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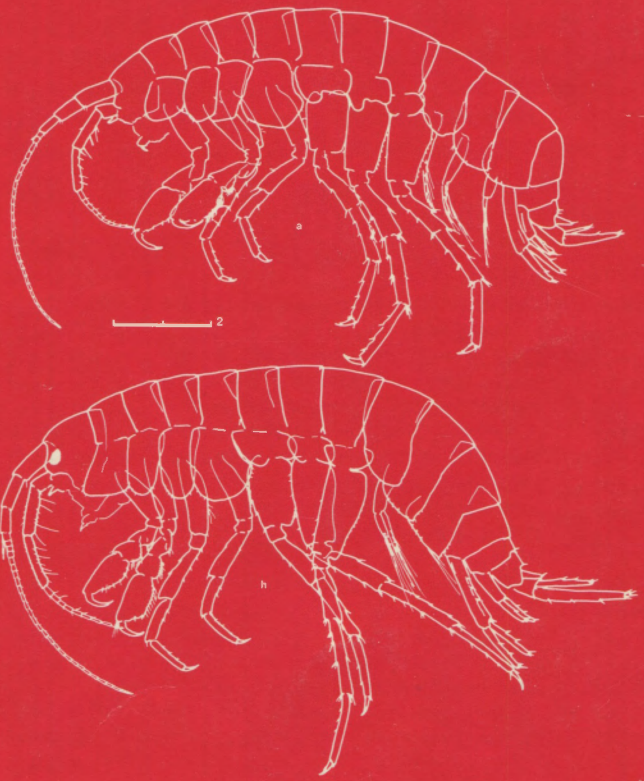

Special Issue
Regressive Evolution

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Cover: Two troglobitic *Gammarus spp.* from
Macedonia, Yugoslavia (see Sket, page 82).

H. H. Hobbs III

INTRODUCTION

The most striking feature of cave organisms, from the point of view of both biologists and sport cavers, is not what they have, but rather what they have lost. Although there are exceptions, species isolated in caves for long periods of time have greatly reduced eyes and pigmentation. The subject of this symposium is how cave organisms came to lose these and other features in the course of evolution. This phenomenon is often termed regressive evolution, or structural reduction.

Regressive evolution is not limited to cave environments. Eye reduction is well known in other aphotic environments such as the deep sea (Poulson, 1971), but other examples of structural reduction are common in more "normal" habitats. These include limb loss in snakes and digit reduction in horses. J.B.S. Haldane, one of the founders of mathematical population genetics, pointed out that for every case of progressive evolution in the sense of descendents being more complex in structure and behavior than their ancestors, there have probably been ten of regressive evolution (quoted by Kimura, 1983). Thus the problem of regressive evolution is of interest to evolutionary biologists in general.

The controversy concerning regressive evolution concerns the role that natural selection plays in the process. Some ascribe to natural selection a major role, while other ascribe a major role to genetic drift and the accumulation of selectively neutral, structurally reducing mutations. Anyone looking to a resolution of this controversy in these pages will be disappointed. A wide variety of contradictory viewpoints are taken up, sometimes with a polemical tone. This is all to the good. Controversy indicates a healthy, active area of research for which many questions remain unanswered.

The papers in this issue can be conveniently divided into three groups—overviews, tests of hypotheses, and case studies. Kane and Richardson provide an important historical perspective to the controversies of regressive evolution, a welcome antidote to the usual scientific view that any work more than a year old is irrelevant. Sket takes a fresh look at the entire selection—neutrality controversy, and Romero points out some of the perhaps unwelcome connotations of the phrase "regressive evolution."

The next three papers by Christiansen, Wilkens, and Poulson, test neutralist and selectionist hypotheses. Wilkens and Poulson feel that cave fish data generally support a neutralist view, but Christiansen, in reviewing the data on cave Collembola, argues that no one theory can explain all the observed patterns. Magniez provides important evidence that regulatory genes, especially those that affect the time of appearance of a trait, are an important mechanism in regressive evolution. Fong and Culver assess the importance of differential migration into caves of eyed and eyeless forms in the same population, a hypothesis championed by Ludwig in the 1940's.

Finally, there are three major reviews of the data on regressive features of cave animals. Parzefall reviews the work on the genetics and reduction in certain aspects of behavior in cave organisms. Huppop looks at the data on metabolic reduction in cave animals. Finally, Lamprecht and Weber present a thorough review of the reduction of clock mechanisms in cave animals. These last three papers are especially important because they expand the range of characteristics of cave animals that need to be considered when discussing regressive evolution.

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REGRESSIVE EVOLUTION: AN HISTORICAL PERSPECTIVE

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Explanations of the evolutionary causes for the reduction or loss of eyes and pigment in cave animals have been controversial since the time of Lamarck and Darwin. The absence of any clear selective advantage to such reduction has led Biospeleogists to consider a variety of mechanisms underlying their existence. During the latter half of the nineteenth century, regressive evolution was used as strong evidence supporting the neo-Lamarckian theory of the effects of disuse and the inheritance of acquired characteristics. At the beginning of the twentieth century, the establishment of Mendelian mechanisms of inheritance undermined this theory, but left evolutionary biologists lacking a mechanism for regressive evolution. Although the neo-Lamarckian hypothesis is no longer tenable, modern theories of regressive evolution—including appeals to energy conservation, indirect effects of pleiotropy, and the accumulation of neutral mutations—can all be seen as developments which emerge as answers to problems formulated in the nineteenth century, and which have nineteenth century precursors.

INTRODUCTION: TROGLOMORPHY AND REGRESSION

American biology in the latter half of the nineteenth century has a decidedly Lamarckian cast. This is due, in part, to attention paid to the fauna specialized for cave life, and, most notably, to the fauna of Mammoth Cave. Observations of troglobites were taken as a paradigm revealing the effect of the environment on organisms as well as the mechanisms of speciation. As A.S. Packard (1839-1905), one of the leading neo-Lamarckians, remarked, "the main interest in the . . . studies on cave life centers in the obvious bearing of the facts upon the theory of descent" (1888, p. 116).

The morphological pattern of troglobites—what biospeleogists often call "troglomorphy"—is striking and simple. Four characteristics are especially noteworthy: there is a loss of pigment; a diminished size for the eyes, with a loss of visual ability; an attenuation of appendages, with hypertrophy of other non-optic sensory organs; and finally, a reduced metabolic rate. Though the pattern is prototypical of troglobites, it is not universal. Some species of the scavenger beetle *Ptomaphagus*, known only from caves, are both eyed and pigmented. The Mexican cave fish *Astayanax mexicanus* exhibits considerable diversity of form. Yet others, e.g. those feeding on bat guano, fit the profile even less well (Culver, 1982). The pattern is nonetheless a common one, and it is reasonable to require that an adequate theory of 'regressive' evolution should explain why the troglomorphic pattern is so common, and not merely how it could come about.

There is little reason to think that all the facets of the troglomorphic pattern should receive a similar explanation. Some are likely to be adaptations, such as the reduced metabolic rate. Some may not be. The most striking troglomorphic traits were held during the nineteenth century to be the consequence of "disuse": reduced visual capacities were a consequence of the fact that eyes are of no use to troglobites. By the twentieth century, appeals to disuse had fallen from favor, largely because the rediscovery of Mendel's results was taken to threaten the associated doctrine of the inheritance of acquired characteristics. In the early decades of the twentieth century, adaptationism—the view that all traits are adaptations wrought by natural selection—gained in prominence. It became fashionable, accordingly, to seek out selectionist explanations of troglomorphic traits. The possibility of appeal to non-selectionist origins for troglomorphic traits has only recently been reconsidered by Wilkens (1971) and Culver (1982).

It is our intention here to provide an historical setting for the continuing controversies over 'regressive' evolution, or what has otherwise been called "degenerative" evolution, and "rudimentation." The literature on the topic is vast and, as can be seen from other papers in this issue, the arguments are still ongoing. We will concentrate on the controversy as it developed in the latter half of the nineteenth century, with particular emphasis on the contributions of neo-Lamarckians in North America.

THE 'ORIGINS' OF REGRESSIVE EVOLUTION

The place to begin is with Charles Darwin (1809-1882). If there are two things characteristic of Darwin, they are, first, his commitment to natural selection as the mechanism of speciation, and, second, his willingness to invoke alternative mechanisms whenever it was necessary to do so. In a passage from *On the Origin of Species* that is as often cited as ignored, he wrote:

> I am fully convinced that species are not immutable; but that those belonging to what are called the same genera are lineal descendants of some other and generally extinct species. . . . Furthermore, I am convinced that Natural Selection has been the main but

> not exclusive means of modification (1859, p. 6).

Natural selection, or differential survival based on "favourable variations," will tend to increase the level of adaptation of species, and simultaneously will tend to eliminate intermediate forms. The result will be divergence, and the creation of new species. Though Darwin never doubted the power of natural selection, he more than once introduced alternative mechanisms—among them sexual selection, use and disuse, as well as what he called "correlation of growth." He wrote, in a discussion of the effects of use and disuse:

> . . . there can be little doubt that use in our domestic animals strengthens and enlarges certain parts, and disuse diminishes them; and that such modifications are inherited. Under free nature, we can have no standard of comparison, by which to judge the effects of long-continued use or disuse, for we know not the parent forms; but many animals have structures which can be explained by the effects of disuse (1859, p. 134).

In a pattern of reasoning that was, as we will see, common among naturalists of the nineteenth century, Darwin explained rudimentation in four steps: immigration, degeneration, inheritance, and compensation (see Table 1). *Immigration* involves the influx of surface forms into the cave habitat. Darwin says little about the causes of migration into caves, and does not say whether it is simply by chance or to gain some advantage. *Degeneration* follows when, within the environment of the cave, lack of use for visual organs results in their reduction. Darwin saw this as a gradual process, taking a number of generations, with intermediate "twilight" forms being incipient troglobites. The *inheritance* of these environmentally induced reductions insures that they are transmitted to offspring and is a prerequisite for their evolutionary significance. While Darwin rightly emphasized that uninherited variation is unimportant, he had an overwhelming confidence that almost everything is inheritable, suggesting we should "look at the inheritance of every character as the rule, and non-inheritance as the anomaly" (1859, p. 13). These reductions will, finally, be *compensated* for by means of an increase in other sensory organs. Darwin attributed this compensation to the action of natural selection.

Though Darwin did not apply the explanations to the evolution of troglomorphy, he simultaneously set the pattern for the principal competitors to rudimentation due to disuse. First, he appealed to "correlation of growth," which includes both pleiotropy and allometry. The genetic structure and dynamics of development impose limitations on what nature can produce (see Gould, 1980). Second, he appealed to immediate selective advantage. As with many more obviously adaptive traits, rudimentation (Darwin's term for degenerative evolutionary change) is in some cases an advantage, as in flightless birds and wingless beetles living in habitats in which flight is dangerous (1859, pp. 135-136). Third, there are indirect selective advantages, due to the principle "that natural selection is continually trying to economize in every part." Economizing in resources spent on one structure allows those resources to be utilized elsewhere, and this economizing in investment in unused structures leads to their diminished size (1859, pp. 147-148).

Even though Darwin always favored explanations in terms of natural selection when they were available, he did not make any appeal to natural selection in explaining rudimentation in cave animals; rather, he favored disuse and the inheritance of acquired characteristics, for the reason that he could see no evident disadvantage to the possession of eyes or pigment in a cave enivonment, and could see no grounds for invoking correlation of growth as an explanation for their loss.

(1) IMMIGRATION: migration of surface forms into the cave habitat

(2) DEGENERATION: lack of use for visual organs results in their atrophy and underdevelopment

(3) INHERITANCE: environmentally induced reductions are transmitted to offspring

(4) COMPENSATION: hypertrophy of tactile and olfactory organs

Table 1: The Darwinian schema for regressive evolution, or rudimentation due to disuse.

Following the publication of *On the Origin of Species* in 1859, evolution and the transmutation of species came to be widely accepted, and controversies focused on the mechanisms of evolution and speciation. The differences between protagonists are unquestionably ones of emphasis rather than principle, but the varieties of evolutionists can be divided, for convenience sake only, into roughly four competing camps (Fig. 1). The more orthodox Darwinians, such as T.H. Huxley, Francis Galton, and George Romanes most nearly followed Darwin. They were united in emphasizing the importance of natural selection, though not wholly dispensing with secondary mechanisms. Their principle differences came in disputes over the nature of secondary mechanisms. Galton attempted one of the few experimental evaluations of the inheritance of acquired characteristics, with negative results. Huxley favored discontinuous genetic change. The Ultra-Darwinians such as A.R. Wallace and August Weismann embraced "panselectionism," altogether denying the importance of secondary mechanisms. Weismann's experiments had convinced him that inheritance of acquired characteristics was illusory. Wallace's extensive work with natural populations convinced him that even Darwin underestimated the effectiveness and importance of natural selection. Accordingly, Wallace wrote,

> . . . none of the definite facts of organic nature, no special organ, no characteristic form of marking, no peculiarities of instinct or of habit, no relation between species or between groups of species . . . can exist, but which must now be or once have been *useful* to the individuals or the races which possess them (1891, p. 35).

No room is left for secondary mechanisms, and the very possibility of non-adaptive characters is denied. When confronted with any trait, we are left with a choice between regarding them as progressive changes or as "less obviously" adaptive. If a selective advantage is not apparent, we are counseled simply to go on looking.

Just as Darwin was not the first evolutionist, the varieties of evolutionism were not restricted to selectionists of either sort. In fact, the most prominent challenges came from the followers of Jean Baptiste de Lamarck (1744-1829). In his epochal work *Philosophie Zoologique* (1809), Lamarck used two mechanisms to explain observed patterns

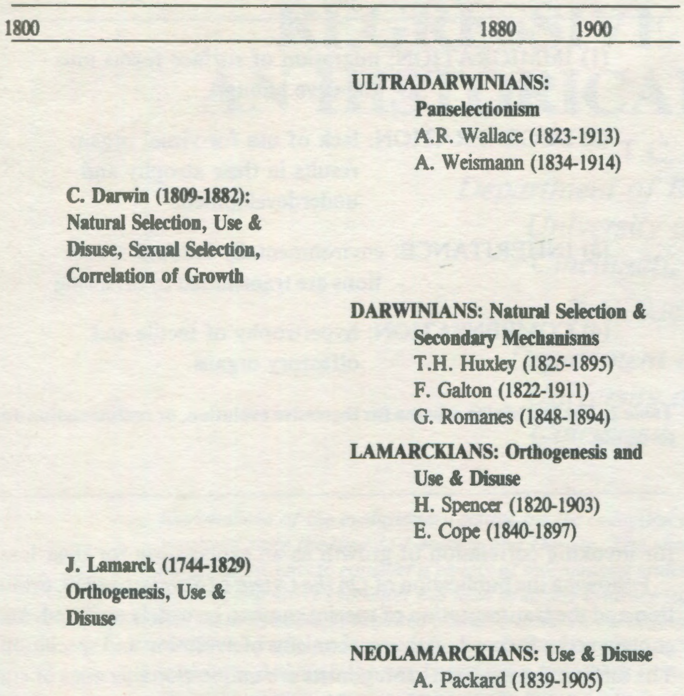

Fig. 1: Schools of Evolutionary Thought in the 19th Century.

in the animal kingdom. The first was orthogenetic: there was an inherent drive toward "perfection" within lineages. In Lamarck's view, spontaneous generation was a continuing process responsible for the production of the simplest living bodies. Once produced, this new life tended by its own nature to progress to a higher level of organization. The orthogenetic trend was held to be responsible for the creation of special organs and the isolation of their functions. Reproduction permanently preserved these perfected structures. This force of Lamarckian evolution is both progressive and unidirectional, moving from the simple to the complex.

The inherent tendency toward perfection was intended to explain the general pattern of continuously increasing complexity observed as one moves from the most "imperfect" to the more "perfect" organisms. It could not, however, explain why these gradations in complexity were frequently irregular, containing deviations and anomalies, in which no order was apparent. The second mechanism incorporated into Lamarckian evolution was to explain just this part of the evolutionary pattern. These are the induced modifications due to use and disuse. Lamarck supposed that "the environment affects the shape and organization of animals" (1809, p. 107). But whereas Darwin's appeal to use and disuse gave them a direct action on the organism, Lamarck held that their action was indirect: changes in the environment could produce changes in the needs of these organisms, which in turn engendered changes in their activities. Should those needs become permanent, the new habits produced would often result in changes in the need and use for the various parts. New needs thus may bring about new structures and new uses for old structures. Conversely, changes in environment or in habit may result in the total disuse of other parts which are no longer necessary, resulting in a gradual reduction of them, and ultimately in their disappearance. Necessity is the cause for the disappearance, as well as the appearance, of structures.

Though use and disuse have parallel underlying etiologies, with both relying on the induction of needs, adaptation is produced primarily through the use of structures leading to their modification. The disappearance of structures from long term disuse, for Lamarck as for Darwin, is in the main adaptively neutral. It is thus the appeal to accentuation and emergence from use, rather than degeneration from disuse, that competes with the selectionist explanations of Darwin and his followers. The appeal to disuse is common to both Darwin and Lamarck.

Lamarck, too, says that changes produced by changes in habit must be inherited if they are to be important for evolution. The recognition of the hereditary nature of induced changes is clearest in Lamarck's "Second Law": "All the acquisitions or losses wrought by nature on individuals, through the influence of the environment . . . all these are preserved by reproduction to the new individuals which arise" (Lamarck 1809, p. 113). This principle, embracing the inheritance of acquired characteristics, is of a piece with Darwin's commitment to a "strong" principle of inheritance: both Darwin and Lamarck held that environmentally induced changes are transmitted to offspring.

Changes in the environment leading to disuse and degeneration provided Lamarck with an explanation for the anomalies of eye reduction in moles and the complete loss of sight in the Yugoslavian cave salamander *Proteus*. The degenerate form is an anomaly, running contrary to the orthogenetic law; both belong to a level of organization in which eyes are a natural part. Yet we do not quite have what is properly *regressive evolution*; for the loss of these structures from their permanent disuse does not result in an overall simplification of the organism's structure, or any regression on the "great chain of being." Similarly, Darwin's appeal to disuse in explaining troglomorphy should not be thought of as a regressive change since, unlike Lamarck, Darwin has no direction for evolutionary change, and can countenance no distinction between progressive and regressive evolution.

THE RESURGENCE OF LAMARCKISM

The latter half of the nineteenth century saw a resurrection of the Lamarckian theory. This was largely a response to perceived difficulties with Darwinism (cf. Moore, 1979). In Britain, the pre-eminent Lamarckian was Darwin's ally, Herbert Spencer (1820-1903). In the United States, the leadership was assumed by Alpheus Hyatt (1838-1902), Alpheus Packard (1839-1905), and Edward Drinker Cope (1840-1897). Initially, this tradition relied heavily on Ernst Haeckel's Biogenetic Law as the driving force of evolution and was accordingly orthogenetic in character. Most of evolution was seen to be progressive and new characters were thought to arise independently of their adaptive nature. By the 1880's, a decided shift from orthogenesis occurred, together with an increased emphasis on the inheritance of acquired characteristics (Gould, 1977). We have again distinguished the Lamarckian from the neo-Lamarckian positions as if it were a principled distinction, even though it too is a matter of emphasis. Neo-Lamarckians are notable for having a greater significance attached to the effects of use and disuse, together with the inheritance of acquired characteristics. Their emphasis was accordingly on adaptation rather than orthogenesis. (As an historical matter, however, many nineteenth century biologists began as Lamarckians, and gradually shifted to a more neo-Lamarckian position later in the century.) Lamarckians and neo-Lamarckians of the later nineteenth century embraced a saltational view, according to which macromutational and dramatic changes were the substance of evolutionary change. This contrasted with the gradualism—the appeal to small and continuous variations—which found its home in Lamarck and Darwin alike. The shift to a saltational view was intended, in large part, to accelerate the amount of variation and thereby to increase the rate of evolutionary change. This would allow the new evolutionists to accomodate the relatively low estimates for the age of the earth which were promoted by William Thomson Kelvin.

Cope, the renowned paleontologist and dabbler in biospeleology, became a leader of North American Lamarckians in the 1870's (for a synoptic expression of his views, see Cope, 1896). He relied heavily on Haeckel's view that ontogeny recapitulates phylogeny, maintaining that all *progressive* evolutionary changes are the result of an acceleration of development. As forms progress evolutionarily, they assume characters at an earlier stage of development which were acquired earlier during their phylogenetic history. Characters attained at an early stage of development reflect those attained by distant ancestors. Traits acquired later in development reflect those modifications gained by more recent ancestors. Retardation of development was also possible under Cope's scenario, and could result not only in a failure to accumulate new characters, but also in a loss of characters previously possessed. The result of this retardation was retrogressive evolution, and a reduction of organic structures and complexity. Evolutionary changes can thus be either progressive or regressive. Eye loss in the cave fish *Typhlichthys* and in the cave crayfish *Orconectes* was explained in terms of retardation. Cave animals were seen, in successive generations, not to develop to the same level of complexity as their ancestors; more specifically, they lose characters which otherwise arise late in development, and which are, correspondingly, relatively recent phylogenetic novelties. However, slower growth, resulting from retardation could not by itself account for the *disappearance* of the visual organs. Since eyes arise early in development, retardation can only produce reduction in these structures. Cope contended that the loss of structures normally acquired early in development such as eyes was a consequence of "retardation and atrophy" (Cope, 1872 p. 417). The attenuated growth rate caused by retardation would diminish the size of the eyes, but they would still persist into maturity. Retardation would become exaggerated in subsequent generations, causing growth in the affected organ (specifically, the eyes) to cease, while growth continued normally in other organs. Once retardation had slowed development sufficiently, atrophy of the organ would occur, and the resources normally used in its development could be diverted.

Cope opposed the Darwinian emphasis on natural selection, on the ground that it could account only for the preservation of some variations in the struggle for existence, and was not an explanation of the origin of variations. According to Cope, the environment could *directly* cause variations in organisms, and could also produce variations *indirectly*, through the influence of habit. It is true that natural selection could not explain the origin of variations; indeed, Darwin intentionally decoupled the principle of natural selection from his speculations concerning inheritance and variation. Since Darwin allowed that the "laws governing inheritance are quite unknown" (Darwin, 1859, p. 13), and professed little confidence concerning the origins of variation or the means of transmission, Cope's is hardly a just complaint.

What is noteworthy are Cope's differences with Lamarck. Cope's theory, though committed to orthogenesis, differs substantially from Lamarck in the treatment of this component of Lamarckian theory. The law of acceleration and retardation, together with Haeckel's Biogenetic Law, give a direction to evolution, but also allow change to occur in the direction of increasing or decreasing complexity. Thus, Cope could allow evolution to be either progressive or retrogressive. This was not his only difference with Lamarck. Both men embraced some appeal to the mechanisms of use and disuse, but Cope, unlike his predecessor, allowed for the direct induction of variations. Later in his career, Cope (1880) was to place more reliance on the effects of use and disuse, and on the inheritance of acquired characteristics, as the driving force of evolution. In doing so, he was able to accomodate the adaptational component of Lamarck's theory. By this time, however, Cope was deeply immersed in paleontology and had ended his brief excursion into the evolution of cave animals. Cope accordingly made no strong appeal to adaptive explanations for the evolution of troglomorphy.

The line of demarcation between Lamarckians and neo-Lamarckians is not a sharp one, and, like that within the Darwinian camp, is finally one only of degree; moreover, the picture is complicated by the fact that individuals such as Cope gradually altered their views, becoming increasingly distant from Lamarckian commitments. It is useful to think of neo-Lamarckism as differing most crucially from strict Lamarckism in placing its emphasis on adaptation. The effects of use and disuse and, correspondingly, the inheritance of acquired characteristics are seen as the critical mechanisms of evolutionary change. Orthogenetic commitments have a secondary place, when accorded one at all.

A.S. Packard's version of neo-Lamarckism is founded on studies of cave fauna, undertaken in collaboration with F.W. Putnam (Putnam and Packard, 1871; Packard, 1871; Putnam, 1872). Packard recognized clearly the troglomorphic pattern, and contrasted it with the pattern exemplified in species such as *Hadenoecus*, the cave cricket, pointing out that this species is not troglobitic, and so should not be expected to exemplify the troglomorphic pattern fully (cf. Packard, 1888, p. 141). He also saw that the troglomorphic pattern allowed for considerable variation. He recognized three main variations in the pattern, which he took to betoken different lengths of time in the cave environment.

> (1) There can be atrophy of the optic lobes and optic nerves, while retaining eyes in at least a rudimentary state. *Adelops*, for example, suffers a reduction in the number of facets with a degeneration of central structures.
>
> (2) There can be atrophy of the visual organs, while retaining the optic lobes and optic nerves. *Orconectes* and *Cambarus* were originally described by George Newport as exemplars of this pattern.
>
> (3) There can be atrophy of both the central structures and the peripheral organs. The arthropod *Scoterpes* and cave trechine beetles are included in this category.

Despite the variations available in the troglomorphic pattern, Packard offered a uniform explanation within a neo-Lamarckian mold. What he says is this:

> Certain Aselli [an isopod] borne into caves or introduced into subterranean streams feeding deep, dark wells, losing the stimulus of light, begin to lose their eyes and the power of sight. The first step is the decrease in the number of facets and corresponding lenses and retinae; after a few generations—perhaps in four or five—the facets become reduced to only four or five;

> the eye is then useless; then all at once, perhaps after only two or three generations, as a result of disuse, there is a failure in forming images on the retina, and those complicated, elaborate structures, the optic ganglia and optic nerves, suddenly break down and are absorbed, though the external eye still exists in a rudimentary state. The imperfect lenses and retinae, like all rudimentary organs throughout the animal world, are like ancient, decayed sign-posts, pointing out some nearly obliterated path now unworn and disused. The result of change of environment, with disuse and atrophy of the organs of vision, together with the inheritance of these defects and their establishment as fixed specific and even generic characters, results in the creation of a new natural genus with its assemblage of species (1888, p. 118).

The explanation of degenerate forms is a variant on the familiar one which we first saw in considering Darwin's appeal to disuse. There is, first, an immigration into the caves or subterranean streams. This results in an isolation which promotes rapid speciation (cf. Packard, 1888, pp. 131 & 140). There is, second, a degeneration because of lack of use. Packard says "it may be laid down as an axiomatic truth that where eyes are defective or atrophied, it is owing to disuse induced by physical surroundings of such a nature as to enable the animals in question to dispense with organs of sight" (1888, p. 132). The initial degeneration subsequent to immigration is explained, in a format due to Richard Owen (1804-1892), as a developmental effect: Individuals born into subterranean habitats lack the stimulation for the development of visual organs, and they are thereby retarded. The result would be a rapid alteration of the visual system rendering it nonfunctional. These developmental defects are then inherited in accordance with Lamarck's second law, and can become established as specific characters. In the final step, there is compensation for the loss of vision: "the atrophy of the eyes and consequent loss of vision have been made up, in part at least, by a corresponding hypertrophy of the organs of touch and smell" (Packard, 1888, p. 123). This can take remarkable forms, as in *Amblyopsis*, one of what Putnam called "the blind fishes of Mammoth cave," which has tactile ridges that are sensitive to motion.

The view defended by Packard is overtly anti-Darwinian. He claims that the mechanism of degeneration through disuse is sufficient to explain the origin of cave species and that natural selection "only plays a very subordinate and final part in the set of causes inducing the origin of these forms" (1888, p. 121). The mechanism of induced degeneration allowed for the neo-Lamarckians to accelerate the pace of change considerably. Packard even suggests at one point that the "adaptations to a life in darkness may have been induced after but a few generations, perhaps but one or two only" (1888, p. 139).

It is true that there are differences of a substantial sort between the Darwinians and the neo-Lamarckians. But they do not lie here, in the appeal to disuse. Darwin clearly allowed for disuse as a mechanism of evolutionary change. Darwin even applied it to troglomorphic characters. The crucial differences lie in the fourth step, in the mechanism for compensation. Both Darwin and Packard accept that there is compensation, that hypertrophy of the sensory organs serves to compensate for lack of sight, and that hypertrophy is a response to the cave environment. Darwin explained hypertrophy as an adaptation produced by natural selection. The neo-Lamarckian approach denies natural selection a role only by pressing that compensation is a consequence of accentuation by use. It is here, over the causes of hypertrophy, that Darwin and the neo-Lamarckians such as Packard differ substantially, just as it was over the causes of adaptive changes that Darwin and Lamarck parted company. Packard's elaborate and developed studies of degeneration, ironically, have no role to play in adjudicating this dispute.

LAMARCKISM IN DISARRAY

With the turn of the century came a wholesale reformulation of the problem of speciation, and the neo-Lamarckian tradition fell into disarray. On the one hand, the rediscovery of Mendel's results was taken to make the reliance on the inheritance of acquired characteristics unavailable to twentieth century biospeleologists. August Weismann's insistence on the independence of germ cells from the somatic cells came to be established biological doctrine. On the other, the discovery of radioactive decay had undermined the principle challenge to Darwinism, by undermining Kelvin's estimates of the age of the earth.

It was in this atmosphere that A.M. Banta (1907) speculated on the evolution of cave animals in discussing the fauna of Mayfield's Cave. The problem, as Banta was to recognize, was how to explain troglomorphy within the framework of natural selection, despite the lack of any clear adaptive function for the retrogressive characters. Banta saw three basic questions pertinent to understanding the origin of cave life: How did animals come to be in caves? What were their morphological characteristics when they first immigrated into caves? How have they arrived at their present condition?

Banta rejected Packard's theory that cave organisms arose from the accidental isolation of small numbers of ancestors, followed by the rapid evolution of cave-associated traits. Banta suggested, instead, that the evolution of cave animals was much more gradual, starting with ancestors which already frequented caves through "voluntary immigration," rather than from accidental isolation. He further postulated that many of these ancestors may have been predisposed to cave life if they favored dark or shady habitats on the surface. (He uses Geodephagous beetles and the California goby as examples.) This provided two potential avenues for cave adaptation. Some of these "voluntary migrants" may begin by living in cave entrances and gradually become further modified until they can survive in the deepest recesses. In addition, Banta suggests that some other species may have already become so highly modified for cave life that they are initially able to survive in the deep cave, even though they are surface dwellers.

Superficially, Banta's scenario may seem to resemble the modern view that cave immigrants are often preadapted for cave life. Closer scrutiny, however, suggests that the view is orthogenetic. Banta states that those species predisposed to cave life are actually "predetermined cave inhabitants." He says this:

> Animals do not possess degenerate eyes and lack pigment because they are cave animals. The eyes have in many cases degenerated and the color disappeared before they entered caves. They are cave animals because their eyes are degenerate and because they lack pigment. . . . They are isolated in caves and other subterranean abodes because they are unfit for a terranean life and caves are among the possible habitats (1907, p. 99).

Thus, Banta denied degenerate characters were adaptations to the cave environment, maintaining instead that they were maladaptations to surface life.

Banta's skepticism about the degree of cave-related modification that animals have achieved prior to entering caves also led him to be skeptical of using the extent of modification in cave-related characters as an index of the age of cave fauna. Banta realized that equating the degree of "retrograde" evolution with the time spent in caves tended to support the theory of disuse, and thus rested on the inheritance of acquired characteristics. This was no longer tenable.

Having dispensed with the direct effects of disuse as an explanation of "retrogade" evolution, Banta's concluding section on the "Causes leading to the modifications of cave animals" presages the modern quandries over the evolution of troglomorphy. Banta suggests that some modifications of cave animals (e.g., attenuated appendages, and increased number and development of sensory papillae and setae) "may be due to individual adaptation [i.e. adaptability] or . . . to natural selection tending to eliminate all individuals which do not possess variations of assistance to the animal under its unusual conditions" (1907, p. 104). The present view, as defended by Barr (1968), Culver (1982), and others, is that these (typically hypertrophied) features are probably the result of natural selection and, as such, reflect true adaptations to the cave environment. Christiansen (1961, 1965), and Christiansen and Culver (1967) have recognized a suite of morphological characters in cave collembola which have undergone extensive parallel and convergent evolution. Behavioral and geographic data support their contention that these "cave dependent features" are adapatations to the cave environment. Poulson and White (1969) also argue that the reduced metabolic rate associated with increased troglomorphy in amblyopsid fishes is an adaptation to life in food poor cave streams (see Heuts [1953] for a somewhat different perspective).

Whereas Banta is willing to accept selectionist explanations for some cave-related features, he feels that "no satisfactory explanation for the loss of pigment and degeneration of eyes has been offered, unless we grant the influence of the environment upon the individual, together with the hereditary transmission of these acquired characters" (1907, p. 104). He goes on, however, to indicate that a growing body of evidence seemed contrary to the inheritance of acquired characteristics. Having found both selectionist and neo-Lamarckian theories wanting as explanations of retrograde evolution, Banta can conclude only that such characters appear to be consistent with "the theory of the cumulative effect of determinate variations," but can provide no mechanism.

SUMMARY AND PERSPECTIVE

Determining the mechanism for explaining regressive evolution has remained an elusive goal for twentieth century evolutionary biologists (for discussion of the many problems, see Christiansen, this volume). Only the Lamarckian and neo-Lamarckian appeals to the inheritance of acquired characteristics have been clearly undermined. This does not mean, however, that the Ultradarwinians have carried the day, or that selectionist explanations stand unchallenged; rather, the Darwinians have diversified (see Fig. 2). Modern views, both selectionist and neutralist, have their nineteenth century counterparts, and the controversy is still fueled by the recognition which guided Darwin: there is no clear adaptive function for the 'regressive' characters.

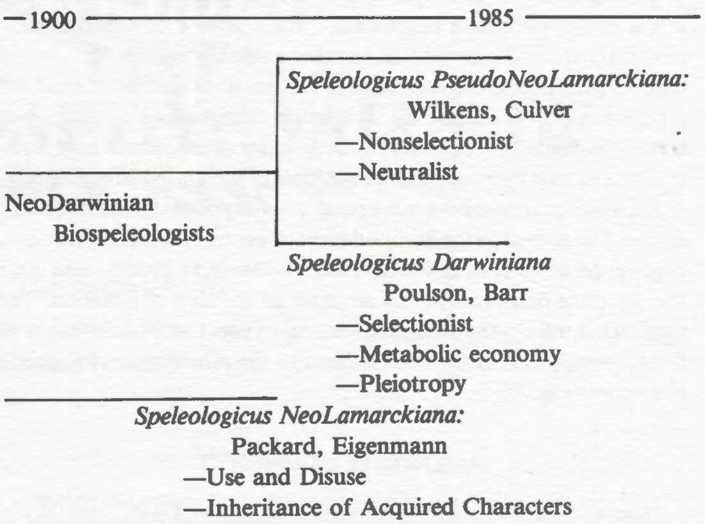

Figure 2. **A Taxonomy of Biospeleologists, Based on the Mechanisms Proposed for Regressive Traits**

The development of mathematical population genetics and the neo-Darwinian Synthesis in the 1930's and the 1940's (see Mayr and Provine, 1981, for a general account) has allowed the modern variants to be stated in a more rigorous manner, and in a fashion consistent with present day knowledge of the mechanisms of inheritance and speciation. The immediate adaptive advantage most commonly appealed to for regressive characters is the appeal to energy economy (see e.g., Poulson and White, 1969). Darwin had suggested that rudimentation in some parasites might occur because "it will profit the individual not to have its nutriment wasted in building up a useless structure" (1859, p. 148). Though he never used this appeal as an explanation for regressive evolution in cave animals, it is clearly an explanation in the Darwinian mold. Alternative selectionist explanations are also available. Barr (1968) has argued that regressive evolution can be explained as an indirect effect of pleiotropy. Genes for selectively advantageous traits (e.g., the hypertrophy of alternative sensory organs, or the attenuation of the appendages) may have negative pleiotropic effects on features such as eyes and pigment, features which are adaptively neutral. Thus, useless structures are lost as a side effect of selection for adaptive cave-related features. Although the structural basis for pleiotropic effects was unknown to Darwin, they would certainly represent one mainfestation of the phenomena he grouped together under the heading of "correlation of growth," and so equally count as a Darwinian explanation of regressive traits.

Recent work has also seen the emergence of non-selectionist accounts of regressive evolution. Appeals to the effects of disuse which played such prominent roles in neo-Lamarckian thought represent Nineteenth Century explanations for regressive evolution which also treat regressive traits as selectively neutral. Wilkens (1971) and Culver (1982) have attempted to explain regressive evolution as the result of the accumulation of neutral mutations. The work of Sewall Wright has provided quantitative estimates of the time needed for the fixation of neutral alleles in a population (the time decreases as the effective population size decreases). Since mutations are liable to cause degenerative changes, the accumulation of "neutral mutations" over time is liable to cause the degeneration of structures with sufficient time. What information we have indicates that the neutralist models are at least in rough agreement with the values we have for the time of isolation and the amount of genetic change required to undergo regressive evolution. Furthermore, one of the major predictions from Culver's neutral mutation model is that "those species that have been isolated in caves for a longer time should show more regressive evolution than species isolated for a shorter time" (1982, p. 70). It is ironic that Banta's reluctance to accept the degree of troglomorphy as an index of the time of cave isolation was in part because such a relationship for regressed characters, in the absence of selective advantage, was support for the effects of disuse. The appeal to the accumulation of neutral mutations similarly depends on an absence of selective value—that is, to disuse—and takes the degree of troglomorphy as an index of the time of isolation. The theory thus mimics the attractive features of neo-Lamarckian explanations, though it lacks the commitment to the inheritance of acquired characteristics.

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WHY ALL CAVE ANIMALS DO NOT LOOK ALIKE*— A DISCUSSION ON ADAPTIVE VALUE OF REDUCTION PROCESSES*

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ABSTRACT: Two antagonistic hypotheses have been postulated to explain reduction of eyes and some other structures in cave animals. The author, reconsidering arguments for "neutral mutation hypothesis," bears evidence of unreliability of some of them. He particularly denies an increased variability of organs in rudimentation in cave animals. On the other hand, some new arguments in favour of the "direct selection hypothesis" are given. The structure reduction grade in different populations of troglobitic species may be sometimes different, probably owing to different severity of ecological factors. A nice evidence for direct selection is given also by clinal variability in some cave animals.

1 INTRODUCTION

The current dilemma of many evolutionary speleobiologists has been caused neither by Barr (1968) or by Wilkens et al. (1979) nor by Culver (1982) who nicely summarized some facts and ideas about possible evolutionary mechanisms in cave habitats. One could say that it dates back to Darwin (1901) who introduced it by the following statements: "If . . . a structure . . . becomes less useful, any diminution, however slight, in its development, will be seized on by natural selection, for it will profit the individual not to have its nutriments wasted in building up an useless structure" (p. 121). " . . . an organ . . . might become injurious . . . and in this case natural selection would continue slowly to reduce the organ, until it was rendered harmless and rudimentary" (p. 350). "Rudimentary parts . . . are apt to be highly variable . . . owing to their uselessness and therefore to natural selection having no power to check deviations in their structure" (p. 123). It seems likely that Darwin, in spite of mostly thinking dialectically, was not aware of the possible controversy of these statements. He recognized the distinction between a well developed organ and its rudiment but failed to appreciate their close connection, and indeed failed to appreciate, that "well developed" and "rudimentary" are relative terms.

Ignoring different kinds of Lamarckian explanations, there are three hypotheses which try to explain the structural reduction (I will show below why I avoid using some prejudiced terms like "regression" or even "degeneration") by genetic mechanisms: the energy economy hypothesis, the neutral mutation hypothesis and the pleiotropy hypothesis (see Culver 1982).

In recent years a great number of arguments for neutral mutation and contra selection have gathered in the literature (see further). This is an attempt to return some balance to this by far unfinished debate. I will try to do this by bringing some new possible evidence for the adaptive significance of reduction processes, not however avoiding their weak points. I will also reconsider some arguments that have been used in support of the neutral mutation hypothesis. The pleiotropy hypothesis tries to be a compromise for people who do not believe in the energetic importance of the structures to be reduced but strongly believe in selection as an almighty pointer of the evolution. I will remark on this lucid idea as well.

2 CAN STRUCTURAL REDUCTION EXHIBIT A POSITIVE SELECTIVE VALUE?

2.1 Unfortunately, weighing of the adaptive value of depigmentation and of the eye reduction is very difficult if not impossible in natural populations. In cave animals these reduced characters are nearly always accompanied (but apparently not genetically linked) with other characters whose adaptive value is more certain. For example, the decreased metabolism is certainly advantageous in the food ("energy") poor environment which doesn't exhibit important seasonal temperature fluctuations. The adaptive significance of long antennae in Isopoda and Amphipoda can only be presumed from indirect evidence.

Turquin (1973) emphasizes a very effective reaction of the cave amphipod *Niphargus virei* Chevreux to chemical and mechanical stimuli. Banta (1910) established a stronger reactivity of *Caecidotea stygia* Packard (in comparison with its epigean relative) to mechanical stimuli. In this case more effective receptors (which might be antennae) can be important for sexual behaviour and/or for a more even dispersal of individuals in an energy-poor environment. The function of other receptors is even less known. In troglobitic Gastropoda the osphradium, as a remote-sensing device, is not enlarged, but rather looks to be diminished (see Bole 1963, fig. 3). The fact that also the modern, expansive, epigean *Asellus aquaticus aquaticus* L. has longer appendages (particularly antennae) than its relic epigean relatives (e.g. *A. a. carniolicus* Sket) indicate an universal biological importance of that character.

Under the pressure of new data even some former partisans of the selection hypothesis had to accept a possible importance of the muta-

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*compare Culver and Fong (in press)

tion pressure for structural reduction at least to some degree (compare Poulson, 1964; 1981; Sket, 1969: this paper). The usual and prejudiced question "may the neutral mutation cause reduction of eyes (and some other structures)?" will, for our further use, be divided into two more precise questions: "are the mutations in question neutral at all?" and "are the structure reductions easier to explain by the mutation pressure or by selection?". One has to bear in mind that both hypotheses are in fact dealing with materially the same mutations!

2.2 The most awkward fact in this discussion is our almost complete ignorance of the energetic requirements of eyes, versus the body's. It is really hard to believe that the regular reduction of the few tiny ommatidia in cave Collembola brings any energetic savings to the animal. The same might be true for the small eyes in Asellidae. However, using published data on the fly *Calliphora vicina* (=*C. erythrocephala*) one can calculate that its complete eyes consume in darkness between 1.9 and 6.4% of the body O_2 consumption, but they account for only 2% of its weight. The relative metabolic needs of the eye might be even higher in fishes. But in this case it is not adequate to deal only with relative values. In a large organism like fish the needs of both the eye and the body are much higher and so is the impact of the energy wasting in the eye for the rest of the body. Probably this may partly explain an extraordinarily fast eye reduction in *Astyanax mexicanus* (in 10,000 yars, Cluver, 1982). We may also take in account that the oxydative metabolism of the (vertebrate-) eye in darkness is even higher than in light (comp. Sickel, 1973).

I have thus far only considered the metabolic needs of an already established eye. But, a higher quantity of energy and material may be used as the eyes develop and grow. Again, the larger eye of larger animals consumes more—the relation of the eye consumption versus accessible food is probably more important than the eye : body relation.

Wilkens et al. (1979) claim, that the persistence of rudiments is not explainable by energy economy. They try to explain it with the high degree of integration of relevant genes into the complex genetic base of the organism. The explanation is plausible, but it is not in discordance with the selection hypothesis. As a rudiment consumes much less than a well developed organ does, the selection pressure for its reduction is very easily to be mastered by the opposite one.

2.3 *Typhlotriton spelaeus* Stejneger is illustrative for many points. As in other (epigean) plethodontids the retina is thinned during larval growth which Besharse and Brandon (1974) explain by an insufficient supply of materials. The same authors also report that the eye in *Typhlotriton* essentially reduces only after metamorphosis, which means with the sexual maturation and with transition to the more rigorous terrestrial cave habitat. Nevertheless the retina degenerated in none of the regularly fed animals in laboratory culture. Females exhibit smaller eyes than males. Also in *Astyanax* a sudden change in the eye development is remarkable and may be linked to "modifications physiologiques qui marquant le debut de la maturite sexuelle" (Peters and Peters, 1973). All these facts indicate certain energy economy problems in an animal, which is not yet physiologically well adapted to the cave environment.

2.4 Fong (1985) claims that the caves are advantageous to those specimens, which are able to "reroute (their limited pool of energy) to enhance development of other characters" (other than unnecessary structures). One could say that it is generally advantageous to reroute energy and material to higher reproduction rate (or larger eggs), or to better osmoregulation etc., anyway, to increase fecundity and/or survivorship by physiological and/or morphological means. Fong formulates this as an *indirect* operation against development of regressive characters. In fact nearly every action of the selection is to some extent indirect. The adaptive value of each genome or of each character in particular is measurable only in comparison with another. Each character is advantageous or disadvantageous (if not fatal/lethal) only in comparison with another. The question of directness and indirectness may be reduced to a mere problem of one's philosophy. Fitness of the cave animal can be raised by a reduction of unnecessary structures (for losing less energy) and/or by some progressive characters (mainly for obtaining more energy). In surface populations also eyes are important and their development positively selected; with abundant food of a high quality an elongation of appendages doesn't require eye reduction.

A special combination of reduction processes occurs in arthropods, which could be designated as a kind of pleiotropy (s. lat.). Eye reduction is mostly combined with depigmentation and with destruction of cuticula and its structures (Sket 1969, 1971a). Because the eyes are in fact integumental structures, it is possible that all these reduction processes are linked mutually either genetically or morphogenetically. In such a case the economisation effect of the reduction is much higher than in the case where only one structure is involved.

3 Is the Eye Reduction Really a Simple Degeneration?

3.1 Like other quantitative traits, eye development is governed by polygenic systems (Wilkens et al., 1979, Kosswig and Kosswig, 1940). The genetic analysis is difficult or impossible for morphogenetic interdependences between different structures in the eye (Wilkens et al. 1979). However, many studies in rats and frogs have shown that inductors for some eye structure development are positioned outside the eye itself (McAvoy, 1980). Therefore the eye reduction is a process which is likely to involve changes in a larger part of the body.

3.2 To treat the above question first from the phylogenetic point of view, let's reconsider the possible genetic mechanisms for structure reductions. A (1) degenerative mutation of structural genes which causes lack of the respective enzymes (Culver, 1982) is probably the most commonly realized possibility. The consequence of a (2) deletion is similar. Another possibility are (3) mutations in the operon or regulator genes which may block the enzyme synthesis for eye development. Mutations can also occur, which cause some (4) new, progressive processes in eye reduction, such as an excessive development of eyelids in *Typhlomolge*. (5) The eye reduction in some Collembola which seems to be fulfilled by fusion of ommatidia rather than by mere reduction of them (Thibaud-Brauner, 1985) is difficult to explain by gene degeneration. The well known (6) "bar" mutation (a duplication) in *Drosophila* is probably only a representative of its own group of mutation kinds which cannot be denoted as simply degenerative.

Last but not least, (7) mutations can cause changes in the competitive dominance of some formative centers during the morphogenesis. Morphological changes can start either by (7a) a reduction in the quantity of the cellular substratum at the disposition of several centers, or by (7b) one of the centers beginning its differentiation before the others and securing the competitive advantage (Spiegelman, 1945, cited by Devillers, 1965). If the development retardation which is very characteristic in cave animals is less expressed in more vital structures, such changes are even easier to explain. In such a case, other structures may retain their normal development on the cost of the eye in spite of a further existence of unchanged genes for eye development.

The reality of such a mechanism is well documented in polymorph epigean animal species, where in some phases (morphs) development of some structures is suppressed, but not in others.

Briefly, the real mutative "degeneration" of genes is only one of the couple of possible bases for the structure reduction. The fact, that in many cases eyes start with a more or less normal development and degrade later in the ontogenesis, may be easier (although not only) understood under the supposition, that the genes for eye development still exist.

3.3 Similar is the situation in the ontogeny of the eye reduction. Some cases, like *Astyanax mexicanus* (Philippi) (Peters and Peters, 1973), nicely illustrate a destruction of the primarily developed eye during its ontogeny. It seems not to be accompanied by any progressive events in the structure. On the other hand, Durand (1976) mentions some active processes in the reducing eye primordium of the larval *Proteus anguinus* Laurenti. Nevertheless, like tissue regeneration mechanisms, the mechanisms of death and selfdestruction of the cell are also built into the cell. Such processes may be provoked by the cell environment (lack of food) which is in turn again influenced by the cell itself. Thus, such processes do not inevitably acquire new progressive mutations.

According to Besharse and Brandon (1974) the eye degeneration in *Typhlotriton* is accompanied by an excessive growth and mostly overlapping of eyelids. Unfortunately, the fact, that "animals with open eyelids had larger eyes than those with closed eyelids" still leaves the question open whether the overlapping of the eyelids is not rendered possible by the diminishing of the eye bubble (rather than only by growing of eyelids).

The implicit supposition of Wilkens et al. (1979) that simply the genes for eye size regulate indirectly also its differentiation grade (". . . können . . . nur Gene festgestellt werden, die für die Grösse des Gesammtorgans verantwortlich sind. Fur diese ist somit charakteristisch, dass . . . (nur ihre) Anzahl für die Grösse und den damit korrelierten Differenzierungsgrad . . . verantwortlich sind") can hardly explain the destruction of the eye in the cave characin when it is still growing (op. cit.!, compare Peters and Peters, 1973). Furthermore, in most epigean animals cessation of growth does not cause a drastic rise in degeneration processes. Therefore, some other, particular factors must be involved in the process of eye destruction.

Thus, the question, whether the ontogenetic mechanism of the eye reduction in at least some cases is combined with constructive novelties, remains open.

4 FLAWS IN THE ARGUMENTS AGAINST THE SELECTION HYPOTHESIS.

4.1 The neutral mutation hypothesis presupposes a high frequency of "degenerative" mutations even in a limited number of genes (within some polygenic systems). In such a case all organisms with a similar genetic background should in darkness reduce their eyes with approximately the same speed. The idea has been also used in attempts to calculate the phylogenetic age of cave animals (Wilkens, 1979). By the selection hypothesis the speed of the reduction would depend on the severity of environmental conditions (food amount) and could be different even in populations of the same species.

4.2 Wilkens (op. cit.) nicely correlates the reduction grade of the Yucatan's cave fishes *Typhliasina pearsei* (Hubbs) and *Ophisternon infernale* (Hubbs) with their possible "cavernicole age." However, it is possible that the higher grade of reduction in *Typhliasina*, which is of the marine provenience, has been caused by higher selection pressure. Marine animals meet in fresh waters additional energetic problems caused by a hypotonic environment.

DeLattin (1938) in Oniscoidea (*Titanethes albus* Koch, *Protonethes ocellatus* Abs. et Strouh.) explicitly refutes the possibility of a correlation between the "cave-age" of the animal and the grade of eye reduction.

In some troglobitic gastropods in the Dinaride Karst and particularly in Slovenia, for which we presume a Pliocene or at least early Pleistocene age (Sket 1971), the eye reduction grades are very variable (Bole 1963). In *Neohoratia subpiscinalis* (Kuščer), which inhabits the prekarstic system of the Pivka river (its parts belong now to three different river systems and even to two seas) only some populations still develop eyes. It is highly improbable that those populations broke their connections to surface habitats essentially later then the others did.

Troglocaris anophthalmus (Kollar) is the most widely distributed cavernicolous shrimp in Yugoslavia. In about 60 known localities, scattered between Gorizia in Italy and Hercegovina, all except two exhibit approximately the same, very high degree of eye reduction. Their eyestalks are uniformly ovoid, and nothing of the compound eye or of its pigment are visible. In the population near Vinica (Jama v kamnolomu) only 6% of specimens exhibit similarly reduced and totally depigmented eyes. Other specimens have mostly a small bulging (its diameter reaches 50% of the eyestalk diameter) in laterodistal parts of the eyestalks, resembling a rest of the corneae. No ommatidia-like structures can be detected on hystological preparations, but the bulging is always underlied by a dense flock of black pigment. Also in a cave near Črnomelj (cave Žopenca) some specimens have very slight pigment flocks in their eyestalks. Both localities are situated in the Dinaride karst of the SE Slovenia (Fig. 1), more remote from its borders than many other populations are. There are even indications that the karstification in that area began earlier (Šifrer 1970) than in some other karstic regions in the *Troglocaris anophthalmus* area. The location of the "oculated" shrimp population is also far from the location of the oculated *Neohoratia* populations. Therefore, the age of those cave populations can not be lower than of the others. As most of the Dinaric cave fauna dates back to the end of Pliocene (Sket, 1971), some small time differences must be unimportant.

4.3 From the selectionist's point of view the *Astyanax: Caecobarbus* relations are not such a problem as Culver (1982) supposes. Breder (1953) claims that, if selection is involved, *Caecobarbus geertsi* Boulanger should have more reduced eyes than troglobitic populations of *Astyanax mexicanus* (Filippi), because it suffers from a greater lack of food and has been in caves longer. First of all, the absence of an epigean ancestor in the area doesn't necessarily indicate a greater age of the cave species. But, more important is the ephemerism of the ecological situation in caves, caused by climatic changes on the surface (particularly during the Pleistocene) as well as by the succession of speleogenetic processes. The initial phase of the speleogenesis is a very long one (Bögli, 1978). We may suppose the same for the next, juvenile phase, as the exposed inner surface of the cave is still very small. The cave spaces become rapidly larger in the last phases of the cave formation when invasions occur. Meanwhile fish may colonize a brook in a relatively young cave, large bat colonies, giving the cave a food-rich appearance, may inhabit them only very late. The Mexican cave characins may well have been under a severe selection pressure until very recent times, which is certainly not the case with *Caecobarbus* in the old (compare Heuts and Leleup, 1954, cit. Thines, 1969) karst of Congo.

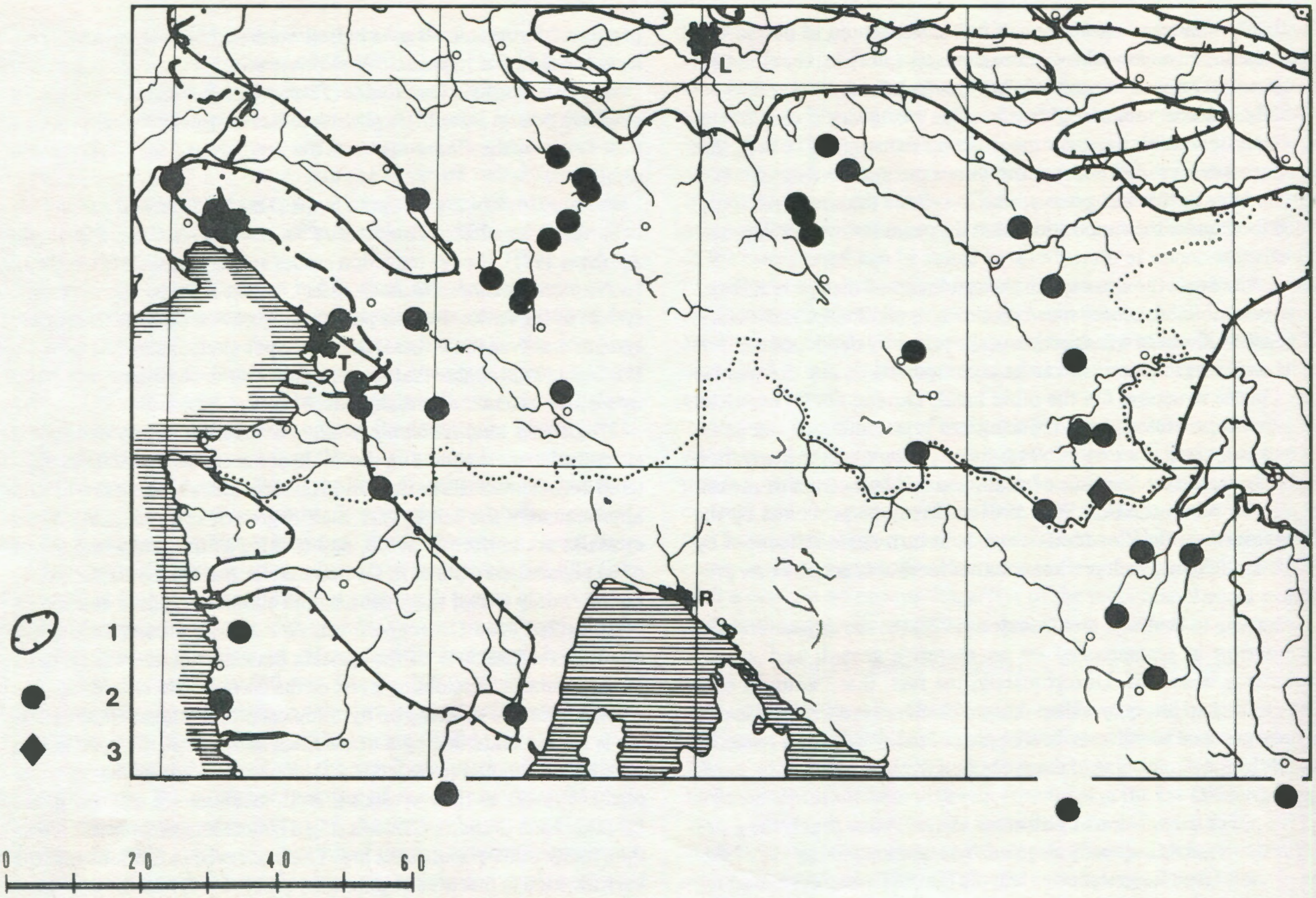

Figure 1. **The NW part of the *Troglocaris anophthalmus* area. L—ljubljana, R—Rijeka, T-Trieste, 1—borders of larger karst areas, 2—"normal" *Troglocaris* populations, 3—"oculated" (see text!) *Troglocaris* population; scale in km.**

4.4 Wilkens et al. (1979) did not find a difference in growth rates in a mixed culture of oculated and eyeless specimens of *Astyanax*, as they would expect in the case the energy economy were an important factor. Unfortunately, they do not mention the culture conditions except for the darkness. In many cases the favourable effect of the adaptive characters may only be expected in really severe conditions. The complex pitfall traps of the insectivorous plant *Nepenthes* spp. are to such a degree unessential in favourable culture conditions, that some gardeners don't even feed the plant (Bailey 1953). Nobody thinks, that the structure in question has been built up by mutation pressure.

4.5 The increased variability of the structures in reduction would be the most convincing argument for absence of selection. A series of such cases have been presented by various authors, which deserve some discussion.

Peters et al. (1973) claim, that in the cave population of the fish *Poecilia sphaenops* (Cuv. et Val.) the diamater variability of the slightly reduced eye is increased while that of the progressively developing genital cushion (Genitalkissen) is diminished. A numerical analysis of their graphical data failed to confirm the statement. The *relative* variability (in % of their own mean relative size) of the genital cushion appears to be the same in both the cave and the surface populations. The relative variability of the eye diameter is augmented in the cave, but only by the factor 2. If one compares *absolute* variabilities of the eye diameters (in mm, not in %), the difference is even much smaller and in its volume (which appears to be a more appropriate measure) the well developed eye is already a bit more variable than the reduced one. Similar, but even much more striking is the situation in the genital cushion.

The variability of lens number per eye in two related forms of Venezuelan cave crickets is also absolutely contradictory to above suppositions. The "microphthalmic type" is less variable than the "macrophthalmic type" (Chapman and Willis, 1981). Similar is the situation in some gammarids in Yugoslavia.

Peters et al. (1973) explain the supposedly augmented phenotypical variability of the reduced eye by an augmented heterozygosity. The expression of single genes in a polygenic system seems to be mostly additive (or dominant or epistatic, both of which are out of question in this case) and only exceptionally multiplicative (comp. Grafius, 1978, Muntzing, 1964). Therefore a high phenotypic *relative* variability in a small character state (where a small number of positively acting genes is involved) may be caused by smaller genetic differences than even a lesser variability in a larger character state. One can suppose that in all studied cases of the eye reduction the differences in the numbers of "active" (or "undegenerated") genes between specimens with partially reduced eyes are smaller than between specimens with well developed eyes.

5 Some New Arguments For Adaptive Importance of Structure Reductions

5.1 If selection for energy economy is involved in reduction processes in cave animals, the lack of reductions could sometimes be compensated for by progressive development of other structures, which enables a more effective utilization of resources. In such a case, the grade of reduction of integumental structures (eye incl.) might be in discordance with the progressive appendages development in some arthropods. On the other hand, integumental structures should reduce to some degree also in epigean habitats, when they are energetically unfavourable. Both cases appear in some *Gammarus* spp. and in some other amphipods.

All hypogean palearctic *Gammarus* spp. (Fig. 2), which are completely eyeless (*G. pulex polonensis* G. Kar. and Pinkst., *G. vignai* Pinkst. and G. Kar., *G. albimanus* G. Kar., *G.* sp. n. from Ohrid) have seemingly unchanged appendages, if we compare them with their nearest epigean relatives. In contrast, all those troglobitic taxa, that possess only partly reduced eyes (*G. pulex cognominis* G. Kar. and Pinkst., *G. microps* Pinkst. and G. Kar., *G. halilicae* G. Kar.), exhibit prolongated antennae and legs.

With the transition from the sea to lower ionic concentrations, amphipods augment their O_2 consumption. Irrespective of the real basis, which is still questionable (Munday and Poat, 1971, Wolvekamp and Waterman, 1960), this phenomenon is certainly related to higher energetic needs of those animals in fresh water. In 11 marine or mixohaline *Gammarus* spp. (subgenera *Pephredo* and *Lagunogammarus*, and *G. duebenii* Lilljeborg) the longest axis of the eye reaches 35-50% of the head length, exceptionally being only 25%. In 21 epigean palearctic species from freshwaters (subgenus *Gammarus* s. str.) the eye reaches only 20-30% and exceptionally 40% of the head length. And still none of that species has succeeded to inhabit such a stressful habitat as the completely blind *Niphargus valachicus* Dobr. and Man. (comp. Sket, 1981, Straškraba, 1972) did. Concerning the abiotic parameters of the environment, this and some other epigean (facultatively hypogean) *Niphargus* spp. are probably the most euryvalent and resistant European amphipods, which is certainly true for the genus *Niphargus* as a whole.

5.2 Another argument for the adaptive relevance is the clinal variability of animals along the subterranean water courses. A series of animal species exist, which in Slovenia (NW Yugoslavia) penetrated hypogean parts of sinking rivers, exhibiting there evident clines in eye, pigment, and cuticular structures reduction.

Asellus aquaticus cyclobranchialis Sket inhabits the hypogean parts of the Šica-Krka River. Its cuticular spines exhibit the first degree of reduction, they are thinned and multiplied. The eyes are still normally developed, but mostly completely depigmented in the downstream (cave Krška jama) specimens. The body of the specimens from the food-rich upstream cave (Viršnica) is palish brown maculated meanwhile the specimens from the food-poor downstream resurgency cave are mostly completely colorless, seldom only very pale. The upstream subpopulation is not influenced by the gene flow from the surface. The subpopulation is very uniform and the surface river is not inhabited by asellids (except for few specimens of the hypogean population, which penetrate through the ponor).

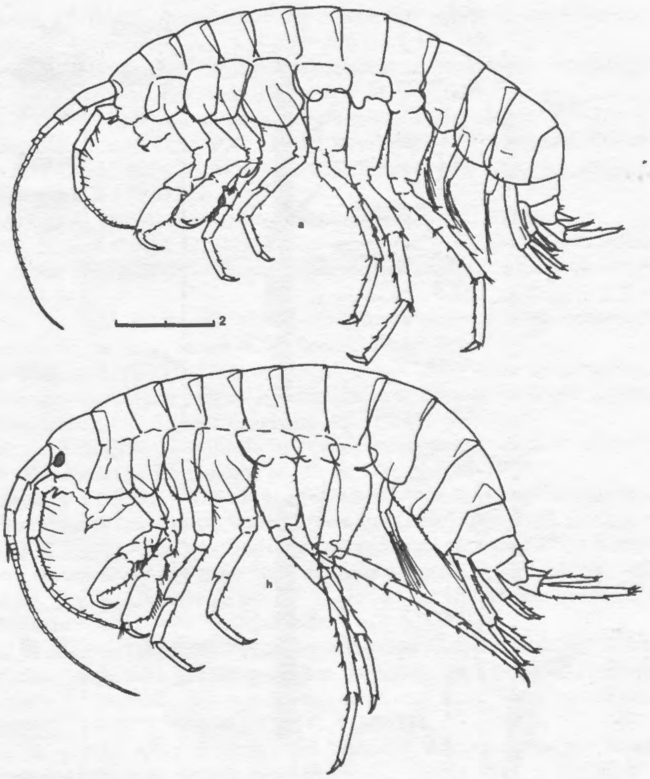

Figure 2. **Two troglobitic *Gammarus* spp. from Makedonia, Yugoslavia. On the top panel (a) is *Gammarus albimanus* (eyes absent, appendages not elongated). On the bottom panel (h) is *G. halilicae* (eyes small but present, appendages elongated); scale in mm.**

The *Asellus aquaticus* L. population in the Postojna-Planina Cave-System has been mentioned several times (Kosswig, 1949, with an error about flow direction; deLattin, 1938; Sket, 1965, etc.). The most troglomorphic specimens from the system represent a subspecies *A. a. cavernicolus* Racovitza, evidently descending from the relic epigean race *A. a. carniolicus* Sket.

A. a. cavernicolus has highly evident progressive and regressive troglomorphic characters; it is completely depigmented, strong cuticular spines are partly reduced to setae which are also rare. The extreme heterogeneity of some parts of the population in appendage morphology, pigmentation, and eye reduction, is a result of a secondary introgression with the modern postglacial invader *A. a. aquaticus*, penetrating downstream in the caves.

The penetration of the non-troglomorph phenotypic characters in this hybrid population downstream is in a nice concordance with the trophic situation along the hypogean river bed (see Fig. 3; comp. Sket and Velkovrh, 1981). The composition varies slightly through the years, but at least the comparison of two representative series which are 40 years apart very clearly indicates also the progression of the non-cavernicolous phenotype with increasing energy input (read: pollution). Only in the poorest (cleanest) downstream waters is the troglomorphic phenotype still able to compete with them.

As *A. a. cavernicolus* exhibits also very striking progressive adap-

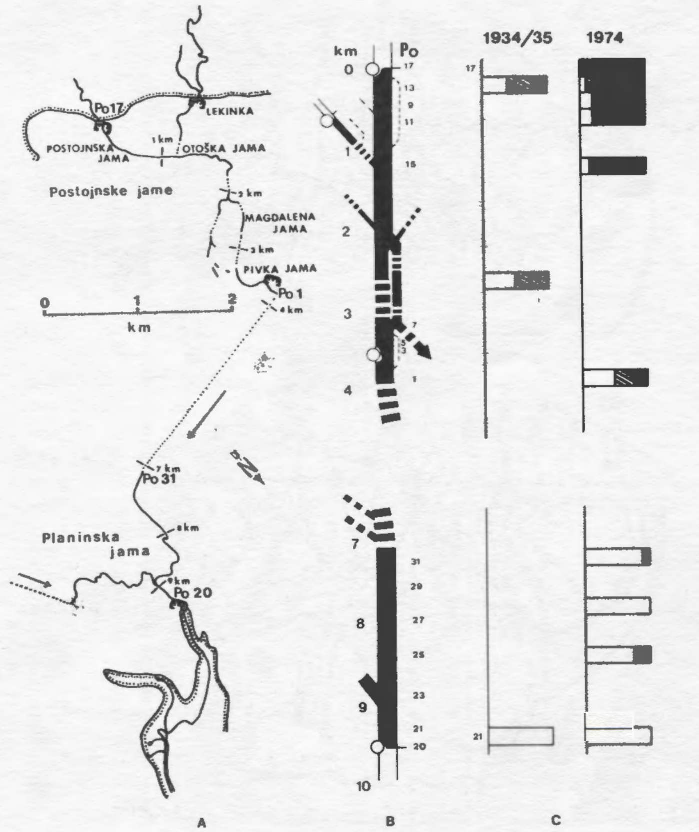

Figure 3. **Penetration of the epigean *Asellus aquaticus acquaticus* along the hypogean river.**
***A*—water courses in the Postojna-Planina-Cave-System (jama—cave, Po—as in B; at Po17 the Pivka River flows underground, at Po20 is its resurgence)**
***B*—diagramatic presentation of PPCS (open circles—cave entrances, Po1-31—sampling localities, km—distance from the sinking point, continuous black belt—accessible parts of hypogean streams, interrupted black belt—inaccessible parts of streams)**
***C*—composition of *Asellus aquaticus* population along the underground river (black—normally pigmented specimens, striped—pale or unpigmented specimens with black eyes, white—completely unpigmented specimens; 1934/35—combined data from Kosswig 1949, 1974—series of samples of 15, 07, 1974, very irregular picture in Po31, 27, 25 is caused mainly by smallness of samples)**

tive characters (very long appendages, some paedomorph characters, changes in metabolism), the case may not be very convincing. But it is supported by two further cases in the same cave system.

Synurella ambulans Fr. Muller seems to exhibit a primary cline in the eye reduction along the same system as its subpopulations are quite homogeneous. In an upstream and an downstream (Po 15, Po 25 in Fig. 3) subpopulations resp. the length ratio ant.I : head is nearly the same in adult animals (the same mean value 3.1, small difference in the growth allometry). The number of ommatidia per eye is about 20 (difficult to count for a thick pigment flock included) in the upstream and 6-12 in the downstream location. In the very clean tributary in the same cave (Rak River), the synurellas possess only 1-11 ommatidia per eye, but slightly (!) longer antennae may make this subpopulation unconvincing.

Similar is the situation in the gastropod species *Ancylus fluviatilis* Mull. (Bole, 1965), with the exception that the lowermost parts of the cave subpopulation exhibit again influences from the epigean parts. (via resurgence). Near the ponor, all specimens are normally pigmented. Other cave parts of the population are variable in pigmentation and eye reduction, in extreme case (around Po 29 in Fig. 3) some specimens exhibit depigmented but normally developed eyes, meanwhile others are "blind." No progressive characters with a sensory compensation function are known in this species.

Chapman and Willis (1981) explain the distribution of two types (species ?) of phalangopsine crickets in Venezuelan caves by the energy economy effect. The microphthalmic type with ca 60 ommatidia in its compound eye prevails in energy poor caves, while the macrophthalmic type (ca 200 ommatidia) in the energy rich ones. From the context we may conclude that differences in appendages lengths are at least not striking.

In all these cases the competitive success of the "reduced" phenotypes in the strong cave environment may in fact be caused by some accompanying physiological improvement, which is not evident in the morphology. Unfortunately, adequate physiological or ethological studies have not been fulfilled.

6 SUMMARY AND CONCLUSIONS

Some arguments for different hypotheses, explaining the reduction processes in cave animals have been presented or reconsidered. As arguments in favour of the neutral mutation hypothesis have augmented in the recent literature, an attempt has been made here to balance the situation.

Although we have no exact data about energetic needs of relevant structures, there are some strong indications for economic reasons of the eye reduction. They even abound in the developmental history of *Typhlotriton*. Also the possible morphogenetic or genetic connections between all integumental structures in arthropods (eye-pigment-cuticula) might be responsible for higher importance of any destructive mutation.

It is very probable that certain reduction processes have been provoked also by other means than by "degeneration" of structural genes. Changes in the physiological dominancy of morphogenetic centers may be very important. It is suggested, that also the ontogenetic eye destruction is not a simple degeneration but an active process, sometimes invoked by genetically based novelties.

The higher variability of the unnecessary structures would be a strong indication for non-existence of a selection for their reduction. Therefore some cases have been reconsidered again, which results in a statement, that those structures are in fact at least genetically usually even less variable than the positively selected and well developed structures.

Some new cases of a clinal variability of the reducing eyes in a cave system are presented as possible arguments in favour of the biological importance of the character. Also a partial reduction of eyes in freshwater epigean gammarids as well as the relation between eye reduction and appendages elongation in the cave species tend to speak in favor of the selection hypothesis.

Both, the "neutral mutation" and the "energy economy" hypotheses suppose a higher grade of reduction in "older" cave inhabitants. The neutral mutation (or, better, "mutation pressure") hypothesis supposes a very high mutation frequency in a limited number of loci (within some polygenic systems). The result would be an equal evolution speed in genetically (structurally) similar animals as well as a high similarity of results, particularly later in time. The energy economy (or "direct selection") hypothesis presumes a small number of mutations, which are therefore very different. As selection favours any of them (if it economises), the reduction of eyes in different populations may go different ways. The equalizing influence of a high mutation frequency is absent. Different severity of ecological factors may cause also differences in reduction grades.

Some further investigations may bring a clearer evidence of importance of selection. First, we need a number of exact data about metabolic needs of structures which are usually reduced in hypogean animals. Secondly, competition experiments in mixed artificial populations (population cages) should be done. Normal specimens of the epigean type should be faced with mutants with reduced eyes and/or pigment but without progressive troglomorphic characters. Light and food quantity and quality are the prime environmental factors to be controlled. This seems to me to be the only reliable method to get direct evidence in the adaptive value of structure reductions in the cave environment.

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ZAKAJ SI VSE JAMSKE ŽIVALI NISO PODOBNE—RAZPRAVA O ADAPTIVNI VREDNOSTI REDUKCIJSKIH POJAVOV

POVZETEK IN ZAKLJUČKI

Redukcijske pojave v razvoju jamskih živali skuša razložiti več hipotez. Tukaj so predstavljeni in ponovno pretehtani nekateri nasprotujoči si argumenti. Ker se v novejši literaturi množijo argumenti v prid hipotezi nevtralnih mutacij, avtor skuša ponovno ustvariti ravnovesje.

Čeprav nimamo eksaktnih podatkov o energetskih potrebah prizadetih struktur, je za ekonomičnost pokrnevanja oči nekaj tehtnih argumentov. Zlasti veliko dokazov najdemo v osebnem razvoju jamskega močerada *Typhlotriton*. Možne morfogenetske ali genetske povezave med vsemi integumentalnimi tvorbami pri členonožcih (oko-pigment-kutikula) lahko močno zvečajo pomembnost destruktivnih mutacij.

Zelo verjetno je, da pokrnevanja struktur ne povzročajo le "degenerativne mutacije" strukturnih genov. Zelo pomembne pri tem so lahko spremembe v fiziološki dominantnosti morfogenetskih centrov. Tudi ontogenetski razkroj očesa najbrž ni preprosta degeneracija, temveč aktiven proces, ki je včasih odvisen od genetsko zasnovanih novosti.

Zvišana variabilnost nepotrebnih struktur bi bila najlepši argument v prid odsotnosti selekcije, ki bi vodila njihovo pokrnevanje. Ponovno preverjanje nekaterih primerov iz literature je pokazalo, da so reducirane strukture v resnici vsaj genetsko celo manj variabilne kot tiste, katerih razvoj vodi selekcija v pozitivni smeri.

Tudi nekateri novi, tukaj predstavljeni primeri postopne (klinalne) variabilnosti delno reduciranih oči v jamskih sistemih, govorijo v prid adaptivnemu pomenu pokrnevanja. Podobno je z delno redukcijo oči pri površinskih sladkovodnih amfipodih (v primerjavi z morskimi) ter z odnosom med pokrnevanjem oči in daljšanjem okončin pri jamskih.

Tako hipoteza neutralnih mutacij, kot hipoteza energetskih prihrankov predvidevata višjo mero redukcije pri "starejših" jamskih živalih. Hipoteza nevtralnih mutacij (bolje: mutacijskega pritiska) predvideva zelo pogosto mutiranje znotraj omejenega števila genov (v posameznih poligenskih sistemih). Posledica bi bila izenačena hitrost razvoja pri genetsko (in gradbeno) podobnih živalih, obenem pa podobnost rezultatov, zlasti po daljšem obdobju. Hipoteza energetskih prihrankov (bolje: neposredne selekcije) predvideva majhno število mutacij, ki so zato lahko zelo različne. Ker selekcija deluje v prid vsaki od njih (če ta ekonomizira organizem), gre lahko npr. pokrnevanje oči pri raznih populacijah po različnih poteh. Izenačujoči učinek visoke pogostnosti mutacij izostane. Različna strogost ekoloških razmer lahko povzroči tudi razlike v stopnji pokrnelosti.

Za jasnejšo podobo o pomenu selekcije boto potrebne nove raziskave. Predvsem potrebujemo zanesljive podatko o energetskih potrebah struktur, ki pri podzemeljskih živalih navadno pokrnevajo. Potrebni bodo tudi poskusi glede izidov kompeticije v mešanih umetnih populacijah. V teh bi morali normalne osebke površinskih vrst sočiti z mutantami ki imajo reducirane oči ali/in pigment, nimajo pa razvitih naprednih troglomorfnih znakov (npr. podaljšansja okončin). V takšnih poskusih bi morali nadzorovati predvsem svetlobo in količino hrane. Očitno je to edina zanesljiva pot k ocenitvi adaptivne vrednosti strukturne redukcije v jamskem okolju.

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CAN EVOLUTION REGRESS?

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Abstract: An analysis of the idea of "regressive evolution" is made under semantic, historic, and descriptive viewpoints and is concluded that the term does not accurately describe the evolutionary trend toward reduction or disappearance of features in, among others, cave and deep-sea animals. Since the trend of reduction can be described and explained using the current evolutionary conceptual framework, it is proposed that we eliminate "regressive evolution" as a descriptive term for what are really only examples for convergent evolution.

INTRODUCTION

The term "regressive evolution" is used to describe the trend toward reduction or disappearance of features. It is frequently applied to the evolution of parasites, deep-sea animals and, especially, cave organisms lacking eyes and pigmentation. Vestigial characters in termites, flightlessness in insects and birds, and limb loss in tetrapods have also been offered as examples. The aim of this paper is to show that from semantic, historic, and descriptive standpoints, the term "regressive" does not accurately denote such evolutionary trend. I will also argue that there is no evidence for "regressive evolution" as a unique mode of evolution, and that for the kinds of changes to which it refers and the mechanisms involved, current evolutionary terminology provides an adquate conceptual framework.

SEMANTICS

Although many authors (e.g. Culver, 1982; Kosswig, 1965; Thines, 1969) have included detailed treatment of regressive evolution, none has concisely stated the meaning of the term. The earliest uses of this term were by Lamarck (1809) and Weissman (1889). They considered any loss in structural complexity to be "regressive" evolution, a not too helpful definition because all lineages lose (and gain) features during evolution. Other authors, such as Heuts (1953), have been even more obscure by defining "regressive evolution" as "any loss of internal stability in the organism."

The Oxford English Dictionary defines the term regressive to mean "returning, passing back, acting in a backward direction." Similar meanings are given in dictionaries of other languages where this word has been used in the same evolutionary sense. Based on this definition, the question "going back to what?" immediately arises.

The term "regressive is also problematic in an ontogenetic sense. If the point is that the adult stage reverts to earlier stages of development, then it should be remembered that some cave organisms show the same features (including eyes) in early stages of development as their "unregressed" ancestors (Cahn, 1958; Durand, 1976; Eigenmann, 1909; Thines, 1969; Wilkens, 1980).

HISTORICAL BACKGROUND

For the last two centuries, evolution has been largely perceived as something "progressive." Lamarck (1809, p. 54) considered evolution a force that "gives to animal life the power of progressively complicated organization." That view was held as a fundamental truth by the first biologists dealing with cave organisms (Agassiz, 1853). Later researchers of cave animals adopted some sort of neo-Lamarckism (Packard, 1894) but orthogenetic (directional) views of evolution rapidly became popular among biospeleologists (Lankester, 1893a,b; Jeannel, 1950). Vandel (1965) adopted an "organicistic" interpretation of orthogenetic evolution in cave animals which held that all phyletic lines pass through four evolutionary stages: rejuvenation, adaptive radiation, specialization, and "phyletic senescence." Vandel (1965) rejected any intervention of the environment during the process: blindness and depigmentation were not common to organisms because they live in caves, but because they were blind and depigmented and represented a "dying phylogenetic line," they invaded the caves (they could not survive elsewhere) (Barr, 1968).

These orthogenetic ideas were extremely popular among European biologists (except in the United Kingdom). Theilhard de Chardin (1959), for example, saw in evolution "only an event, the grand orthogenesis of everything toward a higher degree of imminent spontaneity." However, as Dobzhansky (1970, p. 391) pointed out, "attempts to define what constitutes progressive have met with only mediocre success since mere change is not necessarily progress."

DESCRIPTION PROBLEMS

Clearly the structural reduction (such as blindness and depigmentation) that have been used as the descriptive components of "regressive" evolution do exist, regardless of the inaccuracy of the term employed for phenomenon. When compared with their light-adapted ancestors, deep-sea and cave organisms provide a number of cases in which an increase in the number and/or elaboration of features have been documented. In addition to the classical examples of an increase in the number and size of sensory buds in amblyopsid fishes (Poulson and White, 1969), and in the teleost *Astyanax fasciatus mexicanus* (Schemmel, 1967), the literature of the last several years offers many new examples of increase in size or elaboration of features including hypertrophied chemosensory and tactile organs in Hawaiian lava cave arthropods (Anhearn and Howarth, 1982), new sensory organ in crustacea mysidacea (Crouau, 1978), new amino acid-derived compounds in biochemical pathways of cave sponges (D'Ambrosio et al., 1982), and hyperdeveloped buccopharyngeal membranes in cave salamanders (Serra and Stefani, 1981).

Although there are also cases of troglobites in which neither sensory compensations nor morphological enlargements have been described (Culver, 1982) there is no question that the evolution of cave organisms involves much more than simply a few structural reductions. Emerson (1961), for instance, recognized "associated progressive and regressive evolution" and Kosswig (1973) mentions instances of "constructive" evolution during "regressive" evolution.

THE MECHANISMS

Besides semantic, historic, and descriptive problems, is there a unique mechanism responsible for "regressive" evolution? Experimental studies indicate that well-known mechanisms are sufficient to explain the reduction in features. Selection, pleiotropy, differential migration, "noise" suppression, and ontogenetic buffer mechanisms have been implicated (see Barr, 1968; Culver, 1982; Katz et al., 1981; Lande, 1978; and Regal, 1977). In consequence, no evidence has been provided supporting the assertion that "regressive" evolution is different from other kinds of evolution.

IS A NEW TERM NECESSARY?

The question is if the term "regressive" is inaccurate from many viewpoints, how should we refer to this common trend among many organisms? Previous attempts to solve this problem have not been very successful. The term "degenerative" evolution (Eigenmann, 1909) poses not only the same conceptual problems as "regressive" evolution but also adds a pathological angle. The terms "rudimentation" (Dobzhansky, 1970) and "structural reduction" (Brace, 1963) seem the least problematic terms; however, they do not apply to changes in behavior and physiology, since they refer only to specific structures and do not describe the trend as a whole. The terms "streamlining" evolution (Regal, 1977) and "channel" evolution (Katz et al., 1981) present similar descriptive problems.

Gould and Vrba (1982) have pointed out that in biology "unnamed ideas generally remain unconsidered." However, the trend observed in cave animals has been recognized since the discovery of the first cave organisms regardless of the nomenclature used for it. On the other hand, and for practical purposes, it is convenient to have a term with which one can concisely express a set of ideas or observations. The following attributes characterize obligate cavernicoles (troglobites) and are seen in many lineages: the animals live in a subterranean environment and exhibit at least two common features that are universal (blindness and depigmentation) and others that seem to be widespread at least for organisms at upper trophic levels: enlargement, multiplication and/or appearance of new sensory structures, larger size, and low metabolic rate.

In general, when unrelated species occupying similar environments share a number of characteristics, the term "convergent evolution" is applied. Cave animals provide, in fact, one of the best examples of convergent evolution. Why then is this term not frequently employed by researchers of these organisms? Several reasons can be offered: first speleology has had its major development in continental Europe (especially in France) where Darwinian ideas were not very popular (Vuilleumier, 1984). Second, in continental Europe the defense of evolution as a fact was carried out by Catholic priests such as Theihard de Chardin who envisioned evolution as an orthogenetic perfectionist process in which selection and environment play no role at all. Only in the last 20 years has this orthogenetic and/or neo-Lamarckian tradition been challenged by a few neo-Darwinists and proponents of the neutral mutation hypothesis, and these workers object to the term convergent evolution because, many say, it is the product of directional selection which has yet to be demonstrated to occur during the evolution of cave organisms (Barr, 1968; Culver, 1982; Wilken et al., 1979).

Let us banish "regressive evolution" from our vocabulary. If we use the term convergent evolution in its strict sense (independent acquisition of similar features by unrelated organisms that live in similar environments), then it aptly describes the evolution of cave-dwelling animals. In any event, we should not forget that this convergence is precisely what we are trying to explain in the first place.

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REGRESSIVE EVOLUTION IN COLLEMBOLA

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SUMMARY

Regressive evolution in collembola is examined in two habitats—caves and soil. Four genera are examined: *Friesia, Folsomia, Schaefferia* and *Pseudosinella.* The first genus shows regression only in soil, the second largely in soil, the third mainly in caves and the fourth almost entirely in caves. Six features show clear regression in collembola: 1) eyes, 2) furcula size, 3) tenent hair structure and size, 4) pigment, 5) unguiculus, 6) water retention ability. The first three of these were chosen for analysis. The regression of these features was examined in soil and cave forms with the goal of seeing which of the major theories used to explain regressive evolution best fitted the data observed.

The first conclusion was that there is seldom positive correlation between features displaying regressive evolution. A second is that regressive evolution in caves displays traits quite distinct from those seen in the soil. The result of these, is that different features undergoing regressive evolution behave as if they were under separate evolutionary regimes even when they are subjected to analogous selective forces.

These data do not fit any extant explanation well but are least in conflict with 1) material compensations, 2) evolutionary trap theory and 3) the indirect effect of pleitotropy, if these can be integrated so as not to be mutually exclusive.

INTRODUCTION

Regressive evolution has been a subject of much controversy and little agreement. As the term is used, it is generally understood to involve reduction in structure, physiology and/or behavior through evolutionary time, associated with loss or reduction in function (Emerson, 1961). If the evolution proceeds long enough, the feature may be lost entirely, but during the intermediate stages the process is often associated with vestigial characteristics. Controversy about this topic stems partly from confusion concerning the concept but partly from a question as to the existence of the phenomenon. Thus Romero (this issue) questions whether there is any regressive evolutionary process which differs from normal adaptive evolution. On the other hand Scadding (1981) questions the very existence of vestigial organs and by inference the existence of regressive evolution.

In this paper I shall assume that regressive evolution in the sense that it is defined above does exist and does display some properties which differentiate it from other evolutionary modes. This evolutionary mode involves the loss of function and the existence, for some time, at least, of vestigial organs. I shall concentrate upon the process as it is seen in collembola.

Regressive evolution in collembola has interested many investigators. It occurs largely in three environments, cave and related environments, including the superficial underground compartment, soil, and in interstitial spaces in fine sand (Massoud and Thibaud, 1985). It is not equally common in all families (Thibaud and Massoud, 1973). The families Isotomidae, Hypogastruridae and Entomobryidae show it best. The family Sminthuridae has only one genus showing clear reduction, while virtually all members of the families Neelidae and Onychiuridae appear to be the result of extreme regressive evolution and the only non-regressed forms are seen in the most primitive species of the family Onychiuridae. The regularity of regressive evolution has led several investigators to suggest systems of morphological-ecological classification based partly on the degree of regressive morphological evolution (Gisin, 1947; Christiansen, 1962); however, most studies of regressive evolution have been incidental to large taxonomic works. Works strictly devoted to regressive evolution have largely been concerned with eye structure. In this paper I shall discuss regression in collembola in general, and in some detail in a few characteristics in order to see if the situation found in collembola support or negate any of the various explanations offered to explain regressive evolution. I shall also compare the regressive evolution in caves with that in soil.

FEATURES SHOWING REGRESSION

Regressive evolution, as defined earlier, occurs in a variety of features in both cave and soil forms. Six features show clear regression but all do not show it in both habitat types (see Table 1 and Fig. 1) In addition to these, three other features may show regression 7)

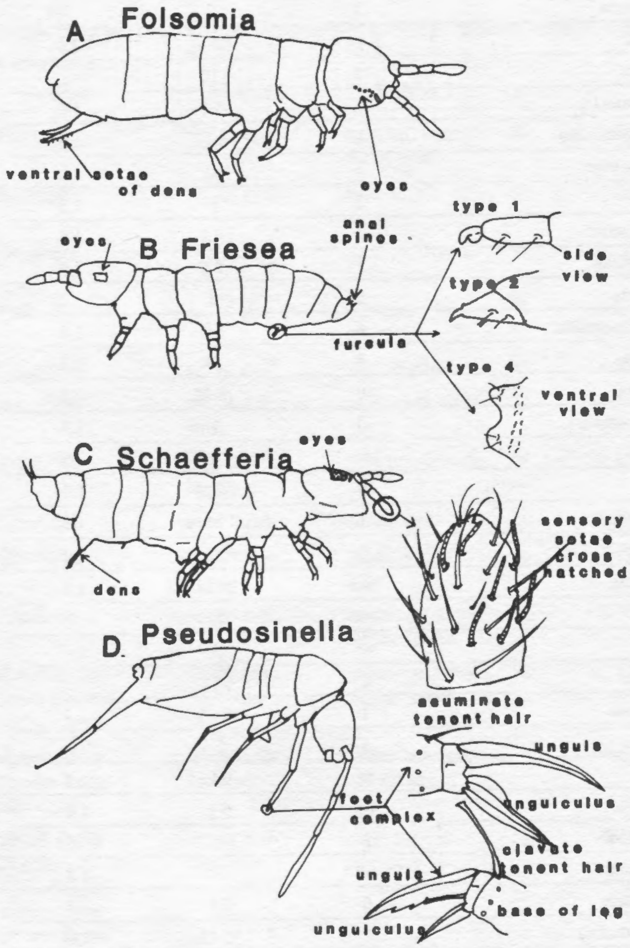


Figure 1. **Diagram of morphological features measured for A. *Folsomia;* B. *Friesea*; C. *Schaefferia*; and D. *Pseudosinella*. See text for details.**

Table 1. Features Showing Clear Regression.

	Features regressing in caves	Features regressing in soil	
1) eyes	+	+	
2) pigment	+	+	
3) furcula	−	+	(see figure 1B)
4) tenent hair	+	?	(see figure 1D)
5) unguiculus	−	+	(see figure 1D)
6) water retention ability	+	+	

chaetotaxy and scale structure, 8) sensory setae and 9) appendages. Below we shall look at each of these features in turn.

Regression in appendages, sensory setae, scale structure and chaetotaxy have not been studied systematically. So little is know concerning the function and systematic change in these features that it is difficult to say whether or not they satisfy our definition of regressive evolution. Chaetotaxy does appear to show regular reductive evolution, but it is not clear whether these changes are regressive evolution. Of the remaining 6 regressive features, the reduction of the unguiculus has not been studied systematically. Thus it is difficult to say much about the regression in this feature. Indeed it is possible that the unguis and unguiculus make a single adaptive unit and therefore the apparently regressive changes represent positive adaptations and not regressive evolution in our sense and I therefore shall not examine this organ further.

The regression in water retention ability has been studied in a series of excellent works by Thibaud (1981), Vannier (1977) and Thibaud and Vannier (1978; 1980). They clearly established that some cave forms lose the ability to control their water loss. There is little I could add to the data already presented and the array of species which has been studied is so limited that it is difficult to apply the data to support or weaken any theory of regressive evolution. The data available does support the idea that regressive evolution in the sense earlier defined does exist and is different from normal adaptive evolution.

A similar statement can be made about reduction in pigment. Reduction in pigment is a regular process in both edaphic and cave forms; however, it is difficult to quantify and there is little data available in the literature. In addition the process appears to follow closely the much more readily analyzable reduction in eye number. For these reasons I will not pursue this feature further. This leaves three features—reduction in the furcula, reduction in eyes and reduction in tenent hair which I shall examine in some detail. In addition I shall look at three putatively adaptive features—numbers of antennal sensillae, unguis length and body size, to see how they compare with features undergoing regressive evolution.

METHODS

In dealing with these questions I attempted to develop some quantitative measures of the features concerned. To make the task workable I limited the study to four genera where data could be obtained with moderate effort: *Friesia, Folsomia, Schaefferia* (S.L.) and *Psudosinella.*

The first genus is not found in caves except as a trogloxene, the second has some common troglophile species but is never clearly troglomorphic. The third genus shows many edaphic and troglomorphic cave species while the last genus is only highly modified in cave habitats. In studying the first three genera I used tabular data where it was available (Grow and Christiansen, 1974; 1976a; 1976b; Thibaud, 1972; Hermosilla et al.; 1984). Where necessary, this was supplemented with standard large taxonomic works. The last genus did not afford adequate tabular data so published drawings of the foot complex were used to measure the relative lengths of the foot base, tenent hair, unguiculus and unguis. Where more than one figure was available but all were similar the one showing greatest reduction of tenent hair was used. Where the difference was large both extremes of tenent hair were used. Analysis of the quantitative data was made using the MINITAB© computer package available on the Grinnell College computer system. In cases not involving *Pseudosinella* where a range of values was present for a given character state, median values were used except in the case of ventral dental setae where the maximum number was used. This was done to avoid error from the recording of immature specimens since dental setae increase with age.

CHARACTERS USED

Before detailed consideration of the data examined it is useful to examine each of the features studied.

The dens appears to shorten in many but not all edaphic and fine sand lineages and the feature is entirely absent in many strictly edaphic forms. A number of workers have shown a general correlation between loss of furcula and edaphic habitat, (Gisin, 1947; Haarlov, 1960) but it is impossible on a basis of present information to establish this quantitatively. It seems logical that organisms confined to small interstitial soil or litter spaces would cease to have a use for this organ. In addition most highly edaphic forms lack the furcula entirely. Personal observation has shown that forms with extremely reduced furcula cannot use this to jump. For these reasons I treat this as a feature undergoing regressive evolution.

Eye regression of both edaphic and cave collembola has been studied in some detail by a variety of workers (Barra, 1969; 1971; 1973; Brauner, 1984; Thibaud, 1967; Thibaud and Massoud, 1973). This work has been summarized (Thibaud, 1976) with an excellent bibliography on the subject. Thibaud notes that eye regression occurs in all collembolan families and is associated with cave, edaphic and similar habits. The regression is associated with both reduction and loss of individual ommatidia. The pattern of the order of loss is usually constant within groups but different between them. He also points out that the regression is generally centripetal in nature with the superficial structures degenerating before the internal nervous apparatus. Thus in *Schaefferia (Bonetogastrura) balzuci*, where cornea number varies from 1-3 per side, the visual nerve center is always developed for 3 + 3 eyes. Since eye number is almost always reported in the literature it is easy to investigate the numerical loss quantitatively.

The tenent hair (T.H.) or hairs are subject to reduction in many groups; however, the normal functioning of these has not been established in most forms. An exception is in the Entomobryinae where earlier work (Christiansen, 1975) established both a locomotor function for the organ and its loss of function in cave forms. For this reason the organ will be studied only in the genus *Pseudosinella* where a large number of species have readily available data.

The same work pointed out the adaptive nature of the elongation of the unguis in this group and relative unguis length will be examined here as an adaptive feature. The other two putatively adaptive features used in other genera are total body length and number of antennal sensillae. While these have not been studied in detail with respect to their adaptive nature, their general association with specific habitat types, as well as their functional significance, makes it highly probable that they are adaptive features. In the following sections I shall examine the regression of some of these features in each of four genera: *Folsomia, Friesea, Schaefferia*, and *Pseudosinella*.

Table 2. Folsomia Features and Species Studied

Species	Eye Number	Ventral Dental Teeth	Total Length (mm.)
achaeta	2	?	.8
agrelli	1	8	1.2
alpha	0	27-29	1.2
alpigena	3	?	?
alpina	6	11	1.5
binoculata	1	?	?
bisetosa	0	12	.84
brevicauda	2	3	.75
candida	0	22-43	2.0
calvipila	5	10	?
contrapuncta	3	6-7	1.0
decaxophthalma	8	8-10	1.5
decemoculata	5	11	1.2
decopsis	5	27	1.3
decopthalma	5	12	1.6
diplothalma	1	7-8	1.3
dovrensis	0	8	.48
duodecimoculata	6	6-9	1.2
elongata	8	7-8	1.6
fimetaria	0	18-22	1.4
fimetaroides	0	?	1.3
garretti	0	?	1.9
giustii	0	26-28	1.14
hasegawai	1	14	1.2
hidakana	0	23	1.4
highlandia	1-4	20-28	1.8
hodgei	0	20	1.0
hoffi	4-5	9	1.0
inoculata	0	8-12	1.2
ksenemani	1	15-18	1.3
loftyensis	3	?	?
lunata	0	11	1.1
macroseta	4-5	6-7	1.0
manolachei	2	3	.8
microchaeta	1-2	?	1.2
miradentata	0	2-3	.4
monophthalma	1	11-12	1.0
montigena	0	12-14	1.25
multiseta	2	16-22	1.6
nana	2	7	1.2
nakajimai	6-7	6	1.1
nivalis	0	31-34	1.6
norvegica	3-4	10-12	1.4
nova-zealandi	0	6	1.0
octoculata	4	15	2.0
ozeana	1-2	8	1.4
penicula	1-2	16-24	1.6
picea	6	7-10	1.3
prima	6-8	6-7	1.3
pusilla	0	3	.6
quadrioculata	1-2	7-8	1.6
regularis	1	7-8	1.0
salmoni	?	?	?
sensibilis	0	?	.6
sedecimoculata	0	6	1.6
setosa	5	10	1.6
setula	4-6	7-11	1.1
sexooclata	3-4	10-12	1.4
similis	1	13-15	1.45
spinosa	0	12-14	1.25
stella	0	15-20	1.40
strenzkei	2	11	1.1
thalassophila	5	12-14	1.6

Table 3. Correlation coefficients (r) among various morphological characters.

Genus	Variable 1	Variable 2	n	r
Folsomia	No. of eyes	max. no. ventral dental setae	54	-0.28*
Folsomia	No. of eyes	max. length in mm	58	+0.22*
Folsomia	Max. length	max. no. ventral dental setae	53	+0.52***
Friesia	no. of eyes	Cassagnau furcula type	70	-0.07
Schaefferia	no. of eyes	total length/dens	28	+0.09
Schaefferia	no. dental setae	total length/dens	28	+0.91***
Schaefferia	total length in mm	total length/dens	28	-0.45**
Schaefferia	total length	no. of eyes	28	-0.13
Schaefferia	total length	antennal sensillae	28	+0.47**
Pseudosinella	no. of eyes	tenent hair/unguiculus length	165	+0.30***
Pseudosinella (acuminate tenent hair spp.)	tenent hair/foot base	tenent hair/unguiculus length	92	+0.85***
Pseudosinella (clavate tenent hair spp.)	tenent hair/foot base	tenent hair/unguiculus length	73	+0.61***
Pseudosinella (species with both cave and surface forms or cave forms only)	*tenent hair/foot base*	*outer unguis/foot base*	*114*	*-0.17**
Pseudosinella (surface species)	tenent hair/foot base	outer unguis/foot base	51	+0.31*
Pseudosinella	no. of eyes	evol. stage	118	-0.67***
Pseudosinella (cave species with clavate tenent hair)	outer unguis/foot base	evol. stage	18	+0.30
Pseudosinella (cave species with acuminate tenent hair)	outer unguis/foot base	evol. stage	59	+0.34**
Psudosinella (surface species)	outer unguis/foot base	evol. stage	32	-0.07
Pseudosinella (cave species)	tenent hair/unguiculus	evol. stage	79	-0.44***
Pseudosinella	tenent hair/unguiculus	evol. stage	31	-0.04

* p<.05
** p<.01
*** p<.001

REGRESSION IN *FOLSOMIA*

Data was available for 63 species. Three items were examined: (Table 2 and Figure 1A) total length, eye number, and number of ventral dental setae. The latter was used as a measure of dens reduction since it is generally reported and appears to be closely associated with dens reduction (Table 3). Not all items were available for species so there will be discrepencies in reported numbers. A number of facts about these features are clear.

First there is a negative correlation between two of the regressive features (Table 3). The significance of this is unclear but it is clear that the two features are not responding to selective release in the same fashion. There is a weak positive correlation between eye number and total size ($P > 0.95$) and a strong correlation between maximum ventral dental seta number and maximum total length (Table 3). A reduction in maximum total length is almost certainly a positive adaptation to increased euedaphic habits and it appears that regression in both eye number and dental setae is associated with this trend, but in different ways.

If we look not at the absolute values of these characteristics but at the amount of recorded variation seen in each feature in each size or number range, a different picture emerges. If we compare the range of dental setae numbers reported with the maximum number we find a clear but not universal, positive correlation (Table 4). There is a similar positive correlation between maximum size and variation in dental seta number. This reduction in variation with decreasing number, is to be expected in any meristic feature merely as a result of decreasing potential for variability; however, if there were a countervailing increase in genetic variability with diminished number this should show up as a leveling factor in the reduction process. No such evidence is seen.

The picture in eye number is a bit different. If we consider the distribution of species with variant eye numbers relative to numbers of species reported with these eye numbers (Table 5), a different picture emerges. The meristic loss of variability should again produce a regular decrease in variability with reduced eye number. Instead we see increasing variability in eye number with reduction of eyes until the eyes are lost entirely, when stability once more occurs.

REGRESSION IN *FRIESEA*

Members of this genus are occasionally found in caves but are probably never true troglophiles or troglobites. The data available for this genus was limited, so only 3 features were used (Table 6, Figure 1B). The anal spines are of questionable adaptive value but show no regressive tendencies either in edaphic or cave environments. Cassagnau in 1958 developed a scheme for categorizing furcula stage from 1 for fully developed organs to 5 for extreme reduction or absence. I follow

Table 4. Difference between highest and lowest number of dental setae reported in **Folsomia.**

	0-1	2-3	4-6	7+
Maximum no of dental setae				
1-8	15	0	0	0
9-19	13	8	0	0
20+	3	3	3	4

Table 5. Eye variability in **Folsomia.**

	Minimum eye number				
	0	1-2	3-4	5-6	7-8
Total Number Species	21	19	8	9	2
Number having varying eye numbers	0	5	5	2	0

this system here. Examination of each of these features in all possible combinations showed no evidence of any correlation. The condition shown in Table 3 is characteristic of all potential correlations.

REGRESSION IN *SCHAEFFERIA* S. L.

Five features were used in this study. These were based on the system developed by Hermosilla et al. (1984; 1985) and are shown in Table 7 and figure 1C. These include three putatively regressive features: relative length of dens, number of dental setae and number of eyes. They also included two putatively adaptive features: total length and number of antennal sensory setae. As in previous genera there appears to be no positive correlation between the regression in eye and furcula structure (Table 3) but there is, not surprisingly, an excellent negative correlation between the number of dental setae and the ratio between dens and body length (Table 3). There is a good negative correlation between relative dental length and total length but none between eye number and total length (Table 3).

The correlation between antennal sensory setae number and total length is positive and much sharper (Table 3) particularly at the higher sensory seta numbers. In none of these cases is the correlation improved if we limit our consideration to only cave or surface forms.

REGRESSION IN *PSEUDOSINELLA*

This genus does not have forms clearly adapted for soil life. There are no species showing clear regression in the furcula or the unguiculus;

Table 6. **Friesia** *features and species studied*

Species	Cassagnau Furcula Type	Eye Number	Anal Spines
acuminata	1	8	6
africana	5	8	0
albida	4	5	3
arlei	2	4	12 capitate
afurcata	5	8	4
atypica	3	6	3
bodenheimeri	4	8	5
brevicaudata	3	3	7
cauchoisi	3	0	4
cera	1-2	8	3
claviseta	2	8	3
coiffaiti	5	8	5
colonavis	5	8	4
decemoculata	2	5	3
decipiens	3	5	3
denisi	4	3	3
duodecemoculata	2	6	3
excelsa	2	8	0
fagei	5	3	many
fara	3-4	8	6
gadeai	5	6	4
geminioculata	1-2	2	3
grandis	3	8	3
griseai	2	8	2
handschini	4	8	6
inermis	3	6	8
japonica	3	8	3
jeanneli	5	8	14
kardosa	5	2	8
laderoi	5	8	6
lammeri	1	5	3
landwehri	2	5	2
littoralis	5	8	11
magnicornis	2	8	3
maritima	1	3	4
mauriesis	3	8	Unique Capitate
millsi	3	7-8	5
mistrali	3	3	2
montana	2	6	5
monteiroi	5	5	2
multispinosa	2	3	+6
nauroisi	3	6	5
nevadensis	4	5	3
nietonis	5	4	5
nigrissima	5	7	0
nigrimontana	1	5	6
octoculata	4	4	3
oligoraphila	3	8	3
pacifica	1	8	5
parva	5	8	6
pentacantha	5	8	5
petiti	1	8	6+
polla	2	8	2
pyrenaica	3	4	3
quadrispina	5	4	4
quinguespinosa	3	3	5
quinta	3	4-5	5
reducta	5	8	0
salti	5	8	0
sexoculata	3	3	6
stachi	2	2	3
subantarctica	1	5	6
sublimis	1	8	3
subterranea	5	0	4
tatrica	5	0	4
tolosana	5	1	6
tourratensis	2	8	5
troglophila	5	2	6
truncata	3	8	3
wilkeyi	2	2	2
Yugoslavica	1	8	5

Table 7. **Schaefferia** *features and species studied. The subgenera are: B* = **Bonetogustrura** *ss* = **Schaefferia**, *T* = **Typhlogastrura.**

Species	Number of sensillae 4th antennal segment	Number of cornea/side	Maximum total length length mm.	Ratio total length + dens	Number of dental setae	Cave only = C Surface = S Both = B
S.T. alabamensis	7-9	4-6	1.5	21	6-7	C
S.ss. ariegica	7	2	1.1	47	3	S
S.T. atlantea	8-14	0	1.7	17	7-8	C
S.B. balazuci	6-9	0-3	2.4	17-18	7-8	C
S.T. breuili	9	2-3	1.8	15.3	7	C
S.ss. canigouensis	5-7	5	1.3	57	3-4	S
S.B. cavicola	6-9	2-8	1.5	20.6	6-7	B
S.T. christianseni	7	4	1.2	15-15.5	7	C
S.ss. coeca	8-12	0	1.7	40-41	3-4	B
S.ss. decemoculata	6-8	4-6	1.8	40-41	3-4	B
S.B. delhezi	8-15	2-3	2.0	14-15	8	C
S.ss. duodecimoculata	7	6	1.0	24	5-6	S
S.ss. emucronata	6-8	3	1.1	47	3-4	B
S.ss. guerrerense	6-7	0	1.5	37	4-5	B
S.ss. lindbergi	5-8	2-4	1.3	40-41	3-4	C
S.ss. maxima	6-8	5	2.1	44	5	S
S.T. mendizabali	8-16	0-1	2.5	13.5	7-9	C
S.ss. pouadensis	7-10	1-4	1.6	40-41	3	B
S.ss. quadrioculata	7-9	1-2	1.5	44	4-5	C
S.ss. scossirolii	7	2	1.3	31-40	5	S
S.ss. sexoculata	6-10	3	1.1	28	3-4	C
S.B. soulensis	7-8	3	3.1	17-18	8	C
S.B. spelicola	7	3-7	1.9	20.5	7-8	C
S.ss. subcoeca	7	0	1.0	52	3	B
S.B. subterranea	11-17	0-4	2.3	14.4	8	C
S.T. topali	5-9	2-5	1.2	27.5	4-7	C
S.B. variabilis	7	1-2	1.2	17-18	7	S
S.ss. willemi	7	4	1.6	40-41	3	B

however many species show regression in eye number, pigment and tenent hairs. Eye regression is probably associated both with microcavernicole and cave habitats. The relationship between the regression in tenent hair and cave life has been studied in detail (Christiansen, 1965).

Following the methods mentioned earlier a variety of data were obtained for over 125 species (Table 8 and figure 1D). Since data were obtained largely from drawings only relative measurements were possible. The tenent hair (T.H.) was measured both against the diameter of the foot base and the unguiculus length. The first was probably a measure of tenent hair size compared to a relatively invariant feature while the second was compared to a putatively adaptive feature without any established cave-dependent tendency. The unguis length compared to the diameter of the foot base has been shown (Christiansen, op. cit.) to be clearly cave adaptive and have parallel and convergent features. daGama's 1984 work made it possible to measure evolutionary or relative phylogenetic position for many of the European species. This array of data allows a number of comparisons and the changes in ratios of these organs show many interesting features.

First, unlike all other regressive features studied, there is a strong statistical correlation between reduction in eye number and reduction in tenent hair (Table 3) although this is not striking when graphed (Graph 1).

The ratio of T.H. to foot base is closely correlated with the T.H.-unguiculus ratio in forms having an acuminate tenent hair (Table 3). This is the highest T value found in the study. The ratio also is statistically correlated in forms having clavate tenent hairs (Table 3) but much less clearly than in the forms with accuminate tenent hairs. Since the loss of the clavate condition was shown to be associated with loss of function in cave forms this seems to indicate a sharp change in evolutionary interaction with loss of function.

This change is also associated with the cave environments. Of the species having acuminate T.H., 86% are found in caves and the great majority of these are limited to cave environments. The few surface forms with acuminate tenent hairs show no evidence of regressive evolution in the structure. In addition, extreme reduction in the tenent hair occurs only in cave inhabiting forms. Only one of the 53 species having a T.H./unguiculus ratio of .75 or less has been found in non-cave environments and this species (*P. immaculata*) is also found in caves. An additional 18 species with ratios between .75 and .90 include only two species found outside caves. In contrast, of the 69 species with ratios greater than or equal to 1.10, 76% are found outside caves. Thus, reduction of tenent hair is largely associated with the cave environment.

If we examine the correlation between tenent hair and relative unguis

***Table 8. Pseudosinella* features and species studied.**

Species	Eye Number	Ratio Tenent Hair + diameter foot base	Ratio T.H. + Unguiculus length	Length outer Unguis + diameter foot base	Cave = C Surface = S Both = B	Clavate Tenent Hair	Evolutionary Stage (daGama '84)
absoloni	2	1.2	0.81	2.18	S	-	4
aelleni	0	1	0.7	2.6	C	-	5
aelocia	0	1.6	1	2.7	S	+	6
aera	2	1.72	1.1	2.67	B	+	
alba	2	1.5	1	2.87	B	+	3
albida	5	2.3		2.8	S	+	3
alpina	1	1.1	0.74	3.22	C	-	9
andersoni	0	1.25	0.91	2.47	S	-	4
annemariae	3	1.6	0.95	2.85	S	+	5
antennata	0	1	0.63	2.89	C	-	12
apuanica	3	1.6	1.1	2.8	S	+	2
argentea	0	1.7	1	3.08	B	+	
aspinata	0				C	-	9
astronomica	1	1.46	0.65	3.23	C	-	8
aueri	0	0.8	0.41	3.2	C	-	7
balazuci	0	1	0.53	3.5	C	-	16
barcelonensis	0	1.05	0.65	3.06	C	-	12
bidenticulata	0	2	1.42	2.68	C	+	4
biunguiculata	0	1.14	1	2.4	S	-	
bohemica	2	1.8	1.35	2.38	S	+	2
bonita	0	0.8	0.29	3.5	C	-	
brevicornis	0	2.4	1.67	3.25	*	-	
bulgarica	4	0.85	0.69	2.37	C	-	1
caladarensis	0	1.38	0.7	2.71	C	-	7
carthusiana	0	0.36	0.5	3.75	C	-	
cassagnai	0	1.65	1	3	C	-	8
cavernarum	0	1	0.4	4	C	-	12
centralis	0	0.65	0.38	2.62	C	-	12
certa	0	0.83	0.5	3.33	C	-	
chapmani	0	0.76	0.38	4	C	-	5
christianseni	0	1	0.57	3.5	C	-	
ciliata	2	1.67	1.11	2.7	S	+	6
collina	6	2	1.5	2.3	S	+	
concii	0	0.78	0.37	3.2	C	-	9
dallaii	0	1.37	0.62	3.63	C	-	9
decepta	0	1.5	0.87	2.81	C	+	4
decipiens	0	1.07	0.6	3.5	C	-	12
delhezi	0	1.42	0.83	4.17	C	-	15
denisi	0	1.25	0.73	3.06	C	-	15
difficilis	5	1.54	1	2.9	S	+	
dispadentata	4	1.42	1.2	2.1	S	-	
dobati	0	1.08	0.75	2.9	C	-	11
dodecopsis	6	1.55	0.77	3.16	C	+	3-4
dodecopthalma	6				C	+	5
dubia	5-6	2.36	1.04	3.26	C	+	
duodecimocellata	6	1.5	1	3	C	-	1
duodecimoculata**	5-6	1.5	1.3	3	S	+	1
duodecimoculata**	5	2	1.11	3.11	C	+	1
edax	3	1.58	1.19	2.23	S	+	8
efficiens	0	0.76	0.54	2.65	C	-	12
encrusae	5	1.25	0.91	2.76	S	+	2
espana	0	0.33	0.25	3.48	C	-	
espanoli	2	2.56	1.15	2.85	S	+	2
fallax	3				B	+	4
fasciata	5-6	2	1.4	2.93	S	+	
feneriersis	0	0.64	0.53	2.29	C	-	

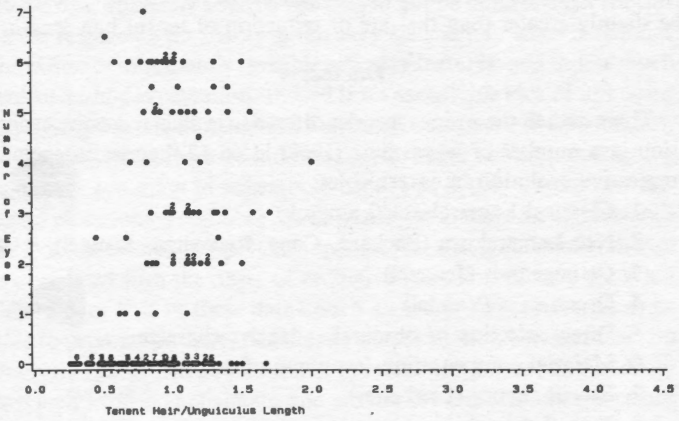
	Eye Number	Ratio Tenent Hair + diameter foot base	Ratio T.H. + Unguiculus length	Length outer Unguis + diameter foot base	Cave = C Surface = S Both = B	Clavate Tenent Hair	Evolutionary Stage (daGama '84)
finca**	0	2.38	1.14	3	C	+	
finca**	0	1.56	0.83	3	C	-	
fjellbergi	5				S	+	3
folsomi	0	2.3	1.46	3	S	+	9
fujiokoi	0	2	1.33	2.23	S	-	
gamae	0	1.43	1	2.79	S	+	9
genghzikhani	2	1.2	1.2	2.48	S	+	
gineti	0	1.04	0.47	3.13	C	-	10
gisini	2-3	1	0.66	3.6	C	+	
gisini	2-3	0.91	0.44	3.6	C	-	
gleycola	0	2	1.27	2.7	S	+	
goughi	6-7	0.9	0.51	3.04	C	-	8
hauseri	3	1.5	1	2.3	S	-	5
hercynica	2	1.54	1.25	2.46	S	+	2
hermanni	3	1.5	1.3	2.5	S	+	7
heteromurina	6	1.04	0.83	2.52	C	-	4
hirsuta	0	1.04	0.55	4.8	C	-	
hrabei	6-0	1.5	1.15	2.8	C	+	
huescensis	6	1.28	0.68	2.95	C	+	2
huetheri	5	2.1	1.26	2.84	S	+	6
illiciens	0	1.54	1.18	2.8	S	+	5
immaculata**	0	0.76	0.47	3.2	B	-	12
immaculata**	0	1.6	0.92	2.89	B	-	12
impediens	0	2.28	1.23	3.14	C	+	9
infernalis	0	0.57	0.27	3	C	-	10
inflata**	0	1.38	1.08	2.6	C	-	10
inflata**	0	1.6	0.95	2.6	C	-	10
infrequens	0	1.26	0.91	2.5	C	+	4
insoloculata	0	1.28	0.9	2.8	S	-	
insubrica	0	1.25	0.71	3.22	C	-	7
insularum	2				C	+	4
intemerata	0	1.35	0.84	2.89	C	-	7
intermixta	3	2.4	1.7	2.75	S	+	
jeanneli	4-6	1.18	0.87	2.63	C	-	
joupani	5	1.58	0.79	2.58	C	+	
kersmaekersi	0	2.05	0.95	4.1	C	-	14
ksenemani	1	2	1.1	2.86	C	+	9
kwartirnikovi	6	1.57	0.89	2.95	C	-	4
lamperti	0	0.87	0.56	2.82	C	-	5
lunaris	0	1.88	1.11	3.47	C	+	15
manuelae	6	2	0.93	3.13	C	+	1
mauli	5	2.23	1.16	2.92	C	+	2
melatensis	0	0.65	0.46	2.71	C	-	11
montis	0	1.55	1.27	2.75	S	-	
nata	0	1.57	1.1	2.54	C	+	
nigra	2	1.63	1	2.62	S	-	
nonoculata	0	1.75	1.17	3.25	S	+	
ocellata	3	1.36	0.84	2.64	C	-	
ophthalma	4	1.42	0.81	2.75	C	+	
octopunctata	4-5	1.66	1.19	2.33	B	+	5
orba	0	1.55	1.03	2.8	C	-	
oxybarensis	0	0.67	0.39	3.18	C	-	12
paclti	0	0.93	0.62	2.8	C	-	11
pallida	2	1.92	1.15	2.75	S	+	2
pecki	0	1.83	1.16	3.04	C	-	
petrustrinatii	0	1.94	1.5	2.41	C	+	
petterseni	0	1.5	0.92	2.71	S	+	5
picta	4	1.64	1.09	3.07	S	+	1
pieltaini	0	1.4	0.78	3.03	C	-	11
praecipiens**	0	1.64	1.29	2.57	S	+	5

	Eye Number	Ratio Tenent Hair + diameter foot base	Ratio T.H. + Unguiculus length	Length outer Unguis + diameter foot base	Cave = C Surface = S Both = B	Clavate Tenent Hair	Evolutionary Stage (daGama '84)
praecipiens**	0	1.43	0.95	2.57	S	-	5
problemartica	6	1.31	0.85	2.92	C	+	1
pyrenaea	0	0.81	0.48	3.54	C	-	13
racovitzai	7	1.46	0.79	2.93	C	-	1
rapoporti	2	1.7	1.5	2	S	+	
recipiens	0	1.85	1	2.88	S	+	8
reddelli	2-3	2	1.33	3.29	C	+	
rolfsi	0	1.38	1.22	2.2	S	+	
salisburgiana	0	0.48	0.3	3.07	C	-	10
sandelsorum	5	1.8	1.56	2.35	S	+	4
selgae	2	1.88	1.25	2.75	S	+	3
sera	0	1.38	1.26	2.3	S	+	
sexoculata**	3	2.5	1.58	2.43	S	+	5
sexoculata**	3	2.31	1.32	2.43	C	+	5
sollaudi	0	0.84	0.53	3.6	C	-	13
soniae	3	1.8	1.38	2.65	S	+	
spelunca	2	1.53	0.88	3.07	C	-	
spinosa	0	1	0.47	4.42	C	-	
stompi	5	1.72	1.06	3	C	+	5
strinatii	0	1.07	0.7	2.62	C	-	
stygia	0	1.05	0.66	3.27	C	-	7
styriaca	0	1.3	0.62	3.3	C	-	
subduodecima	6	2.1	1.3	3.06	C	+	2
subinflata	0	0.53	0.32	3.5	C	-	11
suboculata	6	2.08	1.08	3.5	C	+	7
substygia	1	1.45	0.87	2.85	C	+	1-2
subterranea**	0	1.25	0.66	2.82	C	-	11
subterranea**	0	0.86	0.5	2.82	C	-	11
subvirei**	0	1.71	0.92	3.27	C	-	11
subvirei**	0	1.57	0.78	3.27	C	-	11
superduodecima	6	1.87	0.96	3.52	C	+	5
superoculata	6	1.09	0.58	3.62	C	-	7
tarraconensis**	0	1.08	0.5	3.46	C	-	11
tarraconensis**	0	1.23	0.66	3.46	C	-	11
templadoi	3	1.93	1.12	3	S	+	4
terricola	6	1.5	1.01	2.53	S	+	2
testa	3	1.71	0.98	3.13	C	-	
theodoridesi	5-6	1.84	0.85	3.21	C	-	2.6
thibaudi	2				C	-	7
tridentifera	4	1.28	1.14	2.5	S	-	4
truncata	0	1.35	0.61	3.55	C	-	11
tyrrhena	1	1.15	0.77	2.52	S	+	4
unguiculata**	0	0.8	0.39	3.37	C	-	11
unguiculata**	0	1.06	0.47	3.37	C	-	11
unioculata	1	2.54	2	2.5	C	+	
valis	4	2	1.29	2.85	S	+	
vandeli	0	1.63	0.82	3.6	C	-	8-9
vertamicoriensis	0	0.71	0.56	2.86	C	-	
violenta**	0	1.55	1	2.18	B	-	
violenta**	0	1.63	1	2.8	B	+	
virei	0	0.84	0.48	3	C	-	11
vita	0	1.53	0.96	2.94	C	-	
vornatscheri	0	0.88	0.63	2.67	C	-	7
wahlgreni	5	2.47	1.4	2.94	B	+	
Yosiiana	0	1.46	1.27	2	S	+	
zygophora	5				C	+	1

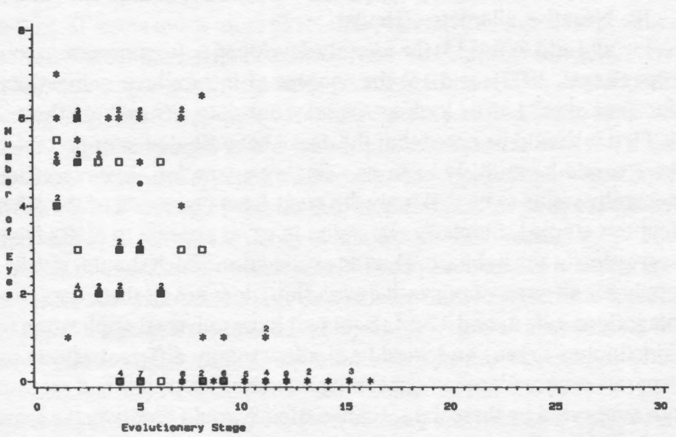
*The evolutionary grade numbers of the *Lepidocyrtus* sp. lineage were raised 1 to make them comparable to the other lineages.

**Where cave and surface forms, or differently reported forms, differ strikingly they are entered separately.

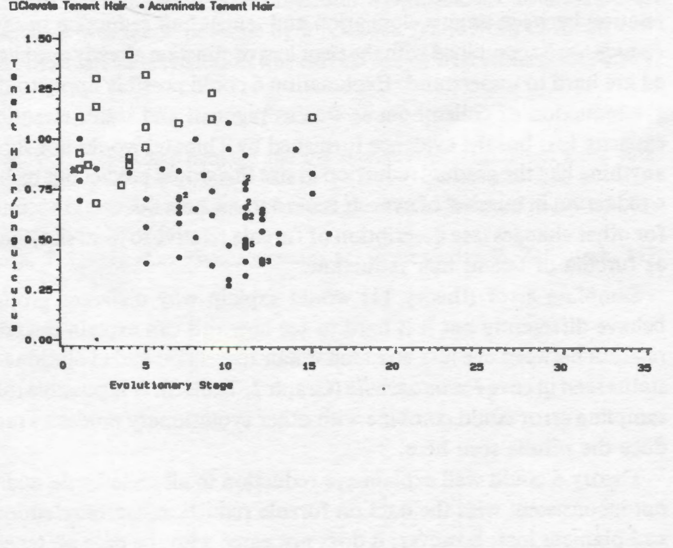
Graph 1 **Pseudosinella**
T–Ratio = 4.02 P very high 165 Observations



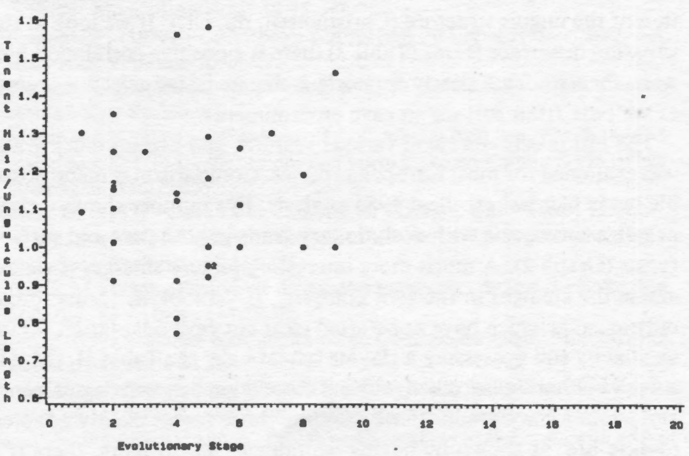
Graph 2 **Pseudosinella**
T–Ratio = –9.69 P very high 118 Observations



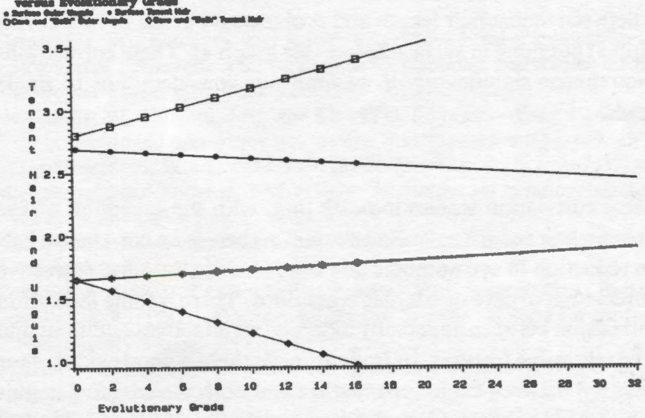
Graph 3 **Pseudosinella**
T–Ratio = –4.27 P very high
Cave Species 79 Observations



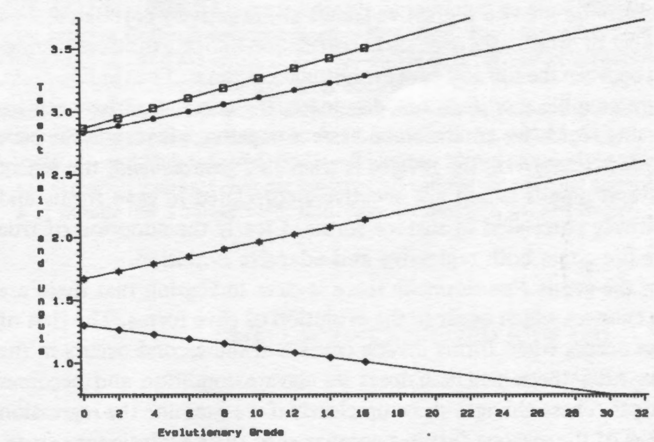
Graph 4 **Pseudosinella**
T–Ratio = 0.24 P<0.60
Surface Species 31 Observations



Graph 5 **All Forms**
Both Tenent Hair Conditions
Tenent Hair and Outer Unguis–Unguiculus Ratio
versus Evolutionary Grade



Graph 6 **Cave and "Both" Forms**
Tenent Hair and Outer Unguis–Unguiculus Ratio
versus Evolutionary Grade



length in forms found in caves (Table 3) there is a weak negative correlation between the two. This supports the idea of a synchronous regressive change in tenent hair structure and adaptive change in elongation of the unguis structure (Christiansen, op. cit.). If we look at the situation in surface forms (Table 3) there is a positive correlation between the two. This clearly supports a change in the selective regime as we pass from surface to cave environments.

The ratios between these various features and evolutionary grade was examined for most European species. Comparison is made possible by da Gamas' excellent 1984 analysis. Eye number shows a clear negative correlation with evolutionary grade in both cave and surface forms (Graph 2). A much more interesting picture emerges if we examine the changes in the foot complex. If we look at forms found only in caves which have not started clear cut cave adaptation (as indicated by still possessing a clavate tenent hair: see Table 3), there is a weak but not statistically significant correlation between unguis legnth and evolutionary grade. If we examine those forms clearly adapted to cave life, as shown by having acuminate, tenent hairs, there is a very strong correlation between the two (Table 3). In contrast, strictly surface forms show no correlation.

In a similar but reverse fashion, there is a strong negative correlation between tenent hair length and evolutionary grade in cave forms (Graph 3) but none in surface forms (see graph 4). These correlations do not change significantly if we limit our considerations to single lineages.

Summary of Results

These correlation studies indicate that, with the exception of eye and tenent hair reduction in *Pseudosinella*, there is no correlation between reduction in eye numbers and the reduction in other regressive features either in cave or edaphic conditions. There is some indication of correlation between apparently independent adaptive conditions and related regressive features. In *Pseudosinella* there is good evidence for correlation between evolutionary stage and both the adaptive unguis length and the regressive tenent hair reduction in cave forms but not in surface forms.

There is a moderate correlation between eye reduction and evolutionary stage in both cave and surface forms in *Pseudosinella*. Most interesting are cases where reverse trends appear to be in operation. In *Folsomia* the two regressive trends are negatively correlated. In a number of other cases reverses in correlation indicate the clear distinction between the soil and cave evolutionary regimes. Thus in *Folsomia*, where edaphic modifications dominate, the correlation between increasing regressive condition and size is negative whereas in the cave adapted *Schaefferia* the reverse is true. In *Pseudosinella*, the tenent hair and unguis length are negatively correlated in cave forms and positively correlated in surface forms. Clearly the adoption of true cave life alters both regressive and adaptive evolution.

In the genus *Pseudosinella* there is clear indication that there are two changes which occur in the evolution of cave forms. The first of these occurs when forms invade caves and the second occurs in the caves after the tenent hair loses its clavate condition and becomes vestigal. These changes show up clearly if we examine the regression curves of the various features compared to their evolutionary stage. Graph 5 shows the striking difference in the adaptive unguis length and regressive tenent hair length in the two habitats. Graph 6 shows that while unguis length elongation appears to go on at a uniform rate before and after cave forms have lost the functional clavate tenent hair, the regression of the organ does not occur until the animals have passed the regressive Rubicon of loss of clavate structure. These graphs also demonstrate that the rate of elongation of the unguis appears to be slightly greater than the rate of reduction of tenent hair length.

Discussion

These data fit the various theories offered to explain regressive evolution in a number of ways. Barr (1968) listed 12 theories to explain regressive evolution in cavernicoles:

1. Classical Lamarckism (Lamarck)
2. Neo-Lamarckism (Packard, Cope, Racovitza, Jeannel)
3. Orthogenesis (Jeannel)
4. Organicism (Vandel)
5. Direct selection of obscurely adaptive characters
6. Material compensation (economy of growth energy)
7. Escape theory (Lankester)
8. Trap theory (Ludwig)
9. Accumulation of random mutations
10. Indirect effect of pleiotropy
11. Genetic drift (sampling error)
12. Negative allometry (Heuts)

I would add to this 13) the recently developed noise suppression concept (Regal, 1977), and 14) the concept of intracellular competition for gene sites. Let us look at the way our data fits each of these.

First it should be noted that the data I have alluded to or presented here would be unlikely to fit any single explanation. Eye regression generally seems to be strikingly different from regression of the other features studied. Similarly regression in caves appears to differ from regression in soil habitats. Thus an explanation which should, if valid, apply for all cases of regressive evolution, does not fit these data. Explanations 1-4, 9, and 12-14 should all have universal application to functionless organs and would not allow totally different effects on separate organs placed under similar selective constraints and are thus not supported by these data. Explanation 7 would hardly make sense under the conditions concerned in this study, as Barr has already pointed out, and can thus be eliminated. If explanation 5 is correct, the negative correlation between eye and furcula reduction in *Folsomia*, in contrast with a positive correlation between tenent hair and eye regression in *Pseudosinella*, is very difficult to explain. In addition, the weak correlation between unguis elongation and tenent hair reduction in cave *Pseudosinella* combined with the clear loss of function already established are hard to understand. Explanation 6 could possibly apply to the eye reduction of collembola as well as pigment and water retention capacity loss but the evidence furnished by Thibaud would not show anything like the gradual reduction in size this would predict but rather a reduction in number of eyes. It is hard to see how this could account for other changes (see description of furcula relative to total size) such as furcula or tenent hair reduction.

Sampling error (theory 11) would explain why different groups behave differently but it is hard to see how this can explain the correlation between eye loss and tenant hair regression and evolutionary status seen in cave *Pseudosinella* (Graph 2, Table 3). It is possible that sampling error could combine with other evolutionary modes to produce the results seen here.

Theory 8 could well explain eye reduction in all collembola and is not inconsistent with the data on furcula reduction, water retention, and pigment loss; however, it does not agree with the data on tenent hair reduction in *Pseudosinella*. It is hard to see how this would apply here since acuminate tenent hairs sometimes occur in surface forms. Pleiotropy (Theory 10) is also not in conflict with our data since the pleiotropic positively selected features might be quite distinct for different features. This theory is not very satisfactory since it requires the existence of presently invisible adaptive features, and thus is much like an ad-hoc explanation. Indeed if we accept this idea of unknown modifying factors, explanations 5 and 6 become possible explanations.

The data here presented indicate that different features undergoing regressive evolution in collembola behave as if they were under different evolutionary regimes even when subject to the same or analagous environmental conditions. It also appears that a clear functional change is associated with the onset of reductive evolution (Graph 6). What theory does best fit these data? Such a theory would have to: a) be able to produce positive, negative or no correlation between features undergoing regressive evolution in the same conditions, and b) differ strikingly in different habitats, and c) allow for great variation in results while showing overall parallel evolutionary features. It is difficult to see how any one extant theory can fit all these criteria. In particular the accumulation of random neutral, or better deleterious, mutations could hardly produce a negative and a positive correlation in different groups. It seems much more likely that regressive evolution is produced by different mechanisms under different circumstances. As Culver (1982) has pointed out, the basic problem in regressive evolution is testing the various hypotheses. It would certainly be possible to establish experimental protocols to test which of these theories best fitted the situations we have seen here. For example, one could measure metabolic efficiency in a variety of species showing different levels of eye reduction seen if increased efficiency is associated with reduced eyes. The number of experiments which would be required makes it highly improbable that such results will be available in the foreseeable future. It will be possible to extend analyses of regressive evolution as phylogenetic studies such as daGama's become more common. Thus it would be very informative to see whether or not the adaptive and regressive changes in *Schafferia* and *Folsomia* are correlated with evolutionary grade.

ACKNOWLEDGEMENTS

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THE EVOLUTION OF POLYGENIC SYSTEMS, STUDIED ON EPIGEAN AND CAVE POPULATIONS OF *ASTYANAX FASCIATUS* (CHARACIDAE, PISCES).

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ABSTRACT: The epigean and cave populations of Astyanax fasciatus *(Pisces) differ with respect to several structures. The regressive features of eye and melanophore/morphological color change as well as the constructive features of taste bud apparatus and the feeding behavior change are genetically analyzed. They are based on polygenic systems characterized by between at least 2 and at least 7 loci.*

The phylogenetically older regressive and the more recently evolved constructive polygenic systems show no difference in the principles of manifestation. The most striking similarity is that after recombination of a minimum number of polygenes the amount of expression a newly added polygene manifests, is considerably increased. General importance in evolutionary progress is attributed to this phenomenon of varying expressivity. Only in phases of high expressivity is directional selection able to enlarge or even manifest the anlagen of cryptogenic structures.

The morphological color change seems to be adaptive specialization in connection with high polygenic expression. In this case this specialization is developed by the fact that environmental influence can suppress high polygenic expression. This type of switch mechanism is probably wide-spread (e.g. phenotypic sex determination).

Monogenic systems are often nothing else but extremes of polygenic inheritance. Monogenic dominance in these cases is caused by loss of a single polygene of high expression. On account of little structural difference, the rest of the polygenic systems mostly remain hidden. This is exemplified by the sex determination system in some toothcarps in which the transition from a poly- to an apparently monogenic mode is found.

INTRODUCTION

The genetic study of evolution as initiated by Kosswig is characterized by refuting the overemphasis of selection (Kosswig, 1948; 1963). Selection has less a constructive role in evolution than that of eliminating those mutations which are non-adaptive (stabilizing selection) or that of just giving a certain adaptational direction (directional selection).

The absence of stabilizing selection regularly occurs during transitional evolutionary phases. These are, for example, initial stages of constructive evolution which may be observed when biotopes with little or no interspecific competition are colonized by an invader. Genotypic and phenotypic variability now arises and equilibria become punctuated, because stabilizing selection for a specific ecological niche which has once been acquired by this species is no longer acting.

An example is the intralacustrine speciation of fishes in Lake Lanao (Philippines) (Wahl 1972; Fig. 1) or Titicaca (Peru) (Kosswig and Villwock, 1964) as well as in East African lakes (Fryer et al., 1983). In all these lakes geological history (Lanao) or abiotic conditions like an unusual ionic composition of the water (East Africa) (Ladiges, 1968) have only allowed one or few species to colonize. The variability of phenotypes is due to the withdrawal of stabilizing selection for certain adaptational features. This stabilizing selection in the original ecosystem was caused by competing species.

Increased variability provides the material for directional selection which radiates such species into open niches. However, these evolutionary processes can only occur when the ancestral species is characterized by a broad preadaptation. Variability will finally lower again under the influence of inter- and intraspecific competition, which slowly increases.

Evolutionary processes such as those described above also occurred when certain oceanic islands were colonized. Based on Lack's (1947) studies of Darwin's finches of the Galapagos Islands, Kosswig (1948) showed that—although already adapted to several new niches—some of them are still characterized by little specialization to the food they live on and by structural variability of their bills.

Furthermore, stabilizing selection will stop acting in features which have lost biological function (Kosswig and Kosswig, 1940; Hubbs, 1936). This can especially be observed in cave animals which convergently show the reduction of eyes and pigmentation in many systematic groups. Except for these organs no considerable phenotypic variability is allowed to develop because of stabilizing selection. This is probably due to the fact that the cave biotope unlike a large lake can only offer one narrow ecological niche which is only slightly different from

*+In grateful memory of Prof. Dr. Dr. hc.mult. Curt Kosswig (*30 Oct. 1903 +29 March 1982)*

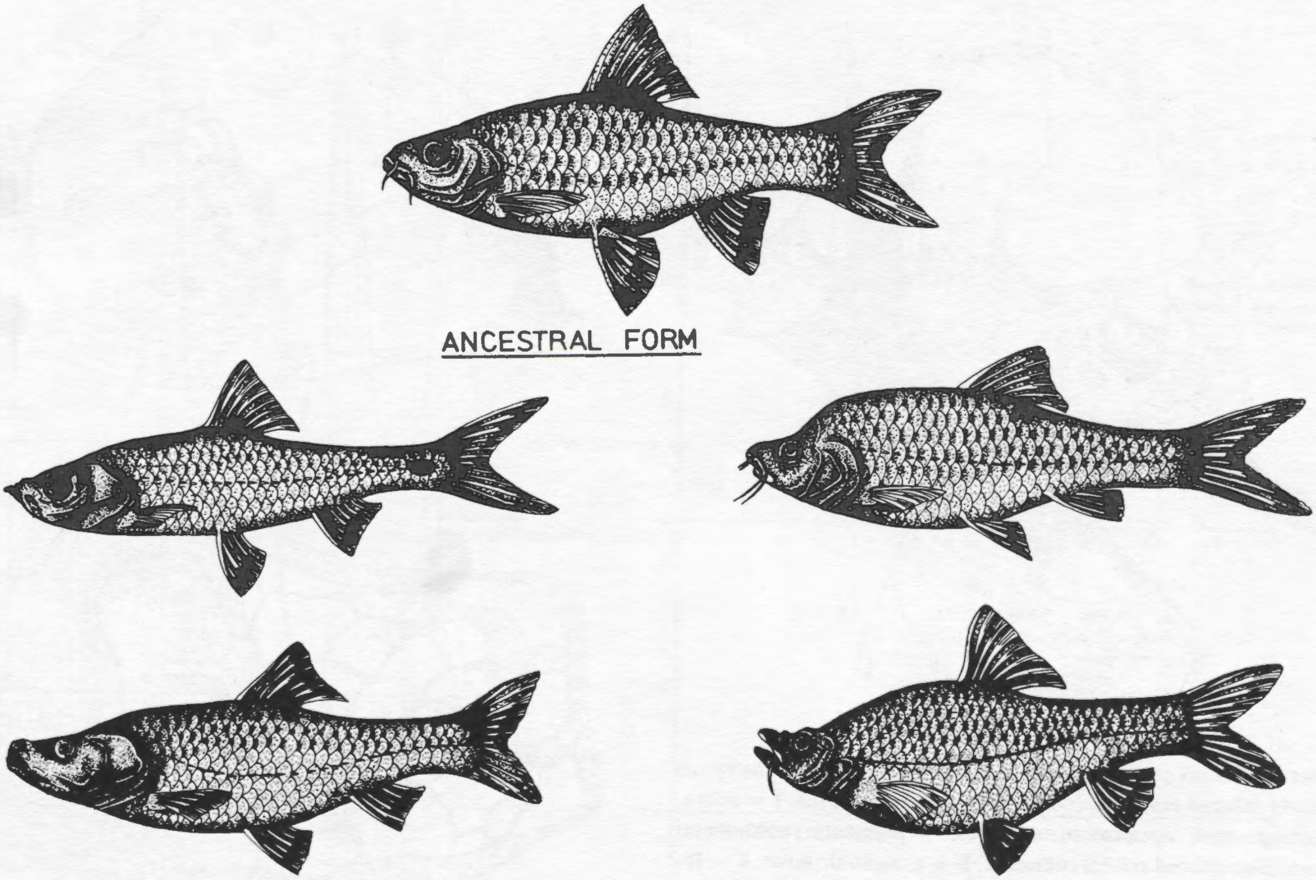


Figure 1: **Part of the species flock of Lake Lanao (Philippines). The ancestral species *Puntius binotatus* (Cyprinidae) divided into several variable forms after colonization of this almost fishfree biotope.**

that of the epigean ancestor.

Cave dwelling animals are particularly suited for the study of evolutionary processes, because in these forms constructive adaptations are simultaneously developed with regressive ones. Both start at the same time and thus enable the determination of evolutionary rates. In few cases a genetic analysis can be performed.

EPIGEAN AND HYPOGEAN POPULATIONS OF *ASTYANAX FASCIATUS*

GENERAL DESCRIPTION

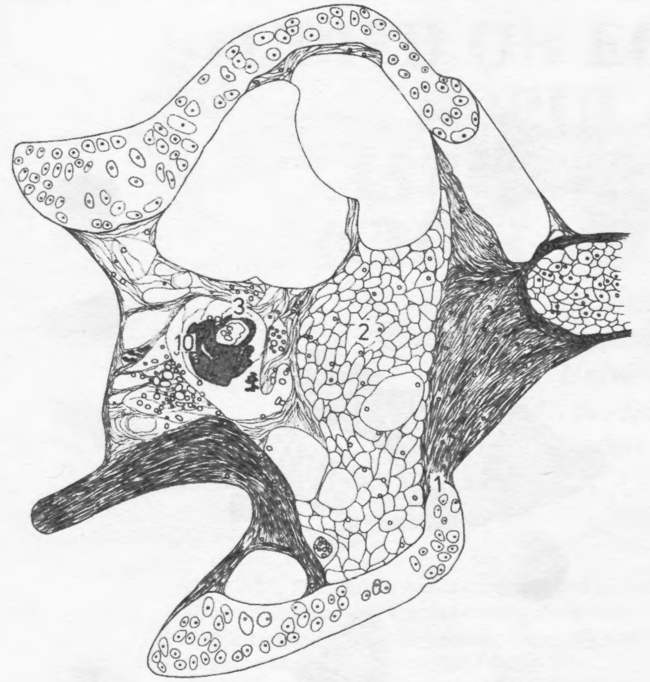
The characin *Astyanax fasciatus,* one of the most common freshwater fishes in Middle America, has developed a series of cave living populations within a geographically limited area in Central Mexico (Mitchell et al., 1977). The largest group of these populations is characterized by the almost complete reduction of eyes and melanin pigmentation. Part of them are populations which were originally described as species of the genus *"Anoptichthys"* (*"A.antrobius"* Alvarez = Pachon-fish, *"A.hubbsi"* Alvarez = Sabinos-fish).

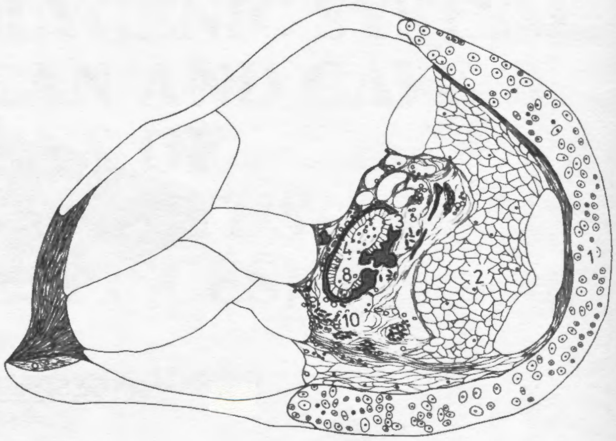
Two further populations phenotypically diverge and show variability of eye and pigmentation. They are the Micos-fish (Wilkens & Burns, 1972), which is phylogenetically young (Peters et al., 1975; Wilkens, 1976), and the Chica-fish (*"A.jordani"* Hubbs) which is a hybrid form between an extremely reduced cave form and the epigean population (Avise & Selander, 1972; Wilkens, 1972).

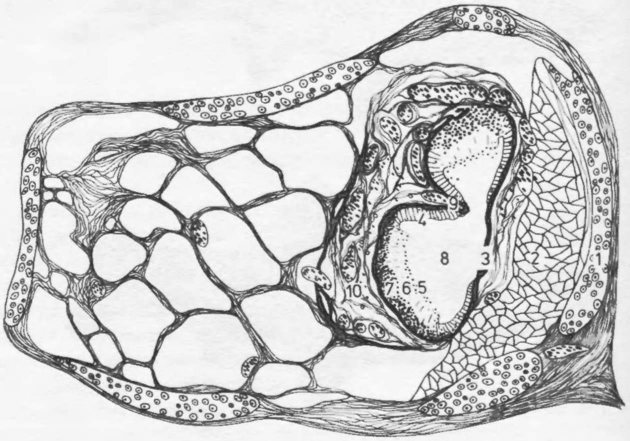
Because of the interfertility of epigean and cave living populations the genetic analysis of a series of morphological and ethological features is possible. It is especially noteworthy that in the case of these fish populations divergences can be studied which are normally only developed between species.

MORPHOLOGY AND GENETICS OF THE EYE

The eye of the epigean population is characterized by a considerable size. In the extremely reduced cave populations its diameter is only







***Figures 2-4:* Stages of different structural differentiation of the eye of extremely reduced cave populations of *Astyanax fasciatus*. 1 = sclera, 2 = spongiosum (ligamentum annulare), 3 = pigmentary epithelium, 4 = undifferentiated retinal rudiment, 5 = ganglionic layer, 6 = inner plexiform and 7 = inner nuclear layer, 8 = vitreous body, 9 = processus falciformis, 10 = chorioid (Wilkens, 1970b).**

20% of the epigean ancestor. There is some variability in the relative size and in correlation of relative size and the degree of structural differentiation (Figs. 2-4). The studies of the phylogenetically young and variable Micos population and of crosses between epigean and extremely reduced phylogenetically old cave forms show that all single structures of the eye-lens, pupillary opening, and retina—are correlated with respect to their size. The larger an eye is, the better it is differentiated. Therefore it is only possible to analyze genetic factors which are responsible for eye size (Wilkens, 1970a; 1976). They are called "eye genes".

The distribution curves of eye sizes to be found in the crossings and in the Micos fish usually show a normal distribution (Fig. 5). The mean eye diameter has an intermediate position. Therefore it can be concluded that the eye genes manifest in an additively polygenic manner. However, the additively polygenic inheritance is characterized by alterations. For example the backcross with the epigean form or the Micos fish show bimodal distributional curves. Furthermore the mean diameters of all crossings are closer to the epigean form than should be expected from numerical calculations assuming additivity (Wilkens, 1970a; 1976).

As shown by the variability of eye size, the gene-pool of the Micos population is heterozygous for the eye genes. Contrary to this the phylogenetically old cave populations seem to be more or less homozygous. However, the remnant eye genes are in part non-allelic. Because of this there are some individuals in the F_1 -crossing between extremely reduced cave forms which possess larger and better developed eyes than the parental populations (Wilkens, 1971). In this case a lens rudiment may even be found.

A minimum number of at least six or seven loci have mutated in the phylogenetically old cave forms.

MORPHOLOGY AND GENETICS OF THE MELANOPHORE SYSTEM

The pale color of the phylogenetically old cave populations is the result of the reduction of the melanophore system and in part of albinism (Sadoglu, 1957; Wilkens, 1970b). The regression of melanophores is due to a reduction of the number of these chromatophores and additionally to a lesser melanin content (Fig. 6). Whereas the physiological color change is intact in all subterranean populations, the morphological one no longer functions.

The phylogenetically young Micos population has an intermediary position. In daylight it will develop a functional morphological color change. In darkness the number of melanophores is much more reduced than in the epigean ancestor. The melanin content of the chromatophores does not seem to be reduced under these conditions (Wilkens, 1976).

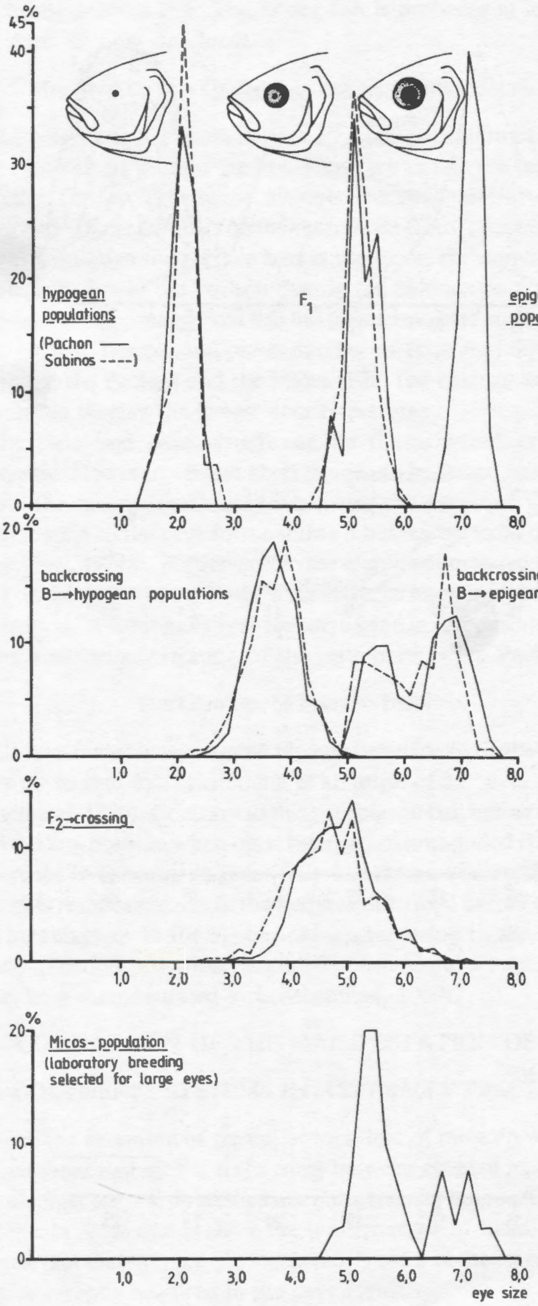


Figure 5: **Distribution of eye size of various *A. fasciatus* populations (Wilkens, 1970a; 1976; 1980).**

The amount of phenotypic variability in most cave forms (Sabinos, Yerbaniz, Piedras) is the same as in the epigean fish. Exceptions are the hybrid Chica population and the phylogenetically old Pachon population. This form was originally heterozygous for an albino gene (Sadoglu 1957; Wilkens, 1970b). It has recently been found that now the whole population is homozygously albinotic (Mitchell et al., 1977; Wilkens, 1984).

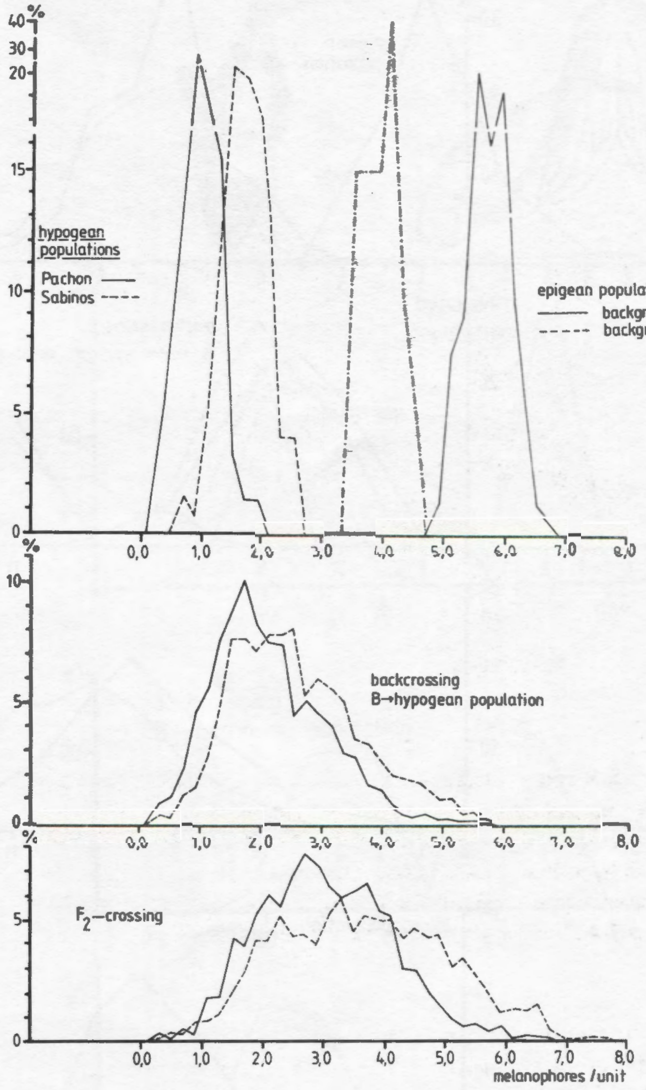


Figure 6: **Distribution of melanophore densities of various A. fasciatus populations (Wilkens, 1970c).**

Both albinism and reduction of melanin content each rely on one single recessive gene. Both mutations have happened at allelic loci in the different populations. They are inherited independently.

The gene system which is responsible for the number of melanophores segregates independently from the features mentioned before. It is additively polygenic. There are also considerable alterations. They are mainly due to the morphological color change. The complete function of this feature is correlated to the genes which are responsible for the number of melanophores. Its functioning is dependent on a minimal number of melanophore genes and the existence of at least one parental haplome, which epistatically provides genetic balance. By this it can be explained that the backcrossings to the cave populations are characterized by some individuals with intact morphological color change. Because of this the distribution of melanophore density shows skewness

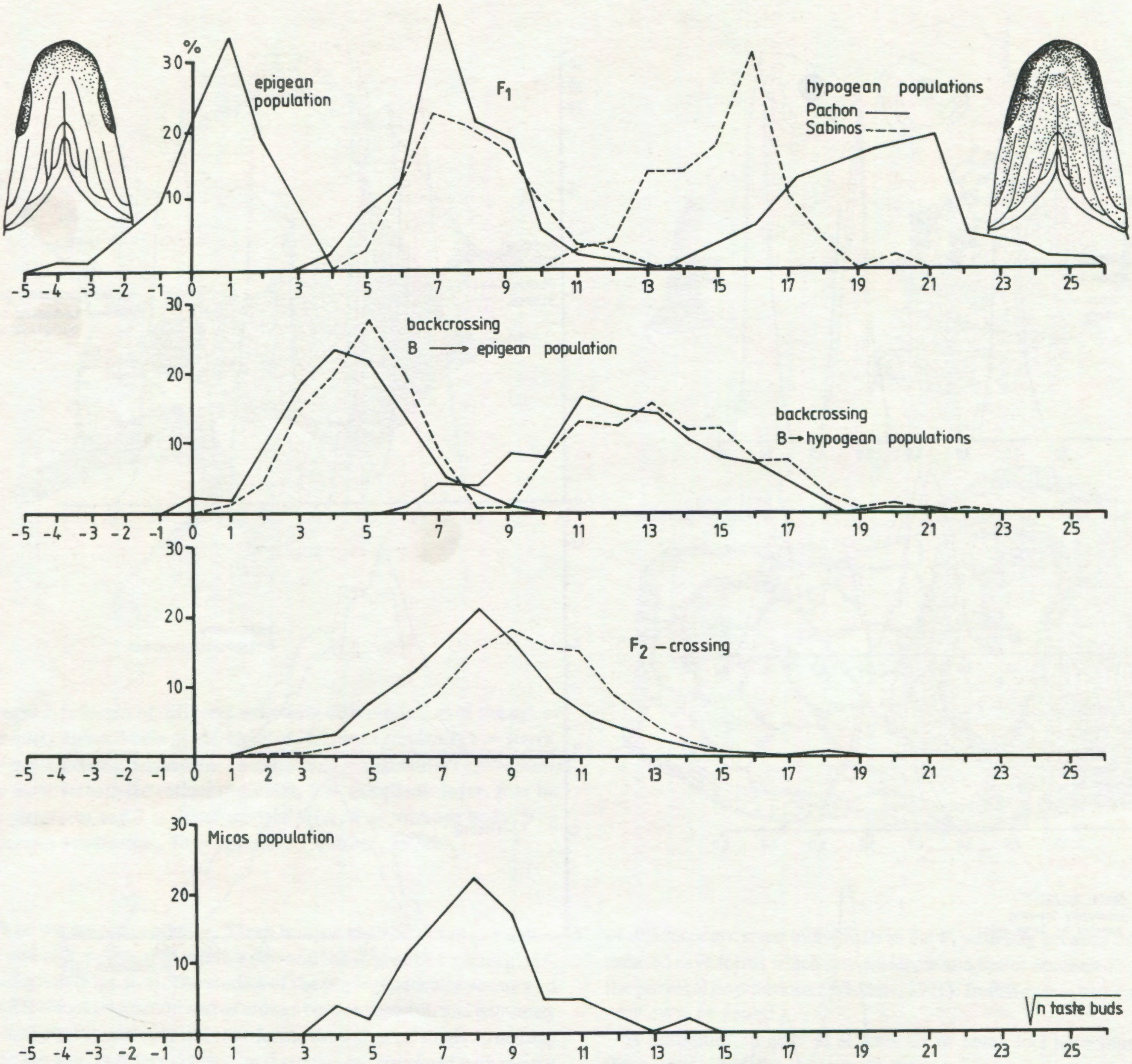


Figure 7: **Distribution of taste bud density of various *A. fasciatus* populations (Schemmel, 1974a and b).**

in the backcross. Contrary to this the F_2-crossings are normally distributed, because they are genetically unbalanced and thus lack individuals with intact morphological color change.

The phylogenetically young Micos fish are able to perform the morphological color change. However, whereas the F_1-crossings between epigean and extremely reduced hypogean populations possess a functional morphological color change, this is not true for the correspondent F_1-crossing of the Micos fish. It is not able to perform a morphological color change and has an intermediate density of melanophores. This is probably due to the fact that some of the melanophore genes of the Micos fish have already mutated.

It can be assumed that the mutated loci in the Micos fish as well as in the phylogenetically old populations are homozygous. The only difference results from the fact that the number of mutated genes increases in the three populations which were previously studied. As a minimum number at least three loci were calculated in the Pachon and two in the Sabinos fish. The Micos fish is probably at least hybrid (Wilkens, 1976) at one locus.

MORPHOLOGY AND GENETICS OF THE GUSTATORY EQUIPMENT

The epigean fish is characterized by a concentration of taste buds in the oral cavity and on the lips. Contrary to this the taste buds in the cave fish are distributed all over the head and are especially developed in large numbers on the ventral side (Fig. 7; Schemmel, 1967; 1974a). Although the surface area is the same, the number of these organs is higher in the Pachon than in the Sabinos population. The phylogenetically young Micos fish has an intermediate position (Schemmel, 1974b). The phenotypic variability of taste bud density is the highest in the Pachon and the Micos fish. The epigean and Sabinos population display the lowest density per area.

The taste bud gene system can be characterized as additively polygenic. However, certain aberrations can be found again. For example the mean densities deviate from the expected values. The backcrossing to the cave forms shows a hidden bimodal distribution (Schemmel, 1974a). With respect to the number of mutated loci Schemmel (1974a) found at least two in the Sabinos and three in the Pachon population. Additionally one recessive gene is responsible for a differing area/density-relation of the taste buds in the Pachon form.

THE GENETICS OF FEEDING BEHAVIOR

All cave forms have adapted feeding behavior to the cave biotope. They try to find food swimming at an angle of 55° over the ground (Schemmel, 1980). Contrary to this the epigean fish has an almost vertical feeding position when optic orientation is excluded (Fig. 8). This difference in behavior is genetically based. An additively polygenic system is responsible. As in the features described before aberrations can be observed. In the F_2- and the backcrossing to the cave forms bimodal distributions are developed. The behavioral divergence is based on at least three mutated loci (Schemmel, 1980).

COMPARISON OF THE MANIFESTATION OF THE POLYGENIC SYSTEMS IN *ASTYANAX FASCIATUS*

With the exception of partial or total loss of melanin which is due to one mutation, all the differences between epigean and hypogean populations previously studied are polygenically based. All polygenic systems in *A. fasciatus* show the same manner of manifestation. It is irrelevant that they are phylogenetically older in the epigean population or recently acquired in the cave forms.

The calculation of the number of genes is difficult, because the phenotypic expression of one polygene is usually considerably influenced by modificability. Careful estimation has revealed that the differences are based on at least two to seven mutated loci. With respect to the number of genes being responsible for the same feature in different populations it could be shown that there may be differences. For example the melanophoral system in the Pachon fish is dependent on 3, in the Sabinos fish on 2, and in the Micos fish on 1 negatively mutated locus. The mutations have convergently happened at allelic loci. Except for the albino gene and to a certain degree also the eye genes, regressive and constructive loci in the phylogenetically old cave populations are homozygous. The phylogeneticaly young Micos form is characterized by genetic variability. These results were confirmed by studies of allozyme variability (Avise and Selander, 1972; Peters et al., 1975).

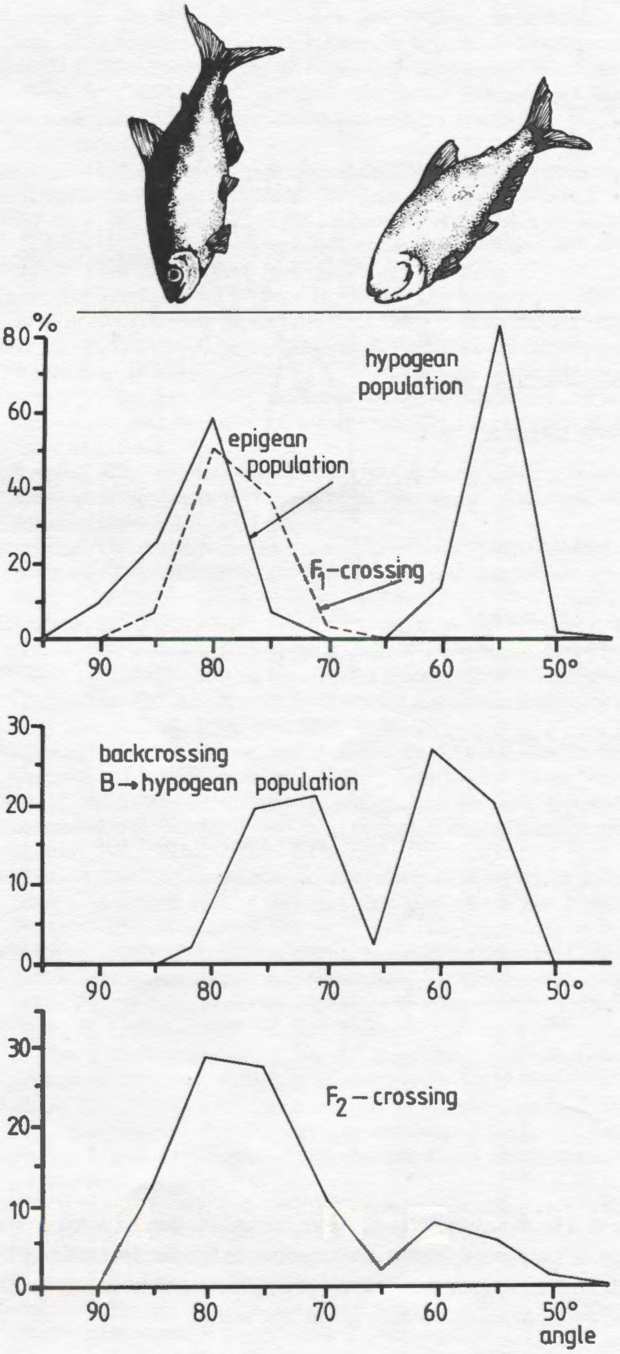


Figure 8: **Distribution of feeding behavior of various *A. fasciatus* populations (Schemmel, 1980).**

In all polygenic systems no single polygenes responsible for special functions could be analyzed. There are no genes which are exclusively responsible for lens or retina formation. The development of the melanophore system and the morphological color change are also not

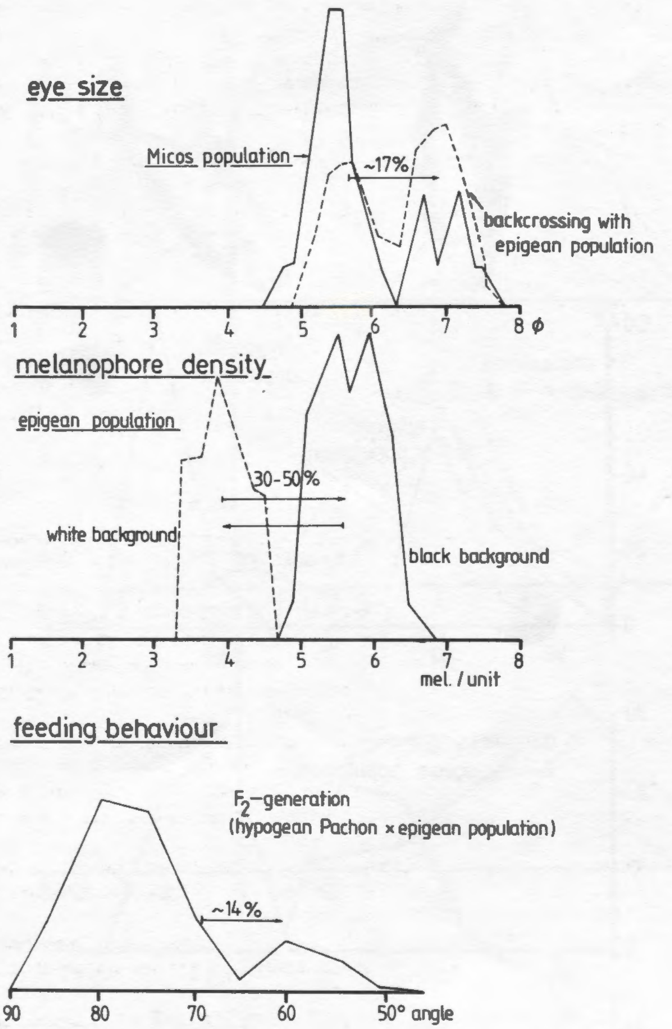


***Figure 9:* The threshold effect. After recombination of a minimum number of polygenes, which are responsible for the formation of a specific structure, gene expressivity changes. A structure may be improved by a considerable amount in one step.**

independent. The relationship of a single structure to the total organ is determined by allometric rules. Very often positive allometrical correlations are found. This is even true for the melanophores and the morphological color change which are correlated: morphological color change will only function after a minimum number of melanophore genes have been recombined.

It is characteristic of all polygenic systems in *A. fasciatus* that an additively polygenic manifestation is shown. However, important aberrations occur: For example the manner of additively polygenic systems, which is characterized by the same phenotypic amount of expressivity for each single polygene, does not manifest in the backcrossings to the better developed parental form. By differing expressivity of polygenes bimodal distributions may be found (eyes, feeding behavior, taste buds). In exceptional cases all individuals of a backcrossing may have the same phenotype as the parental form (melanophore/morphological color change). Contrary to this differing expressivity of polygenes is rarely found in the F_2-crossings. This can be interpreted as due to a disturbed genetic balance of this generation.

The complete identity of the manner of manifestation of regressive and constructive polygenic systems seems to be disturbed in the morphological color change. It is remarkable that this is not found between a regressive system on one hand and a constructive one on the other, but between two regressive ones. Contrary to all analyzed systems it is characteristic of the morphological color change that the enhanced expressivity may be influenced by environmental conditions: Kept on a white background the increase of melanophores can be suppressed. The entire manifestation of the expressivity is only manifested over a dark background.

EVOLUTIONARY ROLE OF DIFFERING EXPRESSIVITY

Phenomena which are similar to the differing expressivity of the polygenic systems analyzed in *A. fasciatus* have been described as "threshold dichotomy" (Wright, 1968) or "quasicontinuous variation" (Grüneberg, 1952) in domesticated mice. The value of the studies presented before is that they rely on naturally evolved genetic systems.

The ability of the polygenic systems of *A. fasciatus* to display threshold effects is obviously not caused by genetic incompatibility. On the contrary threshold effects are very often not shown in the genetically unbalanced F_2-generation. Furthermore it occurs in regressive as well as in constructive features.

It has therefore to be assumed that the characteristic way of manifestation should have a special function. It may play a role during the constructive phase of the evolution of a feature.

It can be shown that phenotypic manifestations of polygenes in the state of low expressivity are usually dominated by environmental modificability. During this phase it is difficult for directional selection to enlarge or improve a structure in a certain direction. This is only possible when there are phases of high polygenic expression. Phenotypic evolutionary rate will accelerate, because directional selection now can promote certain genes.

The mechanisms of polygenic manifestation in *A. fasciatus* elucidate further basic principles of gene manifestation. For example, many monogenic ways of inheritance may be interpreted as follows: They are in reality based on polygenic systems which have lost a single polygene of high expressivity. In the case of a very high expressivity of this gene, manifestation in classical terms is called dominance. The transitional phase between polygenic and monogenic manifestation can also be observed in other examples in nature. Kosswig (1964) could show that within the toothcarp genus *Xiphophorus X. helleri* has a polygenic and *X. maculatus* a monogenic sex determination. *X.m. cortezi* has an intermediate stage: In this form both types of sex determination exist (Zander, 1965). In this case it is a male-determining polygene which causes a high increase of expressivity and thus is the basis for a monogenic system. If this is useful for the species, selection will provide for its distribution in the gene pool.

A further principle of polygenic manifestation may be elucidated by the genetics of *A. fasciatus*. In most features which have been analyzed the high expressivity may not be influenced by environment. However, this is different in the melanophore system. It develops the maximum number of melanophores only over dark background, over a white one the high expressivity is suppressed. It can be concluded from this that the morphological color change has been acquired in fish by modifying genetically based expressivity. Similar "switch-phenomena" may be observed in the environmentally influenced sex determination, as for example in *Bonellia* (Echiurida).

The genetic analysis of different *A. fasciatus* populations has shown that evolutionary progress is based on small gradual phenotypic changes. Lande (1981) calculated 5-10 loci responsible for structural differences between different populations. The studies in *A. fasciatus* described before have shown that at least two to seven genetic factors are involved in the different features.

It is very important to note that the evolutionary process in *A. fasciatus* has only proceeded in certain phases based on genes with an identical small amount of phenotypic expressivity. It is a specific characteristic that during constructive evolution threshold effects of a high phenotypic expressivity occur. One single additional polygene may then increase the eye size by a considerable ratio (Fig. 9).

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EVOLUTIONARY REDUCTION BY NEUTRAL MUTATIONS: PLAUSIBILITY ARGUMENTS AND DATA FROM AMBLYOPSID FISHES AND LINYPHIID SPIDERS

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ABSTRACT

Patterns of change among the six species of Amblyopsid fish are used to argue that evolutionary reduction is based on accumulation of neutral mutations whereas troglomorphic traits are selected. Net changes, for 14 reducing and 17 troglomophic traits, were calculated for the trogloxene-troglophile pair of species, the troglophile vs troglobite with least reduced eyes, and so on along a sequence of increasing eye reduction. The patterns of decrease for reducing traits did not parallel the patterns of increase in troglomorphic traits as expected if reduction were due to indirect selection by pleiotropy. Pattern of change was consistent for troglomorphic traits with moderate net increase for both -xene to -phile and -phile to -bite 1 and virtually no further increase among any of the three troglobite species pairs. In contrast, patterns of decrease for reducing traits were quite variable but all showed continued high to moderate decrease among all the pairs of troglobites.

Natural experiments and calculations of energetic savings for spiders are used to argue that neither direct energy savings nor indirect material compensation are likely bases for evolutionary reduction. There is no trend with more food limitation from low to high trophic levels or from food-rich to food-poor caves at global, regional or local scales. Development, growth, and maintenance cost savings for structural reduction are less than 5 percent of whole organism cost and much less than 1 percent of savings due to troglomorphic decrease in routine metabolism in spiders.

The general discussion deals with alternative hypotheses relevant to structural reduction and troglomorphy, assesses the evidence for loss of physiological compensation for environmental variation, and relates trends in morphological and genetic variation to patterns of reduction and troglomorphy.

INTRODUCTION

Culver (1982) not only has reviewed the evidence for evolutionary reduction based on accumulation of degenerative mutations but has also presented calculations to counter objections to this mechanism. He showed that reduction of structures by neutral mutation is theoretically plausible. An empirical case for reduction by neutral mutations has been persuasively argued by Wilkens (1976, 1980). He based his argument on genetic data obtained from natural and laboratory hybridization among surface populations and both phylogenetically young and phylogenetically old cave populations of the Mexican fish *Astyanax fasciatus*.

In this paper I conclude that structural reduction is more likely explained by the accumulation of neutral loss mutations than by selection. My arguments are based on interspecific comparisons, with the important caveat that one can never know the evolutionary history of any species. I present old and new data on traits that show both evolutionary reduction and troglomorphic increase among Amblyopsid fishes and Linyphiid spiders.

PATTERNS OF CHANGE IN REDUCED AND TROGLOMORPHIC TRAITS

MATERIALS AND METHODS

If interspecific comparisons are to be at all useful for understanding evolutionary trends, then the Amblyopsidae is the group to use. This family includes epigean, troglophilic and troglobitic species that have been exhaustively studied from many points of view (especially Poulson, 1961; 1963; and unpublished). All six species of Amblyopsids were used to obtain measures for amounts of evolution of troglomorphic and structurally reduced traits. The Amblyopsidae includes a potential trogloxene, a troglophile, and, based on degree of eye reduction both phylogenetically young and old troglobites. Table 1 gives the species arranged by ecological stage and the localities for the populations used to quantify the traits. The phylogenetic age of troglobites is based on eye parts lost (Eigenmann, 1909) along with optic lobe and melanophore data to allow inclusion of *Speoplatyrhinus* (Cooper and Kuehne, 1974). I calculated changes in traits by pairs of species: trogloxene to troglophile, troglophile to troglobite 1, and so forth. To compare different traits I used the ratios of the absolute values minus one so that net change across all reduced and troglomorphic traits could be graphed for each of the five species pairs.

I scored 14 reducing and 17 troglomorphic traits (Table 2). With allometric traits the size or numeric measures at 45 mm standard length and *b* values are taken from regressions of body part against standard length or brain part against brain length. The Roquist Creek population of *Chologaster cornuta* was used since it is the only one with 20+ specimens spanning the entire size range and reaching a length of 45 mm needed to compare to the other species. Several populations of

***Table 1.* The fish family Amblyopsidae, populations used for assessing patterns of troglomorphy and reduction, and ranking of phylogenetic ages of troglobites (see text).**

Ecological Stage	**Species**	**Populations Studied**
Troglo-xene	x = CC = *Chologaster cornuta*	Roquist Creek NC
Troglo-phile	p = Ca = *Chologaster agassizi*	Mammoth Cave area KY, Rich Pond KY, Jewel Cave TN Livingston KY, and Pine Bluffs IL
Troglo-bite 1	b_1 = Ts = *Typhlichthys subterraneus*	Horse Cave KY, Cave City KY, Shelta Cave AL, Blowing Caves, TN, Welch's Cave MO, and Midco Cave MO
Troglo-bite 2	b_2 = As = *Amblyopsis spelaea*	Sig Chatlett Cave KY
Troglo-bite 3	b_3 = Ar = *Amblyopsis rosae*	Cave Springs AK
Troglo-bite 4	b_4 = SP = *Speoplatyrhinus poulsoni*	Key Cave AL

Chologaster agassizi and *Typhlichthys* were used since they vary so much. Only one population each was studied for the *Amblyopsis* species since they vary so little among locales. There is only one population of *Speoplatyrhinus*.

Among reducing traits those concerned with vision included eye diameter, the allometric coefficient *b* for that measure, number of retinal, dioptric and nutritive eye parts lost for eyes at their zenith of development (Eigenmann, 1909) and optic lobe volume. For pigment I scored melanophores per 0.1 mm² in the predorsal fin area, the allometric coefficient *b* for this measurement, average melanophore area, range of dispersion of pigment in melanophores of fish kept in light vs dark, and kinds of chromatophores (two possible types of melanophores and a xanthophore). Loss of escape reaction to approach of a simulated predator is a composite of body lengths moved in 5 minutes after a 10 second chase with a net and number of changes in direction per second during escape swimming. Loss of circadian rhythmicity is based on free running activity and entrainability to a 12:12 light:dark cycle during 4-7 days of swimming measured with infrared sensors in a 10 liter aquarium for 2-4 fish of each species, as well as on spontaneous activity and oxygen consumption measured in a 200 ml torus respirometer over 2-4 days for 2-4 individuals per species (Poulson and Jegla, 1969; unpublished). Loss of agonistic behavior was a composite of data from Bechler (1983) on repertoire (kinds of acts), information theory diversity of acts during aggressive encounters, bout length, and the intensity of territorial defense against an intruder. *Chologaster cornuta* had no agonistic behavior and *Amblyopsis rosae* had none for some measures so I arbitrarily added 1, 0.1, 10 and 1 to traits 1, 2, 3 and 4 respectively so that I could plot scores for each species pair.

Troglomorphic categories measured included smell, prey capture and obstacle avoidance correlates, energetic economies, and reproductive adaptations. Surface area of one olfactory rosette was used as an index of improvement in the sense of smell. The rosette's projected area was measured from camera lucida drawings. Neuromast lateral line system is the sense involved with detection of prey and obstacle avoidance. Traits measured were neuromasts in 'stitches' 2 + 3 + 4 on one side mediad of the olfactory openings, the allometric coefficient *b* for that number, exposure of neuromasts beyond the head surface, head length + width and *b* for that number, and forebrain (telencephalon) volume which is a measure of neural capacity to integrate sensory information.

Metabolic economy was quantified by four traits. Standard metabolic rate integrates economies due mainly to decrease in ventilation and cardiac rates and volumes, some reduction in gill surface area, and slight or no reduction in cell level metabolism. Routine metabolic rate reported here is the average for 2-6 one hour periods after 1 day acclimation to a 200 ml torus metabolic chamber. This rate, and the derived cost per day for an individual fish, is ecologically relevant because it includes costs of swimming activity. Body lengths moved per day is an index of foraging distance and was measured with infrared sensors in a 10 liter aquarium over 2-6 days. It increases despite decreases in routine metabolic rate partly because swimming efficiency increases as pectoral fins elongate and stroke-glide swimming is used a higher percent of the time.

Finally there are four traits related to reproduction that show troglomorphic increase. Increased egg size results in greater size post hatching at the end of yolk absorption and is directly selected. Studies on size of herring larvae (Blaxter, 1963) and anchovy larvae (Hunter, 1972) show that even the increase in size at independence from 4 mm in *C. cornuta* to 6.5 mm in *C. agassizi* could increase larval survival by allowing a 2.8 fold increase in maximum size of potential prey, a 3-fold increase in water volume searched per time, and a 3-fold increase in minimum density of prey needed to prevent weight loss. The larger larval size should also confer a longer resistance to starvation and has been shown to be important in herring populations that spawn in winter and so face fewer predators but less dense and larger prey items (Hempel, 1965). Egg size can increase partly because clutch size decreases. The clutch size given is for average sized reproductive age females of 1, 3, 2, 3.5, and 2.5 grams for the five species. Caloric cost of a clutch is eggs per clutch times egg volume, times 1.1 (density conversion factor), times 7300 calories per gram, times 2.5 for cost of production of a gram of egg. Reduction in cost per clutch contributes to decreased risk of reproduction for a female and so allows the repeated reproduction that may be necessary to insure that some young survive. Risk to a reproducing female is clutch calories divided by caloric equivalent for the reproducing female, times months of oral incubation, times standard metabolic rate. Standard metabolic rate is used since females cannot feed curing oral incubation and starvation metabolism is equal to or less than standard metabolic rate.

For both reducing and troglomorphic traits, the data on *Speoplatyrhinus* are incomplete since only some of the measurements have been made (Cooper and Kuehne, 1974; Poulson, unpublished).

RESULTS AND ANALYSIS

Overall: There are cumulative increases in reduction and troglomorphy from first to last species (Cc to Sp) and between related species pairs (Ca vs Ts and As vs Ar) but there are some 'reversals' between species that are not part of the same phylogenetic lines (Cc vs Ca, Ts

***Table 2.* Data for traits showing reduction and troglomorphy in Amblyopsid fish (Table 1). Values for morphological traits are from the allometric coefficient, *b*, and size or number at 45 mm standard length. See text for details.**

REDUCTION		Cc	Ca	Ts	As	Ar	Sp
VISION							
Eye	size (mm)	1.25	0.72	0.18	0.20	0.085	
	slope (b)	.94	.80	.25	.43	.09	
	parts missing	1	2	10.8	12.6	16.0	
Optic Lobe	(mm^3)	2.12	1.55	1.01	1.37	0.94	0.38
PIGMENT							
Melanophores	(no.)	103	50	90	6	0.7	0.6
	(b)	.71	.42	1.50	-1.10	-2.37	
	$(\text{mm}^2 \times 10^{-3})$	1.00	0.83	0.21	0.06	0.15	0.04
Dispersion	(range)	5	3.5	2	1	1	1
Chromatophores	(kinds)	3	2	1	1	1	1
ESCAPE (body length x turns sec^{-1})			252	138	39	31	
CIRCADIAN RHYTHMICITY		1.0	1.0	0.3	0.1	0.1	
AGNOSTIC BEHAVIOR							
Acts	kinds (no.)	0	6	5	6	1	
	diversity (H')	0	2.7	2.1	1.6	0.8	
Territorial Charge	(freq. x Distance)	0	6	3	1	0	
Bout Length	(sec)	0	215	63	290	40	
TROGLOMORPHY		**Cc**	**Ca**	**Ts**	**As**	**Ar**	**Sp**
SMELL							
Olfactory Rosette	(mm^2)	.045	.050	.062	.070	.076	
PREDATION							
Neuromasts	(no.)	21.6	28.5	45.6	37.0	32.5	50.0
	(b)	0.75	1.92	1.49	1.57	0.93	1.10
	(exposure)	0.1	0.2	1.0	1.0	1.0	1.0
Head Size	(w + 1, mm)	21.0	18.2	23.5	24.9	25.9	27.3
	$(bw + b_1/2)$	0.86	0.78	1.00	1.01	0.99	1.07
Forebrain	(mm^3)	0.50	0.90	1.15	1.30	1.25	1.15
Capture Time in 10 liters							
	1 water flea (min.)	21	9	0.40	0.11	0.20	
	1 isopod (min.)	620	41.5	5.0	2.7	8.0	
METABOLISM/ACTIVITY							
Standard	$(\text{ml } O_2 \text{ g}^{-1} \text{ hr}^{-1})$	.060	.028	.016	.018	.011	
Routine	$(\text{ml } O_2 \text{ g}^{-1} \text{ hr}^{-1})$	.116	.087	.061	.022	.023	
Per Fish	(RMR x g)	.140	.121	.092	.036	.058	
Foraging	(body lgths d^{-1})	1000	1854	5454	10980	8082	
REPRODUCTION							
Egg Size	$(\text{mm}^3 \times 10^3)$	0.91	4.19	6.38	6.38	5.58	
Clutch (Eggs)	(no.)	102	154	58	62	34	
	(cal.)	1863	12954	7429	7914	3809	
Risk to Female*		.044	.084	.065	.045	.017	

*cal eggs/cal female x months oral incubation x standard metabolic rate

vs As, and Ar vs Sp). An example is vision. For the Ts vs As pair, 3 of 4 vision traits show increases instead of the expected reductions (Table 2).

The trogloxene to troglophile transition (Cc vs Ca): Both reduction and troglomorphic increase are understandable from the difference in habitat and feeding behavior of *Chologaster cornuta* and *C. agassizi*. Cc lives in streams amongst plant detritus. They take shelter deep in the detritus during the day and at night they feed on passing prey from

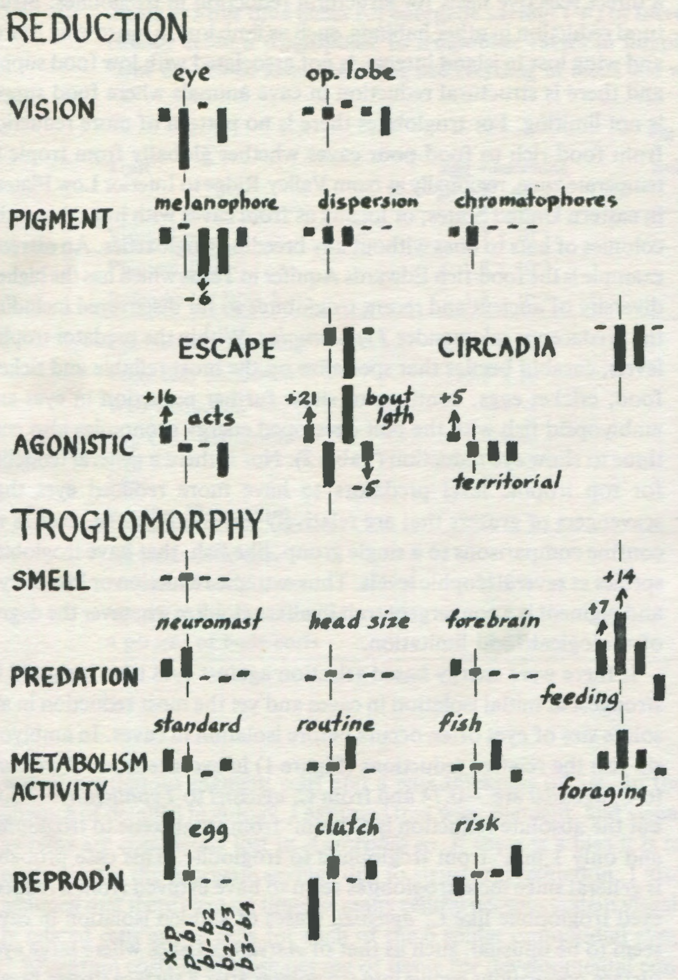

Figure 1. **Trends in reducing and troglomorphic traits by pairs of Amblyopsid fish species, starting with the trogloxene to troglophile transition (x-p) and ending with the transition from the phylogenetically 3rd oldest to 4th oldest troglobites (b_3-b_4, see Table 1). The data shown are average net changes for all components of the traits illustrated (Table 2 and text). Histogram bars above the horizontal represent increases for the species pair and bars below the horizontal represent decreases (the longest bars are for about a three-fold increase or decrease; greater changes are indicated by + or – numbers). The vertical line for each trait is the time of isolation in caves.**

the edge of the detritus. Thus they are sit-and-wait predators. Neither the mobile and plentiful prey nor the 3-dimensional habitat are defendable and they have no agonistic behavior. In fact they often cluster together in aquaria while resting. In contrast, the spring-cave habitat of Ca has prey in well defined patches around rocks and so the locations of scarce prey in the cave part of the habitat are defendable and Ca does show strong agonistic behavior (Table 2) and is highly cannibalistic. The scarcity of prey in the cave is a strong enough selective agent to explain the troglomorphic trends in Ca and the scarcity of predators with dim light in spring and darkness in caves accounts for the reducing trends. The slight decrease in head traits from Cc to Ca

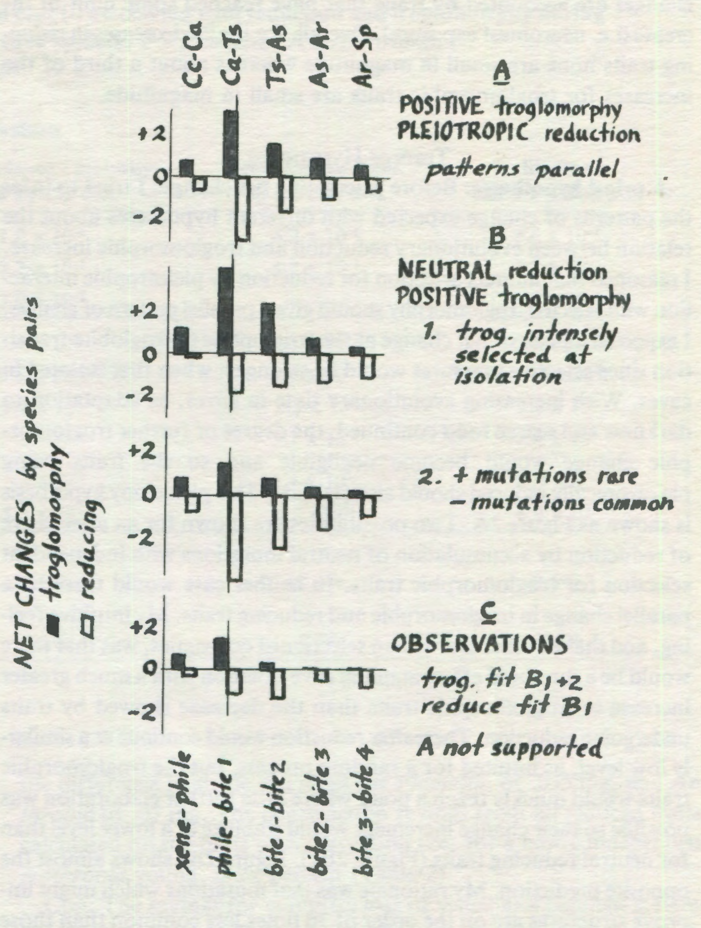

Figure 2. **Overall net changes for reducing and troglomorphic traits (Table 2 and text) by pairs of Amblyopsid fish species (Table 1). Trends predicted by hypotheses about mechanism (A vs B_1 vs B_2) are compared to observed trends (C). See text for details of interpretation.**

is more than compensated by increase in neuromasts and in forebrain 'computer' volume. The retention of circadian rhythmicity is explained by importance of using the food-rich springs only at night when effectiveness of visual predators would be least.

The troglophile to troglobite transition (Ca vs Ts); The most striking trends of reduction and increasing troglomorphy are at this critical transition. All troglomorphic traits increase. Among reducing traits, the increase in melanophore numbers and allometric coefficient (*b*) are more than balanced by the greater decrease in melanophore size (area).

Troglobite vs troglobite species pairs: Reducing traits continue to decrease whereas troglomorphic increases slow, stop, or even reverse from Ts to As to Ar and to Sp (Table 2 and Figure 1). For reduction there are 22 decreases, 6 no changes (± .05 or less for net change), and 4 'reversals'. Three of the 'reversals' are between separate phylogenetic lines (Ts vs As) and all of the no changes are for traits that could no longer change because they attained their limit for reduction at the first troglobite pair (i.e. melanophore pigment dispersion, kinds of chromatophores, and circadian rhythmicity). For troglomorphy there are 17 increases, 7 no changes, and 11 reversals. Seven of the 'reversals' are for related specis (As vs Ar) and only 3 of the no changes are accounted by traits that have reached some limit of increase (i.e. neuromast exposure). Overall, for the 22 decreases in reducing traits none are small in magnitude whereas about a third of the increases for troglomorphic traits are small in magnitude.

TESTS OF HYPOTHESES

A priori hypotheses: Before calculating net changes I tried to infer the patterns of change expected with different hypotheses about the relation between evolutionary reduction and troglomorphic increase. I reasoned that indirect selection for reduction by pleiotrophic interaction with selected troglomorphy should give a parallel pattern of change. I expected a peak of net change at the troglophile to troglobite transition since selective pressures would be strongest when first isolated in caves. With increasing evolutionary time in caves, as adaptation to darkness and scarce food continued, the degree of further troglomorphic change would become negligible and so the traits being pleiotropically reduced should also stabilize. This pleiotropy hypothesis is shown as Figure 2A. Two possibilities are shown for an alternative of reduction by accumulation of neutral mutations with independent selection for troglomorphic traits. In neither case would there be a parallel change in troglomorphic and reducing traits. My intuitive feeling, and that of other Darwinian selectionist colleagues, was that there would be a threshold effect at initial cave isolation with a much greater increase in troglomorphic traits than the decrease showed by traits undergoing reduction. Thereafter reduction would continue at a similarly low level, as intuited for a random process, but the troglomorphic traits would quickly reach a point where little further elaboration was possible so their change increment would stabilize at a lower level than for neutral reducing traits (Figure 2B1). Figure 2B2 shows almost the opposite prediction. My rationale was that mutations which might improve structures are on the order of 30 times less common than those which cause degeneration (e.g. amphipod eyes, Sexton and Clark, 1936). This means that initial reduction at time of isolation would be great but troglomorphic improvement would only be moderate despite strong positive selection. Thereafter this differential should still hold but the increments of change should diminish for two reasons. First, the chances drop for new mutations that could cause further reduction. Second, for vertebrates early eye development is part of brain morphogenesis and early chromatophore development is part of neural crest morphogenesis and so individuals with mutations that stopped eye and pigment development this early would be selected against. (This developmental constraint would not hold for Arthropods where visual and pigment systems are late to develop and we do observe complete loss of both systems in arthropods with extreme troglomorphy).

Tests of alternative hypotheses: Figure 2C shows that observations on amblyopsid fishes are not consistent with the parallel change pattern expected for the pleiotropy hypothesis of Figure 2A. Rather, the observed troglophile to troglobite transition is like that expected if mutations of loss exceed those giving possible improvement (Figure 2B2) and the observed changes among troglobites are like those expected with strong selection for and rapid stabilization of troglomorphic traits (Figure 2B1). This same set of trends is seen for individual systems and their component traits (Figure 1 and Table 2).

IMPLAUSIBILITY OF REDUCTION BASED ON ENERGY ECONOMY AS A SELECTIVE AGENT

PLAUSIBILITY

Natural experiments are not consistent with metabolic savings as a direct selective basis for structural reduction in troglobites. Structural reduction in other habitats, such as limb loss in burrowing lizards and wing loss in island insects, is not associated with low food supply and there is structural reduction in cave animals where food supply is not limiting. For troglobites there is no pattern of more reduction from food-rich to food-poor caves whether globally from tropic to temperate zone, regionally as from Valley-Ridge to Interior Low Plateau in eastern United States, or locally as from caves with huge maternity colonies of bats to ones without any breeding trogloxenes. An extreme example is the food-rich Edwards Aquifer in Texas which has the highest diversity of ancient and recent troglobites so far discovered including the predaceous salamander *Typhlomolge*. Within the predator trophic levels, carabid beetles that specialize on the most reliable and richest food, cricket eggs, continue to show further reduction in eyes and amblyopsid fish with the best developed energy economies also continue to show eye reduction (Table 2). Nor is there a general tendency for top trophic level predators to have more reduced eyes than scavengers or grazers that are relatively less food-limited, even is we confine comparisons to a single group, like fish, that have troglobitic species at several trophic levels. Thus extreme reduction or loss of eyes and pigment is a convergent trait in all troglobites whatever the degree of ecological food limitation.

If there were energy based selection against eyes then it should be strongest at initial isolation in caves and yet the most reduction in absolute size of eyes often occurs before isolation in caves. In amblyopsid fish the relative reductions (Figure 1) in eye size from *C. cornuta* to *C. agassizi* are −0.74 and from *C. agassizi* to *Typhlichthys* −3.00 but the absolute reduction is 5.3 mm^3 from trogloxene to troglophile and only 1 mm^3 from troglophile to troglobite. This case probably is general since most troglobites seem to have evolved from a reduced eyed troglophile like *C. agassizi*. Cases of sudden isolation in caves seem to be unusual, such as that of *Astyanax* fishes where large eyed surface populations evolve into troglobites after a surface stream breaks through a resistant rock bed and is catastrophically captured by a cave system below.

ENERGY SAVINGS RESULTING FROM STRUCTURAL REDUCTION IN SPIDERS

Methods: My estimates of savings for the troglophile to troglobite transition are based on data and extrapolations for linyphiid cave spiders. The data are for a phylogenetically young troglobite, *Phanetta subterranea*, and a phylogenetically old troglobite, *Anthrobia monmouthia*, both from the Mammoth Cave region of Kentucky.

I determined absolute costs by calculating mass for a structure and estimating construction and maintenance cost in mg of glucose, and then got a relative cost by comparing the structure's cost to that for the whole organism. I started by using geometric approximations to calculate volumes of body parts and assumed that 1 mm^3 of structure was 1 mg. For Arthropods of the small size involved each cell is about 8×10^{-6}mg, or $20\mu^3$, and structure mass was then number of cells times this average cell mass. Exoskeleton mass estimated geometrically compared closely to observed molt mass times 1.5 to correct for the inner exoskeleton reabsorbed before molting. Body and egg mass were measured directly with an electrobalance. Web mass was based on Scanning Electron Microscope photographs to get silk width, samples of silk length per area of web from the same photographs, and measures of total web area built by spiders in the lab. Calculations of pigment cell and epicuticular wax mass required estimations of body surface area and the assumption that the condition for an ancestral troglophile

Table 3. **Four time bases for energetic savings (+) or increased cost (-) associated with reduction and troglomorphy during change from a troglophilic to troglobitic facies in linyphiid cave spiders. P = *Phanetta*, A = *Anthrobia*.**
***One time costs, assuming eating and recycling of molts and web silk.**

	mg equivalents			
Trait	**egg - hatching**	**independence - maturity**	**reproductive event**	**lifetime**
	20 d P, 35 d A	**85 d P, 170 d A**	**50 d P, 90 d A**	**365 d P, 2000 d A**
Visual Loss	4.7×10^{-4}	1.0×10^{-2}	4.2×10^{-3}	9.6×10^{-2}
Pigment Loss	1.8×10^{-3}	7.7×10^{-2}	3.2×10^{-2}	7.2×10^{-1}
Epicuticular Wax Loss	3.8×10^{-3}	3.0×10^{-2}	——	3.8×10^{-2}
Exoskeleton Thinning*	2.1×10^{-2}	3.4×10^{-1}	6.5×10^{-2}	6.0×10^{-1}
Body Size Reduction	-3.5×10^{-2}	9.8×10^{-1}	1.5×10^{-1}	-9.4×10^{0}
Web Reduction*	——	1.2×10^{0}	2.3×10^{0}	3.5×10^{0}
Egg Case Reduction	——	——	5.7×10^{-1}	2.4×10^{0}
Eggs	——	——	1.4×10^{-1}	-1.4×10^{0}
Routine Metabolism	——	3.7×10^{1}	1.2×10^{1}	4.9×10^{1}
All reductions as a percent of body costs	22.0%	22.0%	12.0%	8.0%
Visual + Pigment Loss as a percent of body costs	1.9%	4.1%	4.5%	4.8%

was of contiguous pigment cells and a wax thickness of 5μ. In the absence of detailed histology, I assumed that wax, pigment, and visual systems are completely lost in *Anthrobia* so, if there is a bias it is to overestimate the energetic savings due to structural reduction. I also assumed that there were six times as many central nervous system visually related cells as eye cells for each of the 6 spider ommatidial eyes.

Milligram costs in glucose per mg of tissue for developmental construction and per day maintenance thereafter were based on estimates for plant cells and leaves by Merino et al. (1982) incremented to account for higher costs of animal cells. Cost of construction includes both respired glucose and that used in the carbon skeleton and is estimated to be 2 times the structure mass for animals. To estimate per day maintenance cost I assumed twice the value for plants and 67 percent tissue water content to convert plant dry mass data given by Merino et al. to the preserved or live animal masses that I had available. The result in maintenance cost is .02 mg per mg fresh tissue per day. Thus construction plus maintenance cost for a particular time period is ($2 \times$ structure mass) + (mass $\times$ .02 $\times$ days). Routine metabolic rate for sit and wait spider predators is near basal rate and was measured by average daily mass loss of food-deprived spiders that had been prefed enough to produce in the lab the same frequencies and sizes of egg clutches produced in the field.

RESULTS AND ANALYSIS

ENERGY SAVINGS ON DIFFERENT TIME SCALES

There are arguments pro and con for calculating energy savings or extra costs, of a troglophile to troglobite transition, on different time scales (Table 2).

Egg to hatching: This includes cost of construction but no maintenance.

Pro: Savings could be critical in the embryo because material compensation could allow a larger size at independence from yolk supply. The size advantage for newly independent young is on the order of the difference in single egg mass for *Phanetta*, of .035 mg, and *Anthrobia* of .07 mg. The construction savings from all reduced traits, calculated on the basis of construction cost for a newly independent *Anthrobia* young, is 22 percent (Table 2).

Con: The savings for complete loss of visual and pigment systems is only 1.9 percent. Furthermore the cost of yolk is borne by the reproducing female and it would cost her very little more to make the egg enough larger to completely cover savings of reduced traits.

Hatching to maturity: This includes cost of growth and maintenance.

Pro: The early part of this period is a time of high mortality rates in all organisms and is a particularly severe energetic bottleneck for cave organisms. The total savings for spiders is 22 percent.

Con: The savings for eye and pigment loss in spiders is 4.1 percent of troglobite whole body costs for the same time period. The absolute savings for troglomorphic energy economies is many times that for reduced traits. The savings due to smaller body size, smaller web, and lower routine metabolism are 85 times that from reduced traits.

Per reproductive event: This includes only maintenance savings.

Pro: Reproduction is certainly the most crucial and metabolically most expensive event for a female. The total savings of maintenance for reduced traits is 12 percent of whole body maintenance in *Anthrobia*.

Con: The maintenance savings for eyes and pigment in *Anthrobia* is only 4.5 percent and this is 560 times less than savings due to troglomorphic traits.

Lifetime: This includes total costs of development, growth, and maintenance.

Pro: Maintenance costs are a significant proportion of the total and become relatively more important with the increased longevity of troglobites. It even makes the short term savings due to reduced body and clutch deficits instead of savings over the lifespan of *Anthrobia* (minus entries in last column of Table 2).

Con; For spiders the savings due to reduced web, egg case, and routine metabolism are 38 times the savings from all reductions and these are only 8 percent of the total lifetime cost of *Anthrobia*. The savings from not developing eyes and pigment, and so not having their maintenance costs, are 4.8 percent of lifetime costs. In general survivorship is better and energetic stress less as a troglobite, or any organism, gets older and larger and so the small lifetime savings from evolutionary reduction in troglobites are least likely to be selected.

Analysis: The amount of saving differs with the different time bases but the relative contribution to savings is in the same rank order with eyes $\leq$ epicuticular wax $\leq$ pigment $\leq$ exoskeleton thinning $<$ web $=$ egg case $<<$ routine metabolism. Table 2 shows that there is a saving difference of 3 to 5 orders of magnitude among traits and that saving from reduction of routine metabolism alone makes up over 90 percent of the total. This low contribution of construction costs plus cell maintenance cost of organs, compared to routine metabolism, is explained by the fact that cells contribute only 25 percent of basal rate costs, with 25 percent for costs of ventilation and cardiac function and 50 percent due to maintaining muscle tone, posture, and equilibrium, at least for fishes (McFarland, 1959).

To sum up, the calculations do not clearly support the plausibility of selective reduction of 'useless' traits based on energy economy but neither do they falsify it. If there is an energetic basis for selection against 'useless' structures then it is during or just after development when the savings are relatively greatest. It is also at these early stages when any pleiotropic reduction should be sought.

GENERAL DISCUSSION

PATTERNS

I reject a possible argument that the observed average pattern (Figure 2C) is not enough different from the hypothesized pleiotropic pattern (Figure 2A) to compromise its validity. For a specific case, the pattern for a pair of traits most likely to interact developmentally is not at all parallel. Thus amblyopsid fish eye reduction shows a completely different pattern among species than for hypertrophy of the neuromast system (Figure 1). In general one would not expect increments of reduction to be greater for troglomorphy than for reduction if pleiotropic 'hitchhiking' were involved. Nor would one expect increments of reduction for some traits to remain great as troglomorphic traits cease changing if pleiotropy were involved.

The great troglophile to troglobite eye reduction followed by less reduction among troglobite pairs contrasts to the low initial amount of melanophore reduction followed by much greater reductions. This seems explainable by differences in numbers of genes for the two systems. As Wilkens has documented (1976, 1980), there are fewer genes for pigment than eyes and a phylogenetically young *Astyanax* cavefish population has definite eye reduction but scarcely any pigment reduction. This is consistent with random accumulation of loss mutations being more likely with more genes for a structure. It is also consistent with pigment reduction continuing at moderate increments among amblyopsids after eye reduction is much slowed (Figure 1). Reduction of optic lobe volume does not parallel that for eyes, certainly because the optic tectum is only part of the lobe and possibly because visual projection paths could be evolutionarily taken over by tactile pathways as is seen developmentally for experimentally blinded *Astyanax*.

Unlike the case for eyes, reduction in circadian activity is plausibly explained by pleiotropy. The increase in body lengths moved per day during search for food (Table 2 "foraging") is selected in the food-poor cave and could clearly be allowed in part by reduction in circadian activity at the Ca to Ts transition (Table 2 CIRCADIAN..). However, this is the only one of the many reduced traits that seems plausibly related by pleiotropy to a troglomorphic trait.

Evolutionary reduction and loss of behavioral traits is most plausibly due to accumulation of neutral mutations since material compensation or pleiotropic interactions with selected traits are especially difficult to even postulate. Among linyphiid cave spiders the highly troglomorphic *Anthrobia monmouthia* still shows microhabitat selection and startle-jump responses which mitigate predation by carabid beetles. In contrast, amblyopsid fish are the top predators in their food webs and are subject only to cannibalism. Troglobites still show freeze reactions or escape swimming at the approach of larger aggressive individuals (Bechler, 1983) and I think that these reactions can also be important in mitigating cannibalism: freezing is especially prominent in newly hatched and small immature amblyopsid troglobites (Poulson, 1969) which are especially vulnerable. However the escape response to a net used to simulate approach of a very large predator is progressively reduced despite increasing sensitivity of neuromasts to water movement (Table 2), and it is even possible to pick up the advanced troglobites by hand. This loss of escape response is not an energy economy since there is no difference among species for initial rate of return to routine metabolic rate following disturbance (Poulson, 1963). As with escape, agonistic behavior is too rare and transitory for its loss to be an energetic advantage. (Bechler, 1983). I agree with Bechler that troglomorphic metabolic economies resulted in relaxation of selection to maintain agonistic behavior after the Cc to Ca transition of habitats and this allowed loss of agonistic behavior by accumulation of neutral mutations.

PLEIOTROPY AND ALLOMETRY

Fong and Culver (in press) have presented the most convincing evidence for pleiotropic reduction but their data are still open to alternative explanations. They controlled food supply in the lab and demonstrated a weak negative genetic correlation between antennal length and eye size in the amphipod *Gammarus minus*. This negative correlation was only evident after removing the stronger positive allometric effect on both traits using principal components analysis. It seems that this analysis cannot differentiate between negative pleiotropy with (a) common gene(s) and independent genes for increased antennal length, due to directional selection, and genes for decreased eye size, which accumulate due to lack of selection for maintaining normal function. Better insight into mechanism might come from detailed allometric analyses.

Detailed genetic analysis of troglomorphy and reduction among populations of *Astyanax* fish (e.g. Wilkens, 1976; 1980) has shown that 5 to 10 genes account for the most extreme eye reduction. But, as far as I am aware, it is not know whether these eye reducing genes are the same as or linked to genes responsible for increased troglomorphy of tactile and taste sense organs. Again, allometric comparisons might be useful.

LOSS OF COMPENSATION FOR ENVIRONMENTAL VARIABILITY

Loss of physiological abilities to adjust to environmental variation might be expected with the buffered abiotic conditions in caves but clear demonstration of such loss is rare. My preliminary data on 2 individuals each of 3 species of amblyopsid troglobites suggested lower metabolic rates at 10° and 20°C than at 15° but a complete analysis showed little difference among temperatures or species. I included the troglophile *Chologaster agassizi*, used 10-20 individuals of each species, and tested acute and acclimated routine metabolic rate and endurance swimming in an artificial stream at temperatures from 5° to 25°C.

For all species the measures of performance were quite variable among individuals and for the replicated tests on one individual. The overall 1.5 to 2 fold difference in measures of rates and performance is even less than the 4 fold increase expected for biological processes from 5° to 25°C, i.e. a Q_{10} of 2.0. In retrospect it is surprising that all species tended to show slightly lowered performance and metabolic rate above 15°C and this may mean that they all have lost some ability to compensate for temperature change. Even *Chologaster agassizi* lives in springs and spring-cave habitats that can have quite constant temperatures. If anything the spring-cave temperatures are more constant than for cave streams some of which drop from 15° to 5°C during snowmelt runoff and rise from 15° to 20°C during prolonged and heavy summer rains. Some of the troglobite populations studied do come from cave streams with near constant temperature but their metabolic rates and swimming performances were not different. Clearly neither the troglobphile nor the troblobites are compromised by the range of temperatures studied and so they are not evolutionarily 'stuck' in caves by loss of ability to tolerate temperature variation. However, they probably are restricted to caves because vestigial eyes and pigment make them vulnerable to eyed predators.

Terrestrial arthropods do appear to be restricted to caves by loss of ability to tolerate humidities much below saturation. In most cases thinning of exoskeletons has been noted but not quantified for troglobites and an associated reduction in epicuticular wax 'waterproofing' has been presumed but not measured. Both have not only been carefully quantified but also related to significantly greater rates of dessication, even at 90 percent relative humidity, for a lava tube spider compared to a surface relative living on lava fields (Hadley et al., 1981). As we have shown earlier these reductions are most plausibly explained by accumulation of neutral mutations.

VARIABILITY AND ISOLATION IN CAVES

Models for the process of isolation in caves are pertinent to selection vs neutrality since one might predict more variability for traits not under the influence of selection. Barr (1968) hypothesizes an initial release of variation with isolation in caves, then a genetic bottleneck associated with a reduction in variability, and finally a reorganization of the 'epigenotype' with variability again higher. Wilkens (1976, 1980) envisions selection for troglomorphic traits and so less variability than for reducing traits undergoing degeneration due to neutral loss mutations.

The best data for assessing these models comes from cave fish and it fits the Wilkens' model better than the Barr model. For *Astyanax* eyes Wilkens' data (1976, 1980) show that variation around eye - body length regressions for a surface population is much less than for a phylogenetically old cave population and that variation is greatest for a phylogenetically young cave population. Unfortunately he does not present comparable regressions for a troglomorphic trait, such as taste bud density, to show the expected lower variability for a clearly selected trait. However the data on amblyopsids do show this trend. Interspecifically, among amblyopsid fish, the variation within a population around regression lines for neuromasts and cerebellum length is similar and tight with only slight increase in variability for *Chologaster agassizi* and *Typhlichthys* but with great increases in eye and optic lobe length variability both at the Ca-troglophile stage and at the time of initial isolation in caves for Ts (Poulson, 1961). Eyes for the potential trogloxene *Chologaster cornuta* are no more variable than its neuromasts or cerebellum but there is greatly increased variability for eye size in the troglophile *C. agassizi*, a maximum of variability for the phylogenetically youngest troglobite *Typhlichthys*, a decrease in variability for *Amblyopsis spelaea*, and a further decrease in the phylogenetically old troglobite *A. rosae* which has a similar variability for both reducing and troglomorphic traits. Furthermore its level of variability is similar to that seen for the surface species *Chologaster cornuta*. This evidence is from interspecific comparisons and thus is circumstantial but it is consistent with evolutionary reduction by accumulation of loss mutations and independent selection for troglomorphic traits.

Electrophoretic data on 18 allozyme loci for Amblyopsids (Swofford et al., 1981) show a continued decrease in average heterozygosity across the species rather than the restoration of variability implied by the Barr model. Also, reduction in heterozygosity is not just associated with isolation in caves since at least some populations of each species are monomorphic for all loci. For non troglobites the heterozygosities decreased from .040 (maximum .115) for *C. cornuta* to .028 (maximum .061) for *C. agassizi*. Heterozygosities of .019 (maximum .053) for *Typhlichthys* are not much lower and are similar to levels reported for two troglobitic *Astyanax* populations (Avise and Selander, 1972). If isolation in caves always involved genetic bottlenecks then I would expect more than 6 of the 13 *Typhlichthys* populations to be monomorphic or with very low heterozygosities as *is* the case for the two *Astyanax* populations for allozymes and also for eye and pigment genes (Wilkens, 1976). The lower eye variability and allozyme heterozygosities for *Amblyopsis* species, of .006 (maximum .13) for *A. rosae* and none for *A. spelaea*, is more in line with the results for *Astyanax* but the data are not consistent with chance allele fixation due to founder effect or genetic bottleneck. Contrary to the suggestions of Swofford et al. (1981) both *Amblyopsis* species have higher population sizes than for *Typhlichthys* and at 1 to 2 hundred these are not small (Poulson, 1969). Also the monomorphic populations, even for *A. spelaea* on opposite sides of the Ohio River, are fixed for the same alleles, not for different ones as is the case for monomorphic *Typhlichthys* populations. The *Amblyopsis* patterns could not have arisen from founder effect and/or genetic bottlenecking at one cave with subsequent underground dispersal. Rather I suggest that selection for some alleles, and chance fixation of the most common alleles, gave rise to increasing genetic uniformity in the amblyopsid troglobites. Swofford believes that we cannot differentiate between stochastic effects and selection with his data (personal communication).

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REGRESSIVE EVOLUTION IN STENASELLIDS (CRUSTACEA ISOPODA ASELLOTA OF UNDERGROUND WATERS)

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SUMMARY

The morphological features of the family Stenasellidae are strongly conservative. Only a few characters show an evolutionary species in natural phyletic lines. This evolution appears to be bound to the ecological re-adaptation undergone by strains now living in underground waters of temperate regions. These characters are body size and shape, the amount of respiratory blood pigments, the shape of the exopodites of 4th and 5th pleopods and the number of spines in the sternal row of pereopods 2-7 dactylopodites. This evolutionary trend behaves as a progressive drift of the characteristics of these organs and never as sudden modifications in the morphology. So, it seems difficult to assume that classical mutations at structural loci would be responsible for these gradually regressive phenomena.

THE FAMILY STENASELLIDAE

This family of isopod crustaceans consists of some 58 species and subspecies. All are stygobiontic, anophthalmous and possess respiratory blood pigments that give them whitish-rose, rose and even red colors, the exoskeleton being unpigmented. The characteristics of this family are:

1. Pleonites 1 + 2 free, as in Asellota Asellidae, Stenetriidae and Gnathostenetroidoidea, but very large, as in primitive Isopoda,
2. Peduncle of the antenna with a rudimentary expodite *(squama)* on 3rd segment,
3. Maxillipeds without epipodites,
4. Pereopods 1 very strong, forming subcheliform gnathopods,
5. Male 1 + 2 pleopods small, entirely specialized in sexual function,
6. Female 1st pleopods absents, 2nd pleopods small, without function,
7. Pleopods 3, 4, 5 with exopodites sclerotized and endopodites respiratory (gills).

Its global distribution: Iberic peninsula, Southern France, Corsica/Sardinia, Balkan, Turmenistan (extreme South USSR), Thailand, Cambodia, Sumatra, Borneo, West Africa (from Senegal to Zaire), South Morocco and Algeria, Somalia/Kenya, and Mexico/Texas.

The Stenasellidae is an ancient fauna (Magniez, 1981). The species can be considered as old ones, with a very little rate of evolution. Their initial continental biotope is probably the phreatic tropical waters where they dig networks of galleries in alluvial and eluvial clay, i.e., a warm biotope (20°C or more), but poorly oxygenated (1.9 mg/l in Cambodia). The evolution of European strains led them to live in cool underground waters (10°C or less), but richer or even saturated in oxygen (cases of the underflow of Pyrenean torrents and of some karstic waters (Magniez, 1974). The only morphological features varying in these strains are generally correlated with the ecological revolutions that they underwent: size and shape of the body (Figs. 1 and 2), and reduction of the exopodites of respiratory pleopods 4 and 5. I examined the mode of evolution of these last features and also another character: the modalities of formation of the spines of the sternal row of the dactylopodites of pereopods 2 to 7.

EVOLUTION OF THE NUMBER OF STERNAL SPINES OF DACTYLOPODITES

In Isopoda, the 1st pair of thoracopods are maxillipeds, the 2nd (1st pereopods) often are prehensile subchelifom gnathopods, but the last 6 pairs (pereopods 2 to 7, corresponding to thoracic segments 3 to 8) are quite similar to each other and play the ambulatory function.

The primitive condition of these appendanges made these organs suitable for mechanical contact with the substratum: the end of these legs bears a strong curved claw (sometimes called "nail" in French). Racovitza (1923) pointed out that this claw does not represent the morphological extremity of the dactylopodite. The true morphological apex of the segment is the "dactylian organ": a bunch of sensory setae (Fig. 4), one of which is ramified (palm-tree like) and the others smooth. This terminal bunch would have migrated to a tergal position during the transformation of the appendage into a walking leg. Along the sternal edge of this dactylopodite existed a row of spines. The distalmost one enlarged and turned distally, so as to stand now at the topographical apical position: this spine is the claw. The other sternal spines (the number varying from 0 to 5 or 6 with the species and age of the specimens in a single species) have the same structure and morphological value as the claw itself.

In Asellota, these spines appear progressively with the successive molts during the life-span of the animals. Figure 3 shows, in caudal

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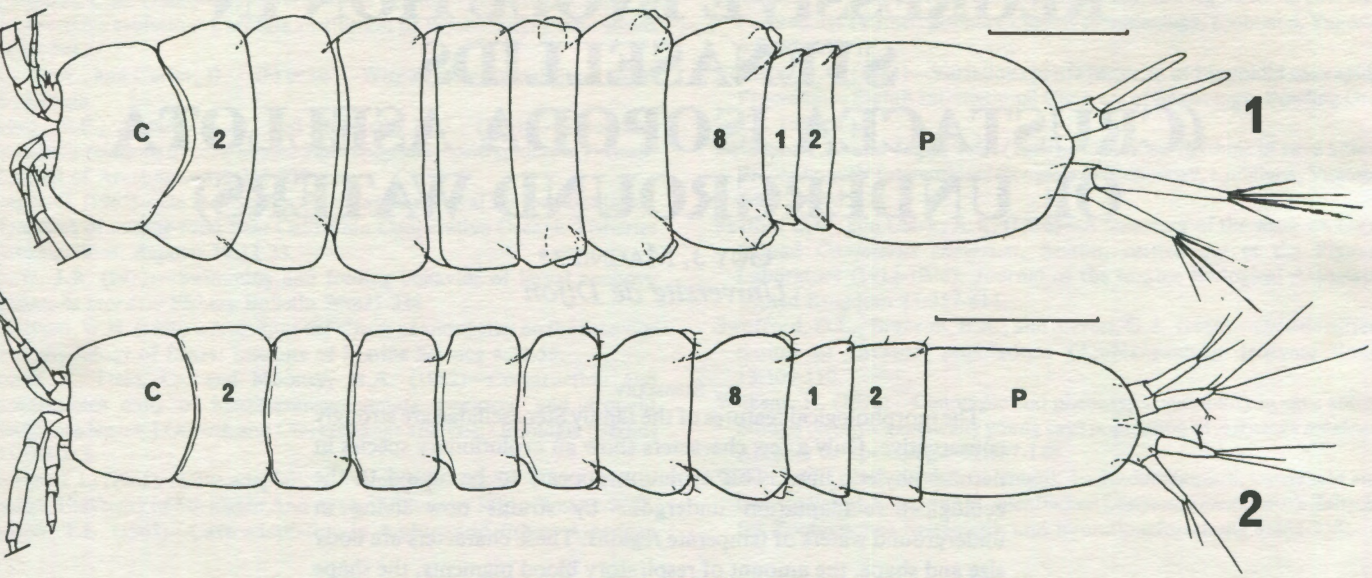


FIGS. 1-2 : COMPARISON OF THE SHAPES OF TWO STENASELLIDS :

1. STENASELLUS CHAPMANI MAGNIEZ, 1982 FROM BORNEO CAVES (FREE WATER) : BODY THICK ; SCALE BAR = 1 MM.

2. METASTENASELLUS LEYSI MAGNIEZ, 1985 FROM PHREATIC WATERS OF SW ALGERIA : BODY SLENDER ; SCALE BAR = .5 MM.

C = CEPHALON ; P = PLEOTELSON ; 1 = FIRST PLEONITE.

view, one right dactylopodite of a large old male of *Proasellus meridianus* (Asellidae). The spines appear from the distal to the proximal: the claw, that appears first, is numbered 0; the subungueal spine (numbered 1) appears next. It is already present in the juvenile Asellid. The spine 6 is the youngest. Generally, one finds several spines in large epigean species, one in stygobiontic small species and rarely no spines (Fig. 6, case of some populations of the little phreatobitic species *Proasellus acutianus* Argano and Henry, 1972 from Italy). In this last case, the claw (spine n°0) is alone.

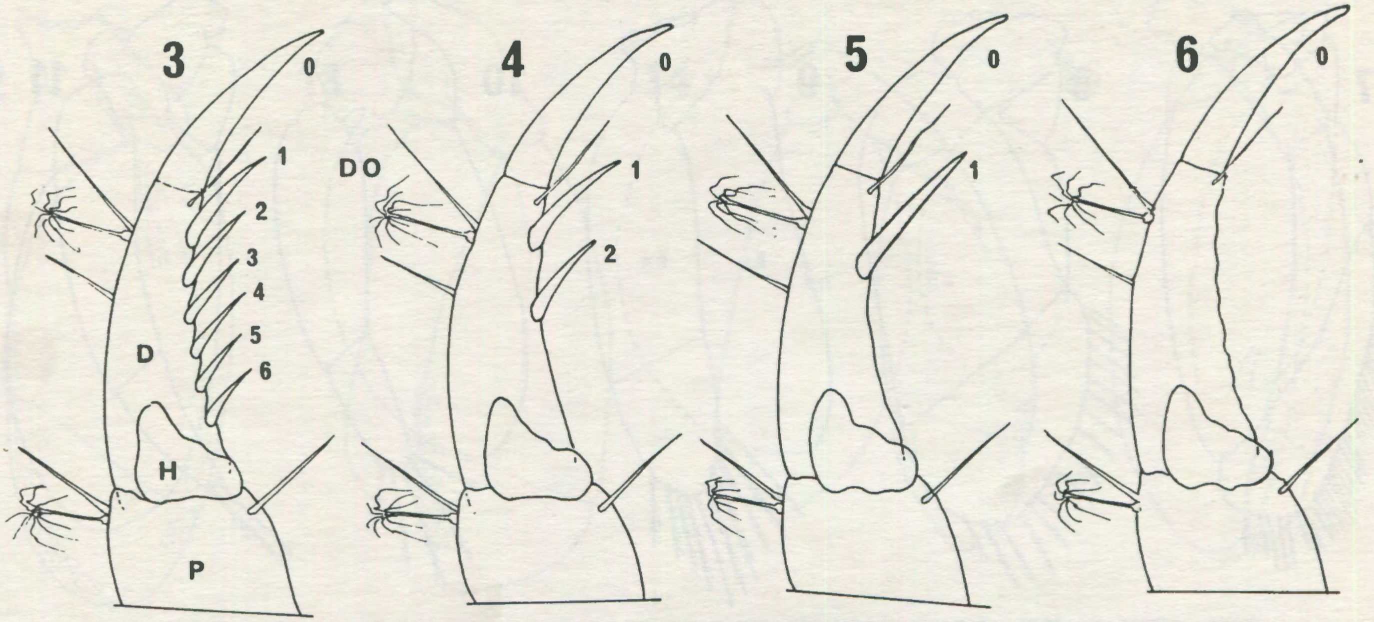
In Stenasellids, the species possess either 2 sternal spines (claw + spine 1 + spine 2), or only one, *Stenasellus virei* from Pyrenean region (Southwestern France and Northern Spain) exemplifies the mode of appearance of the 2nd spine. In the phreatic subspecies *S. virei virei*, all the dactylopodites of the pereopods 2 to 7 bear 2 spines in the adults (Fig. 4). This subspecies can be noted: 2 2 2 2 2 2. On the contrary, the karstic subspecies *S. virei hussoni* is generally of the type: 1 1 1 1 1 1 (Fig. 5). Nevertheless, in some caves of French Pyrenees (Nistos Cave, Hautes-Pyrenees; Sauvajou Swallowhole, Ariege), the populations of *S.v. hussoni* include very old and large specimens (10-12 mm.) They show sometimes but only on some of their pairs of pereopods, 2 sternal spines. So, in these exceptional specimens, the typical formula of the subsp. *S.v.h.* (1 1 1 1 1 1) becomes 2 1 1 1 1 1, then 2 1 1 2 1 1, then 2 2 1 2 2 1 and, in rare cases, this evolution leads to the formula 2 2 2 2 2 2, the same as in the type-subspecies.

So, the difference between *S.v.v.* and *S.v.h.* is not at all the fact that the first bear 2 spines and the second only one, it is the fact that in *S.v.v.*, the 2nd spine appears ***always, in all specimens and early***, but in *S.v.h.* formation of this 2nd spine is very strongly delayed in the ontogenesis. The delay is so great that the 2nd spine appears no more in a large majority of specimens of the subspecies, and appears only in rare exceptionally large and old ones, and generally only on a part of their pairs of pereopods. It appears as if the genetic mechanism responsible for the formation of sternal spines were functional in *S.v.v.* is repressed or inhibited in *S.v.h.*.

The observations of the different cases found in Asellota (Figs. 1-4) point up the fact that, even in species without any sternal spine (Fig. 4), the genetic ability to produce these spines is not lost, the claw being always present. The suppression of the claw, if it occurred, would likely act as a lethal character in these walking Crustaceans. So, the morphological difference between the ssp. *virei* and *hussoni* seems to behave rather as a slow and progressive drift of the genetic expressivity of the character "formation of 2nd spine" in the general chronology of ontogenesis. The character appears so late in *S.v.h.* that only a few specimens can (partially) express it.

REGRESSION OF SURFACE AND CHAETOTAXY OF 4TH AND 5TH PLEOPODS

In Stenasellids, only the 3rd, 4th and 5th pairs of pleopods are specialized in respiration. The exopodites of the 3rd pairs are sclerotized and only the endopodites form exchange surfaces (gills). The exopodites 3 constitute the opercula, covering and protecting their own



FIGS. 3-6 : REDUCTION OF THE NUMBER OF SPINES IN THE STERNAL ROW OF THE DACTYLOPODITES OF PEREOPODS 2-7 IN ASELLOTA . CAUDAL WIEW ; D = DACTYLOPODITE ; DO = "DACTYLIAN ORGAN" POINTING THE APEX OF THE DACTYLOPODITE ; H = "HEEL" OF THE PROPODITE : P = PROPODITE ; THE CLAW IS THE STERNAL SPINE NUMBER 0.

3. *Proasellus meridianus* (Racovitza, 1919), (Asellidae), Western France
4. *Stenasellus virei* Dollfus, 1897, subsp. *virei*, (Stenasellidae), Southern France
5. *Stenasellus virei*, subsp. *hussoni* Magniez, 1968, Central Pyrenees
6. *Proasellus acutianus* Argano et Henry, 1972, (Asellidae), Italy

endopodite and the ipsilateral pleopods 4 and 5.

In all large tropical species, generally living in warm and poor in oxygen water, the 4th exopodites (Fig. 7) and 5th exopodites (Fig. 12) are very large, oval and plane lamellae, the rhythmic beatings of which cause a circulation of new water on their own endopodites. But in Stenasellids strains progressively adapted to live in cool and strongly oxygenated waters (for example, the phyletic line of the European *Stenasellus virei*), one can show a trend toward reduction of the surface and mechanical role of these 4th and 5th exopodites (Figs. 7-11 and 12-15).

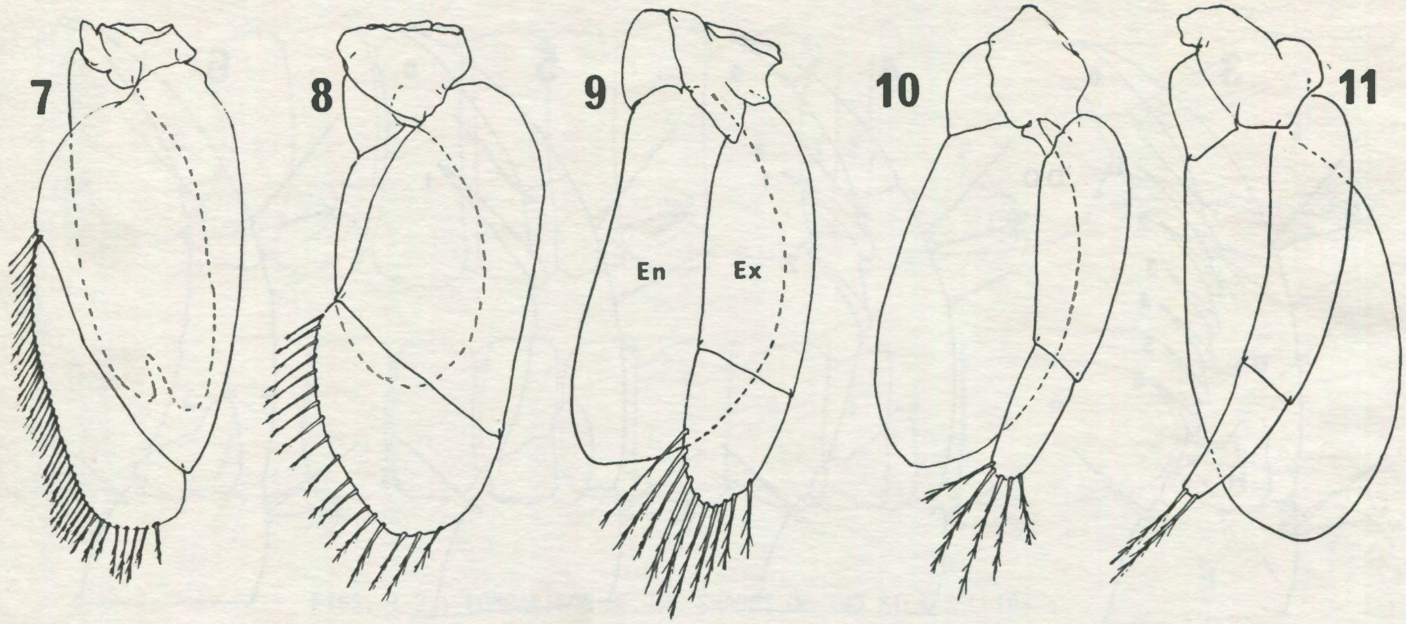
Examine the case of 4th exopodites. They are extremely large in the subequatorial species *Stenasellus kenyensis* (Fig. 7), with numerous marginal setae (30 or more). It is reduced in width in *S. racovitzai* from Corsica and Sardinia (warm Mediterranean climate) and the number of setae falls to about 20 (Fig. 8). In *S. virei buchneri* (living Southwest of Pyrenees) and *S. nobrei* (North Portugal), (Fig. 9), the exopodite is now banana-shaped, narrower than its endopodite and bears only some 10 setae. In *S. virei hussoni* (karst of North-Central Pyrenees), the exopodite becomes still more slender (Fig. 10), with only 5-6 marginal setae. The present end of this evolution is found in *S. virei boui*, an endemic subspecies living only in the underflow of some torrents of the Northern slopes of Central Pyrenees (high basin of Salat River and tributaries), a biotope with very cool and rich in dissolved oxygen waters: the exopodite appears with the shape of an acute stiletto, bearing only 2 setae (Fig. 11). *S. V. boui* is also the form containing the lowest amount of blood pigments: its color is whitish, hardly pale pink, while other forms are rose, intense rose, even carmine or coral red.

We find here a direct logical bound between the regression of the physiological purpose and the anatomical regression of an organ. The same observations can be made on the regressive evolution of the 5th pleopod's exopodite.

Conclusions

In Stenasellidae, most morphological characters show an extreme stability. Only a small number of features present a noticeable evolution in natural strains. The general direction of this evolution is regression. However, it never happens as the abrupt suppression of an organ, as it would have occured if caused by a mutation in structural genes. It behaves rather as a progressive reduction of the expression of the structure of a given organ, or as an ontogenetic delay in the appearance of this organ.

By analogy with some observations in other species (reduction of pilosity in Man, for example), one can explain this evolution as a loss of functioning of genetical mechanisms (architectural or temporal genes) controlling the expression of structural genes responsible for the edification of the organ.



FIGS. 7-11 : LEFT PLEOPODS 3, ROSTRAL WIEW, SHOWING THE REDUCTION OF THE SURFACE OF THE EXOPODITE AND OF THE NUMBER OF MARGINAL SETAE OF THE DISTAL SEGMENT ;

EN = ENDOPODITE (GILL) ; EX = EXOPODITE :

7. STENASELLUS KENYENSIS MAGNIEZ, 1975, FROM KENYA CAVES
8. STENASELLUS RACOVITZAI RAZZAUTI, 1925, FROM CORSICA
9. STENASELLUS VIREI DOLLFUS, 1987, SUBSP. BUCHNERI STAMMER, 1936, FROM SPAIN
10. STENASELLUS VIREI, SUBSP. HUSSONI MAGNIEZ, 1968, FROM PYRENEES
11. STENASELLUS VIREI, SUBSP. BOUI MAGNIEZ, 1968, FROM CENTRAL PYRENEES

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RESUME

La famille des Stenasellidae est très homogène morphologiquement. Seuls certains caractères présentent une évolution, d'une espèce à l'autre dans les lignées naturelles. Cette évolution se fait en liaison avec une réadaptation écologique subie par les lignees vivant dans les régions tempérées.

Les caractères variants sont essentiellement la taille et la forme du corps, la teneur en pigments sanguins respiratoires, la forme de l'exopodite des pléopodes 4 et 5 et le nombre d'épines sternales au dactylopodite des péréiopodes 2 à 7.

Cette évolution se présente comme une dérive progressive des caractéristiques des organes qui se modifient, mais jamais comme une modification brutale de leur morphologie. Il semble difficile d'attribuer de tels phénomènes gradués d'évolution régressive à des mutations de type classique.

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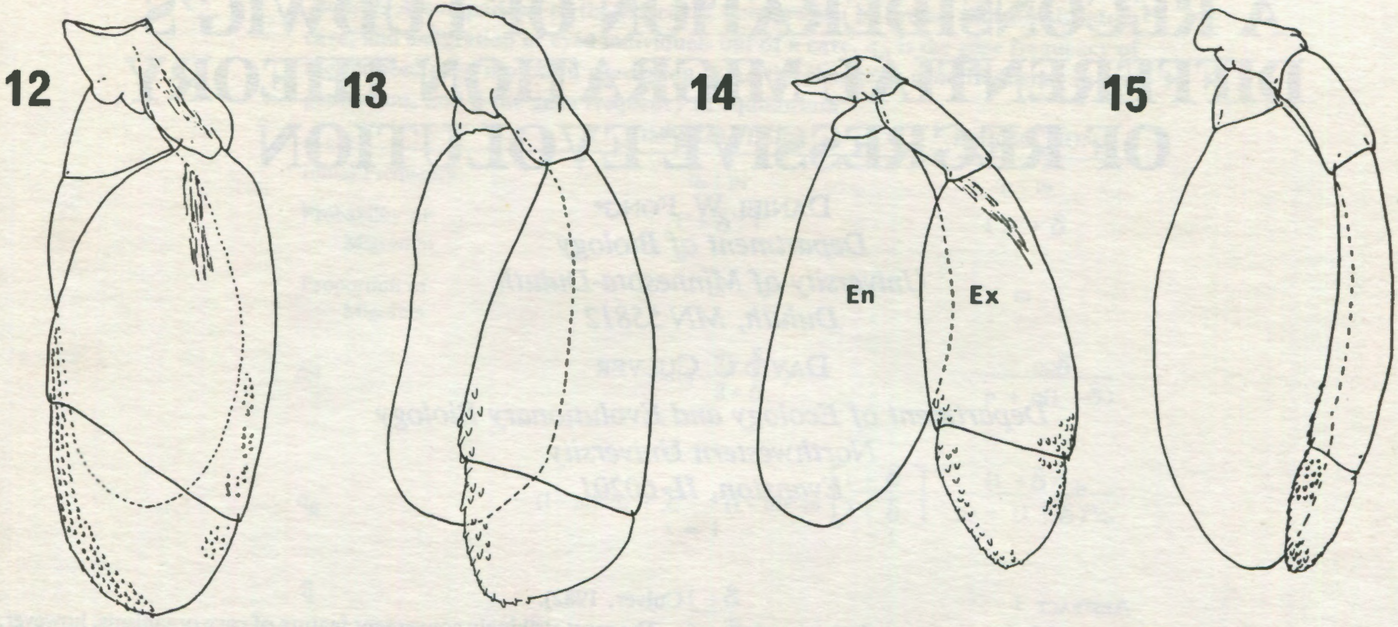

FIGS. 12-15 : LEFT PLEOPODS 5, ROSTRAL WIEW, SHOWING THE REDUCTION OF THE LARGE CHITINOUS EXOPODITE INTO A STYLIFORM ELEMENT, THE EPICUTICULAR SCALED AREAS BEING PRESERVED IN SPITE OF THIS EVOLUTION ; EN = ENDOPODITE (GILL) ; EX = EXOPODITE :

12. MAGNIEZIA GARDEI MAGNIEZ, 1978, SOUTHERN MOROCCO
13. STENSELLUS ESCOLAI MAGNIEZ, 1977, SOUTHERN SPAIN
14. STENASELLUS BUILI REMY, 1949, SOUTHERN FRANCE
15. STENASELLUS VIREI DOLLFUS, 1897, SUBSP. HUSSONI MAGNIEZ, 1968, CENTRAL PYRENEES

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A RECONSIDERATION OF LUDWIG'S DIFFERENTIAL MIGRATION THEORY OF REGRESSIVE EVOLUTION

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ABSTRACT

Ludwig's differential migration model of regressive evolution in cave organisms was examined. Our reformulation of the model shows that only differential emigration can lead to fixation of small-eyed morphs in cave populations, at equilibrium, while differential immigration cannot. However, the rate of approach to equilibrium is very rapid. We specifically studied two predictions of the model. The first is that of a cline of larger-eyed individuals outside caves to smaller-eyed ones inside caves. We found no evidence of such a clinal variation in eye facet number in a population of *Gammarus minus* which spans the entrance zone of a cave in distribution. The model also predicts a negative correlation between eye size and light avoidance behavior. We found extensive phenotypic variation in eye size and phototactic behavior in a population of *Gammarus pseudolimnaeus,* but no significant correlation between the two traits. Analysis of the genetic parameters underlying these traits showed that a large portion of the observed variation is genetic in origin for eye size, but is not genetically determined in phototaxis, and that eye size is not genetically correlated with phototaxis. Our data strongly suggest that Ludwig's differential migration model cannot explain regressive evolution of cave organisms.

INTRODUCTION

Most cave organisms show some phenotypic modifications when compared to epigean relatives. Changes in a series of traits, termed cave-dependent characters by Christiansen (1961), are strongly convergent for cave organisms. In the Arthropoda, with an abundance of cave species, these changes include elongation of antennae and many other appendages, and increases in the number and density of tactile and chemical sensory structures. Examples for other organisms and/or other traits are given in Poulson (1964), Barr (1968), Poulson and White (1969), Culver (1982), and Howarth (1983). In general, these are adaptations for enhanced sensory compensation in the absence of light, for increased metabolic efficiency due to low resource levels caused by the absence of photosynthesis, and, among terrestrial species, for coping with extremely high moisture and humidity conditions (see Culver, 1982).

The most strikingly convergent feature of cave organisms, however, is the loss or reduction of eyes and pigment. Although not common to all cave species, it is found in at least some populations or species of all taxa with cave representatives (Vandel, 1964; Culver, 1982; Howarth, 1983). Such regressive changes also occur in other traits, such as the degeneration of circadian activity rhythms in some species (Jegla and Poulson, 1968), and the virtual absence of hind wings in the cixiid planthopper *Olivarus polyphemus* (Ahearn and Howarth, 1982). Regressive evolution in these traits share a common theme in that they defy explanation as direct adaptations to an aphotic environment *per se*.

No direct selective factor in the cave environment is evident as the cause for regressive changes in eye size and pigmentation of cave species (Maguire, 1961; Sadoglu, 1967; Culver, 1982). The consensus among biospeleologists is that the presence of eyes and pigment is most unlikely to be directly detrimental to a cave organism. Current theories on the mechanism of regressive evolution invoke selection as an indirect agent based on energy economy or pleiotropy arguments, or it is explained as the result of relaxed selection and the accumulation of structurally reducing mutations (see Culver, 1982; and Fong, 1985). These theories rely heavily upon specific assumptions about the genetic variation and covariation patterns of the convergent cave-dependent characters, although few data on these parameters are available; and in fact it is very difficult to frame these theories into testable working hypotheses (but see Fong, 1985; Culver and Fong, 1985.)

In this paper we examine a theory of regressive evolution proposed by Ludwig (1942) that appears to avoid many of the problems of both indirect selection theories and the neutral mutation theory. Except for a brief discussion by Barr (1968), Ludwig's theory of differential migration has gone largely unnoticed in English language papers on the topic. We first summarize and reformulate Ludwig's model, and then discuss two tests of the hypothesis, one from field data and one from an experiment on behavioral genetics.

THE HYPOTHESIS

The core of Ludwig's model is that individuals with different pigment levels respond differently to light. That is, individuals with reduced

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Table 1. **A comparison of the effects of immigration of eyeless individuals into a cave, and emigration of eyed individuals out of a cave. q_n is the gene frequency of the reduced eyed morph in generation n; Δq the change in gene frequency in one generation, and $\hat{q}$ the gene frequency at equilibrium.**

	IMMIGRATION	EMIGRATION
Initial Frequency	$q_0 : p_0$	$q_0 : p_0$
Probability of Migration	$1 + \delta : 1$	$1 : 1 + \delta$
Proportion of Migrants	m	m
Δq	$-m(q - \frac{1+\delta}{2+\delta})$	$\frac{pq\delta}{p + q(1+\delta)}$
q_n	$(1-m)^n q_0 + \sum_{x=1}^{n-1} (1-m)^x m \left[\frac{1+\delta}{2+\delta}\right]$	$\frac{(1+\delta)^n q_0}{p_0 + (1+\delta)^n q_0}$
$\hat{q}$	$\frac{1+\delta}{2+\delta}$	1

pigment (and reduced eyes) are more photophobic than individuals with normal pigment (and normal eyes). The probability that an individual with reduced eyes and pigment will enter a cave is greater than that of one with normal eyes and pigment (differential immigration); and the probability that an individual with normal eyes and pigment will leave a cave is greater than that of one with reduced eyes and pigment (differential emigration). There may be selective advantages associated with this differential movement, but it is not required by the hypothesis. In genetic terms, the model can be restated as follows. Ignoring environmental effects, assume there is an allele 'a' that reduces eye size by the amount v, and whose initial frequency in the cave population is q_0. Likewise, p_0 is the initial frequency of the allele for normal eye size. Let δ be the relative difference in response to light. The calculations of change in gene frequency per generation, gene frequency after n generations, and the equilibrium gene frequency are given in Table 1, for both differential emigration and differential immigration.

Two main points emerge. First, only differential emigration can result in the cave population becoming fixed for reduced eyes. For differential immigration, at equilibrium the frequency of morphs with reduced eyes in the cave population is the frequency of reduced-eyed morphs among migrating individuals, i.e., $[1+\delta]/(2+\delta]$ (Table 1). Second, the rate of approach to equilibrium is very rapid. In the emigration model, for example, if $q_0 = 0.001$ and $\delta = 0.01$, the gene frequency after only 1000 generations is 0.67.

The model predicts, in a populatioin which spans the entrance zone of a cave system in its distribution, a cline of darker and/or larger-eyed individuals outside caves to paler and /or smaller-eyed ones inside caves. There is little evidence to support this prediction. Maguire (1961) finds a cline in pigmentation in the crayfish *Procambarus simulans*, but it reflects environmental variations in carotenoids in their diet rather than genetic differences. Lisowski (1979) describes a cline in eye size in the isopod *Caecidotes brevicauda*, but the genetics of the system is unknown. We will look for clinal variation in a cave population of the amphipod *Gammarus minus*.

A second prediction requires a closer look at the genetics implicit in the model. A mutation that reduces the eye (or pigment) also, in the case of differential emigration, reduces the probability of emigration. The following summarizes the effects (see also Table 1):

Genotype	Eye Size	Probability of Emigration
AA	x	$m + 2\delta$
Aa	x- v	$m + \delta$
aa	x-2v	m

Thus there should be a negative genetic correlation between eye size and probability of moving toward light. We investigate this for a population of *Gammarus pseudolimnaeus*.

MATERIALS AND METHODS

Collections of *Gammarus minus* were made from Coffman's Cave, Greenbriar County, West Virginia. The main stream channel associated with the cave emerges as a spring about 50 meters outside the cave entrance, forms a narrow but deep pool by an artificial dam at the entrance, then flows as a wide and shallow stream into the cave for several hundred meters to a sump. Suitable gravel substrate for the amphipods occurs only in the first 100 m of the cave. Individuals of all sizes were obtained with a 1-mm mesh dip net at 45 and 30 meters upstream from the entrance, at the entrance, and at 50 and 100 meters inside the cave. All collections were immediately preserved in 70% ethanol. In the laboratory, the length of the head capsule (HL) and the number of eye facets (EN) were determined for 40 randomly chosen specimens from each of the five collections. The data were analysed for the parameters 'a' and 'b' from the allometric equation $Y = ax^b$, where y is eye facet number and x is head capsule length.

About 500 adult *Gammarus pseudolimnaeus* were obtained with a 2-mm mesh dip net from the Eau Claire River at Gordon, Douglas County, Wisconsin. Ovigerous females were identified by the dark mass of developing embryos in their marsupia, and were individually isolated in the laboratory in petri dishes containing water and leaf-detritus obtained from the field, and maintained at 11 °C with a 12:12 L:D cycle. Each female was removed and preserved upon complete release of her brood from her marsupium. At between five to ten days from release, four young amphipods were randomly chosen from each brood for the determination of phototactic behavior and measurement of morphological characters.

A behavior index (BI) of phototaxis was obtained from each individual with light-dark chambers. Each chamber consisted of a 2-cm length of clear tygon tubing connected at each end to the bulb of a clear polyethylene transfer pipet, with the bulb at one end wrapped in black water-resistant tape. A specimen was first introduced into the middle of the tubing portion of a chamber, then the chamber was placed in a water bath at 11 °C about 30 cm beneath a 60-watt incandescent lamp. After one hour, the position of the specimen within the chamber was noted; a score of zero was assigned if it was located in the darkened bulb, or a score of one was given if it was located in the clear bulb or in the clear tubing portion of the chamber. This procedure was conducted four consecutive times for each specimen, and its BI was calculated as the number two subtracted from the sum of its scores from the four trials. Thus the possible values of BI are -2, -1, 0, $+1$, and $+2$; a BI of -2 indicated the specimen chose the darkened bulb for all four trials, a BI of -1 showed it chose the dark for three of the four trials, . . .etc. The specimens were preserved in 70% ethanol immediately after the fourth trial.

Three morphological characters were measured: EN-the number of eye facets, N1-the number of flagellar segments in the first antenna, and N2-the number of flagellar segments in the second antenna. Values for these characters were all obtained from the left side of the specimens.

Quantitative genetic parameters underlying the four traits, BI, EN, N1 and N2, were estimated with the standard technique of analysis of full sibs (see Falconer, 1981; Fong, 1985). The total phenotpyic variance (Vp) of each trait was decomposed through analysis of variance into within-family (σ_w^2) and between-family (σ_b^2) portions. The causal components of the between-family portion of the variance are: $\sigma_b^2 = V_A/2 + V_D/4 + V_{Ec}$, where V_A is the additive genetic variance, V_D is the dominance variance, and V_{Ec} is the variance due to common environmental differences between families, especially maternal effects. The heritability of each trait, defined as the proportion of its total phenotypic variance resulting from additive genetic causes, was estimated as:

$$h^2 = 2\sigma_b^2/V_P = (V_A/2 + 2V_{Ec})/V_P, \qquad [1]$$

and is inflated by half of the dominance variance and twice the common environmental variance, if present. The additive genetic covariance between any two traits, X and Y, was obtained through a similar decomposition of the total variance of the sums of the two trait values and then fitting the corresponding between-family portions into equations of the form

$$COV_A(X,Y) = ((\sigma_b^2(X+Y)-\sigma_b^2(X)-\sigma_b^2(Y))/2 \qquad [2]$$

The genetic correlation between the traits was then calculated by fitting the appropriate variances and covariances into the equation

$$r_A = COV_A(X,Y)/((\sigma_b^2(X)\sigma_b^2(Y))^{1/2} \qquad [3]$$

RESULTS

Data from the Coffman's Cave population of *Gammarus minus* are summarized in Figure 1 and Table 2. Individuals of all sizes and age groups were collected, ranging from a head capsule length of under 0.50 mm in juveniles to over 1.25 mm in adults, with correspodning numbers of eye facets ranging from eight to more than 35. There were no significant differences among the coefficients of allometry (b in the equation $y = ax^b$) in the five collections (Table 2B). If all collections are pooled, b is 1.09 ± 0.07, indicating a slight positive allometry. Ignoring the absence of statistically significant differences, one can look at the differences that do exist. The coefficient a shows no pattern, but b is larger for collections inside the cave, indicating that the cave collection contains specimens with larger eyes. This is the opposite of the prediction of Ludwig's hypothesis.

Distance	b ± S.E.	log a ± S.E.	R^2
-100 m	1.22 ± 0.18	1.44 ± 0.02	57.0
-50 m	1.33 ± 0.21	1.38 ± 0.02	50.9
0 m	1.00 ± 0.13	1.38 ± 0.02	62.0
+30 m	1.01 ± 0.11	1.41 ± 0.01	70.6
+45 m	1.04 ± 0.15	1.35 ± 0.03	56.6

***Table 2A.* Analysis of the allometric equation $y = ax^b$ where y is eye facet number and x is head length in *Gammarus minus* from Coffman's Cave. Distances are measured from the entrance to inside the cave (negative values), and to outside the cave (positive values). S.E.'s are standard errors of the coefficients, and R^2 is the percent variance explained by the regression. Sample sizes are 36 for the -100 m distance collection, and 40 for all others.**

Source of Variation	df	SS	MS	F
Variation among regressions (b's)	4	0.036	0.0090	0.97^{ns}
Average variation within regressions	186	1.732	0.0093	
Total	190	1.768		

***Table 2B.* Analysis of Variance of slopes in Table 2A.**

Data from the *Gammarus pseudolimnaeus* population were obtained from a total of 220 individuals representing 55 fullsib groups. These groups showed large differences in brood size, varying from 18 to 54 around a mean of 30; however, no significant correlation was found between brood size and any trait. Frequency distributions of the values and descriptive statistics for the four traits are given in Table 3. The number of eye facets ranged from three to ten around a mode of six and a mean of 6.5. The number of flagellar segments of the first antenna varied from three to six with a mode of four and a mean of 4.1, while corresponding values of the second antenna ranged only from two to four with the mode at three and the mean at 3.3. The three morphological traits were relatively equally variable, showing little differences in their coefficients of variation. The behavior indices varied from -2 to +1 about a mean of -0.9 with the mode at -1, indicating that most individuals chose the darkened side of the light-dark chambers for three of the four trials while no specimen chose the lighted side for all four trials. The behavior index is much more variable than the morphological traits, showing a coefficient of variation more than five times greater in magnitude. The distribution of the behavior indices do seem to reflect phototactic behavior of the organisms. This is because the expected distribution, if the locations of the animals within the light-dark chambers were independent of the presence or absence of light, is 14:55:82:55:14 (at a ratio of 1:4:6:4:1), respectively for BI values of -2:-1:0:+1:+2, which is significantly different from the observed distribution ($X^2 = 294.7$, df = 4, $P < 0.001$).

The heritabilities of the traits and their phenotypic and genetic correlation matrices are shown in Table 4. EN showed the highest heritability value at 1.09, and N1 showed a moderate heritability at 0.63, while

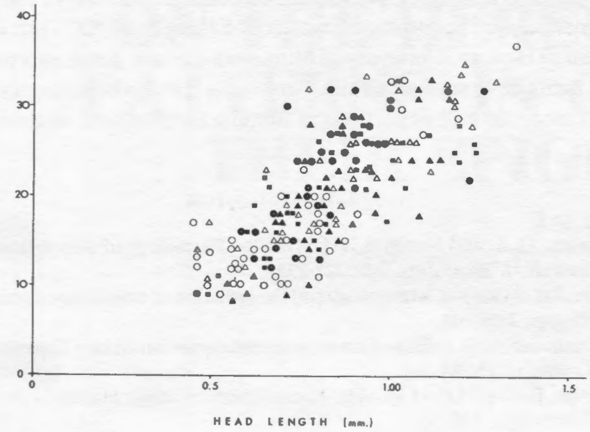

Figure 1. **Plot of eye facet number and head capsule length for five collections of *G. minus* from Coffman's Cave. Solid circles - 100 m inside cave; solid triangles - 50 m inside cave; solid squares - cave entrance; open triangles - 30 m outside cave; open circles - 45 m outside cave.**

values for N2 and BI were not significantly greater than zero. BI was phenotypically negatively correlated with the other traits, but not significantly so. The three morphological characteres were phenotypically positively correlated with each other. The genetic correlations showed a different pattern. N1 and N2 were highly genetically correlated. The other genetic correlations were not significant, but it is interesting to note that eye facet number and the behavioral index were negatively correlated.

DISCUSSION

The heritability values of the four traits in the *Gammarus pseudolimnaeus* population (Table 3) are probably inflated by the common evironmental component of variance, especially maternal effects, because its magnitude is doubled in these estimates (see equation [1]). Maternal variance is caused by variations among dams relative to the environment in which development and rearing of the offspring occur (Falconer, 1981), and is likely an important determinant of phenotypic variance among broods of young amphipods, each brood having developed within a marsupium unique to its female parent. Two other attributes specific to our experimental design may also contribute to overestimation of the heritabilities. Firstly, newly released amphipods from the same brood were kept together in the same petri dish instead of individually isolated, thus increasing the probability and the magnitude of the common environmental variance. Secondly, we were not able to remove possible effects resulting from age and/or size differences among broods prior to estimations of genetic parameters, thus artificially further enlarging the between family component of variance. Inflation from these sources is likely the reason that the heritability of EN exceeded the theoretical limit of unity. It is interesting that even with these potential sources of inflation, heritabilities of N2 and BI were still not significantly different from zero; seemingly the observed phenotypic variations in these two traits were entirely environmental in origin. There is surprisingly very good agreement between the heritabilities of the three morphological traits reported here in five-day to ten-day old *G. pseudolimnaeus* and those of analogous traits found in 40-day old *G. minus* from Coffman's Cave (Fong, 1985; Fong and Culver, in prep.). These heritabilities were 1.06, 0.34, and 0.18, respectively, for EN, N1 and N2 in *G. minus*, compared to corresponding values of 1.09, 0.63 and 0.19 in *G. pseudolimnaeus*. This is unexpected because heritabilities are dependent on gene frequencies, which are expected to vary between populations and even more so between species. The absence of genetic variance in N2 in both species suggests that this character is or has been under selection in both populations, because selection on a trait should reduce its genetic variance (Falconer, 1981). The very large expressed genetic variance in EN in both species, on the other hand, indicates that the trait may have experienced a history of relaxed direct selection. This is explicable for the *G. minus* population because it inhabits a karst window and a large portion of individuals are found in the twilight and aphotic zones within Coffman's Cave; possibly the population had historically inhabited an aphotic cave stream prior to the collapse of a portion of the cave roof which then produced the karst window. A history of relaxed selection on EN is more difficult to justify in the *G. pseudolimnaeus* population, because the species is not found in caves. We suggest that *G. pseudolimnaeus*

Value	EN	N1	N2	BI
-2	—	—	—	67
-1	—	—	—	97
0	—	—	—	32
1	—	—	—	24
2	—	—	9	—
3	2	24	142	—
4	8	150	69	—
5	28	38	—	—
6	72	8	—	—
7	60	—	—	—
8	37	—	—	—
9	12	—	—	—
10	1	—	—	—
x	6.5 ± 0.08	4.1 ± 0.04	3.3 ± 0.04	-0.9 ± 0.06
CV	18.9	15.5	16.2	97.8

Table 3. **Phenoytpic data for *Gammarus pseudolimnaeus*. Upper panel: trait specific frequency distributions. Lower panel: phenotypic means (x) with standard errors and coefficients of variation (CV).**

	EN	N1	N2	BI
EN		0.03 ± 0.24	0.50 ± 0.31	-0.47 ± 0.38
N1	0.14 ± 0.07*		1.07 ± ---	0.07 ± 0.69
N2	0.22 ± 0.07*	0.31 ± 0.06*		0.01 ± 1.19
BI	-0.06 ± 0.07	0.08 ± 0.07	-0.05 ± 0.07	
h^2	1.09 ± 0.27	0.63 ± 0.30	0.19 ± 0.26	0.12 ± 0.25

Table 4. **Genetic data for *G. pseudolimnaeus*. Upper panel: matrices of genetic correlations with standard errors (above diagnal) and of phenotypic correlations with standard errors (below diagnol). An asterisk indicates a significant correlation ($P > 0.95$). Lower panel: trait-specific heritabilities with standard errors.**

is maybe an example of a large group of species that have indeed experienced relaxed selection on traits which function in photic information processing, such as the number of eye facets. As Culver (1982) points out, the fauna of caves is far from being a random sample of the epigean species pool, but includes a preponderance of taxa which primarily dwell in habitats such as forest litter and benthic detritus, some common examples are the Collembola, carabid beetles, millipedes, ampipods and aquatic isopods. It is conceivable that, in these habitats, selection would be relaxed relative to visual acuity and be much intensified relative to chemo-tactile acuity. Further studies similar to this on many more populations and species are necessary to determine whether the pattern of high heritability of eye size traits obtains in general for these animal groups.

The high heritability of EN also indicate that the character is capable of responding to selection directly or show correlated response to selection on genetically correlated traits. The differential migration model predicts a negative genetic correlation between eye size traits such as EN and light avoidance behavior. Our measure of phototaxis, BI, however, showed no significant genetic variance. This is not due to a lack of variation in the trait, it is phenotypically much more variable than the morphological characters (Table 3). This result shows that the *G. pseudolimnaeus* population may be genetically fixed for light avoidance in terms of phototactic behavior.

The negative phenotypic and genotypic correlation between eye facet number and the behavioral index is tantalizing. If it is real, it means that individuals with larger eyes tend to avoid light more strongly than individuals with smaller eyes. Furthermore, this tendency is corroborated by the morphometric data for *G. minus* from Coffman's Cave. In this population, cave collections had slightly larger-eyed individuals than surface populations. This makes perfect physiological sense: an individual with a larger eye probably has a better sensory system to avoid light than an individual with a smaller eye. The evolutionary result would be larger-eyed animals in caves. Therefore, Ludwig's hypothesis seems unlikely to explain regressive evolution.

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ON THE HEREDITY OF BEHAVIOR PATTERNS IN CAVE ANIMALS AND THEIR EPIGEAN RELATIVES

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INTRODUCTION

In 1935 Curt Kosswig, working in genetics and evolutionary biology, drew attention to genetic studies in cavernicolous species. The blind cave fish *Anoptichthys* was hybridized successfully with the epigean *Astyanax mexicanus* (*=fasciatus**) by his research team. So the cave fish of the genus *Anoptichthys* were classified as populations of *A. fasciatus* living in different Mexican caves (Sadoglu, 1958). In the different populations of *A. fasciatus* various morphological characters as the eye, the melanin pigment and the taste buds have been analyzed genetically (Wilkens, 1980; Wilkens et al., 1979; Schemmel 1974). With the increasing knowledge of the genetics of morphological differences behavioral genetics studies of the cavernicolous populations have become especially interesting. This paper summarizes the genetically based behavioral differences in cave animals and their epigean relatives. The data are mainly based on studies of two fish species—*Astyanax fasciatus* and *Poecilia mexicana*. *A. mexicanus*, which is abundant in middle American rivers, successfully colonized various caves in North Mexico (Mitchell et al., 1977). All these populations except the two in Micos and Chica cave show the same degree of reduction for eye and the melanin pigmentation (Peters and Peters, 1966; Wilkens 1970, 1971, 1976). Using the genetic analysis of these characters in hybrids the Chica-fish has been classified as a hybrid population (Wilkens and Burns, 1972) and the Micos-fish as a phylogenetically young cave population (Wilkens, 1972) and the Micos-fish as a phylogenetically young cave population (Wilkens and Burns, 1972). In the second species *Poecilia mexicana* which is also very common in the fresh water of Middle America we only know one cave population. All the fish living in this cave in central Mexico have a diminished eye size only and still show a visual reaction. The eye size and some other morphological characters form a gradient from the entrance to the inner part of the cave. This gradient has been explained by a gene flow from epigean fish living near the cave entrance (Parzefall, 1970; Peters et al., 1973). For the studies presented only the most reduced fish collected in the inner chamber of the cave (XIII after Gordon and Rosen, 1962) have been used.

We first try to demonstrate that the behavioral differences found in the cave fish are genetically based. This step is followed by a genetic analysis and a discussion about the ecological situation which could be the reason for the behavioral differences.

2. RESULTS

2.1 ALARM REACTION

This behavior first has been described by von Frisch (1938), Schulz (1956) and Pfeiffer (1963, 1977). They examined various species and demonstrated that the alarm or fright reaction is widely distributed within the fish order Ostariophysi and also in the tadpoles of some amphibian species. In all these cases the skin releases a chemical when damaged. The conspecifics respond with a specific behavior like rapid swimming or zigzag movements, fleeing or hiding. Pfeiffer (1966) compared the fright reaction in an epigean population of *Astyanax fasciatus* with the reaction of the cave fish from the Chica and the Pachon cave. The cave fish produce an active alarm substance, but they do not react to their own or the alarm substance of their epigean conspecifics. The reaction of the different hybrid generations has been tested by Pfeiffer (1966), and the segregation ratio obtained (Fig. 1) is consistent with a model of two dominent alleles. All F_1-individuals which are heterozygous for the fright reaction have this behavior. In the F_2-generation the segregation ratio of 13 to 197 individuals is within limits of an expected ratio of 1:15. However the result of the backcross to the cave fish (8:47) differs from the expected segregation ratio of 1:3.

Recently Fricke (1985) demonstrated that during feeding the cave populations also avoid the area where the alarm substance has been released (Fig. 2). He believes this avoidance reaction to be the only part of the alarm behavior which is still present in the cave fish. His argument is that all parts of the complex alarm behavior without any biological significance in the darkness have been reduced in the cave fish. This means rapid swimming or zigzag movements and hiding which alarm especially the schooling conspecifics visually are without any importance in darkness. On the contrary avoidance in darkness also prevents the fish from being caught in the hunting area of predators or cannibalistic conspecifics. I have begun hybrid studies with different *Astyanax*-populations for these same traits. In the cave fish *Caecobarbus geertsi* (Cyprinidae) Thines and Legrain (1973) also found an active production of the alarm substance but no alarm behavior.

2.2 FEEDING BEHAVIOR

The epigean *Astyanax fasciatus* schools in rivers and clear fresh water ponds. The fish catch living and dead prey from the surface and in

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*The species name *Astyanax fasciatus* (Cuvier, 1819) has priority but *A. mexicanus* (Philippi, 1853) has been used by most authors.

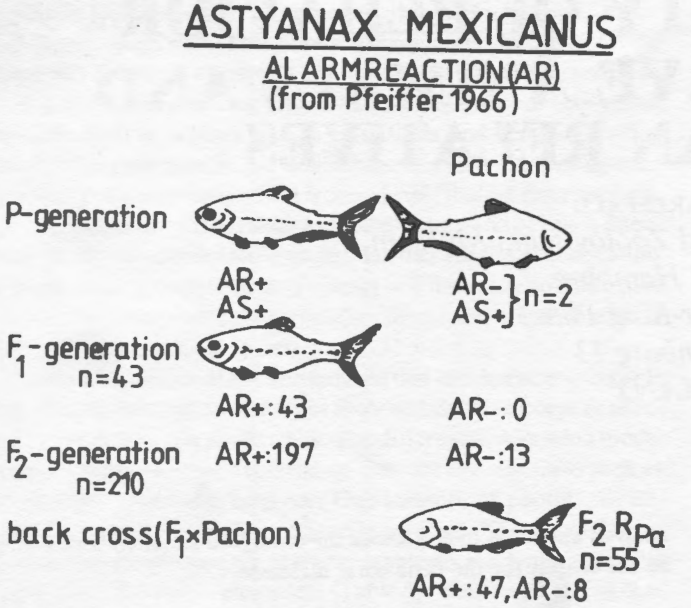

Figure 1: **Alarm reaction (AR) and secretion of an alarm substance (AS) in an epigean and hypgean population of *Astyanax fasciatus* (= mexicanus) and their hybrids. + present, − absent. (from Pfeiffer, 1966).**

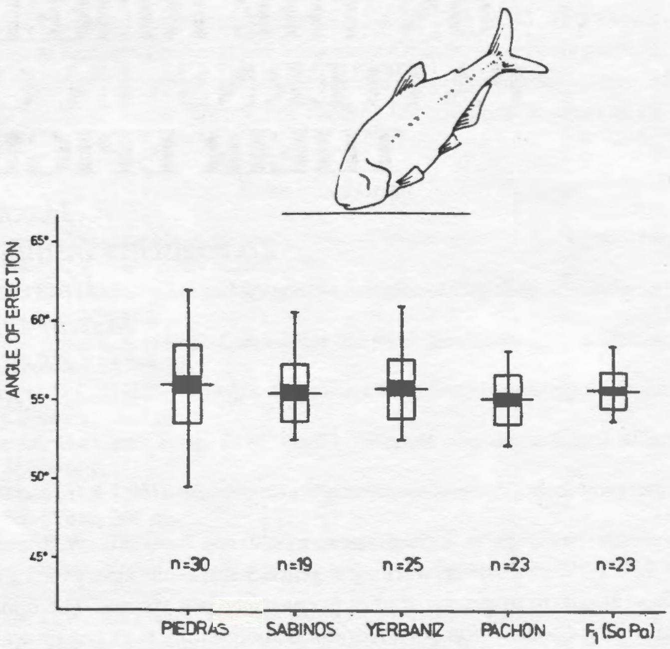

Figure 3: **Mean, mean error, standard deviation and range of variation of the angle of erection in populations of different caves and an F_1-hybrid. Sa = Sabinos cave population, Pa = Pachon cave population (from Schemmel, 1980).**

the water by rapid swimming movements. They also bite off pieces of bigger prey objects by the same movement. Each movement looks like a short attack usually followed by a retreat. The cave fish on the contrary patrols in a solitary manner in a head down position near the surface or the ground of his habitat. When excited by movement or chemical signals of food, the gliding changes into rapid zigzag searching movements (Parzefall, 1983). In laboratory experiments (Schemmel, 1980) the cave fish showed an angle of 45° when he picked up food from the ground. The epigean fish in the absence of visual orientation took the food in a vertical head standing position. Feeding in a standardized situation recorded with an infrared videocamera and single frame analysis of four cave populations and an F_1-generation led to nearly identical results (Fig. 3) for the angle measured. The frequency distribution in hybrids between an epigean and a cave population (Fig. 4) shows dominance in the F_1- and a 3:1 segregation ratio in the F_2-generation. From these data and a 1:1 segregation ratio in the backcross to the cave fish (Fig. 5) one can suggest a monogenetic inheritance and nearly complete dominance of the head-standing posture of the epigean fish. But the data of F_2xP-crossings (Fig. 5) and the variable expression of the trait in different crossings indicate a better accordance with an additive polygenic model of inheritance assuming at least three pairs of genes. Schemmel (1980) did not find any correlation between the headstanding posture and the eye size, the degree of pigmentation and the gustatory equipment. The constructive development of the areas with taste buds on the surface of the head in the cave fish has also been demonstrated by Schemmel (1967, 1974).

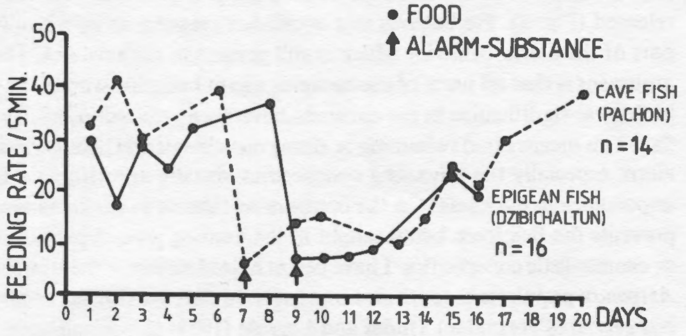

Figure 2: **Mean feeding rate of *Astyanax fasciatus* (*mexicanus*) kept in single aquaria (from Fricke, 1985). Dry food and 25 ml water given in each aquarium, water mixed with alarm substance.**

2.3 Aggressive Behavior

In the laboratory the epigean population of *Astyanax fasciatus* becomes more and more aggressive (Fig. 6) with decreasing tank size: the number of ramming attacks increases but the level of circling remains unchanged. In smaller aquaria the fish tend to give up schooling and defend territories. No differences between males and females have been found. Hungry animals become more aggressive (Burchards et al., 1985). In comparative tests using visible light and infrared there is a significant reduction of ramming attacks under dark conditions in epigean populations only (Burchards and Parzefall, 1985). There

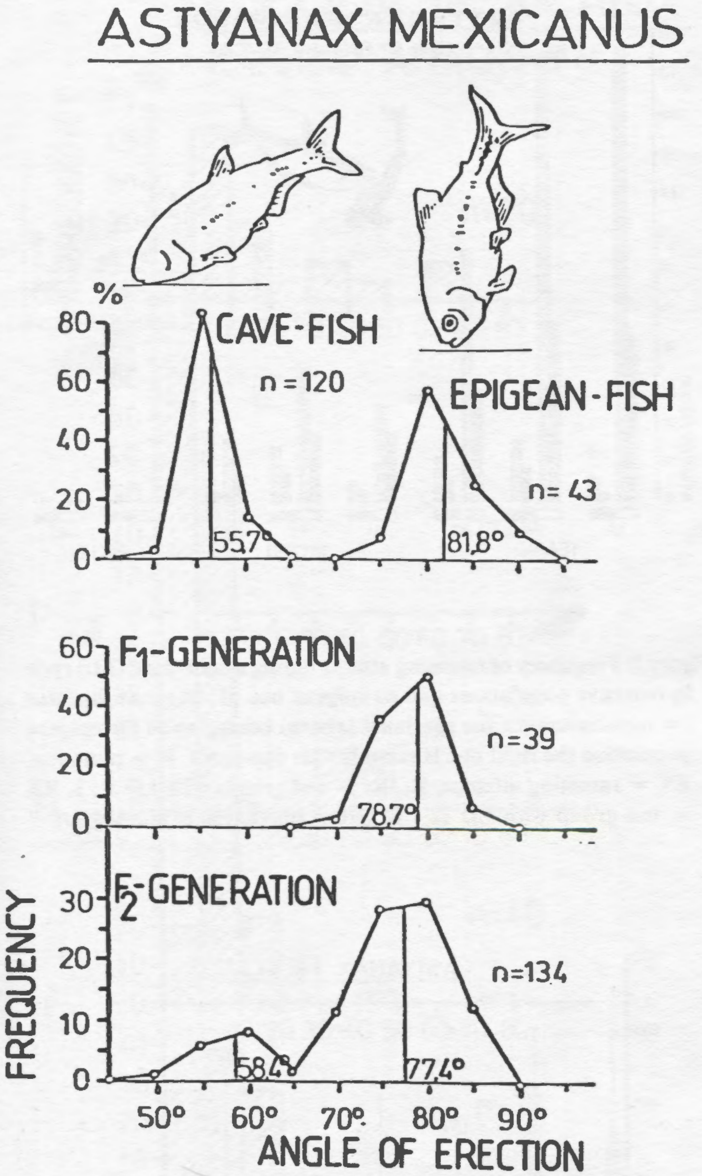

Figure 4: **Frequency distributions of the angle of erection in parental forms and the F_1 and F_2 hybrids. Vertical lines = means. (from Schemmel, 1980).**

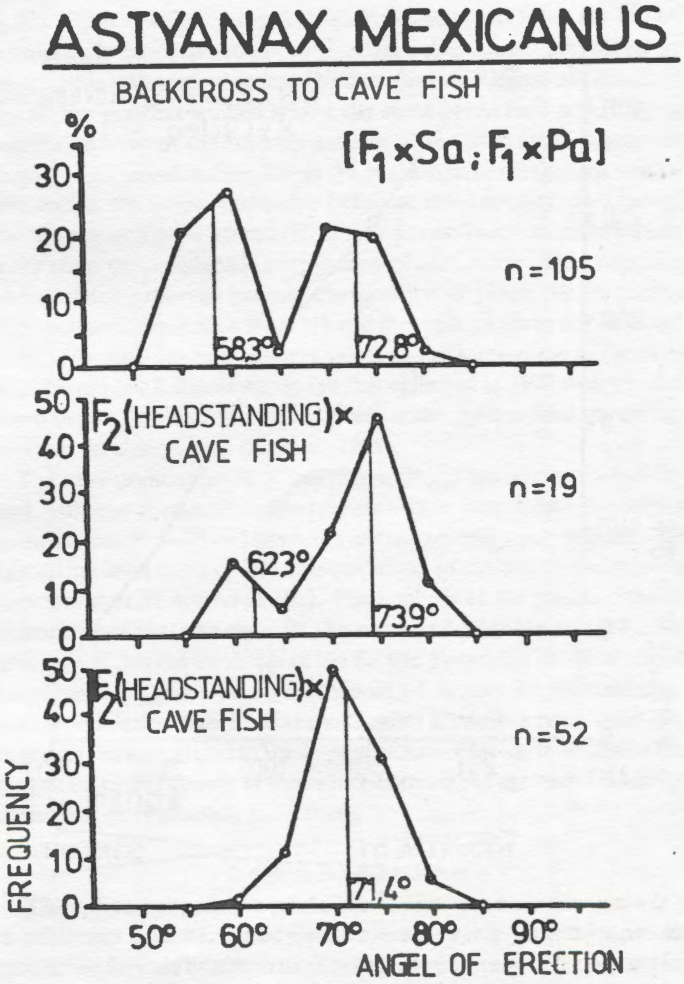

Figure 5: **Frequency distributions of the angles of erection of a backcross to the cave form and two different F_2 × Pachon-backcrosses (from Schemmel, 1980).**

is no difference between the epigean population of the Rio Teapao and the epigean population of the Cenote Dzibichaltung (Fig. 7). Because of the low aggression level in the absence of visual orientation in hybrid-generations only individuals with well-developed eyes have been tested for aggression. The visual orientation of these animals has been measured by means of the optomotoric response. The low aggression level in the blind cave fish may be caused by the absence of the visual orientation only or by a genetically based reduction of these behavior patterns. The significantly lower level of aggression in the F_2-generation, despite a well developed eye (Fig. 8), suggests that the low aggression in the cave fish is based on a genetically controlled reduction of this behavior. The increasing number of attacks in the backcross to the epigean fish is in accordance with this suggestion. In the backcross to the cave fish only blind animals have been obtained. Therefore the low aggression level of these hybrids cannot be used for the argumentation of a genetically based reduction. Theoretically we also should expect a few eyed inviduals in the backcross to the cave fish, too. But up to now the eyed fish obtained showed no visual orientation (Burchards and Parzefall, 1985).

Between hybrids with a good optomotoric response (= minimum separabile 15 ′) and those with less visual acuity there was no difference in the number of attacks (Fig. 8). An optimum for the optomotoric response has been determined in the rainbow trout with a minimum separabile of 14 ′ (Rahmann et al., 1978). From these data we can suggest that the aggressive behavior in *A. fasciatus* can be released visually even in individuals with a relatively weakly developed optic capacity. But on the basis of the present data a final resolution of this prob-

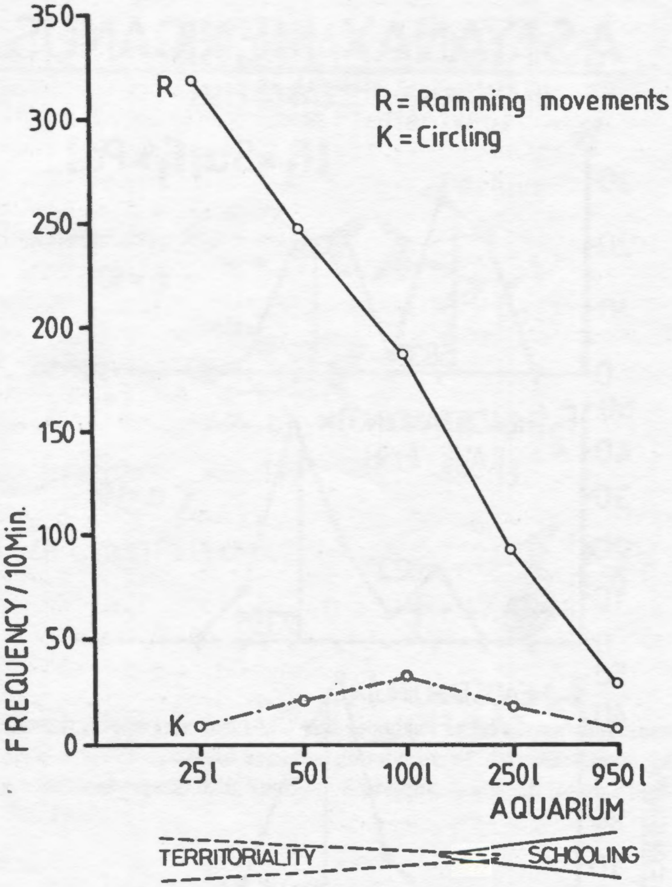

Figure 6: **Frequency of two aggressive patterns and the tendency for territoriality and schooling of two males and two females of the epigean *Astyanax fasciatus = (mexicanus)* in aquaria of different sizes (from Burchards et al., 1985).**

lem is not possible.

More detailed studies have been done about the reduction of the aggressive behavior in *Poecilia mexicana*. Contrary to *A. fasciatus* the cave population of *P. mexicana* develops a functional eye which has a smaller size than in epigean conspecifics. Within the cave population an optomotoric response has been found as in the rainbow trout (Fischer, 1983). In *P. mexicana* the males fighting for females develop a rank order dependent on body size. Aggressive behavior decreases in comparison to a population from the Rio Teapao in the small river near the cave entrance (population P_O) and has the lowest level in the inner part of the cave (Population P_{XIII}) (Parzefall 1969, 1974). The different aggressive patterns have been observed in the P_O-population already with 1 and 5 lux light intensity. In complete darkness only tail beating has been registered by means of infrared (Gagelmann, 1980). However, in the cave fish (P_{XIII}) all aggressive patterns are reduced to the same level of about 5% measured in the population of the Rio Teapao (P_T) (Parzefall, 1979). The data for the population hybrids of the cave fish (P_{XIII}) with the epigean populations (P_O, P_T) for the five aggressive patterns studied more or less show the same frequency

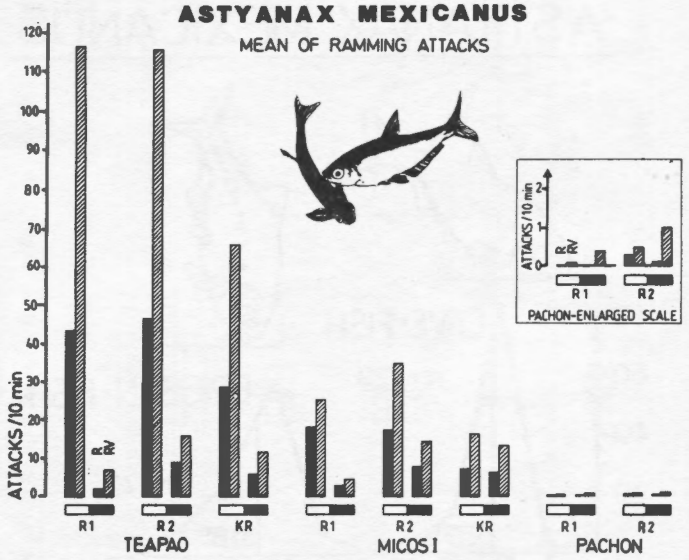

Figure 7: **Frequency of ramming attacks during a light/dark (LD) cycle in two cave populations and an epigean one of *Astyanax fasciatus* (= *mexicanus*). In the specimen (above) belonging to the epigean population the right one is ramming his opponent. R = ramming, RV = ramming attempt, R_1, R_2 = test groups with LD 3 : 3, KR = test group with LD 12 : 12 (from Burchards et al., 1985).**

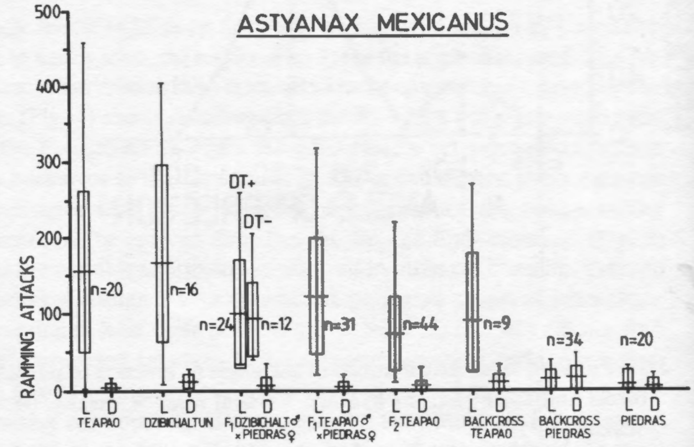

Figure 8: **Ramming and Ramming attempt in light (L) and darkness (D) of two epigean populations (Rio Teapao, Cenote Dzibichaltun), the Piedras cave population and their hybrids. Each fish tested against an opponent of the Teapao river in light (L) and darkness (D). DT = optomotoric response positive (+) or negative (−) (from Burchards et al., 1985).**

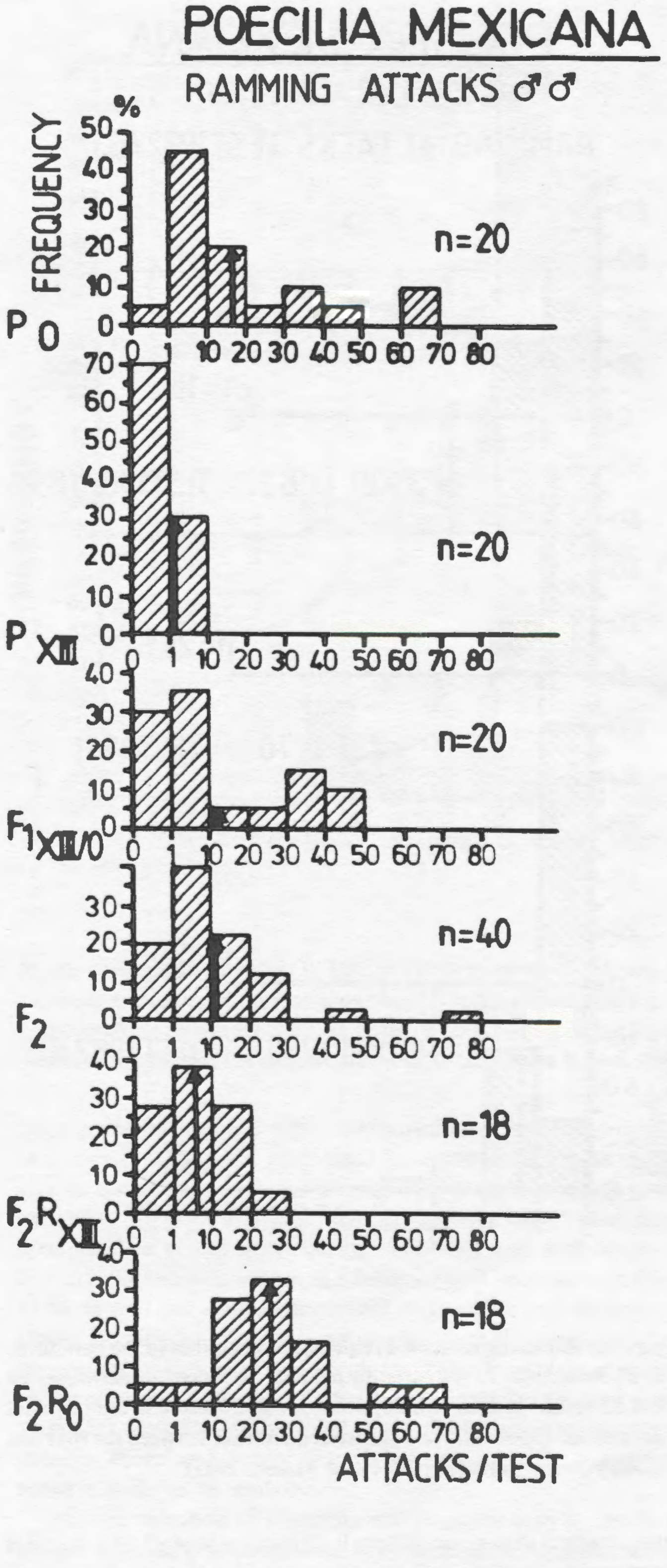

Figure 9: **Ramming attacks. Frequency distribution in the epigean population (P_O, near the cave entrance), the cave population (P_{XIII}) and hybrid generations in *Poecilia mexicana*. The mean is shown by the arrow (From Parzefall, 1979).**

distribution as demonstrated here for the ramming movement (Fig. 9, 10). These frequency distributions can be intrepreted in general by a genetically based reduction of the aggressive pattern controlled by an additive polygenic system. The fact that the degree of reduction for all the patterns studied shows the same percentage and that the correlation between the different patterns still exists in the F_2-generation despite free recombination favors the suggestion of one genetic system controlling the whole aggressive behavior in its totality. Because of the absence of a correlation between eye size and aggression level both traits seem to be reduced independent of each other. The reduction phenomenon observed has not changed for 12 years: the frequency distribution for ramming (Fig. 11) and the other patterns not reported here is identical for two samples taken at the same place in the cave in 1970 and 1982. However, in the fish collected in 1970 which could breed in our aquaria for about 10 years under light conditions the aggression level increased (Fischer, 1983).

The cave population of *P. mexicana* (P_{XIII}) has also been hybridized with the species *Poecilia velifera* which only exists in epigean populations. *P. velifera* shows the same aggressive patterns and the aggression level more or less corresponding to the one of the epigean population of *P. mexicana* (P_T). The analysis of the species hybrids demonstrates that the data fit the model of additive polygenic inheritance. Using the variances of the F_1- and F_2-generation we obtained an estimate of the minimum number of 5-6 factors for the ramming. In the population hybrids there still exists a positive correlation between different patterns in the F_2-generation. This again confirms the suggestion of *one* genetic system which controls the aggressive behavior in its totality (Parzefall, submitted).

2.4 SCHOOLING BEHAVIOR

The epigean population of *Astyanax fasciatus* normally schools in the different habitats. Under special conditions, e.g., in small separated pools, the fish can establish territories. The different cave populations studied neither schooled nor defended territories (Parzefall, 1983). The important role of the visual orientation for the schooling behavior has been demonstrated by Pitcher et al. (1976). A group of epigean *A. fasciatus* exposed to infrared light also changed from schooling to an uncoordinated swimming activity (Senkel, 1983). Therefore the question arises whether the cave fish has already lost this behavior in his dark habitat or only lacks functional eyes to start schooling. To solve this problem in the F_1- and F_2-generation between the Piedras cave fish and the Teapao river fish eyed individuals which showed a positive optomotoric response (minimum separabile 1°) have been tested for the tendency to follow a school of epigean conspecifics. There was a significant lower tendency for schooling in both hybrid generations (Fig. 12). These first results of studies not yet finished favor the explanation of a genetically based reduction of schooling in the cave habitat (Senkel, 1983).

Recently Romero (1984) described an epigean *Astyanax*-population which lives near the cave entrance and does not show schooling behavior. The studies of Jankowska and Thines (1982) demonstrated the absence of schooling behavior in three other cave fish species: *Caecobarbus geertsi, Barbopsis devecchii* and *Uegitglanis zammaronoi*.

There is also an influence of the alarm substance on the schooling behavior (Fricke, 1985): the alarmed fish form a school in higher density. Heczko and Seghers (1981) found the same results in the common shiner (*Notropis cornutus*).

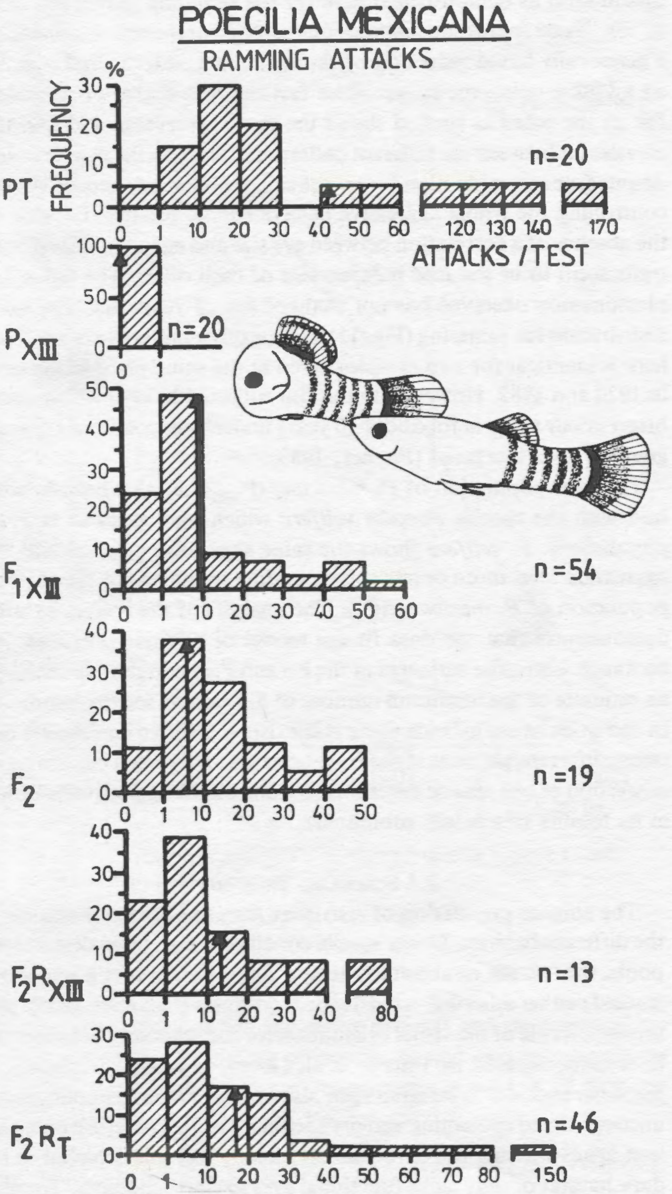

Figure 10: **Ramming attacks. Frequency distribution in hybrids with an epigean population of *P. mexicana* which lives in the Rio Teapao (P_T, about 60 km from the cave entrance). The mean is shown by the arrow (from Parzefall, in preparation).**

2.5 CIRCADIAN CLOCK IN CAVE ANIMALS

In various cave animals a reduction of circadian activity has been described. Among the invertebrates examples exist for cavernicolous beetles, crustacea and diplopods (Weber, 1980). The blind salamander *Proteus anguinus* from the karst region of Yugoslavia and Italy does not exhibit a circadian rhythmic in total darkness (Schatz et al., 1977). In *Astyanax fasciatus* the swimming activity of the Pachon cave fish has been compared with the Teapao river fish under light and dark conditions. The cave fish was only able to perform a free running circadian pattern in darkness after a light dark cycle of 12:12 h. In other experiments after various light dark cycles a circadian rhythm was absent (Erckens and Martin, 1982a, b). Hybrid studies still have to be done.

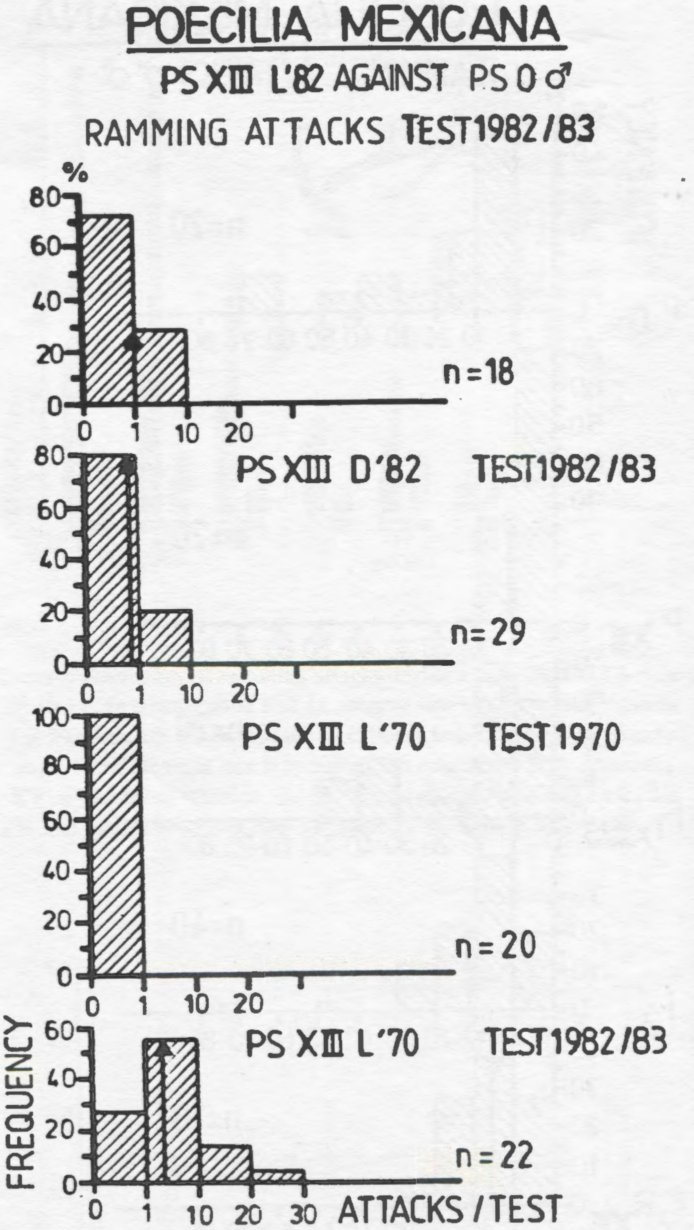

Figure 11: **Ramming attacks. Frequency distribution in the cave form of *P. mexicana*. L = reared in light, D = reared in darkness. 70 and 82 means year of fish importation. all ♂♂ tested in light against an epigean opponent. No significant difference between the four test groups (from Parzefall, 1974 and Fisher, 1983).**

3. DISCUSSION

The reduction of the behavior patterns presented can be explained with two different processes (Kosswig, 1963): the absence of the stabiliz-

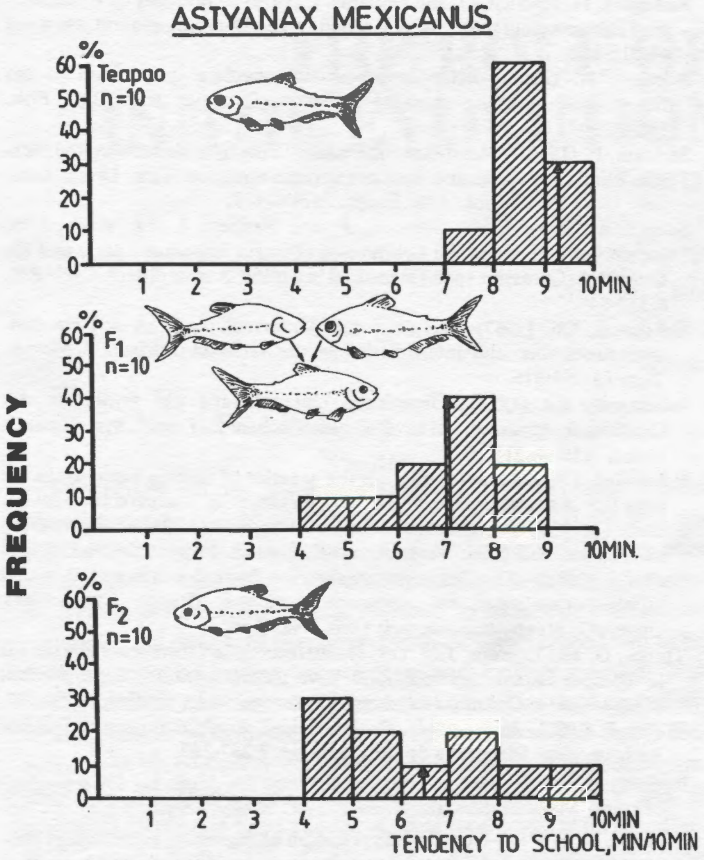

***Figure 12:* Tendency in Min/10 Min to follow a school of epigean *Astyanax fasciatus* (= *mexicanus*) in light. Only hybrids with a good optomotoric response have been tested. F_1 and F_2-generation significant different from the epigean parent ($P < 0,05$) (from Senkel, 1983).**

ing selection on one hand allows the reduction; or a selection pressure in the new habitat on the other hand forces the reduction. In the first case an accumulation of neutral mutations leads to a higher genetic variability expressed in a higher phenotypic variability. The mutation pressure then causes a decreasing variability and with increasing homozygoty the changement of a behavior trait becomes significant. In the second case such a transitional stage with a high variability is absent, because the selection pressure eliminates all phenotypes carrying negative mutations. The difficulty for behavioral traits in contrast to morphological structures is that a differentiation between a high variability caused by motivational or genetic changes is sometimes not possible. Therefore behavior which is independent of the motivational status is easier to be analyzed.

Only the reduction of schooling and circadian activity can be explained completely by the absence of stabilizing selection. For schooling there is a high variability in the F_1- and F_2-generation. This trait is relatively independent of the motivation. So the variability seems to be genetically based. Both behavior patterns need light circadian rhythm as pacemaker and schooling for group-cohesion.

All other traits presented seem to be changed by selection. The great reduction of aggressive behavior in comparison to the eye which is only slightly reduced, favors the explanation of a selection pressure against aggression in *Poecilia mexicana*.

The aggressive males seem to have no more reproductive advantage because of the risk of losing contact with the female in darkness during an aggressive encounter against an opponent. This risk is increased by the fact that only tail-beating, which requires a short distance to the opponent, is effective in darkness. The most successful strategy in darkness should be a quick copulation after a short nipping-contact. Such a behavior already exists among small males in epigean habitats. The absence of bigger males in the cave population supports this hypothesis (Parzefall, 1979).

For *Astyanax fasciatus* more data from different cave populations are necessary in order to understand the reduction of the aggressive behavior. In epigean fish, ramming an opponent occurs even in darkness and increases in hungry animals studied in light (Burchard et al., 1985). So *A. fasciatus* should be able to defend food sources in the cave. But in the Pachon cave the fish fed together without any aggression, and in Chica cave there was no reaction to food offered (Parzefall, 1983). Up to now we can only speculate that the low density of *A. fasciatus* in caves, in connection with enough food in the form of bat guano, does not require aggressive behavior in this habitat.

The changes of headstanding behavior in *A. mexicanus* are surely adaptive. The evolutionary improvement of the gustatory equipment presupposes changes in the head-standing movement (Schemmel, 1980). If the food is mainly distributed on the bottom of the habitat, it seems to be a useful tactic to search at an angle of about 45° in slow zigzag movements (Parzefall, 1983).

These behavioral differences between cave-dwellers and their epigean relatives allow the use of these animals for studies on evolutionary genetics in behavior because of fertile cross-breeding. On the basis of the present data it is very likely in cave animals that, with some important exceptions, such as aggressive behavior, selection may play a minor role compared with the accumulation of neutral mutations. For these reasons field and laboratory studies on cave-dwelling fish seem to hold great promise.

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THE ROLE OF METABOLISM IN THE EVOLUTION OF CAVE ANIMALS

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SUMMARY

This review on the metabolic rates of cave animals includes measurements published by Poulson (1964) and Vandel (1964) and new studies from the last twenty years. All these investigations have been restricted to crustaceans, arthropods, teleosts and urodeles. With a few exceptions, cave animals are characterized by a more or less reduced metabolic rate compared to epigean relatives. Data gained from direct measurements of oxygen consumption and indirect methods (e.g. relative activity and starvation experiments) are discussed separately. In many cave ecosystems the two main characteristics most closely connected with the evolution of a low metabolic rate are: The relative constancy of the environmental conditions combined with a lack of predators on the one hand and the general food scarcity on the other. A schematic figure (Fig. 5) gives an idea on causes and consequences of cave animal characteristics and their interrelations. The discussion whether the deep sea is a parallel to the cave environment is taken up again. Some recent investigations confirm that deep sea animals have a reduced metabolic rate too, caused by conditions similar to those in caves.

INTRODUCTION

Caves present very peculiar but simple and relatively unifom ecosystems and are characterized by two main facts. First, the deep cave environment is relatively stable with respect to abiotic factors such as darkness, high moisture and more or less constant temperature and water chemistry, as well as in regard to biotic factors such as lack of predators. Secondly, subterranean communities are considerably food-limited, with the exception of bat-caves, where bat guano can present an immense source of food for guanobionts. The quantity and even the quality of the food offer in a cave are dependent on the character of the cave. It is of importance whether the cave is dry and nearly isolated from the surface or whether it is strongly influenced by the epigeum, e.g. floodings. Furthermore, the geographical position of a cave can have important influence on the available food (Mitchell, 1969). These two extreme characteristics of reduced food and predators are discussed as the most important reasons for regressive as well as for constructive evolutionary processes in cave animals.

The "organicist" evolutionary theory of Vandel (1964), still supported by a very few biologists, that cave animals are merely survivors of phylogenetically senile species, who found a refuge in the food poor but stable cave environment, can no longer be maintained. There is sufficient evidence that evolution of cave animals really takes place. But, to this day the phenomenon of cave animal evolution, especially regressive evolution, is not completely understood and the discussion continues. It is generally held that a low food supply is the main limiting factor in caves and that adaptation to a decreased food supply has a high selective value in regard to adaptive features, e.g. a reduced metabolic rate. In contrast, the question is still open whether, and to what extent, energy economy represents a selective pressure for the reduction of organs, functions or behaviors, rendered useless as a consequence of the darkness (see Culver, 1982 for a survey). If the reduction of features such as eye size and pigmentation is at least partly caused by food limitation, it would represent an adaptation to the cave environment in a constructive sense as well.

The relative extent of regressive evolution, as far as it is not interpreted as adaptive in energy economy but rather as the consequence of the loss of function, reflects the relative phylogenetic age of a cave form (Wilkens, 1973). At least in taxonomically closely related genera or within one genus or species a comparable rate of reduction as a consequence of mutation pressure can be expected.

In contrast, all adaptive features, including possibly 'regressive-adaptive' ones, evolve at different rates depending on the intensity of selection. Dickson and Franz (1980) showed that differences in metabolic rates of cave crayfish were correlated with food availability: *Procambarus pallidus* from a low-energy cave had lower gill respiration rates and longer ATP-turnover times than *P. franzi* from a high-energy cave. In contrast, Poulson (1963 and 1964) attributed differences in metabolic rates in cave animals to differences in time in caves, but that selection played a major role. Mitchell (1969) agreed, but made one important assumption: 'The degree of cave adaptation . . . as an index to length of time of cave habitation' is useful only 'as long as it is restricted to a group of related species occupying a more or less specific geographic area, an area where the selection pressures leading to cave adaptation could be expected to be similar qualitatively and quantitatively.' He specified that in caves with a relatively high energy input such as tropical ones, where the biomass in the tropical epigeum is great and its production is uninterrupted, the selection pressure can be expected to be not as strong, the evolutionary rate to be not as fast and the appearance of troglobites to be slower than in caves with relatively low energy input such as temperate ones. This holds for the reduction of the metabolic rate too: Since it is a constructive feature and is affected by selection, the intensity of selection may be responsible for the stage of reduction, or the time during which this selection pressure continues may be responsible for the stage of reduction.

RECORDS ON METABOLIC ECONOMY

First examinations of respiration rates of troglobites were made with amphipods by Gal (1903) and Jeannel (1929) and with amblyopsid fish

Table 1:

Species compared (most cave adapted first)			meth.	T (°C)	met.r.1)	author
Amphipoda						
Stygobromus emarginatus (Hubricht, 1943)	:	*Stygobromus tenuis potamacus* Holsinger	1(3)	13	–2)	Culver & Poulson, 1971
Stygobromus spinatus (Holsinger, 1967)	:	"	1(3)	"	—	"
Gammarus minus (cave) Say, 1818	:	*Gammarus minus* (spring)	1	"	—	"
"	:	"	3	"	—	"
Niphargus longicaudatus Costa, 1851	:	*Gammarus spec.*	1	÷3)	much4)	Jeannel, 1929
Niphargus orcinus Joseph, 1869	:	*Gammarus pulex* Linnaeus, 1758	1	÷	1:10	Dérouet, 1952
Niphargus rhenorhodanensis Schellenberg, 1938	:	*Gammarus pulex*	1	12:10	3:4	Troiani, 1954
"	:	"	1	"	3:4	Wautier & Troiani, 1960
" (karstic)	:	*Niphargus rhenorhodanensis* (interstitial)	1	11	—	Mathieu, 1973
"	:	"	1(3)	"	—	Mathieu, 1980
"	:	"	1	"	—	Mathieu, 1981, 1982b, 1983a
"	:	"	1(3)	11,15,19,23	÷	Mathieu, 1982a
"	:	"	3	11	÷	Mathieu, 1983b
Niphargus rhenorhodanensis (interstitial)	:	*other crustaceans*	4	÷		Mathieu & Gilbert, 1980 Mathieu & Taveau, 1984
Niphargus virei Chevreux, 1869	:	*Niphargus rhenorhodanensis* (interstitial)	4	÷	÷	Gibert & Mathieu, 1980
"	:	*Gammarus pulex*	1	15-17 10-11	1:4	Dérouet, 1949
"	:	"	1	10	1:10	Dérouet, 1953b
"	:	"	1	"	1:6-7	Dresco-Dérouet, 1959
"	:	"	1	12:10	1:4	Troiani, 1954
"	:	"	1	"	1:4	Wautier & Troiani, 1960
"	:	*Echinogammarus marinus* (Leach, 1815)	1	10	1:10	Dérouet, 1953b
Niphargus spec.	:	*man*	1	÷	1:8-9	Gal, 1903
Isopoda						
Caecosphaeroma burgundum Dollfus, 1898	:	*Sphaeroma serratum* (Fabricius, 1787)	1	10	1:10	Dérouet, 1953b
"	:	"	1	"	1:6-7	Dresco-Dérouet, 1959
Caecosphaeroma virei Dollfus, 1869	:	*Gammarus pulex*	1	"	1:30	Dérouet, 1952
Decapoda						
Cambarus cryptodytes Hobbs, 1942	:	*Procambarus kilbyi* (Hobbs, 1940)	1	12,22 32	1:3	Caine, 1978
"	:	*Procambarus leonensis* Hobbs, 1942	1	"	1:3	"
"	:	*Procambarus paeninsulanus* Faxon, 1914	1	"	1:2-3	"
"	:	*Procambarus spiculifer* LeConte, 1856	1	"	1:2	"
Cambarus setosus Faxon, 1889	:	*Cambarus rusticus* (Girard, 1852)	1	16	1:3	Burbanck, et al., 1948
Orconectes inermis Cope, 1871	:	*Cambarus bartonii* (stream) Fabricius, 1789 (=*C.laevis*, Faxon, 1914)	÷	÷	much	Weingartner, 1977

Species compared (most cave adapted first)			meth.	T (°C)	met.r.[1]	author
”	:	” (cave)	÷	÷	much	”
Orconectes pellucidus Tellkampf, 1844	:	”	1	÷		Eberly, 1960
”	:	”	1	15-17	1:3	Jegla, 1964
Procambarus erythrops Relyea & Sutton, 1975	:	*Procambarus pictus* (Hobbs, 1940)	1	÷	much	Franz, 1978
”	:	”	5	÷		”
Procambarus franzi Hobbs & Lee, 1976	:	”	1	18:16	1:2	Dickson & Franz, 1980
”	:	”	8	”	1:2	”
”	:	*Procambarus clarkii* (Girard, 1852)	1	18:19	1:2	”
”	:	”	8	”	1:2	”
Procambarus horsti Hobbs & Means, 1972	:	*Procambarus kilbyi*	1	12,22 32	1:4	Caine, 1978
”	:	*Procambarus leonensis*	1	”	1:4	”
”	:	*Procambarus paeninsulanus*	1	”	1:3	”
”	:	*Procambarus spiculifer*	1	”	1:2-3	”
Procambarus lucifugus Hobbs, 1940	:	*Procambarus pictus*	1	÷	much	Franz, 1978
”	:	”	5	÷		”
Procambarus orcinus Hobbs & Means, 1972	:	*Procambarus kilbyi*	1	12,22 32	5:6	Caine, 1978
”	:	*Procambarus leonensis*	1	”	5:6	”
”	:	*Procambarus paeninsulanus*	1	”	—	”
”	:	*Procambarus spiculifer*	1	”	3:2	”
Procambarus pallidus Hobbs, 1943	:	*Procambarus pictus*	1	19:16	1:7-8	Dickson & Franz, 1980
”	:	”	8	”	1:7-8	”
”	:	*Procambarus clarkii*	1	19	1:6	”
”	:	”	8	”	1:6	”
Opiliones						
Ischyropsalis luteipes Simon, 1872	:	*Opiliones* in general	1	÷		Dresco-Dérouet, 1967
Araneae						
Anthrobia mammouthia Tellkampf, 1844	:	*Phanetta subterranea* (Emerton, 1875)	4	÷		Poulson, 1981
Meta bourneti Simon, 1922	:	*Araneus diadematus* Clerck, 1757	1	12-16: 13-14	1:7	Dresco-Dérouet, 1960
”	:	*Nephila senegalensis* (Walckenaer, 1841)	1	12-16: 19-22	1:5	”
Meta menardi (Latreille, 1804)	:	*Araneus diadematus*	1	9-14: 13-14	1:7	”
”	:	*Nephila senegalensis*	1	9-14:19-22	1:5	”
”	:	*Tegenaria saeva* Blackwell, 1844	1	10,24	÷	Dérouet, 1953a
Tegenaria saeva	:	*Araneus diadematus*	1	10,13,25:13-14	1:6	Dresco-Dérouet, 1960
”	:	*Nephila senegalensis*	1	10,13,25:19-22	1:4-5	”
Lycosa howarthii Gertsch, 1973	:	*Lycosa spec.*	1	10,24: 19	2:3	Hadley et al., 1981
Saltatoria						
Caconemobius varius	:	*Caconemobius fori*	1	19:19	—	Ahearn & Howarth, 1982

Species compared (most cave adapted first)			meth.	T (°C)	met.r.	author
Ostariophysi						
'Anoptichthys jordani' Hubbs & Innes, 1936 (=*A.fasciatus* from Chica cave)	:	*Astyanax fasciatus* (Cuvier, 1819)	1	22-30	1:2	Schlagel & Breder, 1947
”	:	”	1	25	slightly	Hüppop, 1986
'Micos-fish' (=*A.fasciatus* from Micos cave)	:	”	1	”	slightly	”
'Anoptichthys antrobius' Alvarez, 1946 (=*A. fasciatus* from Pachon cave)	:	”	1	25	3:4	”
”	:	”	4	”		”
Caecobarbus geertsi Boulenger, 1921	:	*Barbus conchonius* (Hamilton-Buchanan, 1822)	1	23	1:3	Koch, unpubl. data (cited in Poulson, 1964)
”	:	*Barbus holotaenia* Boulenger, 1904	4	÷		Heuts, 1951
”	:	”	5	÷	÷	”
Microcyprini						
Amblyopsis rosae (Eigenmann, 1899)	:	*Chologaster cornutus* Agassiz, 1853	1 (4,5)	13	1:4	Poulson, 1963
Amblyopsis spelaeus DeKay, 1842	:	*Chologaster papilliferus* Forbes, 1882	2	÷	1:4	Eigenmann, 1909
”	:	*Chologaster cornutus*	1(4,5)	13	1:2	Poulson, 1963
Chologaster agassizii Putnam, 1872	:	”	1 (4,5)	”	3:4	”
Typhlichthys subterraneus Girard, 1859	:	”	1 (4,5)	”	2:5	”
Lucifuga dentatus Poey, 1858	:	*Pisces* in general	5	÷		Eigenmann, 1909
Lucifuga subterraneus Poey, 1858	:	”	5	÷		”
Urodela						
Proteus anguinus Laurenti, 1768	:	*Triturus cristatus* (Laurenti, 1768)	7	25	—	Korzhuev, 1950
Typhlomolge rathbuni Stejneger, 1896	:	*Eurycea nana* Bishop, 1941	6	÷		Poulson, unpubl. data (cited in Poulson, 1964)

1) metabolic ratio
2) no difference
3) no statements regarding met.r.
4) met.r. of the left species is lower than that of the right one

***Table 1.* Metabolic ratios between cave animals and less cave adapted or epigean relatives. References arranged in taxonomic order after Barnard & Barnard (1983): amphipods, Hobbs et al. (1977): decapods, Sterba (1970): fish and Gorham (1974): urodeles. Methods (meth.) 1 to 8 are explained in the text.**

by Eigenmann (1909). Their suggestion that cave animals have a low metabolic rate in comparison with their epigean relatives was taken up by many biologists working with a variety of phyla: Crustacea, Arthropoda, Pisces and Amphibia were investigated. Fage (1931), Jeannel (1943), Heuts (1951 and 1953), Vandel (1950 and 1964), Poulson (1964), Barr (1968), Ginet & Décou (1977) and Culver (1982) theoretically analyzed this characteristic adaptation of animals to the cave environment, and in almost all papers on experiments ideas, suggestions and explanations were given. Table 1 summarizes measurements of metabolic rates of cave animals (direct as well as indirect ones) in taxonomic order. Earlier studies were summarized in the general papers of Poulson (1964) and Vandel (1964), which include reviews on metabolic rates of cave animals.

The most meaningful information on metabolic rates is given by the measurement of the oxygen consumption of the whole body or even of parts of it (1 in table 1). Many conclusions were made by using indirect methods such as respiratory frequency (2), relative activity (3), ability to survive starvation periods, body composition (4), growth rate (5), relative gill area (6), oxygen consumption of erythrocyts (7) and ATP turnover times in excised gills (8).

In most comparisons the metabolic rate of the hypogean species was found to be more or less lower than that of their epigean relatives, usually interpreted as an adaptation to food scarcity. Only in a few cases indifferent results or even higher metabolic rates in the cave forms were found. Sometimes they could be refuted, as in Jegla's (1964) critique of Eberly's (1960) studies on *Orconectes pellucidus*. Sometimes the reason was food abundance in the caves where the animals live (Schlagel and Breder, 1947; Culver and Poulson, 1971). Unfortunately, many studies have to be viewed with caution, since they are based on comparisons of less related species, often different genera or even families.

MEASUREMENTS OF OXYGEN CONSUMPTION

Gal (1903) was the first to measure the oxygen consumption of the hypogean amphipod *Niphargus*. He stated a respiratory activity in *Niphargus* of 8 to 9 times lower than that of a man by comparing the relation of consumed oxygen mass to body mass: While *Niphargus* consumed 1/10 oxygen of its body mass in 66 days one man consumed 5/6 oxygen of his body mass in the same time. Jeannel (1929) compared the survival times of the hypogean *Niphargus longicaudatus* and an epigean *Gammarus* species in a closed bottle. *N. longicaudatus* survived 48 hours while *Gammarus* died after a few hours.

These first experiments on respiration rates of cave animals were taken up by Dérouet (Dresco-Dérouet) (1949, 1952, 1953b and 1959), using more precise methods. She compared the hypogean *Niphargus virei* with the epigean *Gammarus pulex* and found an approximately ten times lower metabolic rate in *N. virei*. Troiani (1954) and Wautier and Troiani (1960) found a metabolic rate in *Niphargus rhenorhodanensis* of three quarter, and in *N. virei* of only one quarter of that of *G. pulex*. Recently Mathieu (1973, 1980, 1981, 1982a, 1982b and 1983a) found a higher oxygen consumption rate in cave and interstitial populations of *N. rhenorhodanensis* than Dérouet (Dresco-Dérouet), Troiani, and Wautier and Troiani found for this species and *N. virei*. The differences are probably due to differences in measurement technique, and that only Mathieu measured activity as well.

Caine (1978) used a 'robustness index' (body mass/carapace length) to support his results gained from oxygen consumption measurements of epigean and hypogean crayfish. Oxygen consumption and robustness index both were lower in the hypogean species than in the epigean forms, except *Procambarus orcinus*. From the data it seemed that the cavernicolous *P. orcinus* had a higher metabolic rate than the epigean *P. spiculifer* and *P. paeninsulanus*. But due to size variations, individual oxygen uptake was lower in *P.orcinus* than in all surface species (Caine, 1978). In the case of *P.horsti*, the crayfish with the lowest metabolic rate in the comparison, he interpreted the low metabolic rate not only as an adaptation to low food availability but also as an adaptation to low oxygen tension, found in the spelean water. Caine's trial to determine the critical oxygen level for the seven compared species was successful only in the epigean forms, where it was by 13 to 30 percent oxygen saturation. Hypogean species had no sharp break in the oxygen uptake lines, meaning no critical oxygen level due to variation of the partial pressure of oxygen.

Jeannel (1929), Burbanck et al. (1948) and Dresco-Dérouet (1959) used a more brutal method to detect oxygen consumption and critical oxygen tension of decapods and amphipods: The animals were enclosed in a bottle and the time until they died was measured. In the latter two cases the cave species could not deplete the oxygen in the water to a lower content than their epigean relatives, but survival times differed significantly between cave and surface species. The cave limited crayfish *Cambarus setosus* survived 3.5 times longer than the surface dwelling *C.rusticus* on the same amount of oxygen (Burbanck et al., 1948). It used the available oxygen amount more slowly, suggesting a lower metabolic rate. Unfortunately, no measurements of activity were made, so that it is not known to which dimension the low oxygen consumption rate really can be led back to low energy demand in general or to a reduced activity.

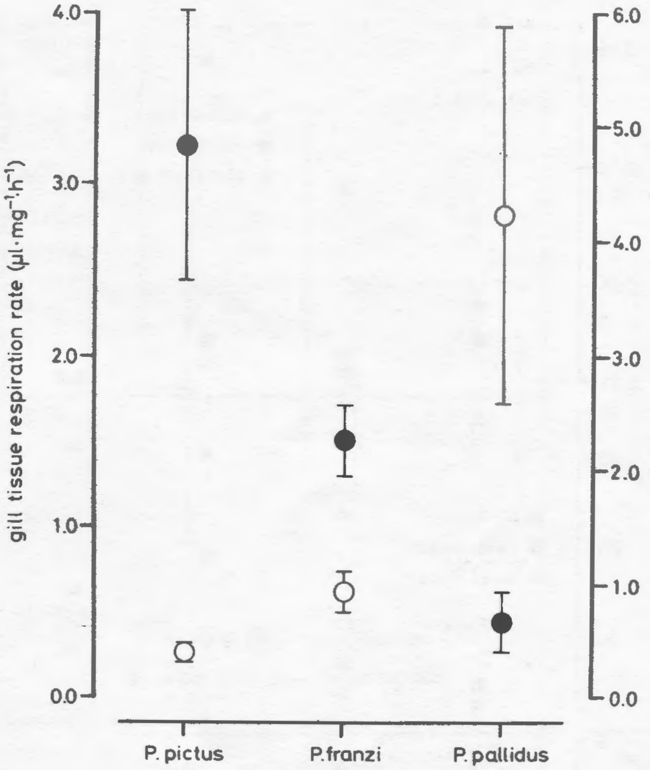

***Figure 1.* Mean gill tissue oxygen consumption rates (±1 s.d.) (closed circles) and ATP turnover times (±1 s.d.) (open circles) of three *Procambarus* species from habitats with different energy contents (after Dickson & Franz, 1980).**

Cambarus bartoni (= *C.laevis*), occurring in cave and surface waters and ecologically classified as a troglophile, was compared with the troglobitic *Orconectes pellucidus* (Eberly, 1960; Jegla 1964) and the troglobitic *O.inermis* (Weingartner, 1977). Although *C.bartoni* had a much higher metabolic rate than both the troglobitic species, it seemed to be able to coexist with them in the cave, competing for highly restricted food sources.

Another very important investigation on metabolic rates of cave animals is that of Dickson and Franz (1980) on *Procambarus* species. Excised gills of surface *Procambarus pictus* and *P.clarkii* and of hypogean *P.franzi* and *P.pallidus* were used. This method provided several advantages, including the lack of variability associated with animal movement, an in vitro analysis of tissue, which under normal conditions was exposed to the external environment, and the potential to estimate energy turnover in a special tissue (Dickson and Franz, 1980). They determined a lower gill tissue oxygen consumption rate in the cave forms than in the surface species, and significant differences between the two troglobitic (*P.pallidus* < *P.franzi*) and between the two surface forms (*P.clarkii* < *P.pictus*)(Figure 1). This association of tissue respiration rates with habitat energy content (*P.franzi* = high energy cave; *P.pallidus* = low energy cave) indicated adaptations in standard metabolic rates in troglobitic crayfish (Dickson and Franz, 1980).

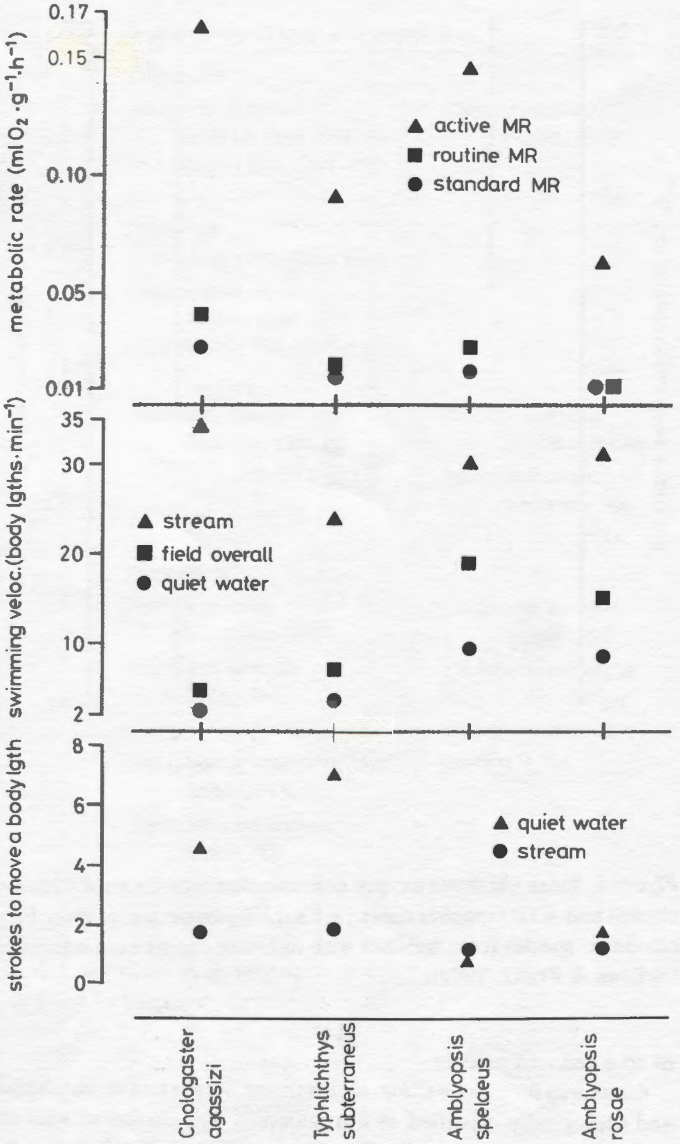

Figure 2. **Metabolic rates, swimming velocity and swimming efficiency of four amblyopsid fish. Increasing cave adaptation from the left to the right (after Poulson, 1963).**

There are only a few investigations on metabolic rates of terrestrial cave animals. Ahearn and Howarth (1982) found no difference in the metabolic rate between epigean and hypogean crickets. However, they related the loss of a circadian rhythm in the metabolism of the cave form to differences in water availability. Derouet (1953a), Dresco-Derouet (1960, 1967) and Hadley et al. (1981) showed a lower metabolic rate in hypogean opilions and spiders compared to epigean ones, but all available data on metabolic rates of hypogean arachnids fall within the range of metabolic rates reported for epigean arachnids. Since these are typically lower than those of other poikilotherms of comparable size, arachnids seem to be preadapted for energy economy, developed by the potential problem of being faced even in the epigean environment with an inconstant food supply as a consequence of an usually predatory life. Food scarcity is stronger in the terrestrial than in the aquatic cave environment. In the case of cave salamander Culver (1982) stated the very likely hypothesis that this might be the reason why neoteny in caves occurs. Nevertheless, the metabolic rate in the terrestrial arachnids is not reduced as much as it could be expected.

Fish represent the greatest number of cave dwellers among the vertebrates. Unfortunately, one of the first investigations of oxygen consumption in cave fish, that of H.J. Koch, has never been published and is only cited in Heuts (1951). Koch found a metabolic rate in the African cave fish *Caecobarbus geertsi* of only one third of that an epigean relative, *Barbus conchonius*.

Poulson (1963) presented a detailed study on cave adaptive features, including measurements on standard, routine and active metabolic rate, on a series of amblyopsid fish. He based these studies on very promising first observations of Eigenmann (1909) on respiratory frequencies in the cave fish *Amblyopsis spelaeus* in comparison to an epigean *Chologaster* species. Poulson was able to show a decreasing tendency in metabolic rate from the epigean *Chologaster cornutus* over the troglophilic *Chologaster agassizii*, the troglobitic *Typhlichthys subterraneus* and *Amblyopsis spelaeus* to the most cave adapted species *Amblyopsis rosae* (Figure 2). In general the amblyopsid fish seemed to be extraordinarily preadapted to live in caves, since even the epigean *C.cornutus* had a standard metabolic rate of 0.9 times of that of the goldfish, *Carassius auratus*, which has the lowest known metabolic rate of non-amblyopsid epigean teleosts (Poulson, 1963). Poulson gave a classical example for the adaptive tendency in cave animal evolution in many revealing observations, e.g. better swimming efficiency of the cave species, increasing hypertrophy of sensory organs with increasing cave adaptation and changes of life history traits.

A more suitable example for the evolutionary trends of metabolism in cave animals is represented by some interfertile (Sadoglu, 1956) Mexican cave populations of different phylogenetical ages of the characid *Astyanax fasciatus* in comparison with epigean populations, ancestors of the cave populations still alive. In my own experiments (Hüppop, 1986) the study of Schlagel and Breder (1947) on a hybrid population from the Chica cave in Mexico was taken up and expanded on two other non-hybrid cave populations. Schlagel and Breder stated a routine metabolic rate in the 'Chica' fish of nearly twice that of the epigean ones. Without doubt, the reason for this is that the Chica cave has a high organic input, caused by floodings and bat-guano (Breder, 1942; Mitchell et al., 1977) and that the fish are not food limited. Furthermore, their hybrid character makes interpretations on their metabolic rate difficult. My studies, in contrast, showed a lower oxygen consumption rate for the 'Chica' fish (Figure 3), but the difference is not significant. The two other groups, the phylogenetically young 'Micos' fish and the phylogenetically old 'Pachon' fish from food limited caves clearly show the tendency towards reduced metabolic rate in agreement with their phylogenetical age (Figure 3).

INDIRECT METHODS

Statements on the metabolic rate of troglobites can also be obtained from other physiological or biochemical measurements and observations on behavior. A relatively valid factor is the activity of the whole body as well as of parts of it, for example the respiratory organs. Lower respiratory movements in *Amblyopsis spelaeus* than in a *Chologaster* species observed by Eigenmann (1909) suggested a lower oxygen uptake per time and consequently a lower metabolic rate. These results were confirmed by Poulson (1963).

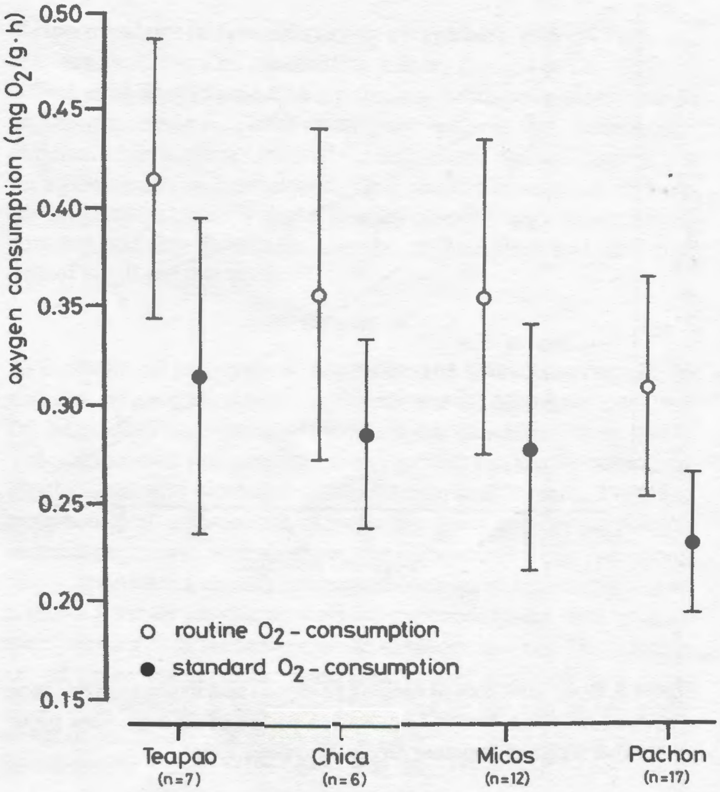

Figure 3. **Mean standard oxygen consumption rates (closed circles) and mean routine oxygen consumption rates (open circles) (±1 s.d.) of one epigean population (Teapao) and three cave populations (Chica, Micos, Pachon) of *Astyanax fasciatus* (from Hüppop, 1986).**

A consequent consideration of activity in metabolic measurements of the whole body would lead to standard metabolic rates, as a basis for the comparison of the locomotion-activity-free stage of cave adaptation. But the degree to which standard metabolism is approached experimentally is difficult to define. Therefore, even differences in so-called standard metabolic rates between cave and epigean forms might actually be less than the data indicate, since troglobites are less active than their epigean relatives, and the consideration or even the exclusion of the activity of troglobites during the measurements might be easier (see also Poulson, 1964).

A very elegant method to exclude activity and to come as close as possible to the standard metabolic rate is that of Dickson and Franz (1980), who used excised gills of crayfish. The already mentioned lower gill tissue respiration rate of crayfish from low-energy caves, indicating a lower standard metabolism, than of crayfish from high-energy caves and from the surface, could be supported by biochemical analyses. ATP turnover times of the gill tissues showed a reverse proportionality to the tendency shown above (Figure 1): This could be the result of adaptations which allow the maintainance of low cellular energy costs (Dickson and Franz, 1980).

For comparative ecological statements the routine metabolic rate, integrating the effects of activity and excitability, is more valid than the standard metabolic rate, since it is the most appropriate index of actual energy expenditure in nature (see also Poulson, 1964). Consequently, the necessity of a separation of activity and metabolic measurements depends on how the question is stated.

Often cave animals have a very changed motion pattern in comparison with epigean relatives, making comparison of total activity difficult. Especially troglobitic fish, and partly even epigean ones in darkness or blinded, show a constant wandering activity. Schlagel and Breder (1947) pointed out that epigean eyed *Astyanax fasciatus* assumed the wandering behavior of the blind animals from the caves when in darkness, correlated with a higher oxygen consumption rate in darkness than in the light. Particularly, Poulson (1963) stated that the highest specialized amblyopsid troglobites were most active, but indicated furthermore that the higher swimming velocities of the cave species were not very important in elevating the routine metabolic rate. Also, in the cave adaptated forms less strokes were necessary to move a body length (Figure 2). The usually increased refractorness to disturbance with increased cave adaptation allowed a gain in activity without loss of the advantage of energy conservation, important in the food poor environment. It enabled the more cave adapted forms to cover more territory in searching for food, resulting in a higher feeding efficiency (Poulson, 1963). Culver and Poulson (1971) showed that individuals of *Stygobromus emarginatus*, inhabiting more open water, had a higher activity than those of *Stygobromus spinatus*, living deeper in the gravels of the stream bed, and Mathieu (1980 and 1983b) found interstitial *Niphargus rhenorhodanensis* less active than the cave adapted ones. All explained their results with the different flow-rates in the compared habitats. It might be possible to derive from the results, that a higher routine metabolic rate exists in the cave animals than in the interstitial ones and that a greater food scarcity in many interstitial habitats might be the reason for this, as Culver (1982) did on the basis of the data of Mathieu (1980). From these examples it is obvious that interpretations of activity measurements alone in cave animals in regard to metabolic economy might be difficult, such as interpretations of solid measurements of oxygen consumptions. Consequently, it is desirable that all studies of metabolism are accompanied by measurements of activity. Unfortunately, many studies suffer from this defect.

Relative conclusions on standard metabolic rates are possible by survival rates or body composition changes during starvation experiments (see also Culver, 1985) and by growth rates. Poulson (1963) elaborated a direct relation of survival rate under starvation conditions to standard metabolic rate in amblyopsid fish increasing with increasing cave adaptation. Greater fat deposits and increased mass per unit length were consistent with lower growth rate, selected by low food supply. Lipids seem to play the most important role as reserves in cave amphipods too (Gilbert and Mathieu, 1980): lipid content was higher in cave *Niphargus virei* than in interstitial *Niphargus rhenorhodanensis*, and during starvation cave *N. virei* depleted lipid reserves to a greater extent from initial content (63%) than carbohydrates (51%) and proteins (35%), whereas interstitial *N. rhenorhodanensis* depleted carbohydrate reserves nearly totally (94%) followed by proteins (53%) and lipids (42%). Since the energy content of lipids is twice as large as that of carbohydrates and proteins, it is concluded that the use of lipids as a reserve is a good adaptation to the subterranean environment (Gibert and Mathieu, 1980; Culver, 1982; 1985). Finally, Mathieu and Gibert (1980), completed from Mathieu and Taveau (1984), established a much slower decrease of lipid, carbohydrate and protein in

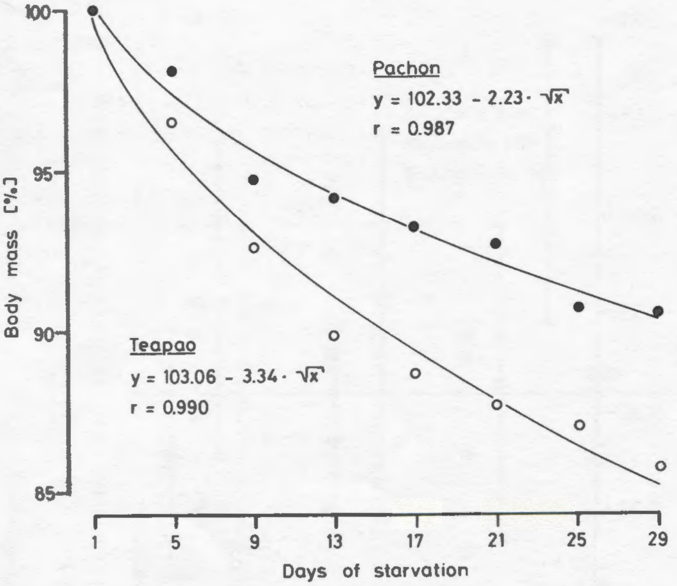

***Figure 4.* Body mass loss of epigean (Teapao) and hypogean (Pachon) *Astyanax fasciatus* during a starvation period of 29 days. One point = six fish weighed together (from Hüppop, 1986).**

N.rhenorhodanensis than in other crustaceans, indicating that even interstitial forms are relatively better adapted to food scarcity than epigean ones.

From differences in body mass loss during food scarcity Poulson (1981) derived a lower metabolic rate in the spider *Anthrobia monmouthia* than in *Phanetta subterranea* and pointed out that this was correlated with the different evolutionary time these two troglobitic species have been isolated in cave, with other life history traits, and with their different habitats.

In my own experiments (Hüppop, 1986) specimens of a hypogean population of the Mexican characid *Astyanax fasciatus* from the Pachon cave showed a significantly lower body mass loss during 29 days of starvation (9.5%) than epigean ones (16.3%) (Figure 4). This agreed well with the relatively lower metabolic rate of the cave fish. Furthermore epigean as well as hypogean *A.fasciatus* were able to reduce their metabolic rate during starvation by nearly the same percentage. The metabolic rate of the surface fish and the cave fish decreased by 32.5% and 34.8% (standard oxygen consumption) and by 27.5% and 28.2% (routine oxygen consumption) respectively. Consequently, the energy demand of the cave fish during food shortage is still lower than that of the epigean ones. In case of competition the cave fish will survive longer, or, at least, will cope better with the food scarcity. Such a situation was described in Wilkens and Hüppop (1986) for a coexistance of epigean and hypogean *A.fasciatus* in a food poor Mexican cave (Micos cave). These differences even between only ecologically distinct populations of one species are a hint for the high value that adaptation has towards energy economy in food scarce environments.

Heuts (1951) confirmed the established low metabolic rate in the hypogean fish *Caecobarbus geertsi* (Koch, unpubl.observ.) by his own observations on starvation and growth rate. *C.geertsi* were not starving but apparently well off. Fat deposits were even present in some individuals, despite the severe and limiting factor of food restriction in the African caves. Heuts, therefore, supported the hypothesis that in conditions of food limitation natural selection favors genotypes determining low growth rates, connected with low basal metabolism and therefore with low food requirements. Also observations from Eigenmann (1909) on the biology of the Cuban cave fish *Lucifuga dentatus* and *Lucifuga subterraneus* were screened and interpreted by Heuts (1951) showing a considerable retardation of growth.

Vandel and Bouillon (1959) cited Kammerer (1912) and GadeauDeKervill (1926) who found an extraordinary starvation ability in the cave urodele *Proteus anguinus* of three respectively eight years. Although these results might be exaggerated (Vandel and Bouillon, 1959), they made the tendency plain. Recent studies on sensory adaptation of *Proteus* for food searching in the food scarce cave environment (Durand et al., 1982) made a metabolic adaptation in these animals also likely. Unfortunately, Korzhuev (1950) could not detect a difference in oxygen consumption rates of the erythrocyts of *Proteus* in comparison with epigean forms as *Triton, Amblystoma* and *Axolotl*. But he found that the blood cells in *Proteus* were larger, suggesting a lower oxygen consumption per weight unit of the cells. Comparative investigations of energy demand of the whole body are urgently necessary before a definite statement on the metabolic rate of this animal will be possible. The same holds for the cave salamander *Typhlomolge rathbuni*. It had a much smaller gill area per gram body mass than the epigean *Eurycea nana*, suggesting a lower metabolic rate for *T.rathbuni* (Poulson, unpubl. observ., cited in Poulson, 1964).

ADAPTATION TO LOW OXYGEN CONTENT

With the exception of Caine (1978), no one has examined the possibility that low metabolic rates of cave animals might also reflect an adaptation to a low oxygen tension in the environmental medium. Caine found that the low metabolic rate of *Procambarus horsti* is not only an adaptation to low food availability but also to low oxygen content, found in the species' habitat. In caves where bat-guano presents a great food supply for many species water pools are often very oxygen poor because of bacterial decomposition of the guano. Unpublished data of J. Parzefall, and H. Wilkens suggest such a situation in the bat-inhabited Chica cave in the Sierra de El Abra in Mexico, inhabited also by a cave population of *Astyanax fasciatus* (= *'Anoptichthys jordani'*). Near the end of the dry season, where there has been no input of fresh water for several months, oxygen content of the pools was found to be only 0.4 - 0.6 mg.l^{-1} while NO_3^- content was extremely high with about 95 mg.l^{-1}. The NH_4^+ and NO_2^- contents were relatively low with 1 mg.l^{-1} each. This suggests that most of the ammonium set free from the guano was oxidized over NO_2^- to NO_3^-, using nearly all available oxygen in the water.

Wilkens (pers. comm.) could not observe any behavior in the fish indicating shortness of breath, neither reduced activity nor aquatic surface respiration. It would be very interesting to investigate if, and when how, the 'Chica' fish are able to use the oxygen supply of the water better than fish from more oxygen rich caves. In contrast, many crayfish (*Cambarus blandingii cuevachicae*) could be observed sitting at the shore with their carapace out of the water, moving air to their gills and wetting them from time to time. They obviously were suffering from the low oxygen content in the cave water (Wilkens, pers. comm.).

COMPARABLENESS OF THE EVOLUTION OF CAVE AND DEEP SEA ANIMALS

The deep sea as a parallel to the cave environment has been the object of several papers. Thines (1979) dealt with this theme, especially with regard to regressive evolution in fish. However, he held to the theory, mainly stated by Vandel (1964), that caves, and correspondingly the deep sea, are refuges for animals which represent phylogenetically senile species and can no longer survive in the geologically and climatically changing surface environment. Nowadays, another interpretation of the colonization process is preferred: The deep sea colonization always, and the cave colonization almost always, occurs voluntarily by animals equipped with preadaptations which allow them to live better in the less rivaled and more stable but also food poorer deep sea environment. As a general rule for both, potential cave as well as potential deep sea colonizers, it must be assumed that genetic variation, especially in metabolic rate, exists and that there are no conflicting selection pressures, such as selection towards rapid growth to escape predation. This was stated by Culver (1982) for cave animals under the prediction that a reduced metabolic rate is the optimal solution for survival in food poor environmental conditions.

Actually, various environmental factors in caves and in the deep sea are comparable, above all the darkness, the relatively stable conditions, and the generally restricted food resources. Since the cave and deep sea organisms consequently face similar selective processes, one would expect parallels in adaptation (Poulson, 1971). Nevertheless, one great difference between these two environments exists: The aquatic cave animals are more or less ecologically isolated while marine species are distributed over a wide range. This difference makes it difficult to give the same importance to the food scarcity in both situations (see also Thines, 1979). For example, adaptation of some deep sea animals to the limitation of food are known, especially in the critical early stage of ontogenetic development. They show a life history involving planktotrophic larval and juvenile stages and deep sea living adults. The advantage of such a strategy is that the early developmental stages are exposed to richer food resources in surface waters allowing more rapid growth. The main disadvantage is increased predation pressure (Smith and Brown, 1983).

Furthermore, caves are less uniform habitats than most parts of the deep sea. The variations in food supply between caves, and occasionally within one cave over the year, may be equivalent to variations in food with depth in the deep sea (Poulson, 1971) even though annual rhythms in food offer may exist in the deep sea environment too, caused by plankton blooms.

Nevertheless, reduced metabolic rates also appear to be a general characteristic of life at greater depths (Torres et al., 1979). Using in situ measurements, metabolic rates of several deep sea animals, including plankton and benthic communities, were found to decline with increasing depth of occurrence. For a summary see Torres et al. (1979), Sullivan and Smith (1982) and Smith and Brown (1983). An exception are vertically migrating fish, which are largely free of limitations imposed on the deeper species and are thus able to sustain higher metabolic rates. The reduction of the metabolic rates with increasing depth of occurrence, in accordance with the situation in caves, is explained as a strategy of low energy requirement, caused by the decline of biomass with depth (Torres et al., 1979). This is said to be a synergetic function of food availability, pressure and temperature (Smith and Hessler, 1974). In fact, the influence of pressure and temperature is relatively small (Torres et al., 1979). The means by which the animals achieve the low metabolic rates remain largely unexplained, as in cave animals, but they probably also involve low activity (Torres et al., 1979).

Fine et al. (1984) observed the smallest brain-body mass ratio of any known teleost in a deep water benthopelagic fish, *Acanthomus armatus*, while gustatory lobes of the medulla and the vestibular senses were particularly well developed. They stated a consistence of brain size and specialization with the strategy of low energy requirements, hovering and slow movement over the deep sea floor and consumption of small benthic prey.

DISCUSSION

As fundamental prerequisites, adaptation and optimization require the presence of genetic variation in fitness and the biological possiblity for adaptation and optimization in general (see also Culver, 1982). The adaptation of organisms to various habitats can involve behavioral, physiological and biochemical (Hochachka and Somero, 1980) and morphological adjustments. Among the great number of possible characteristics cave animals show, the reduced metabolic rate is only one component in a causally interrelated complex of adaptations. Figure 5 shows a survey on this network of responses to the cave environment, making plain the position of the metabolic rate in it. The literature on all these components, integrated in this idealized and partly hypothetic scheme, is immense. It can not be the aim of this paper to list or discuss all of it. Papers giving a review on or dealing in detail with it are e.g. Poulson (1963 and 1964), Barr (1968) and Culver (1982).

Despite the necessity of a truly restricted food source, the great environmental stability, especially the lack of predators and the continual darkness, is inserted into the network (see also Culver and Poulson,

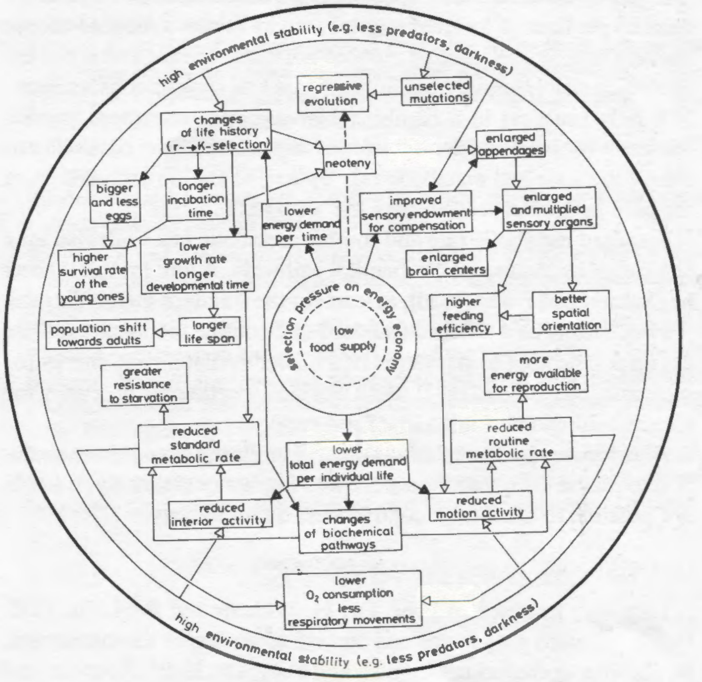

***Figure 5.* Causes and consequences of cave animal characteristics and their interrelations. A proposal (closed arrows require something; open arrows enable something).**

1971). In fact, most of all possible characteristics of cave animals in adaptation to food scarcity can only be realized in stabilized, above all predator poor, environments such as caves (see also Heuts, 1951 and 1953, and Culver, 1982). Finally, as a price for the highly desirable quality of reduced food needs in some cave animals the stabilized cave environment, which allowed this transformation, might become their prison (Heuts, 1951).

A reduced metabolic rate is the response to the selection pressure on energy economy, induced from low food supply. Selection pressure on energy economy mainly results in a lower total energy demand per individual life, in a lower energy demand per time and in an improved sensory endowment for food finding and orientation. In explaining the causalities it is necessary to differentiate between routine and standard metabolic rate.

The routine metabolic rate, usually defined as the mean oxygen consumption per hour over a day, can mainly be reduced by a general diminution of the motion activity, which is the consequence of less escape reactions away from predators and less movements for food finding. That means a regressive component on the one hand, made possible by the lack of a selective pressure on good (effective) escape-reactions, and a constructive component on the other hand, which means a higher feeding efficiency caused by an enlarged and multiplied sensory endowment.

Standard metabolic rate is usually defined as the lowest oxygen consumption per hour measurable or, theoretically, the oxygen consumption per hour only representing the energy demand for locomotion-free body functions. The reduced standard metabolic rate might be led back, at least partly, to a reduced interior activity, possibly including a lowered aggressive behavior, as sometimes found in cave animals (Parzefall, 1982), and to a lower growth rate as the result of life history changes. These are the consequences of the necessity of a lower energy demand per time. A lowered interior activity means a reduced energy demand as a consequence of reduced adrenalin release and is not expressed in visible activity but in heart rate and oxygen consumption. It is only practical in a highly stable environment, where interior readiness for reactions is no longer of importance. The possibility to reduce the standard metabolic rate by torpor can be excluded since the cave animals discussed here are all poikilotherms.

Standard metabolic rate and routine metabolic rate both also may be reduced by changes of biochemical pathways. A hint for this is given by Dickson and Franz (1980), at least for the standard metabolic rate.

Final results of a reduced standard and routine metabolic rate are a greater resistance to starvation or a higher availability of energy for reproduction (Culver, 1982). Both working together are necessary for a successful adaptation to low food supply.

Although many interrelations that occur during cave animal evolution to realize energy economy are well-known qualitatively, it is still not possible to state their contribution quantitatively.

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TIME-KEEPING MECHANISMS AND THEIR ECOLOGICAL SIGNIFICANCE IN CAVERNICOLOUS ANIMALS

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ABSTRACT

The present paper reviews the "circadian literature" in cavernicolous animals. Distinct circadian periodicities, which were reported in locomotory patterns of trogloxenic, troglophilic and even troglobitic species, seem to be ecologically significant. Extremely evolved cavernicolous animals, however, lack circadian periodicities of locomotion. Circadian growth rhythms are also unknown in troglobitic species. It is concluded that during the course of regressive evolution under cave conditions the circadian system degenerates without any residue. This conclusion implies the hypothesis that circadian clocks are not necessary for separating incompatible metabolic reactions in eukaryotic organisms.

The spontaneous locomotory patterns of cavernicolous carabids, including extremely evolved troglobitic species, frequently show structural regularities which can be described as the consequence of control by randomly generated internal signals. Stochastic regularities are also demonstrable in epigean carabids. It is concluded that central random generators, which control behavior, have survived the regressive evolution under cave conditions. A control by stochastic regularities seems to be adaptive. The variable parameters are the probabilities of transition from activity to rest and from rest to activity. - A model is proposed to explain the interaction between the circadian clock and the random generators in animals which possess both controlling systems.

1. INTRODUCTION

Many animals are able to establish a regular sequence of behavioral and metabolic events by *endogenous* time-keeping mechanisms. Such mechanisms enable the animals to measure the time of several hours. The best known time-keeping mechanism is the physiological self-sustained (circadian) clock (Bünning, 1977). Another endogenous mechanism operates by stochastic regularities: transitions from one state to the other (for example activity and rest) occur with time-invariant or regularly time-dependent probabilities (Kaiser and Lehmann, 1975). A further mechanism is comparable to time-measuring by an hour-glass (Pflüger, 1973; Beck, 1980). It has to be distinguished from the phenomenon of damped oscillations, which have been observed after a transition from light-dark cycles to constant conditions (Erckens and Weber, 1976; Erckens and Martin, 1982a). This phenomenon may be identical with that of anticipatory behavior in LD cycles (Aschoff et al., 1983). Possibly, basic structures of some or all of these time-keeping mechanisms are identical. For example, damped oscillations are mostly interpreted as a consequence of increasing desynchronisation within a population of circadian clocks (Gwinner, 1978).

Animals, which react to periodical signals in their environment, make use of an exogenously controlled time-keeping. Usually, endogenous time-keeping mechanisms are synchronized with the periodical changes in the environment; time-keeping is then controlled exogenously as well as endogenously.

The study of time-keeping in cavernicolous animals has intensified in the last decades for two reasons. Firstly, cave dwelling animals may be favorable subjects for studying the biological significance of the circadian clock system; secondly, they may be favorable for studying stochastically operating endogenous time-keeping mechanisms. These two aspects will be discussed in the two main chapters of this article. Time-keeping by the circadian clock system and by exogenous periodical signals will be discussed together.

The term "cave" is used in sensu lato: cave habitats form a continuum extending from the "superficial underground compartment" (Juberthie and Delay, 1981) and the mouths of "true" caves to the depth of the underground. It is not necessary to present the quantitative basis of the results discussed here: for this see the original articles cited. Also the methods used are not described in detail. However, some methodical remarks are given in the legends of the figures. The following abbreviations are used: LD = light-dark cycles; DD = constant dark; LL = constant white light; RR = constant red light.

2. CIRCADIAN CLOCKS AND EXOGENOUSLY CONTROLLED TIME-KEEPING

2.1 *Outline of the problem.*

Physiological circadian clocks have been demonstrated in many eukaryotic organisms including protists, fungi, plants and animals (Bunning, 1977). These clocks reveal some characteristic features: (1) they are self-sustained (i.e. they run undamped and free under constant conditions); (2) their frequency is close to 24 hours and temperature-compensated; (3) they are synchronized by certain daily environmental cycles (for example light-dark or temperature cycles); (4) the complete physiological clock mechanism seems to be localized in the single eukaryotic cells (multicellular organisms, therefore, seem to be multiclock systems in which, because of desynchronisation effects, self-sustainment may be obscured). At present the clock mechanism inside the cellular metabolic network is not yet precisely known (Schweiger and Schweiger, 1977). However, clock mutants have been isolated which show altered characteristics (for example an altered frequency) or even aperiodicity (Bruce 1972; Feldman and Hoyle, 1973; Konopka, 1979). Obviously the physiological clock mechanism depends on the integrity of several genes, and therefore requires the fitting by natural selection.

Four external (ecological) complexes of selection may fit the genetics of circadian clock systems:

(1) the advantage to adapt functions of the organisms (locomotory activities, reproduction, developmental steps) to the temporal changes in the abiotic environment (Remmert, 1977);

(2) the advantage to concentrate or to dislocate interactions between individuals (interactions between mates, competitors, between predator and prey) (Remmert, 1977);

(3) the advantage to develop seasonally ("photoperiodism"); this requires the measurement of the length of the day (Bünning, 1977; Beck, 1980).

(4) the advantage to orientate by an astronomical compass (Hoffman, 1972).

Furthermore, it has been considered that an internal selection factor may be effective, namely,

(5) the necessity to separate incompatible metabolic reactions inside the cells and organisms, respectively (for example the metabolism for rest and activity) (Jegla and Poulson, 1968; Bünning, 1977).

To what extent internal constraints control the genetic fitness of circadian clocks may possibly be clarified by investigations of the temporal organization in cavernicolous animals (Jegla and Poulson, 1968). External factors of selection become more and more ineffective as the binding to cave conditions increases. On the other hand, effectiveness of internal constraints should not be reduced during the phylogenetic adaptation to life in a constant environment. But, does such an internal selection pressure really exist?

If homeostasis in eukaryotes is essentially guaranteed by circadian oscillations, we should find self-sustained rhythms with periods in the range of several hours in at least one important metabolic pathway in every extremely evolved cavernicolous animal. The expected rhythms should be rather precise in *constant darkness conditions*, otherwise they would not fulfil their supposed purpose under true cave conditions. One may demur that our knowledge about the metabolism of cavernicolous animals is to incomplete to test this hypothesis. Indeed, with but few exceptions, we can test the hypothesis only by means of the locomotory behavior. Yet we feel it is legitimate to draw conclusions from the knowledge of activity patterns with regard to the temporal organization of metabolism. The change between rest and activity is accompanied by most drastic changes in metabolic reactions. Rest metabolism and activity metabolism are different to a great extent. If separating long-term oscillations really exist, they ought to control the functions of rest and activity metabolism above all, and, therefore, they ought to be reflected in the locomotory activity patterns. *Therefore, if under natural or simulated constant cave conditions no or only very imprecise rhythms were found, this would make the homeostasis hypothesis very unlikely.*

In case of imprecise rhythms, however, we have to discuss other possibilities: (1) the underlying clock is not completely lost, but degenerated to a residue without any purpose; (2) the underlying clock mechanism is still operating, but, while locomotion is only weakly coupled, other functions may be strictly clock-controlled because of *ecological* advantages; or (3) under other conditions locomotion itself will be effectively guided by the clock - likewise because of ecological advantages.

Ecological advantages of a clock-controlled behavior may be expected in many cavernicolous animals, too, especially in trogloxenes, which rest in caves during the day but periodically return to the surface for food, and in troglophiles, which survive in caves as well as in suitable epigean habitats (compare Barr, 1968). But troglobitic animals may also experience changes in the environment—if they, for example, live in cave entrances, in the ground of sink holes or in the "superficial underground compartment" (compare Juberthie and Delay, 1981). Therefore circadian experiments on cavernicolous animals should include investigation of their behavior in constant darkness and temperature, as well as in light-dark and temperature cycles and in dim constant light.

2.2 Locomotory activity patterns in cavernicolous animals.

2.2.1 Strictly clock-controlled locomotion rhythms are known from trogloxenes, troglophiles and even some troglobites.

In cave-dwelling bats, clear free-running circadian activity rhythms indicating a true clock control have already been demonstrated by Griffin and Welsh (1937), and later in several publications by Subbaraj and Chandrashekaran (1977, 1978, 1981) and Joshi and Chandrashekaran (1985 a,b). Entrainment to 24 hours seems to be due social synchronizing cues inside the caves (Marimuthu *et al.*, 1978; 1981); to exposure to the very dim light during the night, when the bats are flying outside the caves (Joshi and Chandrashekaran, 1982), and by "light sampling" in the cave mouth prior to flying out (Twente, 1955). Timing by endogenous and exogenous signals was described in detail for the cave bat *Hipposideros speoris*. Under the control of an endogenous clock the animals awake about 1-2 hours prior to the actual onset of foraging flight and fly close to the cave mouth "sampling light" (Marimuthu et al., 1978; Marimuthu, 1984). After sunset, the rate of reduction of light intensity seems to trigger the start of the outside activities (Subbaraj and Chandrashekaran, 1977; Marimuthu, 1984). Thus, by a combination of endogenous and exogenous signals an exact time-keeping is guaranteed so that the animals cannot miss the optimal time to start outside activities. Moreover, sampling of light contributes to the daily resetting of the circadian clock system: the animals do not awake continuously later or earlier in the darkness of the caves, but every day at about the same time before the actual start of outside activities. In a similar way, but less precisely returning into the caves before sunrise seems to be controlled by endogenous and exogenous signals. The behavior of cavernicolous bats resembles the behavior of dark-active epigean ground beetles, which rest during the day in darkened hiding places (Weber, 1981).

It may be supposed that, as in bats, in trogloxenic cave crickets the change between resting in a cave during day and foraging outside during night (Nicholas, 1962; Reichle *et al.* 1965; Campbell 1976) is controlled by a synchronized circadian clock system. Indeed, gathering in the cave mouth prior to walking out has also been observed in cave crickets. Moreover, like bats they seem to perceive light of extreme low intensity (Campbell, 1976). Thus, exposure to star or moon light during night could contribute to the synchronization of a circadian clock, too. Hunger may determine the particular night during which

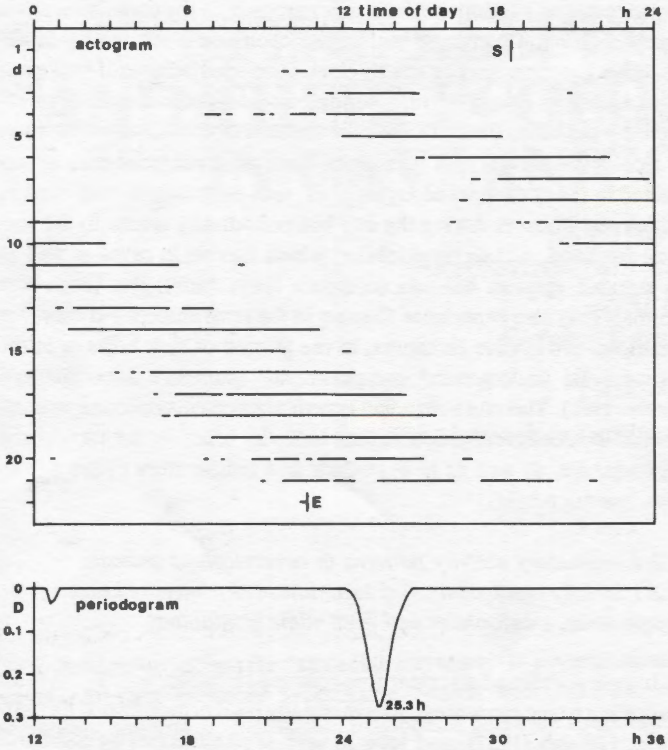

***Figure 1.* Behavior of a specimen of the troglophilic carabid *Ceutosphodrus oblongus* in LL (10^{-2} lux); 18.5°C. Above the actogram; below the periodogram indicating the free-running rhythmicity ($\tau = 25.3$h).**

an individual forages outside (Reichle *et al.*, 1965); a circadian clock, which shows a suitable (positive) phase relationship with the outside LD even after some days in the underground, may determine the time of day when the crickets become ready for activity and begin to move to the cave mouth; an external signal, for example a distinct light intensity, may trigger the actual start of outside activities. Unfortunately, up to now, individual free-running behavior has not yet been demonstrated. Reichle *et al.* (1965) and Campbell (1976) have reported on endogenous time-keeping in North American cave crickets under experimental constant conditions. However, these authors failed to demonstrate individual behavior, but they pooled the activity of several days and several individuals. Therefore, it is not impossible that the observed time-keeping was exogenously induced, and—strictly speaking—a circadian clock control was not proved. Simon (1973) measured the activity of groups of cave crickets in a jar set up in a mine for 27-30 days. To show the frequencies in the activity time series, Fourier transformation was applied. The crickets' activity transform revealed a "diurnal" and a "semidiurnal" peak. The exact frequency cannot be distinguished in the published figures. Simon was able to measure simultaneously lunar and solar earth tides. She suggested that earth tides caused the activity rhythms. In our opinion, however, only the proof of a distinct phase relationship between the geophysical rhythm and the rhythm of the animals would be a hint of such dependence.

All *troglophilic* species so far adequately studied showed circadian rhythms of activity: the amblyopsid fish *Chologaster agassizi* (Poulson and Jegla, 1969), the trechine carabids *Duvalius exaratus, boldorii, wingelmuelleri,* the sphodrine carabids *Pristonychus terricola, Ceutosphodrus oblongus* (Lamprecht and Weber, 1975, 1977, 1979, Weber, 1980) and the agonine carabid *Rhadine rubra* (Wiley, 1973).

In the carabid beetles investigated by us, free-running periodicities of locomotion were recorded in dim constant light, and partly in constant darkness too (Fig. 1). In *Duvalius exaratus, Pristonychus terricola* and *Ceutosphodrus oblongus* it has been proved that the freqency is temperature-compensated (Weber, 1980). In artificial 24 hrs

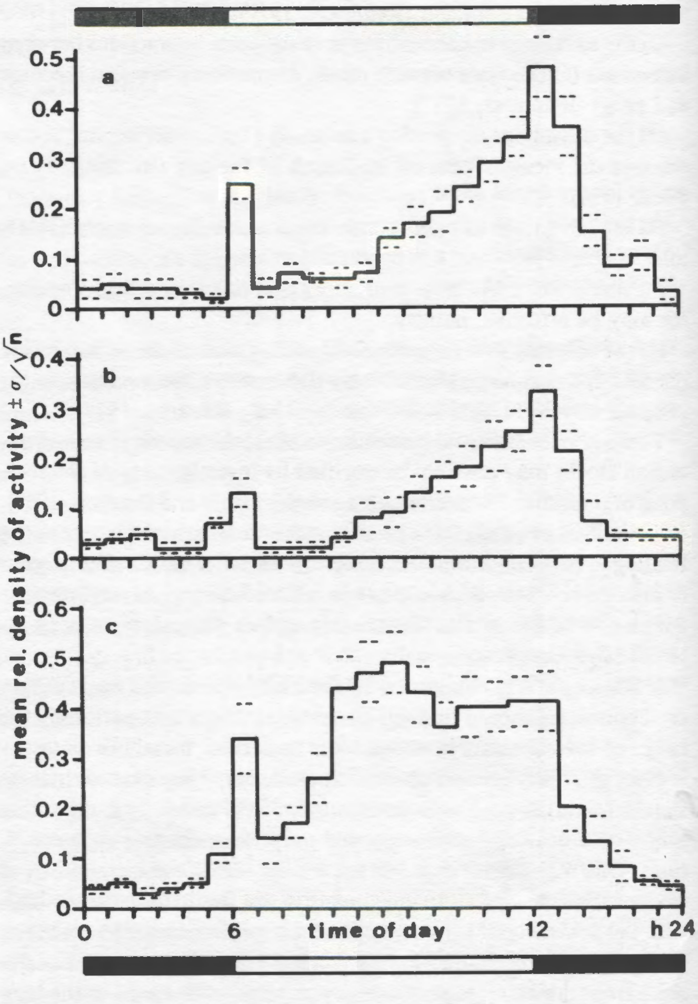

***Figure 2a-c.* Activity distribution of macrophthalmic *Duvalius* species (carabids) in LD cycles (0/1 lux). The "relative density of activity" is 1 for a particular hour if an animal is active in all 10 min-intervals. It is 0.5 if an animal is active at 3 10-min intervals, etc. The "mean rel. density of activity" (ordinate) is the average of n animals. a *Duvalius exaratus*; n = 17; 9 days; 10.5°C. b *Duvalius wingelmuelleri*; n = 15; 10 days; 10°C. c *Duvalius boldorii*; n = 14; 7 days; 10°C. Experiment a was repeated twice, experiments b and c were repeated once. The results were nearly identical (for the *D. exaratus* experiments see Lamprecht and Weber, 1979).**

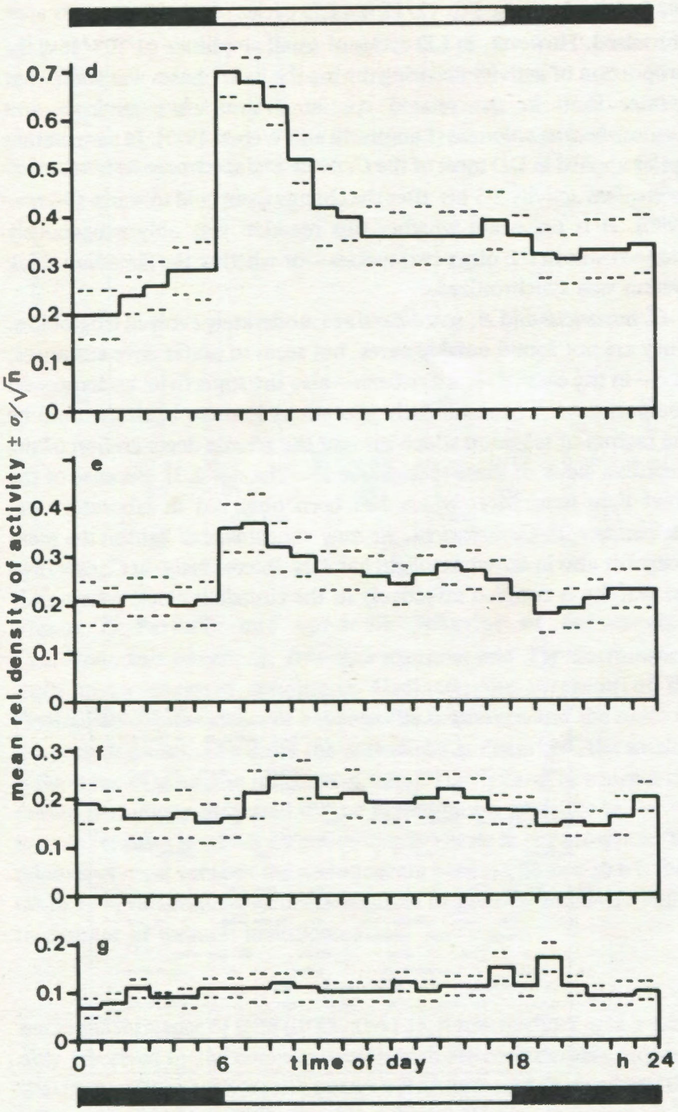

Figure 2d-g. **Activity distribution of anophthalmic trechini (carabids) in LD cycles. Compare Fig. 2a-c. d *Duvalius jureceki*; n = 16; 11 days; 0/1 lux; 9.5°C. The experiment was repeated once. The result was nearly identical. e *Duvalius balazuci* and *delphinensis* (the data were pooled, as these closely related species behaved identically); n = 15; 24 days; 0/1 lux; 9°C f *Aphaenops cerberus* and *pluto* (the data were again pooled); n = 9; 21 days; 0/5 lux; 9.5°C. The experiment was repeated in LD 0/0.5 lux. The result was nearly identical. g *Geotrechus orpheus*; n = 9; 17-21 days; 0/0.5 lux; 8.5°C.**

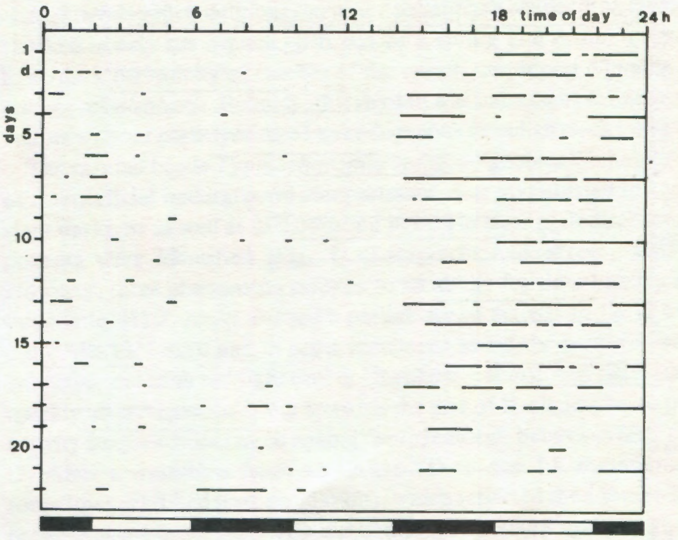

Figure 3. **Behavior of a specimen of the troglophilic carabid *Ceutosphodrus oblongus* in LD cycles of 4/4 hrs ($0/10^{-2}$ lux; 19°C) showing synchronization to a rhythm of 24 hrs. Adapted from Weber (1985).**

LD cycles the rhythm was synchronized. Surprisingly, in the *Duvalius* species, especially *D. boldorii*, a considerable proportion of the activity was recorded during the light phases of the applied LD cycles of 0/1 lux (Fig. 2a-c). This behavior clearly does not resemble that of the animals in natural twilight habitats. On the other hand, *Pristonychus terricola* and *Ceutosphodrus oblongus* were found to be strictly dark-active in artificial LD cycles even when the light intensity was very low ($0/10^{-4}$ lux) (Lamprecht and Weber, 1977) *C. oblongus* was additionally tested in LD cycles of 4:4 hrs ($0/10^{-2}$ lux). In 6 out of 15 specimens the locomotion rhythm was synchronized to 24 hrs (Fig. 3) (Weber, 1985). This phenomenon too, called frequency

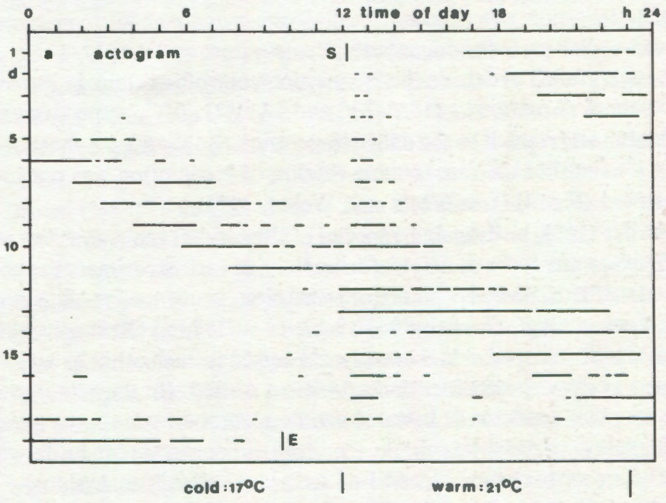

Figure 4. **Behavior of a specimen of the troglophilic carabid *Pristonychus terricola* in RR (10^{-3} lux) and 11/13 hrs temperature cycles (21°/17°C). The period of the free-running rhythm equals 25.8 h, that of the temperature-induced rhythm equals 24.0 h (periodogram analysis combined with a stepwise period elimination according to Lamprecht and Weber, 1970, 1972).**

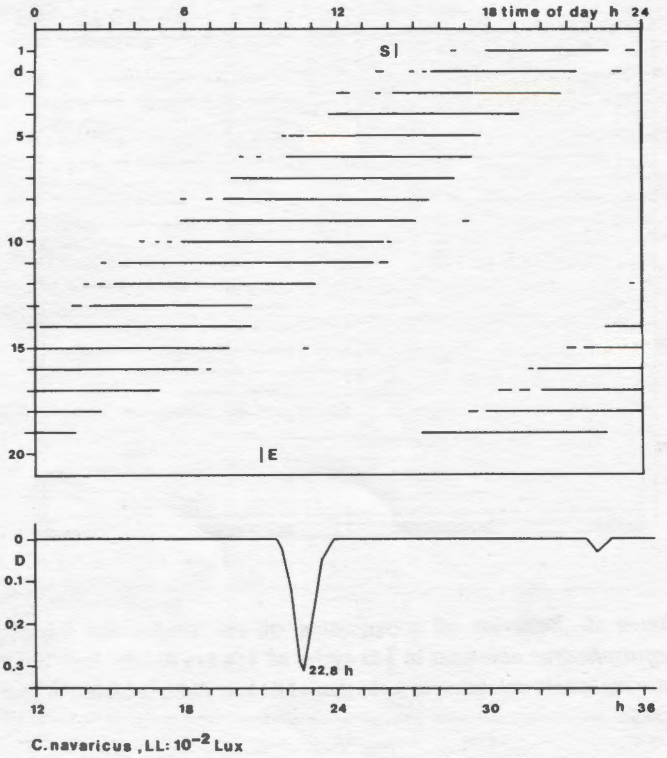

Figure 5. **Behavior of a specimen of the troglobitic carabid *Ceutosphodrus navaricus* in LL (10^{-2} lux); 10°C. Above the actogram, below the periodogram indicating the free-running rhythmicity (=22.8 h). Adapted from Weber (1980).**

demultiplication, points unequivocally to the animals' ability to measure the circadian period endogenously. Temperature cycles of 12/12 hrs., however, failed to entrain the locomotion controlling clock in *P. terricola* and *C. oblongus* (18/5/23° and 17/0/21, 0°C, respectively). The animals reacted to the daily temperature rise showing a short activity burst, but the free-running rhythm of locomotion was not influenced (Fig. 4) (Lamprecht and Weber, 1975).

Wiley (1973) investigated 4 species of the carabid genus *Rhadine* in DD experiments for 96 hrs. Unfortunately, in each experiment the activities of 22 beetles were pooled. Nevertheless, in one species - *R. rubra* - a free-running periodicity was visible (24 hrs.) Obviously, the animals of this species had been synchronized to each other by an exogenous signal just before the experiment started. (In the actograms of the other species—*R. subterranea, tenebrosa, kowdeni* —no clear circadian periodicity was visible, therefore no conclusion can be drawn with respect to the existence of an individual circadian rhythm).

Surprisingly, we found that there are even troglobitic carabids whose locomotory behavior can be precisely clock-controlled (Lamprecht and Weber, 1975; 1977; 1979; Weber, 1980). The microphthalmic sphodrine *Antisphodrus schreibersi* was periodically active in constant darkness as well as in dim constant light. In the similarly microphthalmic sphodrine *Ceutosphodrus navaricus*, the circadian clock controlled the activity in dim constant light effectively, whereas aperiodic patterns prevailed in constant darkness (Figs. 5, 6, 7, 14). The frequency was found to be temperature-compensated in both species (Weber, 1980, 1985). When exposed to 12/12 hrs LD cycles, *C. navaricus* was synchronized. However, in LD cycles of small amplitude (0/10^{-4} lux) the proportion of activity occuring during the light phases was somewhat greater than in the related species *Pristonychus terricola* and *Ceutosphodrus oblongus* (Lamprecht and Weber, 1977). In temperature cycles applied in DD most of the *C. navaricus* specimens tested showed intensified activity 2-3 hrs after the change from cold to warm (Weber, 1980). It is uncertain whether this reaction was only exogenously induced—as in the other two species—or whether the circadian clock system was synchronized.

C. navaricus and *A. schreibersi* are moderately evolved troglobites. They are not found outside caves, but seem to prefer cave entrances, and—in the case of *A. schreibersi*—also the superficial underground compartment. The small daily alterations in these habitats could be the factors of selection which prevent the genetic degeneration of the circadian clock of these species (see also chapter 2.3). Because of the great light-sensitivity, which has been observed in laboratory experiments with *C. navaricus*, we may conclude that light in the main Zeitgeber also in extreme twilight habitats. Interestingly, in *C. navaricus* the activity is coupled effectively to the circadian clock system only

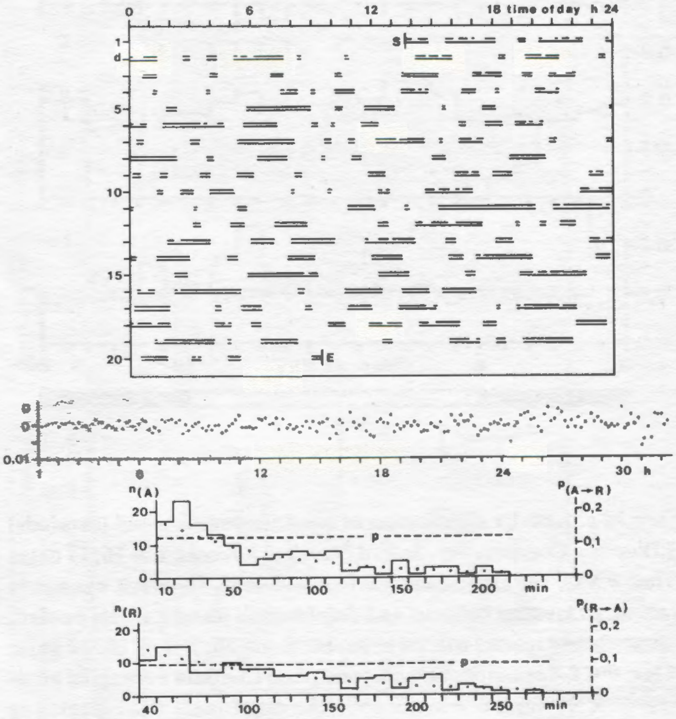

Figure 6. **Behavior of a specimen of the troglobitic carabid species *Ceutosphodrus navaricus* in DD, 10°C. Actogram (above), periodogram (in the middle) and the frequency distributions of the lengths of the activity bursts (A) and rest pauses (R) (below). Double lines in the actogram: above the original recordings, below the activity bursts (intervals ≦30 min without activity were added to the activity bursts). The frequency distributions can be approximated to exponential functions (courses of points) (α_{χ^2} = 0.30 and 0.70, respectively). p: the (time-invariant) transition probabilities. Adapted from Weber, 1980.**

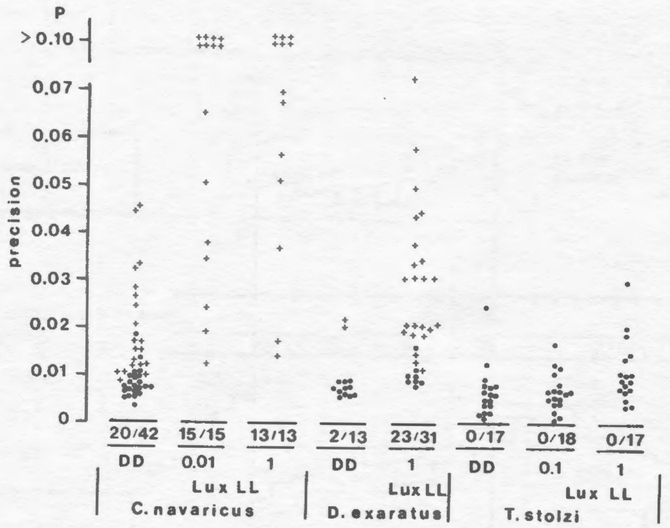

Figure 7. **Periodic and aperiodic behavior of the carabids *Ceutosphodrus navaricus, Duvalius exaratus* and *Typhlochoromus stolzi* under constant conditions. Ordinate: the precision of the rhythmicity (= the distance between the minimum and the noise of the periodogram). The more the periodicity is disturbed, the smaller is the value of precision (maximum value: 1). +: cases in which a circadian periodicity indicated by the periodogram is visible in the actogram. •: cases in which no periodicity is visible in the actogram; the relatively lowest value of the periodogram between 20 and 30 h is then taken as "precision." Quotients: number of periodic animals relative to number of animals investigated.**

under the influence of light (in LL and LD, respectively). Light is probably perceived by the complex eyes, which are considerably reduced; the ommatidia, however, still posses a rhabdom and show adaptation effects (Tscharntke, 1978; Bartkowiak and Weber, in press).

Tercafs (1961) observed the activity distribution of the troglophilic snail *Oxychilus cellarius* in caves as well as in the laboratory. The animals were much more active during night than during day. The external rhythms, that synchronized the activity inside the caves, were unfortunately not investigated.

2.2.2 Locomotion patterns with very imprecise or doubtful periodic components under constant conditions are reported from some more evolved troglobites.

The eyeless amphipod *Niphargus puteanus* was very intensely studied in DD, LD and temperature cycles (Güenzler, 1964). Under DD the animals were active without clear pauses of rest. By means of mere eye inspection, no evident periodicities could be detected in the actograms, but by "progressive Fourier analysis" a number of superimposed oscillations were found with period lengths ranging from 8 to 57 hrs (Blume et al., 1962). However, the statistical significance of this result is unknown. Ginet (1960) did not find any indication of a DD periodicity in the closely related species *N. orcinus*. In *N. puteanus*, LD cycles of various length and 12/12 hrs temperature cycles were found to induce activity rhythms which mostly disappeared immediately after a change to constant conditions (no post-oscillations). Müller *et al.* (1963) checked hourly the organic drift out of a spring and found a clear light/dark periodicity of catches of the eyeless amphipod *Niphargus aquilex schellenbergi*. Possibly, this rhythm was induced by exogenous factors without participation of an endogenous circadian clock system.

The carabid beetle *Typhlochoromus stolzi*, a "geobitic" inhabitant of a superficial underground compartment, was investigated in constant darkness as well as dim constant light. No clear periodical components were identified (Fig. 8) (Lamprecht and Weber, 1983). However, three phenomena pointed to an ability for time-keeping in this beetle. (1) *T. stolzi* showed a periodicity of activity in 12/12 hrs LD cycles (0/1 lux), and in some specimens tested the behavior was not a mere reaction to "light on" or "light off": the activity decreased quickly after "light on", but towards the end of the light phases the activity frequently increased again ("anticipatory" behavior) (Fig. 8). (2) After a transition from 12/12 hrs LD to dim LL the animals sometimes exhibited post-oscillations, which reflected the LD activity patterns for 1-2 periods. The distribution of activity and rest then became aperiodical (Lamprecht and Weber, 1983). (3) In 6/6 hrs LD (0/1 lux) *T. stolzi* was sometimes synchronized to 24 hrs: the animals were a little more active in one of the two dark phases of the 24 hrs period. Frequency demultiplication was indicated by the actogram as well as by the periodogram in which the minimum at 24 hrs was deeper than at 12 hrs (Lamprecht and Weber, 1983). Also 12/12 hrs temperature cycles induced a periodicity: tested in horizontal actographs *T. stolzi* was more active during the cold phases than during the warm phases (12.5/8.5 °C and 12/10 °C cycles were used; Lamprecht and Weber, 1983). In these experiments anticipatory behavior was observed in only a few cases. A possible effective escape reaction to temperature changes was detected in vertical actographs: whenever in 12/12 hrs cycles the temperature increased, the animals moved down and stayed at the bottom of the actograph; whenever the temperature decreased, the animals moved up and down (the following temperature cycles were used: 9.2/11.0°C;8.0/13.0°C;10.5/12.5°C;6/10°C). Sometimes anticipatory behavior was visible (Fig. 9) (unpublished authors' results).

The interpretation of the swimming behavior of the crayfish *Orconectes pellucidus* was contradictory for some decades. Park *et al.* (1941) recorded the activity during LD, DD and LL experiments lasting 24 hrs. They did not find a preference for the outdoor day or night. Brown (1961) re-examined these data and reported the discovery of a 24 hrs rhythm. He was guided by his hypothesis that biological clocks are not grounded on the animals' physiology, but are exclusively regulated by extrinsic geophysical forces and therefore demonstrable in troglobites, too. By the work of Bünning, Aschoff, Pittendrigh and other scientists Brown's hypothesis has been conclusively refuted (compare Enright, 1965).—*O. pellucidus* has been reinvestigated by Jegla and Poulson (1968). Of six animals tested in constant darkness and temperature, three were apparently regular in their rhythmicity (but true free-running was not visible), and three were irregular or apparently active at random. Of the three animals showing apparently regular rhythmicity, two showed statistically significant periodicities.—Moreover, *O. pellucidus* seems to be able to react to LD cycles with activity peaks in the dark phases.

The millipede *Blaniulus lichtensteini* was investigated under DD conditions (Mead and Gilhodes, 1974). No rhythms could be seen in the actograms. But, treated by autocorrelation and periodogram analysis, activity sequences of 200 hrs often exhibited periods lying around 12 or 24 hours. Because the statistical significance of this result was not

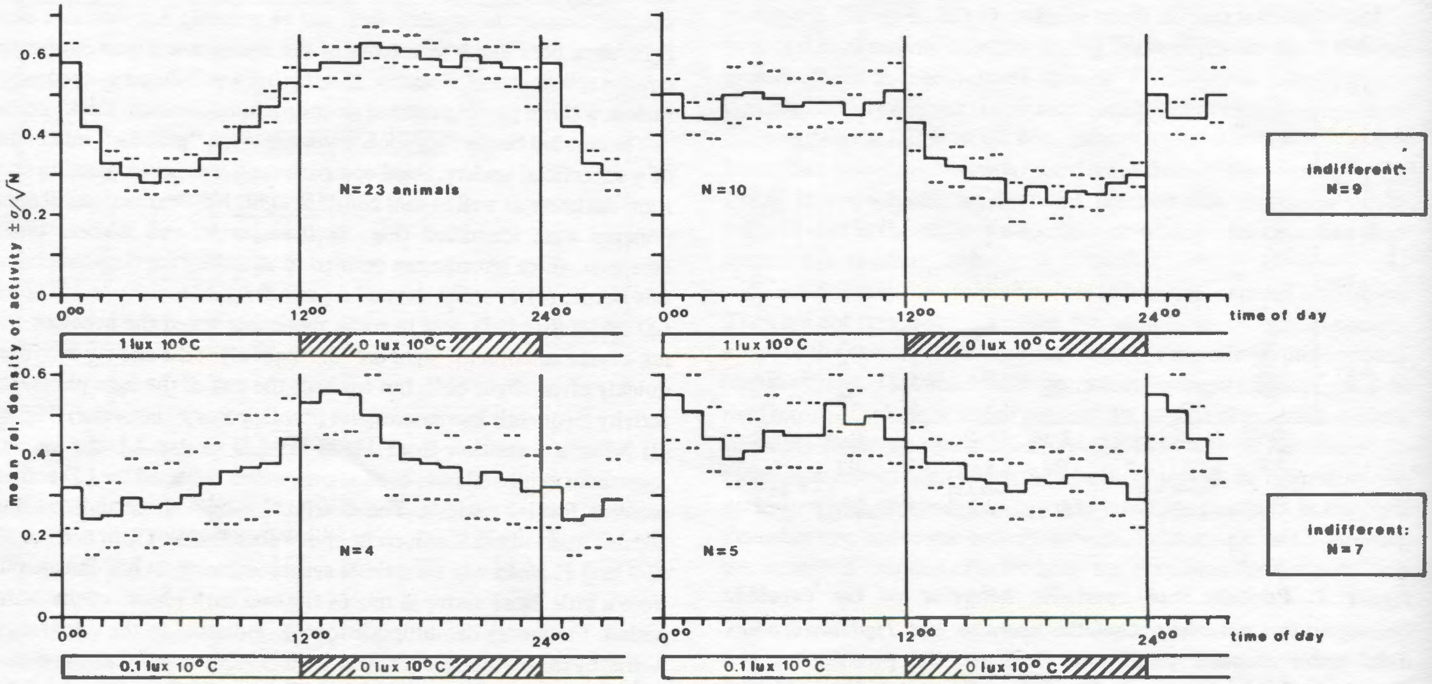

Figure 8. **LD behavior of the "geobitic" carabid beetle *Typhlochoromus stolzi*. N = Number of animals. Four series of experiments which lasted 8, 15 and (2x) 16 days. See Fig. 2 for "mean rel. density of activity." Adapted from Lamprecht and Weber (1983).**

checked, the existence of these periods is doubtful.

The fish *Astyanax mexicanus* offers the possibility to study the regression of time-keeping by a comparison between cave forms and their epigean ancestor (Erckens and Martin 1982a,b). The cave forms possess extremely reduced eyes. The epigean river form is well-eyed. The river form revealed two endogenous time keeping mechanisms: (1) A foreign-sustained one is entrained by LD's of any period length and damps out in DD within one or a few cycles with decreasing amplitude and unchanged frequency. In LD cycles the maximum values of surface activity correspond to the dark phases, those of bottom activity to the light phases. This inverse pattern causes less strong entrainment of the total activity up to a loss of a significant oscillation in extreme cases. The inverse pattern is stronger, the more the period length of the LD's deviates from 24 hrs. In 24 hrs LD's there is a greater flexibility with regard to the phase relation of the maximum values. (2) A circadian free-running rhythm obviously controlled by a self-sustained clock system is demonstrable in LD's deviating from 24 hrs as well as in DD.

In a cave form (Cueva de El Pachon) of *Astyanax mexicanus* the foreign-sustained time keeping of the river form has been reduced to an extremely passive one: (1) Activity is still entrainable by LD's of any period length (the same patterns were observed as in the river form, however disturbances often occured in the entrainment of one activity form, or one form was arhythmic while the other was still entrained), but the rhythms immediately disappeared in DD without post-oscillations. (2) The self-sustained system is also regressive: in no LD with a period length differing from 24 hrs a circadian rhythm could be observed in addition to the induced frequency. However, after LD cycles with a period length of 24 hrs, in DD a circadian free-running rhythm of surface activity was clearly shown (by the mathematical procedure of complex demodulation, significant at the 5% level). In DD after all other LD's, activity patterns were arhythmic. Obviously, only LD cycles of 12:12 hrs are able to stabilize the circadian clock system, possibly by synchronizing multiple oscillators. The several cave forms of *Astyanax mexicanus* seem to be of different phylogenetic age, as they differ with respect to the regression of some morphological and behavioral characters (Peters *et al.*, 1975); probably they differ also with respect to the regression of time-keeping mechanisms: in a group of eyeless fishes, whose origin was unknown, Erckens and Weber (1976) frequently found 1-4 post-oscillations after the animals had been transferred from 12/12, 8/8 or 6/6 hrs LD to DD conditions. Thines *et al.* (1966) and Thines and Weyers (1978) also found periodical LD behavior in cave forms of *Astyanax mexicanus*. The fishes reacted also to temperature cycles (amplitude 3 °C). These authors observed no rhythms in DD. Breder and Rasquin (1947) observed the reaction of the river form and several cave forms of *Astyanax* to the illuminated and the darkened part of an aquarium. Intensity and sign of the reaction were reported to be different: the river form reacted negatively, the most evolved cave forms positively to light. This conflicts with our observation that the reaction to light in the river and cave forms is identical.

A situation analogous to that in *Astyanax* exists in the Brazilian fish species *Pimelodella transitoria,* the epigean river form, and *P.*

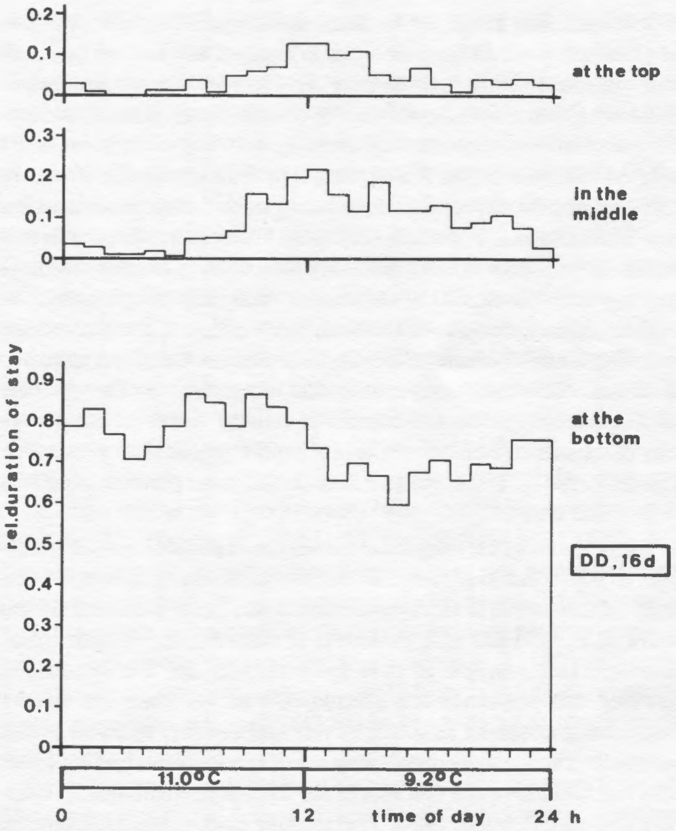

Figure 9. **Behavior of a specimen of the "geobitic" carabid *Typhlochoromus stolzi* in a vertical actograph (140 x 107 x 4.5 mm). Conditions: temperature cycles of 11.0/9.2°C; DD; 16 days. For the definition of "relative duration of stay" see the analogous "relative density of activity" in Fig. 2.**

kronei, the cave-dwelling form, which lacks eyes, and shows a varying degree of reduction of pigmentation (Pavan, 1946). The river form was nocturnal strictly avoiding illumination. By contrast, in the laboratory the cave form showed either an uninterrupted activity regardless of light conditions, or else a reaction to light much less pronounced than in its free-living ancestor. In nature, the animals from one of the caves made foraging excursions outside, but exclusively during the night. It is unknown how the rhythmicity in this cave form is controlled.

In *Proteus anguineus* a persistent daily locomotion rhythm during a DD experiment in the laboratory was reported by Briegleb and Schatz (1972), but repetition of the experiment in a mine could not confirm this result (Schatz *et al.,* 1976).

2.2.3 Locomotory patterns without apparent periodic components under constant conditions are reported from extremely evolved anophthalmic troglobitic fishes and beetles.

Troglobitic amblyopsid fishes (*Typhlichthys subterraneus* and *Amblyopsis rosae*) showed no circadian components in their DD behavior (Poulson and Jegla, 1969). *Amblyopsis spelaeus* reacted negatively to light (Eigenmann, 1909, Payne, 1907). *Typhlichthys subterraneus* has been reported to react indifferently to light (Verrier, 1929). It should be noted that troglobitic amblyopsids are much more cave-evolved than the cave forms of *Astyanax mexicanus.*

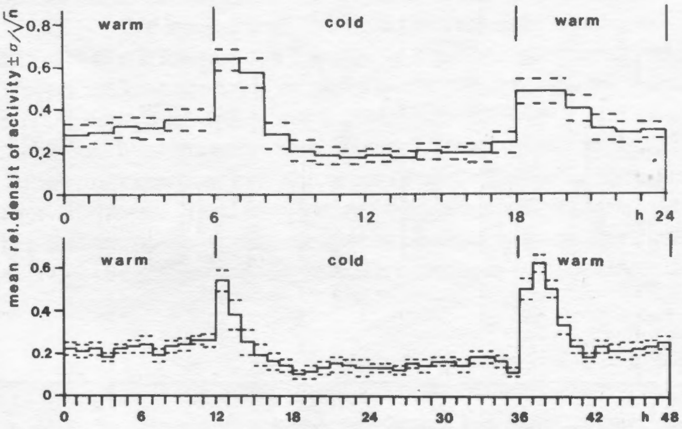

Figure 10. **Distribution of locomotion in troglobitic carabids of the genus *Aphaenops (cerberus* and *pluto)* in temperature cycles of 12/12 hrs (above) and 24/24 hrs (below). 12/12 hrs cycles: 6.8/9.0; 6.0/9.0; 7.2/9.0°C; 15 animals. 24/24 hrs cycles: 6.2/9.0; 6.8/9.3°C; 19 animals. For "mean rel. density of activity" see Fig. 2.**

The catopid beetle *Speonomus diecki* was found to be aperiodically active in DD (Deleurance-Glacon, 1963; Lamprecht and Weber, 1978). The trechine carabids *Duvalius jureceki, delphinensis, balazuci, Geotrechus orpheus, Aphaenops cerberus* and *Aphaenops pluto* were tested in DD, dim LL, 12/12 hrs LD cycles of small amplitude and partly in temperature cycles (Lamprecht and Weber, 1978, 1979; Weber 1980). With respect to the LD reaction, a striking difference was found: the anophthalmic *Duvalius* species showed sudden increase of activity after "light on", but did not react to "light off" (Fig. 2d,e). *Geotrechus* and *Aphaenops* did not clearly react to the applied LD cycles. Some *Aphaenops* specimens seemed to run in the light phases a little more frequently, however, this reaction was so weak, that it could not be considered as significant (Fig. 2f,g,1). Post-oscillations were not evident when the light-sensitive *Duvalius* species were transferred from LD cycles to constant dim light.

Some field observations confirmed our results regarding the different light-sensitivity in troglobitic trechines. Juberthie (1979) observed *Aphaenops cerberus* showing no escape reaction in the entrance range of a cave, where the animals were illuminated by weak natural light. Barr (1968) noticed that North American troblobitic trechines are frequently encountered in lighted areas at the bottom of sink holes showing no apparent phototaxis. On the other hand, *Duvalius delphinensis* has been reported to flee from artificial illumination in its habitat.

In *Speonomus, Aphaenops* and *Geotrechus* temperature cycles induced a clear locomotory rhythm (Lamprecht and Weber, 1978). Activity bursts were induced by the change from cold to warm and usually also by the change from warm to cold; additionally some animals were more active during the warm phases (the following temperature cycles were tested: 6.8/9.0; 7.0/9.0; 7.2/9.0°C) (Fig. 10). The reaction to temperature cycles in these laboratory experiments, however, was not

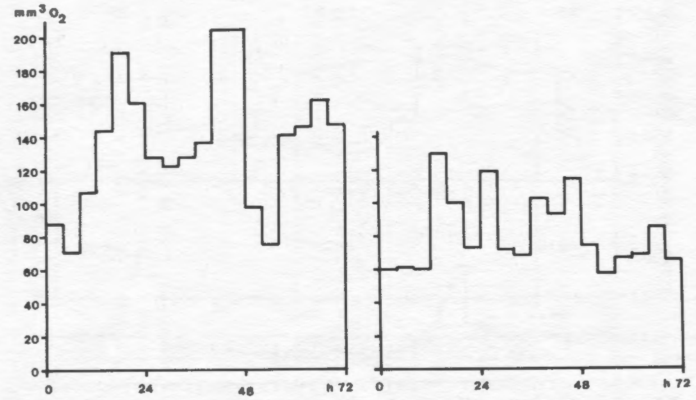

Fig. 11a. **Oxygen consumption in specimens of the troglobitic carabid *Ceutosphodrus navaricus* under dim LL (0.5-2.5 lux), 13°C. On the left: the "best" periodicity, on the right: the "worst" one of 13 experiments. Compare Table 1. Adapted from Lamprecht and Weber, 1982. The 0_2 consumption in mm^3 is reduced to O°C and 760 mm Hg. The ordinate gives the consumption per animal. Warburg's apparatus was used.**

very sensitive. In a cycle with an amplitude of 1.5°C (7.1/8.6°C) about half of the animals tested were not induced to be periodically active, the other ones showed only a weak periodicity. [Compare the astonishing sensitivity of the antennal cold and warm receptors for sudden and slow temperature changes which has been discovered in the cave beetle *Spheophyes lucidulus* by electrophysiological examination (Loftus and Corbiére-Tichané, 1981; Corbiére-Tichané and Loftus, 1983)]. *Aphaenops* species reacted to 24:24 hrs temperature cycles in the same manner as to 12:12 hrs cycles (Fig. 10). That means that the reaction to daily temperature alterations was not influenced by any endogenous circadian time-sense. Also post-oscillations were lacking, when *Aphaenops* or *Geotrechus* were transferred from cold/warm cycles to constant conditions (Weber, 1985).

2.4 Conclusions with regard to the homeostasis hypothesis

This review has shown that, without any doubt, precise periodicities of low frequency could not be demonstrated in DD activity patterns of extremely evolved cavernicolous animals. *Therefore we conclude that metabolic oscillations ranging from a few to many hrs are not necessary to maintain internal stability (homeostasis).* Indeed, this conclusion is merely supported by activity recordings. However, as we pointed out, we feel that it is legitimate to draw conclusions from the knowledge of the activity patterns with regard to the temporal organization of metabolism. It would be desirable, however, to measure directly metabolic processes in troglobites. Up to now, only oxygen consumption has been measured. In *Orconectes pellucidus* the periodicity of oxygen consumption seemed to be as weak as that of the activity patterns (Jegla and Poulson, 1968). Our experiments with *Ceutosphodrus navaricus* confirmed the expectation that measurements of oxygen consumption merely reflect the intensity of activity. In dim LL the periodicity of oxygen consumption was much more precise than in DD (Fig. 11 a, b, *Table I*). This corresponds to the different patterns of activity in DD and dim LL.

The behavior of the troglobitic species *Ceutosphodrus navaricus* supplies us with a fair argument for the conclusion drawn above. The activity of this beetle is clock-controlled under light influence: in dim artificial LL and LD and, probably, in nature under twilight conditions too (for example in cave entrances). Under DD conditions, however, it is predominantly aperiodically active. Therefore we conclude that a circadian structure of rest and activity, and also of rest metabolism and activity metabolism is not necessary, and perhaps even unfavorable inside caves (because of the food-poor environment? Compare Jegla and Poulson, 1968). The circadian clock system of this species did not remain fit because of internal necessities, but because of the

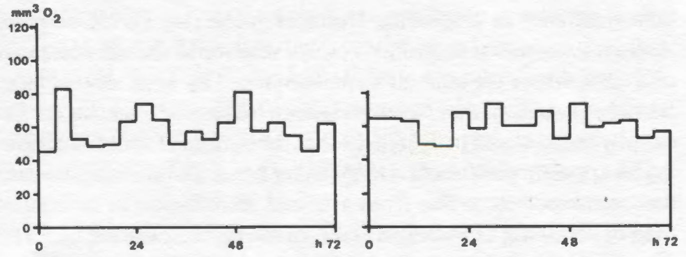

Figure 11b. **Oxygen consumption in specimens of the troglobitic carabid *Ceutosphodrus navaricus* in DD, 13°C. Dim red light was used for reading. On the left: the "best" periodicity of 13 experiments. On the right: an example of aperiodic 0_2 consumption.**

Table 1. **Circadian periodicity of oxygen consumption in the troglobitic carabid *Ceutosphodrus navaricus*. Compare Fig. 11a, b. Figures are the numbers of animals in each of the following categories: A aperiodical; B periodical; B'2 peaks; B''3 peaks in circadian distances; B* periodical, but with additional irregular minima or maxima; B** periodical, without irregular minima or maxima.**

	DD	LL
n	13	13
A	5	0
B	8	13
B'	1	0
B''	7	13
B*	7	2
B**	1	11

efficiency of external selection in cave entrances which are the preferred habitats of this beetle. We suppose that exclusively external factors of selection guarantee the genetic fitness of low frequency (circadian) clocks. The concrete advantage of the circadian clock for cave-entrance dwelling carabid beetles is unknown. Perhaps it is the daily change of the input of prey organisms. The synchronization of the animals' behavior to this or another daily rhythm in the environment seems to be performed by the small daily amplitude of illumination. The precondition—a very high light-sensitivity—is fulfilled (compare Lamprecht and Weber, 1977). The behavior of *C. navaricus* resembles the behavior of certain aquatic animals living near the arctic circle: their swimming activity exhibits a daily rhythm with the exception of the summer (i.e. under nearly LL conditions), when they are swimming aperiodically. In this case it is also considered that aperiodicity is advantageous because of special food conditions (Müller, 1973).

Also extremely evolved cavernicolous animals, for example *Aphaenops* species, have occasionally been observed in cave entrances illuminated by twilight (Juberthie, 1979). There are some imaginable reasons why their circadian clock system was not prevented from degenerating completely. For example, the proportion of the populations living in entrances could be too small to resist the mutation pressure which is effective in the subpopulations living under DD conditions. Furthermore, complete regression could have taken place when the whole population was restricted to the depth of caves, for example during glacial periods.

According to our aims outlined above we have to interpret the possible significance of the imprecise and doubtful locomotion rhythms which have been recorded under DD conditions in some troglobitic species. In the cases of *Niphargus puteanus* (Günzler, 1964) and *Blaniulus lichtensteini* (Mead and Gilhodes, 1974) it is doubtful whether the computed periodicities really existed. Perhaps they are short sequences out of stochastically structured time series (see chapter 3). In any case, however, they are too weak for a verification of the homeostasis hypothesis. *Orconectes pellucidus* seems to be endowed with an endogenous circannual rhythm of molting and reproduction, which may be ecologically significant because temperature and volume of flow of water, in which the animals are living, change throughout the year (Jegla and Poulson, 1970). Possibly, endogenous circannual clocks are based on circadian clocks so that the advantage of a circannual clock prevents the regression of the circadian clock, even if this one offers no direct advantage. In the case of the cave form of *Astyanax mexicanus* we assume that cave-dwelling bats, by leaving the caves every evening and returning every morning, create a daily change in the cave environment, to which the fishes are adapted by a circadian clock. For example, it can be expected that the defecation rate of the bats changes daily. The fishes feed on the guano. Noise and water movement have to be tested as possible Zeitgebers.

Typhlochoromus stolzi lives in a superficial underground compartment on Monte Cavallo, northern Italy. Animals living in this habitat may often reach the upper boundary of the living zone when they are searching for food. It can be assumed that the upper boundary moves daily—being higher during night than during day. Such daily environmental alternation may prevent the complete degeneration of the time measuring ability of the animals living there. In *T. stolzi* light/dark cycles seem to act as a Zeitgeber of its regressive time-sense. Light perception seems also to trigger an escape reaction: in the actograms recorded under LD conditions the activity during the light phases was often rather high, in some beetles even higher than during the dark phases. However, the light sensitivity in *T. stolzi* was rather small: in an LD of 1.0/0 lux 9 out of 33 animals reacted indifferently; in an LD of 0.1 to 0 lux this was 7 out of 16 specimens (Lamprecht and Weber, 1983). Light could be perceived by the very reduced lateral eyes whose surface is free of any faceted structure. However, very small rhabdoms have recently been detected (Bartkowiak, in prep.). Temperature reactions may also be important to keep in the optimum zone. The results of our temperature experiments lead to the assumption that reaching a warmer zone, when climbing up in the superficial underground compartment, as well as a warming up of the abode, triggers a downward movement.

2.4 Growth rhythms in constant darkness dwellers

To test the validity of our hypothesis that circadian clocks are only ecologically important, we have to discuss the small numbers of physiological functions that have been investigated with regard to periodical components in DD dwellers. They all concern growth rhythms: the periodic deposition of endocuticle layers in pterygote insects, of shell layers in molluscs and of sagittae layers in fishes.

All pterygote insects, cavernicolous forms included, seem to possess a periodically structured endocuticle (Neville, 1975). The deposition starts after molting and is controlled by its own oscillator mechanism, which is localized in the hypodermis. This oscillator can be integrated in or controlled by a circadian clock. This has been proved in species of Orthoptera, Dictyoptera, Heteroptera and Hymenoptera. On the other hand, the deposition oscillator in Coleoptera, as far as it has been studied, is not under the control of a circadian clock (Neville, 1975).

In 1965, Neville reported that the cave cricket *Dolichopoda linderi* exhibits a circadian deposition of endocuticle layers after molting. However, he mentioned that this cricket is a carnivore, living quite near to the cave mouth and making nocturnal excursions out of the caves for food. Therefore, it is not surprising that this trogloxenic species still possesses circadian clocks. It should be remembered that we may even expect an exceptionally strict coupling of physiological functions with circadian clocks in trogloxenes (see chapter 2.2.1).

In the epigean ground beetle *Pterostichus nigrita* the period of deposition is about 24 hrs at 21.5 °C, but it is not temperature-compensated, thus lacking one of the most characteristic features of circadian clock control. At constant 21.5 °C (LD or DD) 1 double layer is deposited every day, but at 15 °C the period of deposition is longer, and at 25 °C it is smaller (in DD as well as in 12/12 LD) ($Q_{10} = 1.82$, Althoff, 1979). In the troglophilic *Ceutosphodrus oblongus* and in the troglobitic *Ceutosphodrus navaricus* the clock is still more regressive; even at the optimal temperature (12 °C) the period length of the deposition rhythm is much longer than 24 hours (unpublished results, Fig. 12). Thus, the deposition rhythm of the endocuticle does not point to a maintenance of circadian clocks during regressive evolution. The stability of the (non-circadian) deposition oscillator itself is due to the physical properties of layered endocuticle, which combines high stability with low weight.

Growth rhythms are also known from the molluscan shell. In the bivalve *Mercenaria mercenaria*, which lives in the tidal and shallow tidal zone, the existence of a circadian rhythm in shell growth has been confirmed by mark and recapture experiments (Rhoads and Panella, 1970). The deepwater species *Nucula cancellata* and *Molettia* spec. from 4970 m depths (constant darkness!) has microincrements of remarkably uniform thickness. But nothing really seems to be known about the

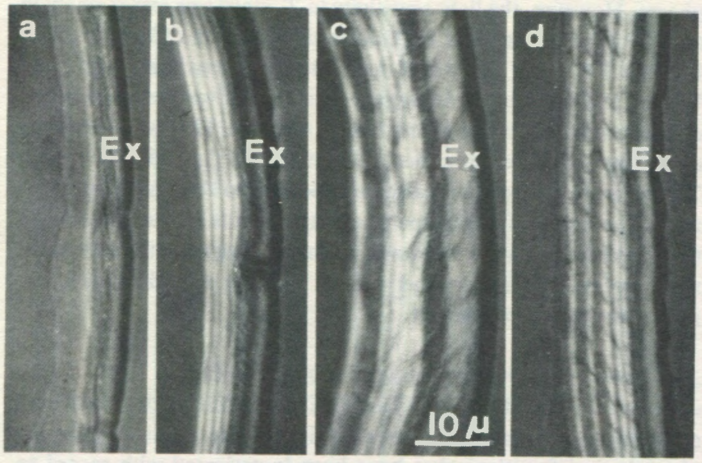

***Figure 12.* Endocuticle growth in a specimen of the troglophilic carabid species *Ceutosphodrus oblongus* under DD, 12-12.5°C. a: x hrs after hatching. b: x hrs + 3 days after hatching. c: 2 hrs + 6 days after hatching. d: x hrs + 9 days after hatching. Cross-sectioned mesothorical and metathorical tibiae.—The first anisotropic layer consists of 3-4 bright lamellae. In d the following sequence is shown: exocuticle (Ex), 1st anisotropic endocuticle layer (3 bright lamellae), 1st isotropic layer, 2nd anisotrpic layer; 2nd isotropic layer, 3rd anisotropic layer, 3rd isotropic layer (altogether 3 periods). Adapted from Lamprecht and Weber (1982).**

period lengths or the significance of these growth rhythms. Lutz and Rhoads (1980) interpreted the patterns as a consequence of spawning events. But what makes spawning rhythms so precise in the constant darkness of deep water? Could mutual social stimulation act as a Zeitgeber, synchronizing an endogenous clock? In this case a clock would be fitted by the necessity to time spawning within the populations (as is realized for many marine animals and plants, Remmert, 1977).

Deposition rhythms are also known from fish sagittae. Panella (1980) speculated that they could be related to feeding activity. Those species known to be almost constantly active showed indistinct or fain separations between increments, whereas those species experiencing rest phases showed marked incremental boundaries. The same correlation was found when growth patterns of surface fishes with daily activity rhythms were compared with those of deepwater species (Panella, 1980).

3. Time keeping by stochastic regularities

3.1 The phenomenon

The question arises, whether aperiodic actograms exhibit structural regularities, which can be interpreted as a consequence of central-nervous control. Such control would be a presupposition for the ability to adapt aperiodic sequences of activity and rest to the physiological and ecological situation an animal is actually living in. Lehmann *et al.* (1974) and Kaiser and Lehmann (1975) showed that aperiodic actograms of fiddler crabs can be described by simple stochastic regularities: by time-invariant or regularly time-dependent probabilities for the transition from activity to rest and from rest to activity. Meanwhile, stochastic regularities have also been demonstrated in the actograms of species living under quite different ecological conditions (Siberian chipmunk, gopher tortoise, vole, human baby: Lehmann, 1976; epigean and cavernicolous carabids: Lamprecht and Weber 1978, 1979; Weber, 1980, in press). In cavernicolous carabids stochastic control of locomotion is widespread: it was demonstrated for DD actograms of the normal-eyed *Duvalius exaratus,* of the small-eyed *Ceutosphodrus navaricus*, for DD- and LL-actograms of eyeless species of the genus *Duvalius* and for DD-, LL- and LD-actograms of *Geotrechus orpheus* and of species of the genus *Aphaenops* (Table 2).

In the test for stochastic regularities only two alternative states of behavior, activity and rest, are considered; differences in the intensity of locomotion are ignored. Therefore, beginning and ending of activity bursts and rest pauses, respectively, have to be defined unequivocally. The methodical difficulties arising from this situation will be discussed in detail elsewhere (Weber, in press).

The mathematical procedure to test *aperiodic* actograms for stochastic regularities comprises:

- —the calculation of coefficients of various correlations between the lengths of the bursts of activity and the pauses of rest following each other in the time-series (compare Weber, in press);
- —the checking for constancy of the behavior pattern throughout the experiment;
- —in cases of aperiodicity, missing of relevant correlations and behavioral constancy, the probabilities of the transition from activity to rest and from rest to activity can then be calculated from the frequency distributions of the lengths of activity bursts and rest pauses, respectively:
- —either by approximating the observed histograms to theoretical functions (to exponential functions of the type $y = a \bullet \exp(-f \bullet x)$ or to other theoretical functions, for example Poisson or normal functions);
- —or (if an approximation is not possible) by computing the probabilities of transitions directly from the observed frequencies of histogram classes.

If a histogram can be described by an exponential function, the probability of transition into the alternative state is time-invariant. That means, for example, that the probability for the transition to rest is identical in each measuring interval of each activity burst (otherwise an exponential decline of the frequency distribution of the lengths of the activity bursts would not result). The same is true for the transition from rest to activity, if the frequency of the lengths of the rest pauses can be described by an exponential function. If, on the other hand, a histogram of the lengths of the activity bursts can be approximated to a Poisson or normal function, the transition probability will continuously increase during an activity burst (Lamprecht and Weber, 1978). However, in this case, too, the transition probability changes in the same manner during each burst, independently of the actual length of the bursts and independently of the lengths of the previous bursts and rest pauses. If the transition probabilities can only be directly computed from a histogram, the alterations of the probabilties are often more irregular with time; however, in this case, too, they show the same course in any burst, for example; i.e. they are independent of the time of the actual transition and of the time of the previous transitions (it is presupposed in all these cases, that there are no correlative relationships between the lengths of the successive bursts and pauses of rest). If the transition probabilities are known, the pattern of activity and rest can be simulated. Such simulated patterns show an amazing similarity to the recorded actograms (Kaiser and Lehmann, 1975; Weber, 1980).

It should be stressed that in stochastically structured time-series (in the observed actograms as well as in simulated ones) by random combinations short sequences can appear that look periodical, especially if the transition probabilities increase with time (Kaiser and Lehmann, 1975). However, sudden and unforeseeable phase shifts indicate the real nature of this phenomenon.

3.2 The proof of stochastic regularities in the actograms of cavernicolous carabids.

The aperiodic actograms of the cavernicolous carabids listed in Table 2 frequently showed no or—occasionally—only very weak correlative relations between the lengths of successive activity bursts and rest pauses ($r < |0.4|$), which therefore can be neglected for the present. *Typhlochoromus stolzi* is an exception: in the analysed DD and LL actograms of this species frequently relevant positive correlations between the length of the activity bursts and the length of the following rest pause were found (r frequently around +0.6) (Lamprecht and Weber, 1983; Weber, in press). That means, in many actograms of *T. stolzi* the length of an activity burst influences the length of the following rest pause to a relevant extent. On the other hand, the length of a rest pause has no or only very little influence on the length of the following activity burst. The length of the activity bursts seems to be a largely independent variable. The actograms, too, showed a remarkable difference between *T. stolzi* and the other cavernicolous carabids investigated: *T. stolzi* actograms frequently exhibited very long activity bursts (sometimes longer than 24 hrs), which are missing in the other species.

In the species without relevant correlations in the actograms, a considerable proportion of the histograms of the lengths of activity bursts, and of the lengths of rest pauses, can be described by theoretical functions, frequently by an exponential function and in some cases by a Poissson or normal function (Table 2, Fig. 6, 13). Thus, it can be concluded that these actograms are structured by stochastic regularities: by transition probabilities which are time-invariant or which change regularly during the course of the activity bursts and rest pauses, respectively. It has been found that such transition probabilities are not temperature-compensated (Lamprecht and Weber, 1978; Weber, 1980).

3.3. The model of endogenous random generators—a first approximation to reality.

It may be supposed that the endogenous signals which determine the transitions from activity to rest and vice versa are produced by central-nervous random generators. This concept is based on two suppositions: (1) The point of time at which a signal stopping activity (or rest) is generated does not depend on the points of time at which the preceding signals were generated. (2) The probability of the generation of a signal ending activity (or rest) is time-invariant or (regularly) time-dependent. If the first supposition is true, we should find a complete lack of any correlation in the actograms. However, a complete lack has been found in no species. Because the significant correlations, found in the cavernicolous species listed in Table 2, were not frequent and very weak, they were neglected. We have to keep in mind that the "random generator" model is only a first approximation to reality. Any improved concept should consider the existence of these weak correlations. However, up to now, no such concept has been elaborated. [It has to be supposed that the transition probability in a distinct burst (or pause) depends on the lengths of the preceding bursts and pauses to an extent which is given by the strength of the corresponding correlations (that means, by sign and amount of the correlation coefficients); the unresolved problem is, how the transition probabilities can be calculated from the actograms under such a supposition].

Table 2. **Description of the frequency distributions of the lengths of activity bursts (A) and pauses of rest (R) in aperiodical actograms of cavernicolous beetles by an exponential function (exp), a Poisson function (Pois) or a normal function (nor). ? distribution function unknown.—The number of analysed A- and R-histograms sometimes differ, as a few histograms had to be excluded because of non-constancy of behavior (see text).**

		activity bursts					pauses of rest				
species	conditions	n	exp	Pois	nor	?	n	exp	Pois	nor	?
C.navaricus	DD a. 10^{-4} lux LL	37	19	–	3	15	37	25	–	1	11
D.exaratus	DD	11	7	1	–	3	11	1	–	–	10
D.jureceki	1 lux LL	15	9	–	–	6	15	4	–	–	11
D. balazuci a. delphinensis	DD a. 1 lux LL	33	17	–	–	16	33	4	–	–	29
A.cerberus, pluto a. crypticola	DD, 10^{-2} lux LL a. LD: 0/0.5 a. 0/5 lux	49	36	1	–	12	52	29	–	–	23
G.orpheus	DD, 10^{-2} lux LL a. LD: 0/0.5 lux	21	7	8	1	5	23	7	–	7	9
S.diecki	DD a. 10^{-2} lux LL	18	8	5	–	5	19	8	–	2	9

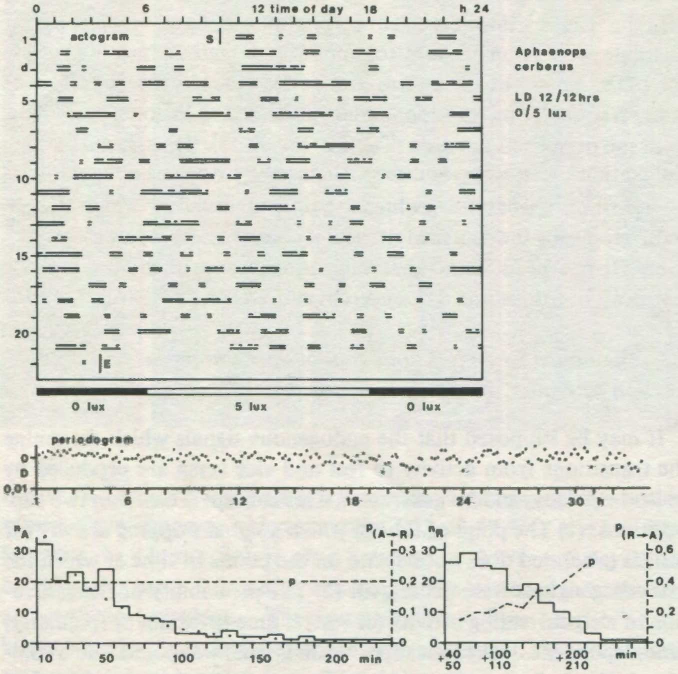

***Figure 13.* Behavior of a specimen of the troglobitic carabid *Aphaenops cerberus* in LD cycles of 5/0 lux, 9.5°C. Above the actogram (for double lines see Fig. 6); center the periodogram indicating no periodicity; below the frequency distributions of the lengths of the activity bursts (A) and rest pauses (R). The A-distribution can be described by the exponential function ($\alpha_{\chi^2} = 0.40$). The probability of transition to rest (p) is therefore time-invariant. The probability of transition from rest to activity was computed directly from the R-distribution. Adapted from Weber (1980).**

The behavior of *T. stolzi* can hardly be described by the simple random generator model. On the one hand, in this species too, no or only very weak circadian residues were found in the DD and LL actograms (Lamprecht and Weber, 1983) (compare Fig. 7). On the other hand, many actograms deviate considerably from a stochastic structure because the lengths of the rest pauses do not vary independently. As the length of the activity bursts are not correlated with the length of the previous rest pauses, at least a stochastic control of the activity bursts could be realized. However, the frequency distributions of the lengths of bursts are very variable, and a simple stochastic regularity was not found. Obviously, *T. stolzi* exhibits a special case of activity control which cannot yet be described by a general model.

3.4 Endogenous Random Generators in Epigean Carabids, and how the Generators may interact with the Circadian Clock System.

There are hints that the stochastic control of locomotion in cavernicolous carabids is not a new "invention," which took place during the evolutionary adaptation to cave conditions, but a heritage of epigean ancestors: (1) stochastic regularities were found in aperiodic DD actograms of cavernicolous carabids, which under LL conditions experience clear circadian rhythmicities (compare *C. navaricus* in Table 2); (2) the epigean carabid *Carabus problematicus* is aperiodically active after removing of both optic lobes, the probable sites of the circadian pacemakers which control locomotion rhythmicity in many insects (Brady 1974; Balkenohl and Weber, 1981). The aperiodic patterns reveal simple stochastic regularities pointing to a control by random generators.—It may therefore be assumed that the locomotory behavior of cavernicolous carabids can largely be explained as a consequence of a stepwise regression of the circadian central-nervous pacemakers, whilst—on the other hand—centers operating as random generators survived during the evolutionary adaptation to cave life.

There are some ideas how circadian clocks and random generators may interact in the control of locomotion. It has been hypothesized that the clock periodically modulates transition probabilities (Weber, 1980). If the modulation is very drastic, a circadian activity pattern results with clearly separated activity and rest phases. If the modula tion of the transition probabilities is more slight, a pattern results with less distinct activity and rest phases.

To test this hypothesis, simulation experiments have been carried out (Weber, 1980; in press). The problem is how to compute the transition probabilities from the recorded circadian actograms. So far, this problem has been solved only for time-invariant transition probabilities. The simulated actograms resembled the recorded ones to a high degree (Fig. 14).

3.5 The Adaptability of Stochastically Controlled Locomotion Patterns.

Can locomotory behaviour be adapted to environmental conditions by means of a stochastic control, which at first sight looks very unbiological? Lehmann *et al.* (1974) and Kaiser and Lehmann (1975) have shown for fiddler crabs living in the tidal zone that random control with time-dependent transition probabilities can produce highly adapted locomotory patterns by adjusting the maximum in the histograms of the lengths of bursts and pauses, respectively, to the time-intervals in the environment. In the case of cavernicolous animals it is imaginable that by varying the transition probabilities (time-invariant as well as time-dependent) the mean length of the activity bursts and the rest pauses can be adapted to the conditions of the habitat, for example to quality, quantity and distribution of food. Moreover it may be speculated that central nervous random generators in animals lacking other time-keeping mechanisms are essential for internal timing of neuromuscular activity and metabolic state (compare chapter 2.1).

4. Conclusions

The present review of the circadian literature on cavernicolous animals reveals that there are extremely evolved cavernicolous animals possessing no or only very weak circadian components in their locomotory patterns. Whenever, on the other hand, strong circadian periodicities are demonstrable in the actograms of cavernicolous animals, it is likely, that ecological advantages have prevented the regression of the circadian clock system. This means that the circadian clock is exclusively ecologically important, but not for internal separation of incompatible metabolic reactions. However, cavernicolous (troglobitic) species, which have lost the circadian clock stepwise during the course of regressive evolution, do not lack any structure in the patterns of locomotion and rest. Indeed, there are structural regularities that can be described as a consequence of a stochastically operating control. Such a control could be adaptable, and—what is more—it could be important for internal timing. Stochastic control does not seem to be a new "invention" in cavernicolous animals, but is also demonstrable

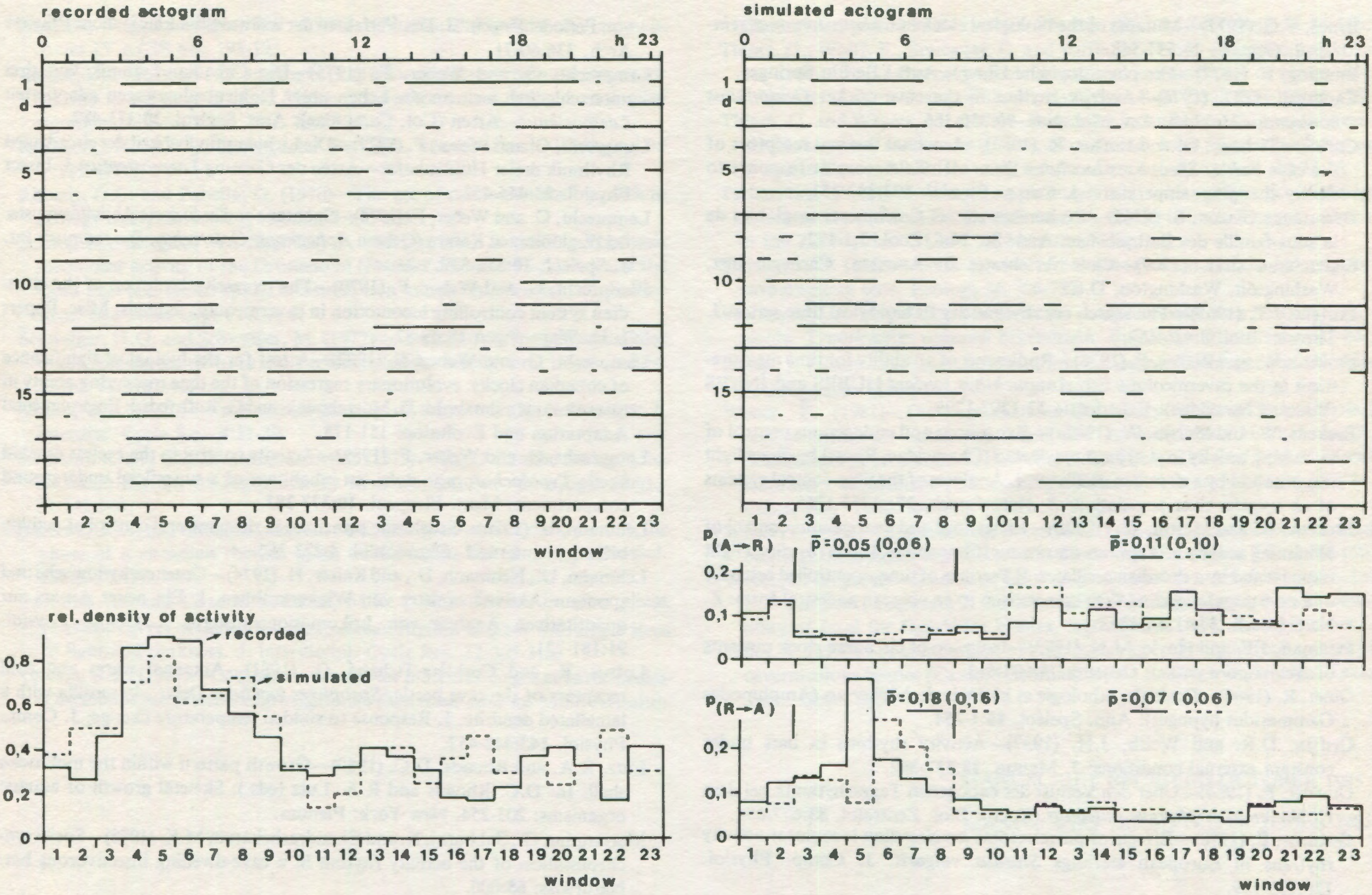

Figure 14. **The simulation of a circadian rhythmicity by periodically changing transition probabilities. Above on the left: the recorded actogram of a specimen of *Ceutosphodrus navaricus* under DD, 15°C. The abscissa gives the length of the period indicated by the periodogram (not shown, $\tau = 23.3$h, precision: 0.033). Above on the right: the simulated actogram. Below on the left: the "relative density of activity" (see Fig. 2) for the recorded and the simulated actogram. Below on the right: the transition probabilities (p) computed for the "windows" 1-23 for the recorded (______) and the simulated (-----) actogram. For the simulation the transition probabilities of the "windows" 1,2,9-23 and the "windows" 3-8, respectively, are assumed to be constant and are therefore averaged ($\bar{p}$). Adapted from Weber (1980).**

in the circadian activity patterns of epigean animals.

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