chimpanzee, or human, is substantially larger than that marmoset.

SIZE AND EXTREMOPHILES

Among extreme faunas, most arid land animals are small in size, and large desertophiles are in a minority (Degen, '97). Several factors may explain this relation. As a rule, the numbers and diversity of the faunas in extreme environments are reduced, drastically in some cases. The marginal productivity of environmental frontiers is a likely major factor in this (part 1). However deepsea soft

sediments seem exceptional in the richness of their fauna, mostly small in size (Grassle and Maciolek, '92; Van Dover and Trask, 2000). Ants, beetles, or small lizards require trivial actual amounts of water and food, compared with most large mammals. Some smallish desert frogs and toads during estivation store modest, but significant, amounts of water available briefly during desert rainy spells. Early in dormancy the urinary bladder, lymphatic system, and even the coelom are distended with urine-like fluid (Warburg, '97). In their own arid emergencies Australian aborigines learned to dig out and drink the water from such local anurans.

In various deserts, seemingly minor water sources, such as dew, water vapor, and fog, may provide adequate external water to many kinds of small animals. A Namibian Desert tenebrionid beetle, for example, adds to its other, more usual, water sources by condensing fog on the back of its body (Nicolson, '80). Despite the area's severe scarcity of rain and ground water, such *fog-basking* depends on frequent bouts of nocturnal fog along the coastal severely desert strip of the Namibian Desert that borders the eastern South Atlantic Ocean.

Many other animals also benefit from this minute supply of water. Quite a few insects and some spiders and scorpions in the Namib make use of fog water formed on the sand surface. One other beetle there, *Lepidochora*, is remarkable because it digs little trenches in the sand to collect fog condensate and dew. Special water- and watervapor-sensitive receptors, clearly useful in locating minor sources, have been identified on desert scorpion legs (Gaffin et al., '92).

Lizards and snakes in the Namib may also imbibe fog condensed on their body surface or dripping from desert plants. Even sizable mammals, such as jackals, have been seen to lick condensate from rocks (Bothma, '98). Yet elephants, giraffes, antelopes, and black rhinos still sparsely inhabit the northern Namibian desert. The survival of such large plant-eating mammals, exceptional in so arid a place, apparently depends on their detailed learned knowledge of just where and when to locate precious pockets of scarce water and food. Also a few individual cheetahs, leopards, and lions may hunt in this desert with the lions sometimes feeding on fur seals at the coast (Louw, '93).

In the stressfully arid coast of Oman bordering the Arabian Sea, dew and fog create a situation somewhat similar to that in the Namibian coastal desert (Spalton, '99). But in this case certain

desert trees, shrubs, and grasses can use traces of water in fog and dew to maintain some photosynthesis-driven growth, despite droughts that may last two or three years. As a result, the large Arabian oryx (hunted to extinction in the wild but reintroduced), an ibex, and two species of gazelles can maintain viable populations there dependent on the water and minimum protein thus available there in plant food. These animals also benefit from digging out underground water-storage tubers produced by some of the desert plants. Recall, too, that dew has been ingeniously exploited by humans for agriculture in the Negev desert apparently back to prehistoric times.

A variety of insects elsewhere can even absorb water vapor from a sufficiently saturated atmosphere without fog. For instance, in the North American southwest, the sand-diving desert cockroach, *Arenivaga*, collects moisture from the air by using a pair of curious little balloon-like bladders everted below the jaws. Apparently their specialized outer surface is wet with a secretion that condenses water vapor present in the air and thus makes liquid water available for transfer into the insect's mouth. Other insects can also absorb water *vapor* directly near the rectal end of the intestine.

Scale, as epitomized by Kleiber's Law, surely has a pervasive influence on animal physiology, behavior, and ecology (Schmidt-Nielsen '84). Measured as body mass, animal sizes range over as much as eight orders of magnitude between minute rotifers or midges and whales. The resulting differences due to size have remarkably large effects on most aspects of the animals' lives. Within the mammals, for instance, the metabolic rate of a gram of muscle in an Etruscan shrew, the smallest of mammals, is about 100 times that of one gram of elephant muscle and may be 400 times that of a gram of muscle in a blue whale, the largest of mammals. Even so, the whale overall needs perhaps *50 thousand* times as much oxygen as the shrew even though the cetacean's weight is about *20 million* times that of the smallest mammal.

In addition, diversity has been reported to be inversely related to the animals' size (mass) for many kinds of animals, ranging from marine invertebrates, to insects and all vertebrate classes, including mammals (Gardezi and da Silva, '99). Also because generation time is often shorter in small animals, their evolutionary rates tend to be faster. The wide extent and strong influence of animal sizes greatly increases the spectrum of ani-

mal types potentially available to evolve into various extreme niches.

However, an important common feature of extreme environments is also relevant here. Food scarcity and the threat of starvation are at least seasonal in most frontier environments, ranging from the polar regions to subtropical deserts. This means that animals' actual metabolic rates at rest and in activity are often important limiting parameters in extremophile fitness. Hence there are major tradeoffs needed between size and regulatory complexity in extremophiles. Despite the remarkably uniform relation between size and rates of metabolism, the rates themselves may differ greatly with the kind of animal and its particular activity levels as well.

Usually, the component species of animal communities and ecosystems include many small species, and successively fewer medium and large types, culminating in a few large predators. Basically this must depend on larger species' need for a greater part of the total local resources and the decreasing efficiency of usable energy transfer with added links in the food web. Extreme environments tend to have limited food webs, because both numbers and diversity may be severely reduced by high stress levels. Also in most severe environments primary production, largely through photosynthesis, is curtailed by shortage of water and light as well as extreme temperatures that also directly stress animals. In addition green plants are themselves stressed on land by soil quality and in the sea by lack of essential substances such as nitrate, phosphate, and iron. This in turn will limit the animal community that depends on plant production for its essential energy source.

In simple food webs specific items may be crucial in sustaining the whole extreme ecosystem, such as the abundant shrimp-like krill in the Southern Ocean around Antarctica (Reid et al., '99). These feed on phytoplankton, and are a pivotal element in the diet not only for fully aquatic animals, such as fishes and squids, but also for large populations of diving birds and mammals, including baleen whales. The long Antarctic night should present krill with a starvation-stressful overwintering problem, in spite of which these crustaceans have flourished in enormous numbers (Hofmann and Lascara, 2000). In the deep sea, the rain of food from productive near-surface waters (Christensen, 2000) and the diurnal vertical migration of epipelagic animals is crucial to almost all deepwater animals. Modest changes in

some seemingly remote component of such large scale ecosystems may have disastrous consequences for certain extremophiles in the food web.

Ectotherms, including invertebrates and most fishes, amphibians, and reptiles, normally have low metabolic rates although minute flying insects, such as *Drosophila*, and some fast swimming fishes, have high active rates. Among vertebrates, there is a marked difference between amphibians and reptiles compared with birds and mammals. The endotherms have resting metabolic rates 5 to 10 times greater than the ectotherms and perhaps 50 times greater energy requirements when vigorously active. Energy food and oxygen needs will escalate directly with these rates, other things being equal.

Yet for animal extremophiles, large size may be maladaptive for desert ants and rodents, but of positive advantage for others, such as the Namibian rhinoceros, the Arabian oryx or dromedary, and Bactrian camels. Yet large, medium, and small animals have all evolved to flourish in extreme environments, even though large size demands more of the scarce frontier items per individual. Fossil evidence indicates that large animals are more susceptible to extinction than smaller ones (Hoffmann and Parsons, '97) implying that they are at greater risk under stress.

Large extremophiles

Because metabolic rates per unit weight are lower in large animals, one animal weighing a kilogram could be more efficient in using resources than a swarm of smaller ones weighing altogether the same amount (Griffiths, '92). On the other hand, reduced population numbers may, by acting as bottlenecks, jeopardize survival because of genetic stresses (Landweber and Dobson, '99). Where scarce food or water occurs in local areas distant from one another, as in desert oases, large size is advantageous because locomotion in large animals can cover more territory and obtain more mileage per unit fuel than in small ones (McMahon and Bonner '83). As a result, camels and antelopes demonstrate how such interacting factors may have played out in deserts; yaks and llamas do the same for high altitudes.

Range-effective locomotion also favors birds and mammals, such as many in arid parts of Africa, that have to migrate long distances seasonally to escape or mitigate intolerable local shortages of food and water (Waterman, '88). The same relation also holds in the Arctic. Snowy owls, crows, ptarmigan, polar bears, musk oxen, and reindeer/

caribou are among numerous arctic birds and mammals that actively inhabit high latitudes above the Arctic Circle throughout the year.

Because of their high metabolic rates and insulation, these endotherms, including both herbivores and carnivores, are far more capable of long-distance rapid geographic movements than most terrestrial ectotherms. Seeking scarce food they tend in winter to wander widely in longitude as well as south over the tundra. If prey is scarce, snowy owls may occasionally reach quite surprisingly distant lower latitudes well outside their usual subarctic range limits. Also, large-sized predators and prey may benefit because greater size, up to a moderate point, can increase running speed as in gazelles and cheetahs (Alexander, '89).

Note also that among breath-holding divers in pursuit of prey, the largest living penguin (the emperor penguin) and the largest seals, the Northern and Southern Hemisphere species of elephant seals, regularly dive furthest and longest (for their respective families: Spheniscidae and Phocidae) into the deep sea. Typically, more work effective locomotion, lower metabolic rates per gram and larger oxygen storage capacity favor diving prowess in the larger species.

However, the largest whale, the blue whale, filter feeds on zooplankton, more abundant nearer the surface and in shallower water. Consequently its normal diving behavior is modest. Yet the toothed whales, which include dolphins and the sperm whales do pursue prey at deepsea depths. Repeated rapid descents and ascents are made to 400m to 600m by some dolphin species and to as deep as 1200m by the sperm whale (Berta and Sumich, '99).

In addition, large- and medium-sized high-latitude terrestrial endotherms that do not seasonally leave their extreme habitat, are often quite capable of maintaining viable internal body temperatures in the face of frigid polar conditions. Some mammals can also avoid winter stresses by denning and by hibernation, a special kind of dormancy. Interestingly, *classic* hibernation, discussed later in this article, occurs only in some small mammals, mostly at subpolar temperate latitudes. Even some moderate-sized high-latitude mammals such as the arctic fox, ermine, wolverine, lemming, and arctic hare, are nonhibernators. Yet they flourish in the subarctic and arctic winter.

Important responses to this thermally severe life are often reflected in thicker and denser fur, as well as heat-conserving regulation of blood cir-

ulation to the skin and appendages. Some species, such as the collared lemming, may even seasonally lose as much as 50% of their body mass, apparently to reduce the total maintenance metabolism they need (Nagy et al., '95). Yet reindeer, ptarmigan (Blix, '89), and polar bears are also known to reduce their metabolic rates substantially in severe winter weather, largely by minimizing locomotor activity and thereby energy needs, but they do not become dormant.

The large hoofed mammals, musk oxen and reindeer/caribou, are plant eaters feeding all winter on lichens, mosses, and various other plants that are then sparse, but usually protected against freezing by snow cover. Massive long-range treks are usually required to find enough nutritious plant material in the coldest weather. Such geographically long-range high-latitude movements are hardly feasible for small animals such as rodents or ants.

In addition, large size is often an advantage in surviving both high and low environmental temperatures. Transfer of heat into or out of an animal's body depends on the temperature gradient between inside and out, on the thermal resistance of the body surface (whether bare skin, or skin underlain by blubber or covered with feathers or fur) as well as on the area of the body surface (Schmidt-Nielsen, '97). According to simple geometry, the surface area of solids of similar shape varies as the square of their linear size while their volume varies as the cube of linear size. This means, among other things, that large animals heat up more slowly in a hot environment and cool off less quickly in the cold. On the other hand, in large animals, the surface area for evaporative cooling is smaller relative to the volume to be cooled.

Also, a large animal, such as a dromedary, can take up much more heat for a given rise in body temperature than a small one. In fact, the camel and some other desert endotherms relax their thermoregulation to warm up a few degrees above normal by day and cool off through dry heat loss at night. In this way the radiative heat gain is reduced by the weaker heat gradient between the hot surround and the animal's hyperthermic body temperature. Also, the increased body temperature conserves the water otherwise needed for sweating to maintain the normal body temperature strictly.

Although they lack sweat glands, birds in the desert tend to lose precious water through the skin and, as mammals do, by respiration (Tieleman and

Williams, '99). Most birds are diurnal and do not burrow, so they tend to experience the desert's heavy heat load fully. Also, their metabolic rates and body temperatures are somewhat higher than those of mammals. This reduces the safety margin between their normal levels and lethal levels. As a result, thermoregulation must be closely controlled for their survival. Like mammals in a hot environment, they can conserve water effectively by allowing a small rise in body temperature. This works well for small and medium-sized birds but apparently not over long periods (more than an hour) for large ones.

In the cold, similar relations mean that for a large animal, its body temperature falls less for a given loss of body heat (calories) to the environment. These size relations no doubt contribute to the fitness of yaks, llamas, ibexes, and bighorn sheep in high-altitude habitats; musk oxen, polar bears, and emperor penguins at high latitudes; sperm whales and elephant seals in deepsea dives, as well as of large antelopes and camels in deserts. Generally large species of birds and mammals are less common at low elevations in the tropics and subtropics than they are at moderately higher elevations and latitudes.

The prospects for large animals in hot deserts are obviously related to how they cope with heat and scarce water stresses. This depends not only on size but also on whether they are ectotherms or endotherms, an element of complexity discussed further later in this article. To ease the heat load of hot deserts, large mammals, such as rhinos and elephants may rest at midday on bluffs and other elevated points where cooler and stronger breezes often blow (Reardon, '86). Medium-sized animals, including leopards, may avoid heat by sheltering in caves or ravines as well as under the shade of trees or shrubs.

Shelter in extreme habitats

Seeking shelter in a broad sense is a widely used way of living in an extreme environment without bearing the full brunt of its stresses. As just mentioned, it usually is limited to small or moderate-sized terrestrial animals. For instance, a desert spider has developed conspecific aggression because suitable shelter against which to construct their webs is scarce (Riechert and Hall, 2000). In most cases shelter is used daily, seasonally, or occasionally to avoid periods of potentially lethal high or low temperatures, extreme dryness (Adamczeszka and Morris, 2000), and intense short wave solar irradiation, as well as

shortage or absence of food and the attacks of predators. Although small birds and mammals under arctic snow and desert ants in their underground nests may remain active and feeding, decreased activity, slowed metabolism, and remarkable resting stages are often dramatic aspects of such stress avoidance (discussed later in this article). Although referred to elsewhere in this essay, a more coherent account of the frequent importance of shelter in extremophile biology seems in order here.

In the nests of ants and the burrows of small mammals, temperatures are usually thought to be significantly cooler or warmer than surface air temperatures in daytime deserts or high latitudes. Yet relatively few detailed and well-controlled measurements have been made of the oxygen and carbon dioxide levels as well as temperatures and moisture in various desert and high-latitude animal shelters. Available evidence implies that in subtropical deserts severely stressful conditions are often present even in the burrows (Walsberg, 2000) and probably in frigid high latitudes even under snow.

In geographic regions subject to year-round permafrost (French, '99), burrows in the ground and caves are usually unavailable for animal shelter. Great areas with perennially hard frozen ground include all of Antarctica plus the South Orkney and South Shetland Islands as well as most of the high Arctic lands except northern Scandinavia and Finland (warmed by the Gulf Stream). In substantial parts of the Arctic Ocean's margins even the sea bottoms are frozen solid with permafrost, a remnant of earlier geological periods. Yet rather bizarre thaw lakes occur widely in Arctic permafrost basins of various sizes and depths (Fogg, '98). Their animal inhabitants may be limited to one copepod species and one fish species. Interestingly, even very high mountains, except those located at high latitudes, lack permafrost if they have a daily warm up by sunlight.

An animal burrow or snow shelter can be important, for instance, in the storage of seeds or other food by desert ants and rodents to tide over periodic or occasional shortages. Unless they have stored body fat for energy, or seeds, grass or tubers, collected when available, animal activity must be reduced or stopped to conserve energy in such shelters. In some cases, such as in lemmings, voles, and pikas, edible plant material, plentiful in the summer, may be gathered together, sometimes dried out, and stored for use during the winter. Arctic foxes also may, similarly, cache their prey or parts thereof.

Although high-latitude terrestrial stresses may be present all year, as they are in central Antarctica and Greenland, they mostly peak only during the local winter. Then the percentage of individual animals that survive may be marginal in the most extreme locations. In Antarctica there are no vertebrate herbivores, or indeed any native terrestrial vertebrates at all. In contrast, plant-eating hares, reindeer, musk oxen, and others flourish in the Arctic as mentioned. Yet even in this less frigid north polar area, these herbivores as well as the carnivorous polar bear and arctic fox are often subjected to seasonal near or literal starvation.

In addition to burrows, other sorts of shelters allow some small animals living at high-latitude and high-altitude to survive disabling cold without tolerance of or resistance to freezing. They can do so by retreating into reliably frost-free refuges locally present in the otherwise inhospitable habitat. A variety of these retreats, such as under bark or deep in decaying wood, provide livable places in which either active or dormant stages of a variety of rather minute animals can overwinter. Temperatures near 0°C may prevail when it is much colder outside.

Deepsea animals in open water have no equivalent to the shelter offered by burrowing to marine bottom animals and many terrestrial types. Yet at least one benefit of such shelter is offered in the camouflage/bioluminescent system of many mid-water shrimps, squids, and fishes of modest size (Herring et al., '90). During the day, light organs and their sometimes elaborate related optical systems (Denton, '90) tend to make these creatures invisible, or nearly so, to their predators and potential prey. Less optically elaborate but functionally comparable camouflage, sometimes seasonal, is common in the white fur and plumage of ermine, snowshoe hare, arctic fox, polar bears, snowy owls, and ptarmigan. Yet crows are common at snowy high latitudes and show no hint of winter change in their jet-black feather color.

Cold ectotherms

Although endotherms are the main concern of the preceding discussion, ectotherms also can survive and flourish at high latitudes and high altitudes. In addition to cold and scarcity of food, these habitats also usually lack liquid water and are particularly stressful for insects (Sinclair, 2000). Yet a surprising number of invertebrates, including insects (Strathdee and Bale, '98) and some fishes, are active in or on snow and ice as well as flying

in air or swimming vigorously at temperatures close to freezing. Rather unexpectedly the cold but quite sheltered subenvironment under high arctic snow (discussed previously for endotherms) is also inhabited by many insects and various other arthropods. In most cases the body temperature is essentially that of their close surroundings.

As a result, they can function actively only within a rather narrow band of cold temperatures. A remarkable variety of ectotherms are active at temperatures a few degrees above and below 0°C. The ice worm (*Mesenchytraeus solifugus*) offers a striking example. This small oligochaete, a remote relative of the familiar earthworm, actually lives in and on subarctic glacial ice as, for instance, high on Mt. Ranier in Washington State. There, at temperatures close to 0°C, it feeds on windborne pollen and microorganisms.

Also a number of small insects and spiders, like iceworms, manage to be active and reproduce at near-freezing temperatures on permanent glaciers on high mountains. Although warming by sunlight is often thought of as a prerequisite for sustained activity at such marginal temperatures, a number of high-latitude beetles and spiders take shelter during the day and emerge to feed at night. However, their activity is limited to nights when air temperatures do not fall too low. Clearly such ice-living animals, even though they lack the ability to raise their body temperature above that of the surroundings, have been able to keep their enzymes and membranes functioning at icy temperatures either by preadaptation or evolutionary adjustments (Leather et al., '93).

A quite different group of worms, the nematodes, are common at high latitudes both as parasites of other animals and also free-living in tundra. Surprisingly, one Arctic species has been proved to survive substantial amounts of *intracellular* ice formation (Womersley et al., '98). This has usually been thought to be lethal. Certain high-latitude nematodes may stay frozen eight months in the year. Some sort of below-zero hardiness is not unusual in high latitudes in both terrestrial and marine invertebrates.

In polar marine environments large numbers of minute flatworms, nematodes, rotifers, crustaceans, and other animals actually live within the Arctic pack ice (Gradinger, '99). In Antarctica, nematodes and rotifers apparently are absent in this habitat, even though they flourish, along with tardigrades, in some of the continent's severe cold desert valleys (Treonis et al., '99) and in alpine soil up to 3000 m or so (Thaler, '99). Various mi-

croscopic algae flourish abundantly in the ice pack environment and provide ample forage for herbivorous invertebrates there. In addition, larger animals, such as amphipods, krill, and some fishes with antifreeze in their tissues, live actively swimming among the sludge ice and at the water-ice interface. This is particularly daunting because contact with ice crystals tends to seed internal ice formation in a supercooled organism.

Ice-tolerant ectotherms allow, or specifically encourage by seeding, ice formation in their bodies, usually only extracellularly. Terrestrial arthropods, frozen in this way, may survive extreme temperatures down to -100°C. Many temperate- to high-latitude marine intertidal animals, such as barnacles, are also freeze-tolerant, as are some overwintering frogs and turtles. Alternatively, freeze-resistant (freeze-intolerant) ectotherms avoid internal freezing by supercooling.

Without antifreeze, this requires ridding the body of small particles, including bacteria and fine bits of food in the gut, that tend to seed ice induction. Collembola may survive in a chill coma *without freezing* at temperatures of -30°C or even -45°C (Eisenbeis and Meyer, '99). Note that teleosts with antifreeze develop a much more modest supercooling temperatures in accord with the freezing point of seawater at a nominal -1.86°C or at -1.97°C near the surface around Antarctica.

Antifreeze proteins, mentioned several times elsewhere in this essay, curiously lower the freezing point of body fluids without changing their melting point. Apparently they act by coating tiny incipient ice crystals, thus blocking their further growth. In this way they can depress the freezing point of body fluids and tissues far more than their usual colligative effects could account for. In insects antifreeze proteins are stored within fat cells during spring and summer, then activated and released into the hemolymph in the fall and winter. Accumulation of sugars, such as trehalose, polyols, such as glycerol, that may reach concentrations of three to five molar, and amino acids (collectively termed cryoprotectants) as well as substantial dehydration also typically contribute to the total cold hardiness of terrestrial arthropods.

Aquatic thermal stresses are generally far less severe than aerial and terrestrial surface habitats. Usually, deepwater temperatures, while cold, are not extreme as are the icy near-surface waters in polar regions (Somero, in Pörtner and Playle, '98). With a few local exceptions, deep ocean temperatures in recent times range monotonously within a few degrees above zero centigrade.

Deepsea ectotherms (invertebrates and most fishes) must function effectively at steady temperatures just above 0°C and at high pressures depending on depth (as mentioned in part 1).

Obviously, protein function, metabolic reaction rates, and so on, must be geared to these factors. Yet marine fishes living in high-latitude near-surface waters need antifreezes (Bargelloni et al., '94) to prevent internal ice from forming in their blood, which freezes, unlike that of invertebrates as a group, at higher temperatures than seawater does (Jia et al., '96). Strong differences between the concentrations of osmotically active substances in the various tissues are responsible for this important distinction between most marine invertebrates and vertebrates generally.

Warm ectotherms

Cold-blooded animals can avoid the metabolic slowdown inherent in low internal temperatures, by somehow acquiring or retaining the heat that they need to be more active. If so, their body temperature may be significantly higher than that of their close surroundings. Although most invertebrates, fishes, amphibians, and reptiles are cold at cold temperatures, there are a number of fascinating exceptions to this broad rule. Despite their basic vulnerability to low temperatures, some of these animals can maintain high levels of activity, and even significantly raise their body temperatures above their cold surroundings.

There are two known kinds of such warm-up devices. One depends on the animals' internal heat production, a natural, and thermodynamically inevitable, byproduct of metabolism. The other depends on heat acquisition from some external heat source in the environment and can significantly warm the blood of many cold-blooded types. It typically depends on behavior that promotes the transfer of external heat into the body, usually by controlling body surface exposure to sunlight. Thermal adjustment by this means is common for terrestrial animals both in ectotherms, such as insects and lizards and also in birds and mammals that use it where possible to control heat load or radiative cooling. The physical principles which permit such *external* heat acquisition are usually not applicable in aquatic environments.

Reptiles, such as snakes and lizards, are well known to seek out and bask in warm sunlight as a means of increasing their internal temperature by absorbing radiant energy. At night they also

may keep warm on previously sun-heated rocks or asphalt highways. The resulting increase in body temperature could sharpen their ability to escape predators or speed up their digestion, their growth, or their reproduction compared with the rates that would prevail without warming. All of these could facilitate their invasion of colder habitats than would otherwise have been possible. Locusts, grasshoppers, and some other insects also regulate their temperature behaviorally by seeking or avoiding exposure to sunlight. Behavioral thermoregulation of this kind is practiced almost universally by terrestrial animals both cold- or warm-blooded (Jacobs, '96).

The alternative, *internal* way of warming up cold-blooded animals usually requires a high level of metabolism rare in invertebrates and the lower vertebrates (Block, '94). Yet leatherback turtles *Dermochelys coriacea*, with low metabolic rates, can maintain their body temperature at 25.5°C in cold seawater (Paladino et al., '90). This ability, which allows them to swim north of the Arctic Circle, is due to the species' large size and the effective thermal insulation of their shell. Unlike this turtle, nearly all warm cold-blooded animals have high metabolic rates dependent on strong muscular activity mainly related to flying in many insects and swimming in a few fishes. In most cases the flight or swimming muscles provide the required internal heat source.

For instance, actively flying insects are also exceptional in their internal capacity to raise their body temperature above that of a cold environment. Some moths, for instance, regularly fly in falling snow at air temperatures near zero (Heinrich, '93). Winter moths of the family Noctuidae are particularly interesting. They can fly at air temperatures down to about 0°C but at lower temperatures become inactive, sheltered under leaves and snow. Many species of such winter moths actually spend the *summer* in a dormant state as pupae (Heinrich, '93). More familiar temperate latitude moths, of course, *overwinter* as pupae.

The key to this seeming "warm-bloodedness" for most insects involved lies in their muscle tissue, particularly the powerful flight muscles (Block, '94). These muscles sustain the highest known rate of energy turnover in any animal. Yet insect flight muscle is only about 10% efficient in mobilizing mechanical energy from chemical energy, so that 90% of the energy used appears directly as heat. Hence heat produced just by *shivering* can raise an insect's body temperature to a level permitting lively activity otherwise impossible at

cool or cold air temperatures. For instance, a number of insects, such as nectar feeding sphinx moths are like old fashioned piston engine aircraft that must warm up before taking off at low air temperatures.

Usually though, such insect warm-ups can be effective only if the outside temperature is 15°C or warmer. Yet, in contrast, certain noctuid moths seem to have flight engines that can be warmed up from a considerably lower winter starting level, provided that they do not actually freeze. One remarkable geometrid moth can even start to fly without warm-up at air temperatures no higher than -2°C to 0°C. Their unusual ability involves thermal changes in wing muscle biochemistry that shift its most effective temperature range to an overall lower level.

More generally, active insect fliers, such as *individual* honeybees, have special heat exchange systems even in temperate environments. These allow the insects to regulate the distribution and level of their body heat to make their flight as efficient as possible. Otherwise overheating would threaten them during their normal summer flights. In cool weather honeybees cannot fly unless the temperature of their thorax, where the flight muscles are, is 27°C or over. The actual body temperatures achieved by these warm-bodied insects are comparable to the 35°–40°C levels steadily maintained by birds and mammals. Bumblebees, common in the Arctic, and dragonflies, as well as a number of moths, also produce enough heat with their flight muscles to reach high internal temperatures that they can regulate over short periods (Heinrich, '93).

Insect social warmth

Although ants usually reach their peak of diversity and ecological importance in warm climates, a few species of the genus *Leptothorax* flourish at surprisingly high latitudes (Heinze et al., '96). One species has been found in the steppes close to the Siberian cold pole and another occurs further north than any other ant in North America. Survival of their small overwintering colonies has been recorded down to -20°C and internal antifreeze compounds may protect the ants to even lower temperatures. Their social behavior allows them to survive winter low temperatures more successfully as a colony than as individual ants, presumably due to food sharing.

Group behavior also keeps overwintering honeybees, for instance, from freezing in the cold (Seeley, '95). These bees cannot feed in cold

weather and ordinarily do not fly then. In winter the colony shrinks to about 60% of its summer population of worker bees, but if 15,000 or more are present, they can keep their nest well above lethal low temperatures. Together they do so with heat produced by their flight muscles contracting isotonically. The sustained *social* thermoregulation by a honeybee colony can maintain its nest temperature fixed within 1°C over a whole day. Even when outside temperatures fall to -30°C or less, the core area of the nest can be kept above 10°C. Below 8°C to 10°C, honeybees become torpid and die in a day or two.

While heating the nest (and keeping themselves warm) as a group, the workers come together in a roughly spherical cluster. It is rather loosely organized when the amount of heat needed is modest but may contract to a much more compact ball if the temperature within the nest falls toward zero. Then the core honeybees in the cluster produce most of the heat while the outer ones facing head inward form an insulating shell, comparable to fur, blubber, or feathers, which greatly slow heat transfer in various vertebrates. Clustering, which also occurs in quite a few species of cold-stressed mammals and birds such as musk ox and penguins, greatly increases heat conservation, in the case of this social insect by a factor of perhaps 20–30 times over that for individual bees.

Actually, the honeybee's way of surviving may be rather precarious. Its low resistance to cold may, perhaps, be inherited from tropical evolutionary origins, where low temperature stress was rarely encountered. Among other things, their overwintering is energy-expensive. As much as 20 kg of honey may be needed by a colony to keep warm during the winter. Even in the moderate latitudes of New England, the winter survival rate of first-year honeybee colonies is low.

Honeybees' overwintering shows two surprising parallels with the winter denning of bears. In both quite unrelated animals, a high body temperature is maintained and reproduction takes place during a time when environmental temperatures are below freezing and external food is not available. Curiously, the much more closely related *bumblebee* survives winter quite differently than the honeybee by becoming dormant rather than keeping warm socially. The bumblebee actually has a more northerly range limit than the honeybee, commonly extending as it does into the tundra well above the Arctic Circle. Yet in bumblebees the summertime large colony may dwindle down by

midwinter to only a few dormant fertilized females who seed the next generation when spring returns.

Warm fishes

Surprisingly, a few large fishes can maintain internal temperatures warmer than cool or cold seawater. For example, large tunas as well as lamnid and alopiid sharks are able to hold their body temperature several degrees above that of the water in which they are swimming. This is quite unexpected because the high heat capacity of the surrounding water rapidly conducts heat away from a warmer body, as human swimmers and divers well know (de Vries and van Eerden, '95).

Normally in fishes such heat loss is almost total across the large efficient exchange surface of their gills. For proper respiration a rapid current of aerated water must circulate over them. The warm-blooded tunas and sharks avoid the usual rapid heat loss to cool or cold water by means of a special heat exchanger in the swimming muscles (Block et al., '93). The exchanger in question functions rather like that in the feet of birds, such as sea gulls, exposed to cold water. Numerous parallel small arterial and venous blood vessels making close contact and containing blood flowing in opposite directions, are arrayed within the fish's centrally located swimming muscles.

This system acts to conserve body heat by passive heat flow from venous blood, warmed by the muscles, into cooled arterial blood coming from the gills. This in turn lowers the heat gradient across the gills and so substantially reduces the overall heat loss. Effectively, this arrangement dynamically provides "insulation" analogous to that of fur or feathers in terrestrial mammals or birds. Field data show that in the bigeye tuna (*Thunnus obesus*), the effect of the heat exchanger may be adjusted to match the fish's thermal needs (Holland et al., '92). Interestingly, muscle heat conservation in tunas may get out of hand and damagingly overheat the fish. What fishermen call "heat burn" in tunas apparently results from the violent struggles of stressed individuals caught on hand-line hooks (Block, '94). Presumably potential sashimi gets "cooked" as a result.

The normal success of such a heat-conserving device is no doubt aided by the large size of the fish concerned, such as a giant tuna. It is also correlated with the continuous vigorous heat-generating swimming of such fishes that drives rapid water currents into the open mouth and out over the gills. This capacity to raise their whole body temperature, exceptional in fishes, allows the few

species concerned to remain vigorously active, migrating and feeding, in colder, deeper water or at higher latitudes than they otherwise could.

Ultrasonic telemetry has shown that large bigeye tuna in French Polynesia at dawn can follow the sonic scattering layer, presumably including the fish's prey, from its nighttime location in the top 100 m down to its daytime level at 500 m or more (Dagorn et al., 2000). The tuna remain in such depths during the day, with some interruptions, and then return toward the surface as the scattering layer moves up several hundred meters in the water column at dusk. The interruptions mentioned involve several daytime excursions upward into shallow depths. These apparently are necessary responses to two stressful aspects of the deepsea location.

For one thing the fish's thermoregulatory system seems overloaded by the temperature decrease with depth that may reach 10°C or more. So the brief trips up into warmer water presumably serve to warm the fish's body temperature back to a higher level. Also they may serve to pay off an oxygen debt caused by the presence of a stressful oxygen minimum common at intermediate depths, particularly in the Pacific (Dagorn et al., 2000). If so, this deep diving fish behavior shows a remarkable similarity to that of diving mammals and birds that must return, in their case, to the water surface periodically to breathe (as discussed in part 1).

Another group of bony fishes, the billfishes, which include the swordfish and marlin, as well as a single species of mackerel, have a different heating system specifically for the eyes and brain (Block, '94). The heat-producing organs, located in these fishes' heads, are modified eye-movement muscles just beneath the brain. They have lost much of their contractile machinery and have amplified the membrane systems normally involved in triggering muscle contraction. As in many other heat controlling systems, a capillary countercurrent heat exchanger is present and acts to retain within the head much of the heat produced. As with the tunas' body warmers, these head warmers apparently allow the fishes to expand their vigorous activity into cooler waters. Swordfish, for instance, can regularly swim down to mesopelagic depths of 600 m or more.

Conserving energy

In response to sustained stress, many kinds of animals survive by conserving energy or increasing energy efficiency, particularly because over-

coming stress itself usually requires extra work (Parsons, '99). Although some large mammals, such as bears, share such slowdowns, a few small hibernating ground squirrels and innumerable small ectotherms have evolved the most drastic ways of aiding passive survival by this means. [Further examples of such parallel or convergent evolution (Hodin, 2000) will be given in part 3.]

A large fraction of the living material and biodiversity in terrestrial extreme environments usually consists of many small-scale invertebrates flourishing in the soil. Nematode worms and minute arthropods, such as mites, springtails, and ants are dominant faunal elements there (Thaler, '99). As in the typical problem of terrestrial shelter in extreme environments, modest to small size favors the availability of security for inactive and dormant animals.

Energy conservation in extremophiles may occur variously in response to low temperatures at high latitudes and altitudes, to hypoxia at high altitudes, to low humidity in semiarid environments, to osmotic challenges and/or to the semistarvation typical of the most stressful environments. This generalized antistress response can be observed most obviously in animals' reduced physical activity and absence of unnecessary behavior. Physiologically, conservation is reflected in a reduction of the metabolic rate (Guppy and Withers, '99; also part 1).

For instance, oxygen consumption rates for 10 species of benthic decapod crustaceans decreased with their depths of maximum abundance between 200 m and 750 m despite the characteristic absence of a temperature gradient with depth in their western Mediterranean deep-water, habitat (Company and Sardà, '98). The amplitude of such reductions may, in various cases, be modest or powerful in many marine, freshwater and terrestrial species. These include desert, temperate, and high-latitude free-living nematodes, earthworms, mollusks, tardigrades, crustaceans, insects, and other invertebrates, as well as a few fishes, amphibians, and reptiles.

When stressed, they can partially shut down their metabolism, sometimes to a remarkably small fraction of its normal resting level (Womersley et al., '98). Torpor and hibernation in birds and mammals, diapause in many invertebrates such as insects, and estivation and overwintering in a number of vertebrate ectotherms, such as lungfish and desert toads and frogs (Warburg, '97), are all examples of elaborate metabolic shutdown. Some nematodes (Womersley et al., '98), as well

as tardigrades and *Artemia* resting eggs, remarkably, can enter anhydrobiotic states with immeasurably low metabolic rates and with the capacity to survive severe desiccation and high temperatures for long periods.

In some cases the scarcity of food and low temperatures at high latitudes slow down the life cycles of animals there. As a result, maturation may take two or more years instead of the one growing season usual at lower latitudes. Cave animals, living in the dark, usually with sparse food, also tend to have slowed life cycles and reduced fecundity (Culver et al., '95). Comparable attenuated life cycle may also occur at high altitudes for reasons similar to those cited for high latitudes.

For instance, in a species of the alpine snail, *Arianta* postembryonic development to maturity takes five years in a population at 2600 m elevation, while at 1220 m only two years are required (Thaler, '99). Also larvae of some salamanders living in mountain ponds overwinter for one or two years before structural metamorphosis occurs, instead of the normal annual developmental pattern at lower altitudes (Iwasaki and Wakahara, '99). Interestingly, the molecular metamorphosis of larval hemoglobin to adult hemoglobin occurred in first-year overwintering larvae despite their delayed morphological maturation. In contrast, differences in rates of development between two copepod species in a small high (nearly 1800 m) Austrian lake have been interpreted as niche differentiation (Luger et al., 2000).

But frogs with wide altitude and latitude ranges in their distributions have a number of life history features (some genotypic, others phenotypic) that are correlated with increasing stresses in their environment. In *Rana temporaria*, for instance, fecundity decreases, but age at maturity, size at maturity, and longevity apparently all increase with altitude up to 2300 m, near the upper limit of its range in the French Alps (Miaud et al., '99). There the short activity season between late spring thaw and early fall freeze of alpine lakes and ponds is only three months.

Despite lower than normal temperatures, the rate of development is increased in this case, and the time to metamorphosis from tadpole to froglet shortened (McDiarmid and Altig, '99). This is similar to, but less extreme than, the greatly speeded up development and larger size at metamorphosis of some desert anurans dependent on temporary pools of water to complete their life cycle (mentioned in part 1 and Warburg, '97). With the seasonal or sporadic arrival of rain, arousal from

dry season dormancy is typically coupled with rapid egg-laying and accelerated development of aquatic larval stages in ephemeral pools of water as also in desert dragonflies (Corbet, '99).

In any case, the behavioral, physiological and life cycle changes that allow many animals to avoid strong stress by copping out are alternatives to the ways in which other species or types may develop by directly submitting to or opposing the stress. Such direct fitness increasing responses include a whole arsenal of molecular alterations (Szilágyi and Závodsky, 2000) special protective proteins, and numerous structural and regulatory mechanisms discussed elsewhere in this essay. Remarkable correlations between lowered metabolic rates, increased stress resistance, and slower aging are considered in part 3.

COMPLEXITY INCREASES

With regard to increasing *complexity* as a trend in evolution, there is much to be said provided definitions can be agreed upon (Lewin, '92; McShea, '96; Sanderson and Hufford, '96; Pettersson, '96). Complexity, like size, greatly affects the diverse ways in which animals could function in extreme environments. Abstractly, complexity can be assayed by considering components in a system:

- How many are there?
- Are they the same or different?
- Are they hierarchically arranged one within the other or not?
- What are the components' dynamic interactions, including signaling and information transfer?

Practically, both definitions and useful quantitative measures of complexity are elusive, although a number of formulas have been proposed based on morphological, physiological, or genetic data. For instance, one can compare the functional Shannon information content of the genomes to be evaluated (Adami et al., 2000). The resulting analysis implied that there is a Maxwell Demon-like force driving evolution toward greater complexity.

With regard to morphology, analyses have been developed for the degree to which various animals have evolved all possible structural patterns that might be available to them (McGee, '99). For instance, on the basis of 21 hard skeleton structural criteria (including only three or four elementary complexity measures) the Burgess Shale fauna (part 1) was found already in the Cambrian to have exploited more than 80% of the estimated available morphospace (Thomas et al., 2000).

Also, analysis of the appearance of novel morphological character states in animal fossil history indicates that novelty appears to have been exhausted fairly early in the evolution of long phylogenetic branches (Wagner, 2000). Based on a large number of trilobite, mollusk, echinoderm, and vertebrate groups, these data imply that, like molecular characters, anatomy must have a limited number of possible states. Obviously, this complicates the identification of homologies and imposes major problems for deriving phylogenies, for systematics and for understanding the constraints or possible directionality of the evolutionary processes (discussed later in this article).

Even the simplest prokaryote or animal is, of course, a highly complex organized entity with hundreds or thousands of components elaborately interacting. Animals are open-ended, self-organizing, homeostatic, self-replicating, work-doing, entropy-producing systems. As such, they are far from thermodynamic equilibrium. They also contain substantial internal information about themselves, as well as about their environmental niches. These features are inherent in our definition of life (part 1) and so, no doubt, have characterized living things from their beginning.

A number of distinct steps or hierarchies can be identified in this overall process of complexity increase (Maynard Smith and Szathmary, '85; Pettersson, '96; Arthur, '97). As with size, it is maximum and mean complexity that increases, while many of the simpler and less complex types have continued to flourish all along. To begin with, the evolutionary step from prokaryotes to single-celled eukaryotes involved major increases not only in the size of their cells but also in the cells' complexity, as well as that of their component molecules (Lake and Rivera, '96) (but see Forterre and Philippe, '99, for a different polarity).

Archaea and bacteria share a scarcity of structural variations along with a wealth of metabolic patterns that are, among other things, important to biologists for identifying the various kinds of microbes (Postgate, '94). In contrast, the eukaryotes, which include both single-celled and many-celled animals as well as fungi (Cavalier-Smith, '98b) and plants, are marked by an exuberance of structural variations on which their species are mainly based. Eukaryotes seem far more species-rich than microbes, even though the nature of their respective species may be quite different. Animals have far more structural complexity, than bacteria and archaea. This starts at the molecular level, as mentioned, and continues to the intracellular domain.

A membrane-enclosed nucleus (Lamond and Earnshaw, '98) containing a number of linear chromosomes and their genes became an early feature of almost every eukaryotic cell as did a variety of other cell organelles and programs (Netzer and Hartl, '97). Thus nucleus, nucleolus, the Golgi, the endoplasmic reticulum, the cytoskeleton, meiotic sex, exocytosis, endocytosis and mitotic cell division, among other things, became characteristic of eukaryotic cells although the evolutionary steps involved are poorly known (Roger, '99). Also, the amount of genetic material (nucleic acids) in each cell greatly increased as prokaryotes evolved into eukaryotes. For instance, the nematode *Coenorhabditis* has more than 20 times as much DNA in each of its cells than does *E. coli* in its one cell. The prokaryote-eukaryote transition was a major one and no doubt was also made in a number of stepwise changes.

Symbionts

Some primitive eukaryotes experienced another major increase in complexity; they acquired *mitochondria*, perhaps two billion years ago (Philippe and Andoutte, '98). Almost certainly, this happened through *symbiosis* with a particular kind of bacterium that came to live within eukaryotic cells (Margulis, '96). The resulting organelles, which still contain some of their own old prokaryotic genes, are the powerhouse for mobilizing energy in most well-developed nucleated cells (Vellai et al., '98). Consequently they are central to nearly all that animals can do, such as grow, move, reproduce, and evolve as extremophiles. The chloroplasts of green plants were also acquired in a similar fashion from a kind of photosynthetic blue-green bacteria.

These two major complicating steps in evolution seem crucial for the later history of all multicellular organisms. Note that such giant steps are quite distinct from the gradual changes typically involved in Darwinian and neo-Darwinian speciation. They are less like results of modest gene mutations than they are like those of substantial, less orthodox gains, losses and exchanges of genetic material not uncommon in microbes (Doolittle, 2000). Note, too, that an intracellular nontransient symbiont, or its genes, is presumably a Lamarckian acquired character that can be inherited, like a mitochondrion.

However, in both animals and plants, symbiosis provides a pervasive means of cooperation among different kinds of organisms. In a sense this gives the resultant *superorganism* the potential advan-

tage of a second full set of substantially different genes acquired from another kind of organism. Obviously, such partnerships may support major evolutionary changes. Countless examples can be cited at various levels (e.g., Moran and Telang, '98; Nardon, '99; Ruby and McFall-Ngai, '99).

For instance, the far-reaching importance of the symbiosis of reef building corals and other rather simple invertebrates with intracellular green algae has apparently evolved independently a number of times. It has long been studied in some detail (Maruyama et al., '98). Much more recently discovered, several kinds of invertebrate extremophiles living around deepsea geothermal vents (Desbruyères and Segonzac, '97) have used such foreign species' cooperation to tap an abiotic energy source not usually available to animals (Vetter, '91.)

This involves the capacity of certain bacteria to oxidize hydrogen sulfide or methane flowing out in hot vent or cold seep water and to use the energy released to reduce carbon dioxide to organic compounds the bacteria need for growth and reproduction. This light-independent system of primary production has allowed some deepwater extremophiles to acquire food, but not oxygen, independently of photosynthesis. That photon-requiring process, which releases oxygen, can usually occur only in the upper 100 m or so of the sea because of the rapid absorption and scattering of sunlight penetrating seawater. Yet chemoautotrophic symbiosis has been reported deeper than 7300 m in the Japan trench (Fujikura et al., '99).

Typically the symbiotic types in such benthic communities are accompanied by a surprisingly varied fauna of bacterial mat and detritus feeders, scavengers, and predators (Sarrazin et al., '99). Present day deepsea vents seem to be ephemeral, but associated faunas of such habitats may have existed in Paleozoic and Mesozoic times (Shank et al., '99). However, molecular estimates of divergence times imply that recent members of deep vent faunas are all less than 100 million years old and for some types less than 20 million years old.

Some deepwater vent animals filter-feed on the chemotrophic bacteria suspended in the vent water and thus obtain both the energy source as well as the organic raw materials they need in the usual way. Other animal types in the vent community have developed an intimate symbiotic relation with closely similar bacteria, which they cultivate within the cells of their gills or a special internal organ (Wittenberg and Stein, '95). Oxygen and reduced sulfur or methane are picked up

from the environment by the host animal and transported by its blood to their internal bacteria. The latter, in turn, like the free-living ones, can oxidize the reduced substrate to generate ATP. Part of this energy can be used to drive the host animal's metabolism that may also depend on organic compounds produced by the bacteria. One deepsea hydrothermal vent mussel, a species of *Bathymodiolus* living on the Midatlantic Ridge, apparently relies for its nutrition on both sulfur and methane oxidizing bacteria that it harbors internally (Pond et al., '98).

In the large tube-worm, (adults 2 to 3 m long) the mouth, anus, and whole digestive tract are quite absent when this best-studied deep hydrothermal vent animal is mature. Hence, this worm depends entirely on its in-house sulfur bacteria for energy as well as for most, if not all, of the necessary organic raw materials in its own diet. At the same time, the worm must provide raw materials needed by its symbionts and eliminate their waste products (Goffredi et al., '99). The microorganisms themselves differ from their free-living relatives found in the surrounding seawater. Hence, living within the worm may be necessary for their survival, too. Yet, each individual seems to acquire its symbionts from the surrounding seawater (Nelson and Fisher, 2000).

This mutually required collaboration between bacteria and the adult worm raises some puzzling questions. In such intimate interdependence, are the two quite different organisms involved fused into one? If so, is that one a distinct new entity, like the many species of lichen that result from various pairs of fungi and algae living cooperatively together? A new species so produced would provide a distinct and rapid evolutionary step toward greater complication. Some lichens, for instance, are extremophiles that serve as the last animal forage available to herbivores, such as yaks and musk oxen, at the frontiers of extreme high altitudes overall and high arctic latitudes.

An Antarctic sponge may provide a remarkable case where an Antarctic stress has induced a symbiotic diatom, typically cooperative at low latitudes, to become a parasite (Bavestrello et al., 2000). Presumably because of darkness under surface ice and the prolonged polar winter, the green alga concerned, instead of producing extracellular polysaccharides that nourish the sponge, themselves take up some of their host's metabolic intermediates to prevent the diatom from starving in the dark.

The evolution of *many-celled* animals immediately

allowed a complexity increase through the subsequent specialization of many cell types along with a resultant overall increase in body size (Bell and Mooers, '97). Multicellularity also permitted the emergence of a division of labor that organizes cells into tissues, tissues into organs, and so on, to various levels of social organization (Sendova-Franks and Franks, '99). Specialization and the division of labor in turn could increase efficiency (fitness).

Also, the number of different cell types in an individual animal may provide another, rather simple but significant, gauge of an animal's complexity. Like life itself (part 1), cells are remarkable for their basic unity as building blocks and at the same time for their marked diversity of structure and function. Counts of cell types clearly indicate a persistent increase over evolutionary time (Valentine, '95). For example, according to Valentine's estimates:

- The earliest many-celled animal may have had as few as 2 cell types, 600 million years or more ago
- Early arthropods may have had 55 cell types, 520 million years ago
- Early amphibians may have had 150 cell types, 320 million years ago
- Humans at present have at least 210 cell types and probably many more, depending on definitions.

Self-regulation

As animal size and complexity increased during evolution, a growing degree of regulation was required. The escalating numbers, kinds, and interrelations of the organism's components had to be integrated and controlled to assure their vital survival and reproduction. Cooperation and the resolution of conflicts were important requirements of evolution at a wide range of levels (Keller, '99). Of course, the first cellular life already needed a plasma membrane and genome to regulate their existence as well as a cybernetic energy-yielding metabolism to permit work to be done (Morowitz, '92). Yet when many-celled eukaryotes evolved, the need for coordination became much more complex. Both their internal maintenance and regulation as well as their sensitivity and appropriate responsiveness to the relevant features of the external environment and its changes require capable control systems.

At every level, from signal protein molecules to familial and social behavior, control and regula-

tion gradually became pervasive and interactive functions. Between molecules and behavior numerous important intermediates have evolved widely. For instance:

- intercellular communication, as with tight junctions
- multiple cascades of gene action; gene networks; proteomics (Pandey and Mann, 2000)
- rhythms and biological clocks
- sense organs, both internal and external
- endocrines, pheromones and neurohumors
- immune systems
- nervous systems, autonomic and systematic
- locomotor systems
- whole animal behavior

Indeed, so elaborate and effective have such stabilizing mechanisms become in the more complex metazoans that how leeway can be found for more than minor evolutionary innovation is a serious problem (as discussed in part 1). It possibly could explain the fact that no major new body plan (phylum) has evolved for hundreds of millions of years (Erwin, '99). To test this effectively, much more needs to be learned about bauplan genes (Tautz and Schmid, '99). However, fish did evolve into tetrapods and tetrapods into whales, as already documented as post-Cambrian macroevolution.

Self-regulation has to be a defining feature of cellular life because the living organism is locally quite different from its nonliving surroundings. But the organelles and other elements involved in maintaining a steady state have multiplied and grown in complexity as metazoans evolved. Basically, organisms must freely acquire essential raw materials, including energy, from the environment and discharge waste and excess byproducts externally. Movement of particular ions and molecules both into and out of each cell as well as selective impermeability to others are central functions of plasma membranes. These organelles also respond to both internal and external molecular signals vital to the animal's survival and reproduction. These plasma membranes, because their proper functioning is quite sensitive to stresses, such as temperature, or pressure, are usually critically involved in survival in extreme habitats (Viarengo et al., '99).

In prokaryotes the obvious importance of the plasma membrane for these basic functions goes back to the beginning of cellular life (Morowitz '92). With the much later evolution of animals,

the numbers and specialized diversity of such cellular organelles has greatly increased the organism's complexity. Also, specialization of tissues, organs, germ layers, and functional systems, such as the circulatory system, have emerged on a more complex level to stabilize the animal in many ways. The skin, capillaries, gills, trachea, lungs, GI tract, and kidneys are typical dynamic elements that function to maintain the internal steady state of the organism as different from and, to a considerable extent in terms of fitness, independent of its surroundings.

The modern roots of these regulatory ideas reach back to Claude Bernard's notion of the fixity of the *milieu interieur*, conceived around 1870. Walter Cannon's *homeostasis* and Norman Wiener's *cybernetics* were important 20th-century contributions. Well-developed self-regulatory abilities allow an animal to remain fit by counteracting and repairing, as by blood clotting, wound healing, and regeneration, the many effects of environmental stresses affecting it, rather than just passively enduring or behaviorally avoiding them. Obviously internal stability is particularly critical for extremophiles because their frontier habitats test the limits of their self-regulation as well as their capacity for effective stress resistance and avoidance.

Such regulators include, as major types, the genetic, respiratory, circulatory, excretory, neurosensory, neuromuscular, central nervous, endocrine, and immune systems plus hundreds or thousands of protein signals and their receptors (Kliwer et al., '99; Ray, '99). Some such stabilizing mechanisms were present in the earliest animals, but their number, complexity, and scope have greatly increased during historic evolution. Remember, however, that both stability and flexibility are inherently antagonistic properties of life at many levels including the genome (Becskei and Serrano, 2000).

As a rule, the presence of internal body temperature control, cell volume regulation, stabilization of water content, balancing specific ion distributions (Hazon et al., '97) and so on, broadens the animal's tolerance of corresponding otherwise stressful environmental challenges in changing or new habitats. For example, the lack of adequate osmoregulatory mechanisms in the whole phylum of echinoderms would seem to be a critical factor in this major group's failure to expand out of their sole marine aquatic habitat. Even so, various quite distinct classes of this phylum are prominent benthic types down to extreme deepsea depths.

Buoyancy

One striking example of such internal self-regulation relates to buoyancy in mid-water deep-sea animals. Buoyancy is a challenge to all pelagic organisms larger than microscopic or not small enough for extended surface areas (parachute effect) and turbulence to keep them in place. Buoyancy for others becomes physiologically more difficult in deep water because of interactions with the rise of hydrostatic pressures with increasing depth. Underwater pressures are high compared with the atmosphere because water is about 800 times denser than air at sea level. As a result, every 10 m of water depth adds one atmosphere of pressure to that in air just above the water surface.

Marine animals, as already mentioned, are subject to pressures ranging from two atmospheres at 10 m depth to over 1000 atmospheres in the deepest trench. The average pressure in the ocean as a whole is about 380 atmospheres. Such pressures do not have grossly obvious biological effects, except on gas-filled cavities such as lungs or swim bladders (Macdonald, '75). However, because liquids and solids are relatively incompressible, the pressure at 10,000 m depth would reduce a given liquid volume, including an animal's living tissue, only by about 4% of that at the surface. As a result, an animal without any internal gas-filled cavities will be just slightly reduced in volume in the deepest seas but certainly not squashed. High pressure is surely stressful for life, but its influence, except on gases, seems to lie mainly at molecular, biochemical, and membrane levels (Somero, '93).

Most pelagic animals, whether plankton or nekton, normally occur over rather limited depth ranges. To remain in place within such water layers, neutral buoyancy can obviously conserve swimming energy otherwise needed to counteract gravity (Power, '89). In between floating and sinking, when an animal's average density just equals that of the medium, it will, of course, not rise or sink spontaneously, but will merely be suspended at whatever depth it happens to be. At the same time compression due to depth reduces the volume of unsupported gas-filled parts of the body, and hence decreases the animal's buoyancy with increasing depth.

Accordingly, to maintain neutral buoyancy, gas pressure in such a float must be adjusted to that in the immediate environment. Regulating buoyancy in this way is a widespread capacity among

midwater animals. Several intriguing mechanisms have evolved for this purpose. Even organisms as watery as jellyfish and ctenophores fairly quickly respond to changes in the density of their medium by osmotically altering their own density.

Yet most animal tissue, especially something as heavy as calcified bone, is denser than water. As a result, neutral and positive overall buoyancy are possible only if lighter body components, such as ammonium ions or blubber, act as floats to counterbalance the weight of heavier ones. Internal spaces containing gas are the least dense floats that animals can use to buoy themselves up. The gas-filled swim bladders of many bony fishes and a pelagic octopus, and the gas-filled spaces of siphonophores and of the pearly nautilus are striking examples.

A major physical effect of pressure is to compress any gas present to an extent proportional to its intensity (Boyle's law). A liter of air at the sea surface would contract to one milliliter in the deepest ocean trench. Increasing depth strongly compresses an animal's lungs, gas-filled swim bladder or other air-filled cavity. Such a collapse would usually lessen or block normal function or otherwise damage the animal. As a result, the lungs of diving mammals, such as seals or whales, are believed to be virtually collapsed and hence useless for respiratory gas exchange at depths greater than about 100 m. Even so, many species regularly dive far deeper than that without apparent stress.

Remarkably, the gas-filled swim bladders of most bony fishes are fully able to counteract external pressure changes (and hence circumvent Boyle's law) by actively secreting and reabsorbing gas, mostly oxygen, into and out of the bladder's closed space. As a result, swim bladder volume, and hence its lifting force, can remain unchanged over a range of depths. Essential for maintaining nearly neutral buoyancy, this remarkable organ lets the fish stay at a given depth or rise or fall in the water column without the effort of continual swimming. Obviously this is valuable asset for energy-poor deep-water pelagic life.

The swim bladder in some species can secrete oxygen from the blood into the swim bladder against a pressure gradient of up to 100 atmospheres (equivalent to a water depth of about 1000 m). Both grenadier fish (macrourids) and rose fish (the percid *Sebastes*) have gas-filled swim bladders, despite living at habitat pressures of more than 100 atmospheres.

Furthermore, other fishes with gas-filled swim

bladders have been caught living as deep as 7000 m, which would require about 700 atmospheres in the float to match the outside water pressure. This remarkable feat depends on some unique properties of bony fish hemoglobin (Mylvaganam et al., '96) and on respiratory gas transfer from blood to swim bladder in a small specially organized part of the circulatory system. To raise such great internal pressures, a length of closely parallel arterial and venous capillaries forms an efficient countercurrent exchange mechanism.

This can maintain a strong partial pressure gradient between ordinary arterial gas and the level needed for transfer to the gas gland. In addition, the system uses a recurrent multiplier to gradually build up increasingly high levels of respiratory gas in the capillary blood as it approaches the gas gland. In some species that peak level apparently allows gas pressure in the swim bladder greatly to exceed a 100-atmosphere limit. The length of the countercurrent capillary system is a major factor in that accomplishment. In a number of deepsea fishes its length increases roughly with their usual habitat depth.

To float upward in the water without having to swim, the volume of gas in the bladder must be increased. To do so, the gas gland allows oxygen at high concentrations in its capillaries to diffuse into the float. To sink passively downward, a second capillary area in the swim bladder wall becomes active and allows oxygen under pressure inside to diffuse back into the blood stream and so deflate the float. To maintain neutral buoyancy at different depths the pressure of gas in the swim bladder must be adjusted to match that of the surrounding water, so that the gas float volume does not change.

Some mesopelagic fish, such as moderately deep-living lantern fishes, have swim bladders filled or invested with oil or fat instead of with gas. The oil and fat are less dense than fish blood and thus serve as floats. At the same time they are relatively incompressible as liquids and solids and so provide constant buoyancy over a wide range of depths. In this way the fishes avoid the need to secrete and reabsorb large volumes of gas to adjust buoyancy for daily vertical migrations of several hundred meters.

Another internal control mechanism, physiologically somewhat similar to the teleost swim bladder, but used for excreting highly concentrated urine by desert rodents, is discussed later in this essay. Parallels lie in using countercurrent exchangers by the mammalian kidney and the

buildup of a steep osmotic concentration gradient that limits the maximum urine concentration.

Floats in the form of oil droplets occur in various types of plankton, such as copepods, as well as in the large livers of pelagic sharks and in more generally in the body of the Antarctic ice fish and the lobe-finned fish *Latimeria*, all of which lack a swim bladder. Typically deep pelagic animals decrease their density by having reduced and less mineralized shells, carapaces, and skeletons than their benthic relatives (Company and Sarda, 2000) and generally substituting less dense materials in their structure.

Genetic systems

Ordinarily, animals' genetic systems are primary overall controls that also program development. They themselves show remarkable evolutionary growth in complexity and the scope of its regulation (Osawa, '95; Nagy, '98). Yet, the "universal genetic code" which functions efficiently in nucleic acid replication and protein synthesis (Freeland and Hurst, '98) was already established by the time of the earliest known bacteria and has remained largely unchanged throughout two or three billion years of evolution, like a living fossil, as will be discussed in part 3.

Overall, complexity usually is positively correlated with the number of genes but not the total quantity of DNA in the genome (Graur and Li, 2000). A major complexity increase occurred in the vertebrates during their origin from earlier chordates and subsequent history. Comparing nematodes, fruit flies, and tunicates with vertebrates show a large evolutionary increase in estimated protein-coding nuclear genes from about 15,000 in the nonvertebrates cited to 70,000 or more in the vertebrates (Martin, '99). This substantial coding gene increase has been attributed by some biologists largely to genome duplications in the early history of vertebrates (Greer et al., 2000).

Yet an analysis of the phylogeny of 35 vertebrate gene families, variously active in a wide variety of levels and functions, indicates that this massive increase in genetic complexity seems to have occurred mainly through the accumulation of numerous small-scale piecemeal changes (Martin, '99). No evidence was found for a few large-scale coherent events.

The phylogenetic occurrence of Hox genes also shows significant evolutionary growth in complexity at least in their numbers and clustering. Available data are, no doubt, still rather sparse but according to a count in the late 1990s, the bilater-

ians may have more than twice as many Hox genes as the prebilaterians (sponges and cnidarians). Also, the deuterostomes, including echinoderms, hemichordates, and vertebrates (Bromham and Degnan, '99), may have somewhat more than the protostomes, including annelids and arthropods (Valentine et al., '99). In addition, questions have been raised about whether Hox gene *clusters*, apparently characteristic of the triploblasts, are even present in the diploblastic phyla (Davidson and Ruvkun, '99; Holland, '99).

Among chordates, amphioxus has a single Hox gene cluster, as do the protostomal arthropods and the pentaradial deuterostomal echinoderms (Mito and Endo, 2000). Primitive jawless fishes, such as lampreys, have three. Tetrapod vertebrates, from amphibians to mammals, including humans, have four such clusters, each on a different chromosome, suggesting to a number of scientists a sort of octoploidy. Yet multiple clusters probably arose by individual gene duplication or by duplication of chromosome sections containing the genes (Wolpert et al., '98; Martin, '99).

Curiously, teleost fishes have various numbers of Hox clusters with *eight* presumed to be the basic number, implying further evolutionary duplications (Longhurst and Joss, '99). Possibly the high number of teleost Hox clusters may be causally related to their substantially greater number of species than the other vertebrate classes (Gregory and Hebert, '99). Since the amphibians arose from early jawed fishes, the number of Hox clusters in lungfish or coelacanths seems crucial to understanding the core evolutionary pattern. In the Australian lungfish, *Neoceratodus* Longhurst and Ross identified the same four clusters, (A, B, C and D) characteristic of tetrapods.

This is consistent with the notion that the further duplications and later losses of Hox gene clusters in teleosts took place after their split with the lobe-finned fishes. Yet the exuberant array of teleost Hox genes seems surprising in view of the apparent lack of much differential growth along their anteroposterior axis. Does this mean that the high numbers indicate that axial differentiation of teleosts is more complicated than it seems in this species-rich group? Or does it mean that teleost Hox genes have at least in part evolved to control other functions than those usually attributed to them?

Social complexity

Another important aspect of the increasing complexity in animals' evolution emerged in the so-

cial relations that appeared among individuals of a species, although its scope was long obscured by the traditional isolation of scientific disciplines, (Wilson, '75; Gadagkar, '97). Breeding groups, such as families or swarms, obviously have strong, but often overlooked, effects on population biology (Sugg et al., '96). *Cooperation* among individuals within a species in families, schools, flocks, herds, prides, gaggles, and eventually in societies, gave rise to unprecedented new evolutionary prospects (Parrish and Hamner, '97).

This, of course, had already achieved remarkable levels in the early emergence of colonial invertebrates such as corals, siphonophores, bryozoans, and salps. Such ideal intraspecies cooperation seems possible only in quite simple organisms. Yet strongly social insects have evolved repeatedly, mainly in bees, wasps, and ants, which are all in the Order Hymenoptera (Wilson, '75). In such insect groups individual survival depends closely on their colony's survival. The termites are the only other kind of strongly social insects. However, some beetles and other insects as well as certain spiders have less strongly developed social behavior (Choe and Crespi, '97).

Colonial animals and strong insect social systems (Seeley, '95) seem to form a sort of superorganism. Birds and mammals have also evolved various degrees of sociality, but they are less far-reaching than in the invertebrates mentioned. Individual survival in the warm-blooded vertebrates is less closely bound to their group's survival. Finally in humans and other primates, social organization, including families (Davis and Daly, '97), has increased strongly again. Its evolution has led to the development of learning, externally stored memory, and more effective communication systems.

To date, these culminate in language, stored records, problem solving, exploration, self-awareness, and human culture in general, including myth, ritual, art and science (Maynard Smith, '99). Such developments have evolved much faster than traditional genetics-based evolution, and the resulting changes can be spread widely with remarkable speed (Bonner, '88). Even so, phylogenetic methods can be effectively applied to some of these topics such as the evolution of human languages (Gray and Jordan, 2000). Certainly behavior and social structure can be crucial for animal fitness in extreme environments as in desert ants, emperor penguins, and musk oxen, not to mention humans. However, the extension by sociobiologists, of genetic determinism to the evolution of

human behavior has been bitterly controversial (Sterelny and Griffiths, '99).

Extremophiles and complexity

As in the case of size, animal extremophiles run a wide gamut of complexity from sponges and cnidarians to numerous kinds of insects, crustaceans, cephalopods, and vertebrates in various far-from-average environments. Despite seemingly endless capacities for sustained evolutionary trends, even the most evolvable organisms may be unable to change in certain directions, like echinoderms confronted with fresh water. Increasing complexity may also conflict with some other evolutionary tendency that acts to constrain it. This could have been the case for ammonoids, extinct cephalopods well known from extensive fossils, including 588 genera that lived during a period of more than 140 million years in the Paleozoic and early Mesozoic (Saunders et al., '99).

Ammonoid history was marked by a strong overarching trend for increasing complexity in the sutures joining the septa in the animals' chambered shell to its outer wall. Yet this well-documented and taxonomically important anatomical detail reversed its basic trend and became more simple during three extinction episodes in the group's overall history. Apparently the factors increasing extinction rates during these severely stressful episodes were selectively more destructive at shallower depths than in deep water.

Presumably, ammonoids began moving into the depths to avoid this threat of destruction. But this increased the hydrostatic pressure on the spiral chambered shell, gas-filled for buoyancy, rather like that of the living, but not closely related, pearly nautilus (Wells, '99). As a result, simple septal sutures became more effective than complex ones, because the latter are more vulnerable to collapse under high pressures. Avoiding extinction in shallow water would seem to have won out in this conflict, accounting for the observed decrease in suture complexity.

On a much larger scale the insects seem to have been blocked somehow from subsurface pelagic marine waters and the deep sea broadly, as part of their striking near-absence from most of the marine environment. Even so, a number of aquatic types, such as water bugs and whirligig beetles, are familiar in fresh water, as are water striders. This last group also has open ocean surface species and many coastal marine ones. Insects, such as springtails, mosquitoes, caddis flies, and midges, are common in the upper tidal zone, man-

grove swamps, salt marshes, and even saline lakes. Hence salinity *per se* would not seem to be the barrier.

Yet most of the great volume of the world ocean has no insect inhabitants whatever, shallow or deep. Explanations are speculative, but perhaps commitment to a tiny air-filled tracheal respiratory system, limiting them to shallow depths (Maddrell, '98), was a factor in this strange failure of the most speciose animal taxon to invade the world's largest living space, including the deep sea. Perhaps, too, insects' rather late evolutionary arrival was influential, several hundred million years after the ocean had been richly colonized by numerous other animal groups, such as polychaetes, mollusks, crustaceans, echinoderms and fishes.

No doubt, the explanations and consequences are different for amphibians, which also are nonmarine except marginally for one or two frog species. In contrast to insects and amphibians, cephalopods and echinoderms are all obligatory marine animals and hence precluded from freshwater and terrestrial extreme habitats. The special eggs of reptiles, birds, and mammals (collectively, the *amniotes*) have, among other things, allowed them to invade arid terrestrial frontiers. But rather unlikely types without such eggs including a few crabs, frogs, and toads also live successfully in various severe deserts (part 1 and above), by undergoing periodic sheltered inactive phases having strongly reduced metabolic rates during dry periods that may last 8 to 10 months per year or longer. One Australian species of desert frog in the genus *Cyclorana* can survive as many as five years of dormancy (Warburg, '97). Also exceptional are some high-latitude amphibians and reptiles that in winter undergo a prolonged inert subzero diapause, and a few that are even freeze-tolerant.

Despite being ectotherms, reptiles may also succeed well in extremely hot desert environments. Typically they tolerate considerably greater heat stress-induced changes in their internal environment that would be lethal to birds and mammals (Bradshaw, '97). Also reptiles' metabolic rates are so low that their energy food requirements are significantly less than those of endotherms. Their water needs are also modest because, like insects and birds, they typically excrete nitrogen as uric acid that, being relatively nontoxic to process, can strongly spare urinary water loss.

Reptiles' dry and water-impermeable skin reduces water loss by evaporation as does the waxed chitinous exoskeleton of desert insects. Recall, too,

that some reptiles and birds have salt glands that can reduce salt loads without sacrificing much water (Schmidt-Nielsen '97). Yet mammals, particularly many small desert rodents, excrete nitrogen as urea, which usually requires considerable water loss. Even so, mammals can conserve water because of their kidneys' unique ability to produce strongly hypertonic urine, as discussed elsewhere.

Such generalizations from comparative data reinforce the idea that stressful environments can be responded to in many different ways. Even animal types that have not yet evolved many complex physiological self-regulatory mechanisms, can commonly survive on the environmental frontiers. What some ecologists refer to as trade-offs can be made at a number of alternative levels and by various means. Human beings seem an obvious exception since they can penetrate and at least briefly survive the most extreme habitats in the world, such as the highest mountains (West, '98).

Yet humans' sustained survival in extreme habitats is only made possible by behavioral or social means such as bringing oxygen, water, and food into extreme environments, using insulated clothing, and setting up shelter, along with heating or air conditioning and protecting themselves from high or low pressures, as needed (Marriott and Carlson, '96). All of this survival gear can be marshaled nearly instantaneously compared with the thousands or millions of years needed for any comparable Neo-Darwinian animal adaptations. Such behavioral, social, and cultural assets for survival are surely supported by remarkably complex brains.

A naked human being, without clothes, water, food, or other support, is actually far from an effective extremophile. Such an individual dies rather quickly on any of our four major habitat frontiers. Its metabolism, its hardiness, and most of its means of self-replication fail in the most extreme habitats. Space travel, which requires long-term self-maintenance without outside access aside from take-off stocks of fuel, food, and so on, plus solar power in flight, brings the difficulty of practical solutions to such problems into sharp focus. Despite much research, feasible bioengineering and social protocols remain to be worked out for viable interplanetary travel and the colonization of the moon or other potential extraglobal home sites. All of which obviously require sustained survival under extreme, usually strictly lethal, conditions. The whole prospect faced by various space agencies of terraforming severely

hostile extraterrestrial environments is indeed challenging (Fogg, '95).

DIVERSITY CHANGES

Increasing *diversity* in the kinds of organisms on earth has, like increasing size and complexity, been a continuing powerful trend in their long evolutionary history (Magurran and May, '99). At the level of total species, genera or families, maximum and mean diversity of animals have greatly increased overall from the end of the Precambrian to recent times. But like life's growth in size and complexity, its diversity changes are many-layered and have a number of special features. Morphology, physiology, genetic, nervous and endocrine controls, behavior, lifestyle, as well as family, group and population patterns—these are all diverse elements already mentioned under complexity. Here animal taxa at various levels from species to the animal kingdom are the central topic.

In any case, diversity, like complexity, presents problems of definition and measurement beyond simple counts of individual phenotypes or taxa. Diversity, also, since the late 20th century, has taken on urgent components of public policy, ethics, and natural resource ecology and economics because of its central place in the conservation debates (Abe et al., '97). Even at the headcount level, diversity's overall rate of increase (discussed in more detail below) has varied markedly over time and has even been repeatedly reversed during major periods of extinction. (Mass extinctions and rates of evolution, including bursts of species and higher taxa will be discussed in part 3).

In addition, the taxonomic diversity of animals and its changes with evolution is quite unevenly spread among the various phyla and their subgroups. One phylum, for instance, has only a single species whereas another has more than a million. Why, for instance, have some phyla, such as the priapulid and poganophoran worms, survived but remained undiversified and minor over some hundreds of millions of years? Both the worm phyla mentioned have only one closely-conserved body type and only a few, solely marine, species. Among more than 30 generally recognized animal phyla, nine have only 100 species or fewer (Margulis and Schwartz, 1998). The fossil record for a number of these groups implies that never in their history have they diversified notably either at the species level or higher.

In contrast to such weakly diversified phyla, quite a number, particularly the arthropods, have gone to the other extreme, with estimated species

numbers reaching into the millions. Identified arthropod species, even excluding insects, number well over 500,000; mollusks number over 110,000, and the chordates, which include the vertebrates, 45,000. Among the diversity-rich monophyletic arthropods, the probably paraphyletic crustaceans, for instance, can be subdivided into many groups to separate the shrimps, krill, lobsters, crabs, barnacles, wood lice (isopods), beach fleas, and so on (Spears and Abele, '98). Each of these subgroups can be separated into smaller ones until the species level is reached. Current opinions suggest that malacostracan crustaceans, at something like an isopod level, may have given rise to insects (Abzhanov and Kaufman, 2000) and independently to other arthropod types (Brusca, 2000).

Other phyla, highly prolific in an evolutionary sense, include nematode worms, annelid worms, mollusks, echinoderms, and chordates (Margulis and Schwartz, '98). Of these, the species numbers cited for the nematode roundworms range from about 20,000 currently identified to many times that number still unknown (Malakhov, '94). About one-third of these are parasites in plants and animals; the rest are free-living in a wide range of environments, including various stressful frontiers.

Species are widely used as basic units in Linnaean systematics, population genetics, biodiversity, ecology, evolution, and comparative biology. Yet some researchers consider *species* to be quite an arbitrary term with little "real" meaning and in need of replacement (Bachmann, 2000). Darwin himself seems to have been rather ambiguous on this point (Depew and Weber, '95). Even now, quantified species discrimination has rarely been practiced (Wiens and Servedio, '99). Yet for the classic Linnaean systematic biologist and many others, the species is taken to be a stable and distinct kind of animal (de Quieroz, '98), quite generally used as a measure of biodiversity (Claridge et al., '97).

But even among biologists who accept species as key evolutionary entities, the concept of species has been defined variously (Harrison, '98). Systematists, paleontologists, ecologists, and population biologists all have rather distinct definitions. Consequently, several different meanings of "species" must be acknowledged when discussing evolution (Gosling, '94). Definitions often become even more troublesome when dealing with higher taxa in classification, such as genera, families, orders, and so on. These groups tend to be less clearly definable than species and vary con-

siderably in character for different major animal groups, typically studied independently by specialists (Nielsen, '95; Cavalier-Smith, '98b).

If we refer here to speciation as grist for evolution, we are referring to the structural and functional differences between various kinds of rather similar evolving animals, whether or not reproductive isolation has been, or even could be, tested. Yet we do know that persistent changes in animal populations, heritable over generations, require some reproductive isolation, even if it may be partial or temporary. In fact the *genetics of species formation* may be taken to be the *genetics of reproductive isolation* (Coyne and Orr, '98). Some sort of genetically controlled traits that at least partially block gene exchange must be involved in such isolation.

The factors that promote speciation and hence the species richness of particular animal groups are still uncertain (Price, '96). Ecologically, species diversity and productivity are correlated, but explanations are still needed (Waide et al., '99). Even the genetics of species formation remains in a rather elementary state (Via and Hawthorne, '98; Gavrilets, '99). Underlying this ambivalence are the more than 20 different concepts of species (Mayden, '97) and widely varied estimates of species importance, mentioned later in this article. Also complicating discussion are the quite distinct ways of identifying taxonomic units used by the Linnaean systematists (most of the many species counts and other references to species in this essay fall into this category) and the phylogenetic cladists (Nixon and Wheeler, '90).

Later discussions of rates and mechanisms of evolution will expand on this topic (part 3). Particularly interesting is the question of whether stresses themselves can accelerate or positively affect the direction of evolution (Hoffmann and Hercus, 2000). Various earlier hypotheses about speciation remain mostly untested. Comparison of evolutionary sister groups, identified by cladistic analyses, may offer a promising future approach to this problem (Barraclough et al., '98). For instance, data on plant-feeding by insects and on sexual dimorphism in birds show that rates of genetic change and speciation rates can be correlated.

As might be expected, there is also considerable correlation between *species* diversity and *adaptive* versatility among various animal groups. However, some *exceptions* are clear. For instance, tardigrades are known from the early Cambrian Chengjiang fauna (Brusca, 2000, but their cur-

rent morphological and species diversity are minor, with only 600 or so recent species (Kinchin, 2000). Yet they seem ecologically remarkably versatile, occurring among other places in the deep sea, in hot springs, in highly stressful Antarctic desert valleys, and on glaciers up to 5600 m in Nepal (Thaler, '99).

As discussed in part 1 of this essay, animal diversity at the phylum level has usually been thought to have already reached its peak by the early or mid-Cambrian and then apparently declined (Mayr, '91). If so, phyla seem to be exceptions to the usual trend toward continual increases in taxon diversity over time. Certainly all of the major animal phyla, except apparently flatworms, have been known as fossils dating back to the late Proterozoic or early Paleozoic (Valentine et al., '99).

Certain of the minor sparsely diversified phyla, such as the comb jellies (Ctenophora) and priapulid worms, neither expanded from the sea into other major habitats nor evolved widely even into various sub types of marine environments. Instead they have remained, since the Cambrian at least, as a few species of marine "conservative," yet distinctive, major types. More recently proposed phyla, of which there are a few, seem to be increasingly minute, poorly known types not yet found as fossils (Brusca, 2000).

Extreme diversity

The most versatile and diverse present day phylum, the arthropods, apparently has been so for at least 600 million years (Brusca, 2000). As a result, much of the variation needed for animal speciation, as well as their evolvability itself, was concentrated in one class of the most diverse phylum in earth's history. That class of arthropods, the insects, far outnumbers all other arthropods together and constitutes about half the total known number of species of recent animals (Kristensen, '98). Such remarkable diversity has stimulated considerable speculation about how it came to be (Fortey and Thomas, '98).

The beetles, as mentioned earlier, include about 300,000 known species, although they form only one order among the 25 to 30 orders of insects. The fruit fly family to which *Drosophila* belongs has about 3,200 named species. In ancient times, insect rates of evolution apparently were not exceptionally high, but as discussed later in this article, their extinction rates were quite low. Insects' early evolutionary success, no doubt, was also tied to the then-burgeoning evolution of early land

plants, including seed-bearing conifers and cycads (Kenrick and Crane, '97).

Although usually associated particularly with flowering plants, insects' great diversification began well before those green plants evolved (Lambandeira and Sepkoski, '93). Instead, the typically inverse relation between size and diversity, just cited, may have been involved in insect species profligacy. In turn, the insect tracheal respiratory system, unsupported by effective circulatory transport, must impose a likely restraint on size, as well as on insects' severely restricted marine occurrence, mentioned below. However, long after insect origins, plant-eating beetles, co-evolving with flowering plants, may account for perhaps 100,000 new species in that insect group just during the Cretaceous and early Tertiary (Farrell, '98).

More broadly, a number of insect features seem crucial for their great evolutionary success on land including extensive extreme habitats (Gullan and Cranston, '94):

- Acquisition of a waterproof external skeleton made of chitin. Especially because of their small (but not minute) size, water conservation is critical for insects to function well in any aerial-terrestrial environment not saturated with water vapor.
- A largely impervious exoskeleton, which helps insects survive in extreme habitats with water shortages.
- The special excretory system of insects, which also seems particularly relevant to terrestrial life and water conservation. Insect urine, during water shortage, can be eliminated as a paste or even a dry powder, mostly of uric acid. As just mentioned, this provides a clear fitness gain by minimizing water needs in deserts or high on mountains.
- Development of an effective respiratory system not dependent on a heart and circulation. It functions mainly by passive diffusion of gaseous oxygen and carbon dioxide within a fine branching tree-like structure of tiny air-filled tubules that reach all active tissues. This so-called tracheal system works very well on a small scale but, presumably because gas diffusion is only fast enough over very short distances, has kept insects quite small. Some fossil dragonflies had wingspreads of half a meter, but we know few details about their flight or respiration (Wootton et al., '98). Perhaps a sufficiently sustained pulse of higher than usual percent of oxygen

in the atmosphere and greater overall air densities may have permitted such giants to flourish for a limited period only (Dudley, '98; Harrison and Lighton, '98).

- Tracheal respiration is also limited in its effectiveness to breathing in air because respiratory gases diffuse so slowly in water that trachea would not work if water-filled. Aquatic insects mostly breathe at the surface or take bubbles of air down with them, features that limit them to shallow water depths, as cited.
- The remarkably prolific evolution of modern insects has also been attributed to *flight* that allows them quickly to exploit varied and extended habitats despite the animals' small size.

Another insect feature particularly effective in periodically or occasionally extreme environments is their widespread ability to enter a resistant dormant state. During extreme stress, most ectotherm survival requires, as in many insects, being inactive and dormant as well as being freeze-tolerant or freeze-resistant in extreme cold at high elevations and latitudes (Fogg, '98; Sømme, '99). Dormancy in ectotherms typically reduces metabolism markedly and may strongly heighten resistance to stresses. The dormancy concerned may occur in various phases of the life cycle from egg, to larva, to adult. It permits survival during the more or less temporary absence of otherwise viable temperatures, supplies of water, oxygen, or food. During seasonal stresses at moderate to high global latitudes and elevations, dormancy is crucial (Chapin and Körner, '95; also mentioned in part 1 and later in this article).

Clearly, some diversity of both animals and available environments is basic to the evolution of *extremophiles*. Habitat diversity for the large taxa also varies widely, as already mentioned. Consider, for example, the six animal phyla (following Nielsen's '95 analysis) that have more than 10,000 living species. Among these only the flatworms (Phylum Platyhelminthes) have scarcely moved into extreme environments except as parasites of extremophile hosts or minute soil types. The other five species-rich groups, arthropods, mollusks, vertebrates, nematodes, and to a lesser extent, annelids (with about 15,000 species), have shown substantial evolutionary versatility. Yet the starfish, sea urchins, and other echinoderms remained solely in their marine environment. Others, such as arachnids, just moved from the sea onto the land (Dunlop and Webster, '99) whereas mollusks stayed substantially in the sea, where they

are mainly deployed today. But, except for certain taxa such as cephalopods, some mollusks also radiated into freshwater as well as onto the land.

All echinoderm and most mollusk extremophiles are restricted to the deep seas. One clade of the Aplacophora, a poorly known class of cylindrical, rather strange spiny worm-like mollusks, has mostly been found at depths between 40 m to nearly 6,000 m, with most of the species diversity below 200 m. At least 250 species have been described and perhaps 1,000 seem likely to be found (Scheltema, 2000). Could they be candidates for an evolutionary species burst? Mollusks are also extraordinary because they evolved the octopuses and squids, which are among the most active and complex-behaving animal types with numerous deepsea species (Pörtner et al., '94)

Cephalopods are rather unexpected relatives of slugs, oysters, and abalones. Many species in this class are prominent bathypelagic or benthopelagic carnivores. For instance, the giant squid *Architeuthis* is the largest invertebrate, a fast-swimming, powerful deepsea predator, a proper model for sailors' tales of sea monsters (Lordan et al., '98). The prominence of squid beaks in the stomachs of deep diving whales proves that deepwater squids are a major element in some cetacean diets. Above all, the nematodes and arthropods are outstanding as versatile phyla that have strong representation in deserts, high latitudes, high altitudes, and the deep seas as well as plant and animal parasites. The vertebrates are close behind them but have not evolved true parasites.

Birds and mammals, particularly, with their well-developed thermoregulation and high-level energy budgets can in many cases manage to keep vigorously active under extreme stresses. Trans-himalayan flight paths and the deep diving programs of certain large fishes and various large marine mammals are discussed elsewhere. Aside from a few minor taxa, many marine species in nearly all major animal phyla have effectively invaded the deep sea. This did not require a change in medium but only involved spreading into a huge, but likely stressful, habitat always adjacent to more benign inshore and near-surface pelagic marine areas.

NATURAL SELECTION

Between the "push" of phenotypic diversity, population pressure or chance and the "pull" of environmental opportunity (Schluter, '98) lies the keystone of Darwin's theory of evolution: *natural selection* (Futuyma, '98). Natural selection acts on

phenotypic variations in living organisms in the field so that some unfit variants are eliminated and other fit variants persist (Price and Yeh, '99). Since the earliest stressful and tentative biotic times more than two billion years ago, selection of some sort must typically have favored the persistence of accidental complex quasi-stable cooperative self-replicating systems that were potentially alive and the disappearance of those that were not.

An additional feature of natural selection is that it acts ultimately on the reproductive rate of individual animals (Bell, '97). That rate may depend indirectly on various organisms' size, morphology, and physiology. Yet these in turn are basically determined not only by the animals' genes but also in complex ways by many other influences, such as environmental signals (Van Buskirk et al., '97; Tollrian and Harvell, '99), diet, acclimation, parasites, and learning. These apparently casual influences sometimes can affect the phenotype dramatically (Huey and Berrigan, '96) in ways that may significantly affect natural selection (Greene, '99). Also, an organism's everyday evolutionary path may be partly shaped, sometimes decisively by chance factors (discussed in part 3).

The basic course of Darwinian evolution is set by natural selection interacting with inherited phenotypic variations in a given population responding to environmental change of whatever sort (Rollo, '94). Sexual selection (Cunningham and Birkhead, '98) is a variant of ecological selection (Schluter, '98) and may strongly facilitate speciation (Boake, 2000), as suggested to account for the cichlid fish species burst and retreat in Lake Victoria (upcoming in part 3).

Depending on circumstances, selection can stabilize a population (thus greatly slowing or blocking evolutionary changes in phenotype), or it can move the population toward a state of higher fitness, or it can divide the two "tails" of a population's variation, such as the smallest and the largest individuals, into two new populations (Bell, '97). On the other hand, natural selection can also function in extreme environments to move a population in an evolutionary direction opposite to that expected.

Instead of conserving energy in response to cold stress, for example, an endothermic taxon's capacity for metabolic work and thermogenesis may be increased in a way that would allow it to thrive at higher latitudes or altitudes, provided that adequate energy food is available. In such a case, *exploitation* rather than conservation is the word. An interesting example has been reported for deer mice that live at about 3800 m on White Moun-

tain in California (Hayes, '89). In addition to the increasing cold stress with altitude, these small rodents, like other montane mammals, face reduced maximum metabolic output because of the decrease of oxygen partial pressure with elevation (West, '98).

To study this, the maximum aerobic capacity in local wild deer mice was measured in a mountain laboratory's refrigerated wind tunnel for a substantial number of individuals collected by trapping on three separate occasions. The animals were first measured, marked, and then released to their natural environment. Two months later the survivors were recaptured and tested again for their maximum aerobic capacity. Strong directional selection for an increase in this factor was statistically significant in the largest of the three data sets. This appears to be an important instance of Darwinian selection for an extremophile. One gene has been associated with maximum aerobic capacity in deer mice, but other components of the overall control mechanism remain to be identified.

During life's long history, natural selection has evoked substantial amounts of apparently parallel or convergent evolution (will be discussed under "chance" in part 3). These are widely evident at the level of orders and smaller taxa, at the nucleic acid level within organisms (cited previously), as well as at the enzyme (Kreitman and Akashi, '95) and perhaps gene levels of evolution (Goodrich et al., '97; see also part 1). For instance, rapid parallel evolution through natural selection has been reported at the speciation level for stickleback fishes (Rundle et al., 2000) and at the level of geographic clines for a species of *Drosophila* (Huey et al., 2000). In the latter case, the genetic mechanism of the parallel geographic size increase with latitude is different in the two fly clines, one from Europe and the other from North America.

An extremophile example within what is usually considered a single species occurs in the evolution of some cave animals. In the Mexican characin fish *Astyanax fasciatus*, both subterranean cave-living and nontroglobitic surface populations of the same freshwater species are well known. Cave forms of this fish occur in eight groups of limestone caves. Some of these populations, estimated to have been cave inhabitants for perhaps a million years, are as adults *pale*, *pigmentless*, and *blind*, with much reduced eyes and visual centers in the brain. Imperfect eyes form in the embryo, but apoptosis of the early lenses apparently sig-

nals regression in much of the rest already present (Yamamoto and Jeffery, 2000).

A different cave population, believed to have been trogloditic for a much shorter time, has moderately developed eyes, intermediate between normal above-ground fish and the severely reduced blind types. Such variable structural features of these cave fishes, as well as certain behavioral traits, are apparently all under genetic control. As a result, hybrids between the above ground *Astyanax* and its various cave-adapted populations have provided remarkable insight into the genetics and fitness-related evolution of complex organs such as eyes (also cited in part 1; vision and eyes in the deep sea will be discussed in part 3).

Less well known is the amphipod crustacean *Gammarus minus* that has repeatedly and independently adjusted to cave life after invading different cave systems in various limestone (karst) basins in middle Atlantic and middle central United States (Culver et al., '95). Compared with surface-dwelling populations, each such invasion resulted in resident populations with compound eyes having a reduced number of facets and underlying ommatidia, a shift in body pigment from brown to blue or white, as well as lengthening of the antennae and other paired appendages.

At the same time, the optic centers in the central nervous system were reduced in cave populations and the antennal centers enlarged, presumably representing a shift in sensory modes correlated with cave darkness. All of these features showed heritable variation in general and directional changes in the cave habitat. Curiously, *extreme* changes of this sort, such as reduction in ommatidial number from 25–30 to 3–4, were observed only in a few isolated large caves in West Virginia and Virginia. In addition, evidence for the reversal of these cave correlations was found in populations of *Gammarus* living in collapsed cave areas open to daylight. This suggested that the genes involved were still present in the dark cave forms and that their expression could be resumed over generations with exposure to day light.

Essential stability

In contrast to such labile features, any random tendency to change or displace stable features that are essential for animal survival must be curbed either by selection or by the organism's self regulatory systems (Bell, '97). Presumably this constraint underlies the classic conservatism of mutation rates in particular proteins and

nucleic acids (and their component amino acids and nucleic acids), a conservatism correlated with their inherent functional importance in the organism. Alternatively, to retain their essential presence, their genetic control may be shifted to new or alternative mechanisms, as may be the case for the stasis of some living fossils, discussed in the forthcoming part 3. While somewhat reminiscent of the old, rather fusty, idea of archetypes, these complex-system characteristics actually can be described in more contemporary and plausible terms (Van de Vijver et al., '98), including developmental and evolutionary modules.

The characters on which natural selection may act range from parts of molecules to large-scale components of anatomy, development, life history and behavior. Despite some hubris on the part of a few molecular biologists and genetic engineers, genes, as fragments of information, ordinarily do not surface directly in the phenotype. Hence *individual organisms* that carry the genetic information and, no doubt, higher levels, such as *social groups* that interact cooperatively or antagonistically between themselves and the environment, comprise the *units selected* (Reeve and Keller, '99; Weiss and Fullerton, 2000).

Darwin originally conceived of natural selection largely as related to highly productive ecosystems, such as tropical lowlands, where crowded inhabitants must compete, tooth and claw, within and among species for essential available resources. In such abiotically nonstressful resource-rich environments, populations seem to operate well below the carrying capacity of the environment. Accordingly, positive evolutionary factors tend selectively to favor fecundity and population growth. Yet natural selection reins in organisms' tendency toward reproductive exuberance and, as far as possible, molds their inherent diversity to match available habitats including extreme frontiers.

Natural selection may be considered as the complex outcome of an increase in an animal's or a population's fitness at one or more levels. Eventually it results in greater reproductive power, if that is not already limited by some other factor, such as aging, that reduces reproductive capacity and ultimately individual survival. In addition, natural selection, under severe stress, may reduce the number of healthy offspring of less robust animals so far that their death or extinction results (Gems, 2000). But for any given organism, the emergent opportunity of moving into the potential living space of a new uninhabited extreme environment is reduced by the organism's

vulnerability to the stresses there and any inability to respond to them effectively.

In the evolution of extremophiles, natural selection's evolutionary force acts on populations so that they can survive and reproduce under various abiotic conditions that would have previously been stressful or even lethal. Yet to reach a sustainable steady state, this positive force for growth and expansion must be contained by an opposing force that consistently eliminates the less fit and the unfit.

Darwin himself recalled that a serendipitous first reading of Malthus's essay on population (1798) provoked him to think about what forces must prevent animals from propagating until they are literally piled up one on top of the other (Depew and Weber, '95). He realized that offspring typically differ from their parents and siblings from one another. As a result, some are fitter to survive than others, where fitness is ultimately measured by the number of viable offspring produced by the organism or taxon (Kozlowski, '99).

Selection in extreme environments

Survival of the fittest in Darwin's sense may not be directly relevant to life in extreme environments. Yet the evolution of fitness in such habitats is exactly the point of departure of this essay. Even so, it has been argued that *nonsurvival of the unfit*, including their failure to reproduce and their ultimate extinction, is a more appropriate maxim than the classic one. In either case the survival to maturity of one male and one female for sexually reproducing animals and at least two viable offspring, one of each sex is the theoretically lowest limit for continuity. More practically, a minimum population number of reproductive individuals of both sexes is needed for likely sustainability and evolution.

By definition, such evolution moves potential extremophiles away from the middle-of-the-road, sometimes to become specialists, but more often to become hardy generalists (Parsons, '99). For instance, extremophiles flourish with reduced energy-food supply in general, with increasing hydrostatic pressure at increased water depth, with decreased temperatures at higher latitudes and altitudes, with increased temperatures near hydrothermal vents and so on, as already discussed. But also the numbers of individuals and taxon diversity decrease to an ultimate limit where no taxa or individual animals survives more than briefly, let alone reproduces. Presumably the limit of survival and zero fecundity are usually imposed

by starvation-inducing low primary productivity as well as by extremely severe abiotic factors, such as anoxia at extreme elevations, acting directly on the animal.

In contrast to mild highly productive habitats, extreme environments near the Malthusian limit have resources that are reduced to a minimum. As a result competition may be largely secondary to mere survival as in the interior of severe continental deserts or the Greenland and Antarctic ice caps (Labropoulou and Kostikas, '99; Parsons, '99). The polar bear on the Arctic ice sheet and the Bactrian camel in central Asian deserts provide extreme examples in which coexistence rather than competition would seem to mark the sharply limited animal communities (Takeshi, '99).

Also, the high Andean habitats of two camelids, the guanaco and the vicuña, overlap between 3000 m to over 4000 m without apparent interspecies competition (Lucherini et al., 2000). Communities of the birds and fish of northern Canadian lakes seem to have evolved with minor predation and competition between them (Paszkowski and Tonn, 2000). In any case, evolution's usually assumed central dependence largely on biotic factors, such as competition and predation, may have been exaggerated by Darwin and by many ecologists after him (Hengeveld and Walter, '99).

Note that in some less stringent frontiers having clusters of closely related animal types, interspecies competition for scarce resources no doubt does occur more widely. Examples are evident among certain fishes (Haedrich, '97) and crustaceans (Company et al., 2000) in the upper 1,000 to 2,000 m of the deep sea as well as among species clusters of desert darkling beetles (Sømme, '95), desert lizards (especially in Australia; Vitt and Pianka, '94) or desert rodents worldwide (Kelt and Brown, '99). Yet competition is known to be important in some deserts for certain taxa, but not for others.

On the other hand, intraspecies competition in the form of *cannibalism* may erupt in an extreme environment if survival becomes desperate (Polis, '81). For instance, tadpoles of some desert toads and frogs begin to eat one another if the temporary pool of water on which their accelerated larval development depends begins to dry up before they can reach metamorphosis (cited in part 1; Greene, '99). Obviously a few individuals (sometimes carnivorous oversize phenotypic morphs) might survive in a small puddle, while none may survive in a writhing mass of hundreds or thousands. Experiments have shown that poorly nour-

ished treefrog tadpoles from an ephemeral drainage ditch reached metamorphosis more quickly and grew to greater size when fed conspecific tadpoles than did well-nourished, but not cannibalistic, controls (Babbitt and Meshaka, 2000).

Cannibalism is common, also, in Arctic char, the salmonid, living close to the limits of survival as the only fish species in some high-latitude lakes in northern Svalbard (Hammar, 2000). In addition, desert scorpions indulge in intraspecific prey. As in the further instance of the arctic fox, such behavior can be considered as a *homeostatic mechanism* keeping calories within a population and regulating the population's size within the reduced carrying capacity of a stressful environment.

Presumably cannibalism is under genetic control but is facultatively triggered by environmental, or more likely, internal signals, such as hunger. More generally, cannibalism is remarkably common in many animals ranging from flatworms to mammals, stressed by food scarcity. With some limitations, cannibalism can increase individual fitness, and probably group fitness of nearly starving extremophiles (Polis, '81). Under such conditions noncannibals starve to death.

However, Darwinian fitness over time and space is usually some sort of unspecified integrated long-term concept, rather than an abrupt quantum leap (den Boer, '99). For one thing, the animal itself varies considerably from moment to moment, with time of day, asleep or awake, hungry or satiated, and so on. Also populations and species are made up of individuals whose many properties, including number and size, vary over space and time.

The environment, too, is patchy in space and variable over time; extreme spatial focus occurs in desert oases and in deepsea sulfurous hot springs. As a result, an individual animal's overall fitness fluctuates with stress levels. Also in populations, individuals vary slightly, too, so that effective natural selection is probably considerably weaker than in a simple diagrammatic model. Quite often, too, the individual victims of predation and accidents seem to be selected independently of their classic genotypic or phenotypic fitness.

Limits to selection

Most evidence amply supports natural selection's central role at the phenotypic level. Yet a number of factors, both internal and external (Arthur, 2000) limit its action. Because natural selection:

- does not respond to *neutral* evolutionary variations,

- but does *over time* efficiently eliminate *negative* phenotypes with decreased viability and fecundity, it
- supports the persistence of *potentially positive adaptive* variations, including those relevant to extreme environments, and
- acts on traditionally overlooked internal factors such as development and other regulatory controls, mainly independent of the environment (Arthur, '97). Such internal, largely nonDarwinian factors are discussed below.
- requires for adaptation, as it does for speciation, some reduction in gene flow between the normal and the stress resistant populations (Riechert and Hall, 2000).

The notion that most mutations are *neutral* seems, like the notion of evolution by chance, at odds with the Darwinian view that natural selection is the pervasive motor of evolution (Bell, '97). Current analysis indicates that a purely neutral theory cannot even account for all of molecular evolution (Kreitman and Akashi, '95). The increase in genetic variation with population size, predicted by that theory, is not observed. For instance, nucleotide variation in humans was found to be 4 to 10 times lower than in three *Drosophila* species. Yet fly and human protein variations were about the same despite the presence of far more individual flies. At present the role of natural selection in the evolution of DNA seems to be minimally, or at least poorly, understood. Clearly natural selection is more relevant to protein molecules, such as visual and respiratory pigments, than it seems to be to nucleic acids.

However, rare mutations may be beneficial and, as proved in massive experiments with bacterial cultures, can become fixed by natural selection in populations that exhibit a sort of punctuated evolution (Torres et al., '96; see also Coyne and Charlesworth, '96; Elena et al., '96). Yet the neutralists argue that weak or no natural selection *could* have the opposite effect by keeping a species orthodox. Neutral mutations could do this by lowering variation, rather than allowing a species to wander away from the established middle of the road.

Instead, only when ecological or biological changes open up new or freshly available habitats or conditions could natural selection activate evolution in a long-static species that has accumulated many previously neutral features. In this way a surge in evolutionary change could occur. Such a possible mechanism is related to the no-

tion of "preadaptation" (Futuyma, '98) in which some cryptic feature, such as tolerance to cold (Margesin and Schinner, '99), may be already present in a tropical animal. Yet the potential asset is not obvious or put to the test until the climate cools or the animal migrates to high altitudes or higher latitudes.

Preadaptation, or perhaps more aptly recruitment, also is frequently relevant at the molecular and genetic levels (Le Guyader, '96). For instance, a number of ancient proteins long used in *heat shock responses* or as certain enzymes in intermediary metabolism were recruited, perhaps by gene duplication and modification, for structural use as crystallins in the lenses of various vertebrate and squid eyes. Apparently, the molecules concerned "happened" to be transparent to visible wavelengths of light and otherwise suited to the visual optic needs of various animal groups (Wistow, '93).

A number of comparable switches in function are likely among UV-B screening pigments that are widespread in exposed organisms ranging from the earliest types of prokaryotes to fungi, plants, and animals. Various pigments serve passively to reduce radiation damage in exposed organisms (Cockell and Knowland, '99). Animals in most deserts as well as at high latitudes and altitudes usually are strongly exposed to such potentially harmful rays. They may also be important in the upper 50 m of the open ocean generally.

In high alpine or Arctic freshwater ponds zooplankton exposed to high summer light and UV-B intensities typically are bright red from carotenoids that act both as light-shielding filters and as antioxidants (Hessen et al., '99). *Daphnia* in such clear water ponds occurs as a morphotype with a black melanin pigment screen in the carapace. An alternative melanin-free morphotype occurs in less transparent, or shaded, ponds.

Another example relates directly to extremophile biochemistry. As mentioned in part 1, Antarctic notothenioid fishes year-round have antifreeze glycoproteins in their blood that prevent internal ice formation at seawater temperatures, modestly below 0°C. Apparently, the genes controlling antifreeze synthesis evolved from those classically responsible for trypsinogen (precursor of the enzyme trypsin) synthesis. In the process, much of the trypsinogen molecule was discarded, numerous repeats of a retained threonine-alanine-alanine tripeptide were generated, and two sugars attached to each threonine (Chen et al., '97a,b; Cheng and Chen, '99).

Available evidence implies that notothenioid antifreeze evolved just once in their history and did so between 5 and 14 million years ago (Eastman and Clarke, '98). Such a transformation of an old molecule to a new function seems rather like the more upscale conversion of a terrestrial tetrapod limb into the flipper of a seal or whale (part 1). Interestingly, quite independent evolution of a closely similar antifreeze molecule in the Arctic cod (cited in part 1) took place by a different mechanism that did not involve trypsinogen (Chen et al., '97b). This implies that some biochemical constraint was involved here (Hodin, 2000).

QUESTIONING NATURAL SELECTION

Natural selection, although a key element in Darwinian evolution, is nevertheless a topic of disputed importance among biologists. However, well-documented examples of evolution, observed in nature and in experiments in both the laboratory and the field, prove that natural selection can act dramatically and quickly to change the phenotypes of microorganismal, plant, and animal populations (Bell, '97; Amzallag, 2000; discussed further in part 3). Even the extensive changes involved in tetrapod and whale evolution (part 1) appear from the fossil evidence to have proceeded by cumulative modest Darwinian steps. In contrast, notions of macroevolutionary jumps, or abrupt transitions, are implicit in the probable effects of regulatory gene mutations (part 1). Also, such major steps could perhaps account for some of the frequent "missing links" in the fossil record as well as in punctuated evolution (Schwartz, '99; further discussed in part 3).

Some major differences of professional opinion depend on the level at which natural selection is believed mainly to act (Sober and Wilson, '98). Molecules, genes, individual organisms, populations, species, higher groups, ecosystems, and even a global ecosystem (Gaia?) (Huggett, '99) or some, or all of these together, have been taken by various biologists to be effective units in evolution. Obviously, such a wide spectrum of opinions weakens the probability of reaching a broad consensus (Williams, '92; Depew and Weber, '95; Jablonski et al., '96; Claridge et al., '97). Most often, *species* are taken to be the *key units* of selection.

More broadly, various biologists emphasizing neutralist, punctuationalist, antiadaptationist, haphazardist, and other points of view, have raised critical questions about some basic Darwinian beliefs. For instance, molecular biologists generally

embrace the neutral mutation theory (Kimura, '91; Skibinski, 2000) and believe that natural selection has a small, or even insignificant, role in molecular evolution and hence presumably in phenotypic changes (Rollo, '94). Yet the working connection between genotypic and phenotypic evolution, no doubt, depends only in part on natural selection. To different degrees in various cases (Ridley, '97), it also involves chance drift of mutations to fixation or loss and on numerous factors that affect and control gene inheritance and expression (Fernandez and Hoeffler, '99).

In the late 1990s a number of techniques became available for exploring the relations of phenotypes to genotypes (Streelman and Kocher, 2000). These may be relevant variously at the levels of the genome, the transcriptome, or the proteome. Single gene analysis is rarely adequate. Yet microarrays of DNA, for instance, can be used as sensors to measure the activity of a thousand or more genes at once (Marshall, '99; Lockhart and Winzeler, 2000),

Such results are beginning to be correlated with different kinds of cells and organisms engaged in various types and levels of activity, including development and disease. Also, these data could demonstrate changes in response to extreme stresses, as well as any distinctive patterns of gene expression (Niehrs and Pollet, '99) characteristic of species, such as camels, long established as extremophiles. One barrier to applying these methods to evolution, especially to that of frontier animals, lies in the importance of a detailed linkage map on which to locate genes likely to be involved.

So far such information is limited to relatively few mesophilic animals and some prokaryotic extremophiles including hot spring and deepsea species. Another hurdle, Streelman and Kocher argue, is that data scans at both the genome and transcript levels are needed (plus their linkage to the phenotype) (Vukmirovic and Tilghman, 2000) as well as functional tests preferably in the field. The scope of this challenge is suggested by a DNA micro array study of the effects over 250 cultured generations of yeast partly starved for glucose (Ferea et al., '99). By the end of the experiment, several hundred genes, clustered into groups related to metabolism, respiration, and glycolysis, had changed their expression. The responses of *Drosophila* (for which a large amount of the required molecular data is available) to multigenerational starvation, mainly studied at the phenotypic level, will be discussed in part 3.

During the 1980s and 1990s, probability, various physical and chemical constraints on evolu-

tion, and emergent properties of complex systems (Kauffman, '93) gained some support as evolutionary mechanisms at the expense of fitness, adaptation, and natural selection (Depew and Weber, '95). Yet, the neo-Darwinians and ecologists, as well as some molecular and developmental biologists, seem often to have underestimated the probable significance of such inherent alternative vital elements for evolution.

Most dramatically, these are components of the basic unity of life mentioned near the beginning of this part of this essay. Many of these are not just "frozen accidents" (Weiss and Fullerton, 2000) but seem largely dependent on the nature of the universe. Also, the evolutionary consequences of physiological self-regulation appear not to have been factored in effectively. Such rather neglected features may be quite distinct from the natural selection-responsive factors usually considered relevant to evolution. They tend, in fact, to define life's essential components plus its emergent complex-system properties. Some of these inherent elements were in place in the solar system long before living beings arose.

Thus the basic physics and chemistry of life are remarkably the same as for nonlife. In their crucial energy handling, organisms are subject to laws of thermodynamics (Prigogine and Stenger, '84) comparable to those of Willard Gibbs for reversible abiotic chemical reactions. For its substance, life is made up of the same chemical elements as the rocks, the sea, and the atmosphere, but in a highly selective pattern. The properties of the particular chemical elements essential for life (initially, mostly carbon, hydrogen, and oxygen, along with nitrogen, phosphorus, sodium, potassium, chloride, iron, and a few others) and of simple inorganic molecules (mainly water and carbon dioxide) seem to be fundamental in all prokaryotes and eukaryotes through phylogenetic time (Henderson, '13).

Some of life's complex inherent properties, such as the core of its energy metabolism, have been firmly in place since the early prokaryotes (part I). Of its three major components in animals, the citric acid cycle has been proposed as the universal ancestor of intermediary metabolism (Morowitz et al., 2000). Also, the lipid plasma membrane, enclosing each cell, was a necessary component from the start of cellular life. Even rhodopsin, the photon-driven proton pump, familiar in vision, was apparently already present in ancient purple bacteria (Edman et al., '99). Certainly since animals evolved, many such ancient vital features up to now seem

to have been largely insulated from any ordinary action of natural selection and the environment.

At the level of complex organic molecules, extensive analyses of single-stranded RNA indicate that clear nonrandom biases in their nucleotide composition (not sequence) are largely independent in widespread data sampling of many functions and phylogenetic affinities (Schultes et al., '97). Such remarkable broadly occurring close similarities in nucleic acid composition probably depend in this case, on biophysical constraints, such as specific molecular folding (known to be important for extremophiles) that may augment thermodynamic stability (Schultes et al., '99a).

These data imply that more than half of RNA's secondary structure relates to self-organization and less than a third is available to be acted on by evolutionary selection, with the remaining balance accounted for by random mutations (Schultes et al., '99b). Failure to allow for such substantial limitations may explain some of the inconsistencies in phylogenies derived just from nucleic acid sequences or even from coded proteins (part 1; Foster and Hickey, 2000). Selectively analyzing extremophiles in Schultes and colleagues' massive data sets for possible correlation of their RNA composition with their specific stresses may provide some interesting leads.

Despite problems and controversy, considerable additional evidence continues to pile up to support the relevance of natural selection to most levels of evolution (Culver et al., '95; Givnish and Sytsma, '97; Graur and Li, 2000; Huey et al., 2000; Rundle et al., 2000). These include phenotypic molecular changes, as in the hemoglobin of high-altitude birds (Butler, '91) and the quite special hemoglobins of deepsea vestimentiferan worms inhabiting hydrothermal vent areas (Zal et al., '98). Yet the time may be ripe for a fresh formulation that will effectively integrate the burgeoning molecular data into a new comprehensive theory of evolution (Carroll, 2000). The rapid, multiple divergence of some large, as well as some small scale, evolutionary changes are clearly challenging (forthcoming in part 3). Among other things, the present day relationship between Linnaean species and contemporary species defined by molecular and population genetics or cladistics needs to be resolved.

The basic definition of the gene and its relation to development and evolution no doubt needs some revision or replacement (Beurton, '98; Keller, 2000). For instance, despite the one-on-one conservatism of classic coding genes as units, much of the active genome functions in

complex interacting networks in which gene expression, suppression and release from suppression play critical roles (Nagy, '98; Burton et al., '99). In addition, organization into compartments or modules (Hartwell et al., '99; Raff and Sly, 2000), involving control genes, and signaling systems, may provide the flexibility needed for evolvability.

Also, the relation between genotype and phenotype, discussed further in part 3, is in need of aggressive research. How a genotype can change substantially despite an apparently stable phenotype (phenogenetic drift), and how apparently conservative genes, such as Hox, can switch from one classic function to a quite new and different one (Eizinger et al., '99; Abzhanov and Kaufman, 2000) are challenging events. They surely attest to our rather shallow understanding of such crucial relations (Weiss and Fullerton, 2000). This would seem to be an example of the dilemma already cited more generally: how can the basic unity of life be maintained despite a built-in antagonistic tendency toward diversity of many kinds that includes the wide spectrum of the world's extremophiles. Perhaps the drifting genotype is a given feature of life comparable to the Malthusian population pressure. Not the least of the problems involved is to deal scrupulously with the subtleties of homology that are crucial for tracking evolution at various levels from molecules to behavior (Nilssen, '96; Abzhanov et al., 1999).

In the third and last part of this essay the influence of several major factors on extremophile evolution will be discussed—genetic and possible extreme environmental sources of animal variations, rapid bouts of diversification, as well as chance and extinction.

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