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Causes and Consequences of Excess Resistance in Cryptobiotic Metazoans

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ABSTRACT

Despite more than 200 yr of recognition that some microscopic metazoans survive environmental conditions far beyond those experienced in nature while in a cryptobiotic state, this phenomenon has received little attention from evolutionary biologists. The excess environmental resistance exhibited by cryptobiotic organisms cannot be viewed as an adaptation within current evolutionary biology. Rather, excess resistance may have evolved as a by-product of natural selection for tolerance to desiccation or other naturally occurring environmental agents. The combined effects of desiccation, metabolic arrest, effective stabilization of dry or frozen cells by protectant molecules, and efficient DNA repair mechanisms may have led to a protection of the organism against conditions far beyond those experienced in nature.

Introduction

Cryptobiosis (latent life) is the collective name for a state of life used by some organisms to overcome periods of unfavorable environmental conditions (Keilin 1959; Crowe 1975; Wright et al. 1992; Kinchin 1994). Cryptobiotic organisms are known from both the plant and animal kingdom, but in animals mainly among invertebrates (Keilin 1959; Hand 1991). Cryptobiosis may be entered either within a specific (ontogenetic) life stage (e.g., larvae and eggs within Arthropoda, Crustacea, Brachiopoda, spores of various fungi and bacteria, and pollen and seeds of some plants) or over the entire life cycle (e.g., in species of Protozoa, Rotifera, Nematoda, Tardigrada, Arthropoda, mosses, lichens, and algae, as well as some higher plants; Keilin 1959;

Crowe 1971; Hand 1991; Wright et al. 1992; Alpert 2000; Gutiérrez et al. 2001).

From the environmental mechanism inducing the entering of a cryptobiotic period, four main forms of cryptobiosis have been defined: anhydrobiosis (desiccation), cryobiosis (freezing), osmobiosis (elevated solute concentrations), and anoxybiosis (low oxygen levels; Keilin 1959; Wright et al. 1992). At the organism level, the distinction between the first three categories often may be unclear because more than one process may be involved in the entry of cryptobiosis. For instance, low temperature inducing extracellular freezing in the organism will have a desiccating effect on the cells.

The most important biological effects of cryptobiosis are a dramatic reduction of metabolic processes to undetectable levels (Pigon and Weglarska 1955; Clegg 1986); a complete cease of reproduction, development, and repair; and a dramatic increase in the organism's resistance to extreme levels of various environmental factors. The evolutionary and life history consequences of such dramatic changes in the organism's activity and energy turnover have yet to be investigated (Jönsson 2001). I will concentrate on the last effect, which I will call excess cryptobiotic resistance (ECR). A great number of studies have documented an astonishing capability of cryptobiotic (mainly anhydrobiotic) metazoans to survive environmental conditions much more extreme than ever encountered in their natural habitats. This ability is unexpected from the perspective that natural selection should favor capacities of organism structures adjusted to the functional needs under natural conditions, not in far excess of those needs. It is therefore of some interest to consider the background and consequences of this phenomenon.

In this article, I first discuss the adaptive significance of cryptobiosis generally, then briefly review the evidence of ECR in cryptobiotic invertebrates, and finally discuss ECR from an evolutionary perspective, putting the apparent phenotypic overcapacity in resistance in the light of the current theory of adaptation.

Definition of Evolutionary Adaptations

The question of what criteria to use to define an evolutionary adaptation (in the sense of the outcome of an adaptive process) has received much attention from evolutionary biologists and philosophers of science over the years (e.g., Williams 1966; Gould and Lewontin 1979; Gould and Vrba 1982; Sober 1984;

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Brandon 1990). Two main concepts of adaptation, one historical and one nonhistorical, have been distinguished (Amundson 1996). According to the historical definition, supported by many philosophers of biology (Sober 1984; Brandon 1990), a phenotypic trait (morphological, physiological, or behavioral) is an adaptation only if it arose in the past due to natural selection for that trait. The nonhistorical definition instead considers a phenotypic trait an adaptation if it contributes to the organism's current fitness, regardless of its historical background (Bock 1980). A combined historical and nonhistorical definition of adaptation was formulated as a trait that arose in the past due to natural selection for that trait and that persists in current populations by virtue of its current effects on fitness (Amundson 1996). An important component of the historical definition is the distinction between selection for and selection of traits (Sober 1984). Selection for a trait occurs when natural selection acts directly on the properties of a trait, through the effects of the trait on fitness. Selection of a trait is the indirect selection of a trait, that is, its spread in a population, caused by a genetic correlation (arising, e.g., from pleiotropy) between the trait in question and another trait on which natural selection acts directly. Such "hitchhiking" of traits is relatively common and represents an important part of quantitative genetics theory (Falconer 1989) and life history theory (Lande 1980; Rose 1982; Roff 1992). Obviously, traits whose spread in a population is due to natural selection acting on another trait should not be considered adaptations. Rather, they are by-products of selection.

Lauder (1996) discusses the problems of verifying that a biological structure is an adaptation. From the nonhistorical definition of adaptations, verification is relatively straightforward, requiring only a documentation of positive fitness effects of the trait in current populations. From the historical definition, verification of adaptations becomes much more problematic since proofs must be sought in processes of the past.

The Adaptive Status of Cryptobiosis

Is the phenomenon of cryptobiosis (i.e., the ability to survive in an ametabolic state) an adaptation? To answer this question we have to make a distinction between the factors and processes leading to the cryptobiotic state and the cryptobiotic state per se. Clearly, the ability to tolerate complete desiccation, freezing, or anoxia has a paramount positive impact on fitness in current populations of organisms exposed to these factors. The fact that these kinds of tolerances have been found in widely different taxa but restricted to habitats where organisms are exposed to such factors (although current data on this are admittedly rather limited) suggests convergent adaptive evolution (Hinton 1968). Whether the cryptobiotic state per se should be considered an adaptation promoted by natural selection is much less clear. I will touch on this problem more when I discuss the benefits of a complete metabolic arrest as opposed

to partial arrest. For now I conclude that tolerance to the factors that induce the cryptobiotic state must plausibly be considered adaptations, although more in-depth analyses of selection processes in the past would be desirable. It is also believed that tolerance to desiccation and anoxia played a crucial role in the assumed evolutionary transition of some ancestral marine invertebrate forms to their terrestrial descendants (May 1951; Pilato 1979).

Even if tolerance to naturally occurring factors inducing cryptobiosis does not provide a problem in light of adaptation theory, the extreme resistance to environmental factors far beyond their natural ranges (discussed subsequently) seems to provide a challenge. The apparent overadaptation of cryptobiotic organisms has received some attention from popular science (Copley 1999; see discussion in Jönsson and Bertolani 2001). Creationists have also readily adopted it as evidence against the theory of evolution by natural selection (Vetter 1990). However, as will be concluded, although ECR cannot be considered an adaptation, it may nevertheless be understood and explained by genetic and physiological mechanisms within current evolutionary biology.

Excess Resistance in Cryptobiotic Metazoans

The ability of some micrometazoan groups to survive biologically extreme conditions while in a cryptobiotic, mainly desiccated, state is well documented. These studies include exposure to biologically extreme high (>100°C) and low (down to -272°C) temperatures (Doyère 1842; Broca 1860; Rahm 1921; Becquerel 1950; Hinton 1951, 1960; Nakanishi et al. 1963; Skoultchi and Morowitz 1964; Iwasaki 1973; Ramløv and Westh 1992), vacuum (Rahm 1921; Crowe 1975), pure alcohols (Hinton 1968; Ramløv and Westh 2001), high levels of ionizing radiation (May et al. 1964), pesticides (Freckman et al. 1980; Jönsson and Guidetti 2001), and high hydrostatic pressures (Seki and Toyoshima 1998). In addition, cryptobiotic organisms have proved to survive in their inactive state for considerably longer periods than experienced in their natural habitats (Steiner and Albin 1946; Fielding 1951; Clegg 1967; Sømme and Meier 1995; Jönsson and Bertolani 2001; Guidetti and Jönsson 2002).

These observations clearly establish that while in the cryptobiotic state, organisms reach a resistance to environmental conditions that goes far beyond those experienced under natural conditions. Although most documentation of ECR originates from desiccated (anhydrobiotic) organisms, in some cases ECR seems to exist also in the hydrated state, as documented by some cryobiological studies (Sayre and Hwang 1975; Ramløv and Westh 1992), where hydrated tardigrades were shown to survive exposure to -196°C.

Optimal Phenotypes and the Problem of Excess Capacities

Neither the historical nor the nonhistorical definitions of an adaptation seem to allow us to view ECR as an adaptation. Even if the abiotic conditions under which cryptobiotic organisms have lived and evolved in the past were different from conditions today, the levels of temperature, radiation, and other conditions that these animals seem to survive are far beyond the levels that these factors may have reached in the past. Also, because such conditions do not exist today, ECR cannot have a fitness value in current populations. Consequently, considered as a separate phenotypic trait, ECR must be classified as a nonadaptation (or nonadaptation *sensu* Gould and Vrba 1982).

Excess capacities in performance, such as ECR, are not generally expected features of organisms that have been shaped by natural selection. This is because the potential for excess capacity will generally require more energy to maintain than a capacity corresponding to the actual needs, and therefore imposes an energetic cost. This reasoning is inherent in the hypothesis of structural symmorphosis (Taylor and Weibel 1981; Linstedt and Jones 1990) in optimally designed organisms. Symmorphosis suggests that natural selection should favor a close fit between organism structure and functional requirements and disfavor costly excess structures. Another case in which the problem of excess capacities has been discussed concerns the sophisticated intellectual capacities of humans, such as complex mathematical understanding, which is hard to consider a result of natural selection (Sober 1984). The problem was discussed already by Darwin (in a dispute with A. R. Wallace), who proposed that such apparent excess capacities could be by-products of selection for simpler mental abilities that were the actual target of selection (Gould 1980). This kind of explanation may represent a viable candidate also to the ECR problem, as will be discussed subsequently.

In an evolutionary context, excess capacities represent a problem only if such capacities are connected with some fitness costs. Without any costs, energetic or any other kind, natural selection will not act against excess resistance. It may then persist in a population by a linkage or pleiotropic relationship with another trait under selection. The problem with cost-free capacities, however, is that we seldom consider structures and capacities as free of costs.

Capacities of organism function above those normally used may be incorporated within an adaptive explanation if they represent safety margins (Alexander 1982; Linstedt and Jones 1990). When the fitness costs of a functional failure are very high compared with the costs of keeping an excess capacity, natural selection may favor a considerable margin of safety (Williams 1992). This may apply to cryptobiotic resistance, since failure to provide secure survival in a periodically unfavorable environment would be devastating to a cryptobiotic metazoan. A reasonable criterion, however, for considering a high level of capacity as a safety margin is that the level of

capacity should not largely exceed the natural level in the environmental factor. This criterion is not fulfilled in the case of cryptobiosis. The levels of resistance reported in several cryptobiotic organisms are far beyond the environmental levels ever found in nature, so there is no support for the safety margin explanation of ECR.

ECR as an Evolutionary Spandrel

Could ECR be an evolutionary spandrel *sensu* Gould and Lewontin (1979), that is, a by-product of the evolution of another adaptive trait? This view was hinted at by Crowe (1971) and was discussed more thoroughly by Puchkov (1988). According to Puchkov (1988), selection of mechanisms that increase the resistance to naturally occurring conditions (e.g., desiccation) may simultaneously give rise to resistance against other unnatural conditions. No special explanations are therefore required to explain excess resistance. There are currently very few other studies specifically addressing this question for cryptobiotic metazoans, but evidence available from some other organism groups does suggest that resistance to nonnatural conditions may in fact be functionally and genetically correlated with traits that are of importance for fitness. Three supposedly general characteristics of cryptobiotic organisms may be of particular interest in this respect: metabolic arrest, desiccation tolerance by cell stabilization mechanisms, and DNA repair mechanisms. The first two characteristics relate to resistance against cell damage and the last one to postcryptobiotic repair of damage. In addition, the severe loss of water exhibited by anhydrobiotic organisms may in itself lead to a high resistance against potentially damaging conditions mediated by the presence of water.

Resistance to one kind of stress is often positively correlated with resistance to other stress factors. For instance, Hoffmann and Parsons (1993) have shown that strains of *Drosophila melanogaster* selected for increased adult desiccation resistance (a trait of supposedly adaptive value) also have increased resistance to toxic ethanol levels. Similarly, desiccation-tolerant plants also show tolerance to various other environmental stresses (Alpert 2000). Such associations in resistance may arise from a common mechanism, such as reduced metabolic rate, as discussed by Hoffman and Parsons (1991). In cryptobiotic organisms, metabolism is completely arrested, and any factors of stress that rely on metabolic activities will have no effects on the animal. For instance, fumigants such as methyl bromide, which imposes its toxicity by affecting the respiration system of the animal, have little effect on anhydrobiotic tardigrades (Jönsson and Guidetti 2001). Thus, metabolic arrest *per se* may explain excess resistance to some environmental factors.

Hochachka and Guppy (1987) discussed the dual effects that metabolic arrest has on an organism: not only does it lead to a protection against unfavorable environmental conditions but also it increases the organism's biological time relative to as-

tronomic time. There are several important implications of this. One is that with reduced metabolic rate, the time over which a given amount of stored energy may support the organism's metabolic processes increases. Thus the energetic costs of surviving the unfavorable period decline with the degree of metabolic arrest. Another implication is that if the rate of senescence increases proportionally with metabolic rate (Finch 1990), a reduction of metabolism during inactive, nonreproductive periods will increase the effective (reproductive) life span of the organism. An interesting perspective then is that if a period of reduced metabolic rate is a necessity due to hazardous environmental conditions, natural selection should favor as low a metabolic rate as possible. This conclusion may be formulated more generally: once reproduction and development are prohibited due to environmental factors, natural selection should favor a slowdown of metabolism during the period of nonreproduction or inactivity.

In cryptobiotic organisms, the metabolic arrest has in fact been taken to its limit. Was this necessary for surviving the naturally occurring harsh conditions? If this question can be answered in the negative, then ECR could indeed be a by-product of direct selection for reduced metabolic rate.

Another field of research that strongly relates to cryptobiotic organisms is the study on stabilizing mechanisms of dry cells (Crowe et al. 1997). This field originated from work on anhydrobiotic organisms such as crustacean embryos (Clegg 1962), nematodes, and tardigrades (Crowe and Madin 1974; Madin and Crowe 1975) but has then developed into a large research area with its main applications within the medical and food industries (Tablin et al. 2001). However, the relevance of this research for understanding natural populations of anhydrobiotic organisms is obvious. How do anhydrobiotic organisms manage to protect their cells with sometimes less than 1% water in the body? According to the water replacement hypothesis (Clegg 1986; Crowe et al. 1998b), polyhydroxy compounds replace the structural water in membranes, DNA, and proteins and maintain a stable cell structure throughout the dry phase. Disaccharides seem to be especially important in this respect, and trehalose in particular has proved to be one of the most efficient membrane stabilizers (see reviews in Crowe et al. 2001 and Crowe 2002). Trehalose is produced by many anhydrobiotic organisms at the induction of the dry state and sometimes reaches levels of 20% dry weight (Madin and Crowe 1975; Westh and Ramlöv 1991; Crowe et al. 1998a). Trehalose also forms a glassy (amorphous) state at low water contents under natural temperature conditions, which may provide additional advantages for dry cells in terms of structural stability (Sun and Leopold 1997; Crowe et al. 1998a, 1998b). The stability against dehydration provided by polyhydroxy compounds may, as a "by-effect," also protect the cell from other potentially damaging factors. In the desiccated state, the animal has withdrawn into a "biological crystal" (Ramlöv and Westh 2001) that escapes the impact of many environmental agents.

Efficient DNA repair may be another important factor explaining ECR. Studies in the radioresistant bacterium *Deinococcus radiodurans* have shown that resistance to ionizing radiation is functionally linked to tolerance to desiccation (Mattimore and Battista 1996; Battista 1998). The important factor behind tolerance to desiccation in *D. radiodurans* is an amazing ability to repair the DNA damage that results from dehydration and long-term storage in the dry state. This repair capacity apparently also explains how *D. radiodurans* is able to cope with massive doses of ionizing radiation.

Even though there currently seem to be no corresponding studies on DNA repair in cryptobiotic micrometazoans, it is tempting to speculate that similar mechanisms as documented in *D. radiodurans* for radioresistance may underlie the excess resistance of tardigrades, rotifers, and other cryptobiotic metazoans. It is well established that the time for recovery after a period of cryptobiosis in micrometazoans is positively correlated to the time the organism has been inactive (Jacobs 1909; Baumann 1922; Crowe and Higgins 1967; Bhatt and Rhode 1970). A similar lag phase in recovery is also observed in *D. radiodurans* and is positively related to the level of irradiation, or time in a desiccated state, that the bacterium has experienced (Battista 1998; Battista et al. 1999). This lag phase probably arises as a consequence of DNA repair processes (more damage requires more time to repair) and thus may be explained by the same mechanism in both bacteria and metazoans. An efficient system for DNA repair provides the organism with a general and efficient tool for coping with cell damage arising from a variety of environmental factors.

Which of the previously described characteristics of cryptobiotic organisms is most important in explaining ECR remains to be evaluated. The importance of a specific mechanism will naturally also depend on the factor of stress. It is not unlikely, however, that ECR may arise as the combined effects of dehydration, metabolic arrest, cell stabilization, and efficient postcryptobiotic DNA repair.

Most studies on ECR have been done on organisms in anhydrobiotic (dry) states. Studies on resistance of cryptobiotic organisms in nondesiccated states (e.g., in cryobiosis or anoxybiosis) will be important to reveal the extent to which desiccation per se contributes to documented ECR. Long-term anoxybiosis under hydrated conditions lasting for several years or decades has been documented in embryos of copepods and in the brine shrimp (Marcus et al. 1994; Hairston et al. 1995; Katajisto 1996; Clegg 1997, 2001). In the brine shrimp (*Artemia franciscana*), a heat shock protein (p26) seems to be involved in providing structural stability of the anoxic animal (Jackson and Clegg 1996; Clegg 2001). The extent to which these organisms are able to cope also with nonnatural stress, such as high doses of ionizing radiation, in the anoxybiotic state apparently has not been studied.

Even if we accept the hypothesis that ECR has evolved as a by-product of selection for more modest levels of resistance,

the question remains whether or not ECR is a cost-free by-product. This question may be formulated more precisely: Does the ECR impose a cost above that which is necessary to obtain the normal (adaptive) level of cryptobiotic resistance? If this question can be answered in the negative, then ECR has no cost. It is important, however, to remember that in evaluating this question, it is not the energetic costs of actually obtaining (physiologically) a resistance to, say, -200°C (e.g., in terms of the postcryptobiotic DNA repair processes required) that are at stake because these costs are never imposed under natural conditions. Instead, it is the costs of keeping a potential for ECR, without having a use of it, that are relevant.

ECR and the Adaptability of Cryptobiotic Organisms

The extreme resistance against a wide range of extreme physical environments means that cryptobiotic metazoans have a large potential for persisting under dramatically changed environmental conditions. The adaptability (Dobzhansky 1968; Endler 1986) of these organisms is therefore very high with respect to various environmental factors, and ECR in the future could become an exaptation sensu Gould and Vrba (1982), a trait that did not arise by natural selection but that turned into an adaptation by selection later on. However, it is important to remember that survival through unfavorable conditions is not a sufficient condition, although it is a necessary one, for persisting cryptobiotic populations. Regular periods of active life during which new individuals are entered into the population by reproductive processes are necessary, since cryptobionts do not have an eternal life in the inactive state. If the environmental conditions in which the fitness benefits of ECR could be realized will be accompanied by more harsh conditions also for active life, the evolutionary importance of ECR as a component of adaptability may be limited.

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