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Contents

EDITORIAL

Dear Reader,

Biology International, the voice of the International Union of Biological Sciences (IUBS), has become a purely electronic journal. This change aims at giving this voice a broader distribution and thus to improve interactions among scientific and ordinary members of IUBS, as well as to reach the larger community of scientists from biology and related disciplines.

Biology International has now reached its 46th volume and the journal is focused on proceedings of IUBS conferences and symposia that are generally linked to our scientific programmes, i.e.

- DIVERSITAS
- Human Dimensions of Biodiversity
- Systematics Agenda 2000 - International
- Towards An Integrative Biology – TAIB
- Biological Education
- Bioethics
- Bionomenclature

This volume includes three plenary lectures given at the IUBS General Assembly in Cairo, Egypt on 18-22 January, 2004. These papers are entitled: "Integrative Biology: The Nexus of Development, Ecology, and Evolution" by Marvalee Wake, "Sciences for Health and Well-Being" by Mark Wahlqvist, and "Sustainable Development, Scientific Development, and the Development of Peasant Farmers" by Bertrnad Hervieu. It includes also the reports of the TAIB symposia in 2003, held in Hayama, Japan, on the "Biology of Learning" and on " Biological Significance of Stochastic Events" by Motonori Hoshi, as well as the report of the TAIB meeting held in Quebec, Canada, on “Stress Proteins in Natural Environment and Populations” by Subhash C. Lakhotia.

In addition to the journal’s changes, IUBS has recently agreed to strengthen the day to day scientific discussion among its members and the scientific community. In order to achieve this ambitious goal, we will launch electronic conferences and a discussion forum on the IUBS website in the months to come.

Electronic conferences will discuss topics from our scientific programmes and stimulate a broader participation than traditional meetings usually allow. In keeping with previous meetings, e-conferences will be organised and moderated by a chairperson. We would appreciate suggestions for e-conferences from scientific and ordinary members of IUBS, as well as from scientists linked to the IUBS programmes.

The forum aims to discuss important and sometimes urgent cross-cutting themes suggested by IUBS members. Such discussions also will be moderated by a chairperson, who will have been nominated by the IUBS President.

Both, e-conferences and forum discussions will be announced to the scientific and ordinary members asking for further distribution among the

scientific communities. At the end of these electronic events a collection of the contributions including a summary will be compiled and made available through the IUBS website.

Because IUBS' mission is to reach scientists from all over the world, including areas off the information highways, we will base our work on low-end electronic tools such as electronic conferences by e-mail instead of video-conferences.

It is our hope that these steps towards the present day communication style in science will stimulate interaction among our members and the scientific community in biology. However, only **YOUR** contribution will ensure that IUBS is the platform of Biological Sciences.

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INTEGRATIVE BIOLOGY: THE NEXUS OF DEVELOPMENT, ECOLOGY, AND EVOLUTION

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A plenary lecture given at the IUBS General Assembly on 18 January 2004, in Cairo, Egypt.

It is becoming increasingly apparent that 'integrative biology' provides both a philosophy and a mechanism for the amalgamation of expertise from different but relevant fields of science to be brought to bear on complex questions. Often, when the term is mentioned, people ask "What is integrative biology?" It is both an attitude about science and an approach to the practice of science. It seeks both diversity and inclusiveness. It deals with questions across all levels of biological organization, and includes physical and social dimensions as appropriate to the questions. The conceptual basis of 'integrative biology' requires a hierarchical approach to the exploration of complex questions/problems, the use of multiple techniques, and novel but relevant analyses that lead to syntheses of appropriate arenas of the sub-disciplines of science, biological and otherwise, to give broader and more innovative insights into the consideration and even resolution of major questions. It has applications to issues in biodiversity science (Wake, 1995, 1998) and many other areas (see Wake, 2001, 2003). At the same time, it cannot be successful until it becomes a framework for education and training, so that the practice of science is truly changed (Wake, 2000). Similarly, if the promise of integrative biology is to be realized, it must be explicitly developed as a framework for research on the complexity of life. An integrative approach may then become the paradigm of 21st century biology, if not all of science.

Integrative biology promotes several principles and goals:

- the delineation of complex questions,
- the organization of expertise to tease apart the questions hierarchically,
- the extension of expertise into non-traditional arenas, and
- the development of new educational/ training modes.

It is these principles and goals that IUBS seeks to promote with its adoption of its program called "Towards an Integrative Biology".

An emerging arena for the integrative approach to major questions in biology is the developing interest in exploring the interaction of ecology, development, and evolution in organismal biology, including such questions as the basis of developmental change, the origin of new body forms, the nature of evolutionary processes, and the effects of environmental factors on development and evolution. It is an area of science ripe for synthesis because new and 'old' questions can now be dealt with differently, as a consequence of the employment of new tools and approaches. 'Eco-evo-devo' is rapidly adopting integrative principles, as I will illustrate.

The realization of such a paradigm shift has a history. In 1973 *(Science,*

180:488), Leigh Van Valen, with his usual prescience, stated that "A plausible argument could be made that evolution is the control of development by ecology." It has taken many years for that insight to be appreciated, but it is now being exemplified in the current wave of research that is exploring the interface and interactions of ecology, development, and evolution. I will consider the integrative and hierarchical properties of that research.

More than two decades ago, a synthesis of research in development and evolution began to develop, with the tools of molecular genetics facilitating the investigation of mechanisms of development, and of the mechanistic bases of changes in developmental programs. "Evo-devo" has become a successful research paradigm, represented by at least two international journals, a division of a major biological society devoted to it, and many researchers espousing it. The molecular biology of the genetics of development, the organization of phenotypes, and insights into mechanisms of evolutionary change are all being explored. Now, that synthesis is beginning to integrate an additional, significant, component---the environmental factors that both regulate and disrupt differential gene expression, affect rates of development, determine reproductive and developmental patterns, and provide selection mechanisms that drive evolutionary change, as Van Valen advocated. "Ecological developmental biology is the meeting of developmental biology with the real world. It involves studying development in its natural context rather than only in the laboratory" (Gilbert, 2001). 'Eco-evo-devo' includes a host of areas of investigation, including density-dependent morphological polyphenisms, predator-induced alteration in developmental rates, context-dependent life-cycle progressions (e.g., temperature/photoperiod dependent metamorphosis), egg protection against radiation, hormone mimics, and many others. Work on these diverse problems is highly integrative of research at all levels of the hierarchy of biological organization, and the genetics of development can complement the ecology and physiology of development, so that a new understanding of those elements that both stabilize populations and provide for evolutionary change is emerging.

In fact, the broadening of developmental biology into evolutionary developmental biology, ecological developmental biology, and medical developmental biology represents in part a return to questions that embryology had abandoned as it became developmental biology. Historically, ecological concerns had played a major role in the development of experimental embryology in the late 19th century, but the ecological paradigm was overtaken by the physiological one, as experimental embryology became an investigation of the causal physiology of development in the early 20th century. Integrations are now occurring among many 'disciplines'—molecular genetics, molecular development, organismal development, morphology, biochemistry, physiology, endocrinology, ecology, behavior, systematics, population biology, and evolution. This integrated network is changing our ideas about evolution, variation, and morphogenesis, and it is highlighting questions that have long been at the margins of developmental biology. As Scott Gilbert declared at a recent symposium, an expanded evolutionary developmental biology should become the central processing center to integrate the cellular and molecular levels of biology with the organismal and populational areas of the biological sciences.

An 'emerging value' of the new synthesis is that, with new approaches to complex interactions, biologists are again focusing attention on the *organisms* that are involved, as well as their interactions and effects. Organismal biology is returning to the forefront of investigation because of better understanding of the history of lineages and the responses of species, populations, and individuals to biological processes. I write as an evolutionary biologist who focuses on the biology of organisms, and who employs the techniques and approaches from many areas of biology in order to be hierarchical in my approach—that is, an integrative biologist.

Many examples of the integrative biology of 'eco-evo-devo' involve research on amphibians. Members of that class illustrate many of the issues, questions, and problems involved, ranging from the evolution of development to the ecology of extinction. Also, not only are several amphibian taxa 'model animals' for developmental, evolutionary, and ecological studies, but recent research on the biology of amphibians is re-exploring the interface of development, ecology, and evolution. Tools and techniques from molecular and cell biology, genetics, development, physiology, morphology, ecology, behavior, systematics, and evolutionary biology are being brought to bear on complex interactions. Research on such interactions is conducted hierarchically, often from molecular to ecosystem and evolutionary levels.

I will illustrate the integrative and hierarchical approach to the effect of ecology on development, and of development on evolution and vice versa, with four examples, all from recent research by several scientists on the biology of frogs, mostly, and also salamanders. All of these examples involve research that is truly integrative, in that questions from the molecular to the ecological, behavioral, and evolutionary are explored; multiple techniques and analyses are employed; major implications for science and in some cases for public policy are involved. The examples are:

- Limb development in direct-developing frogs,
- The effect of parasites on limb development,
- The effect of predation on hatching time and success, and
- The effect of UVB on hatching success.

Limb development in direct-developing frogs

Eleutherydactylus coqui is a species of small leptodactylid frogs that inhabits Puerto Rican forests. There it is ubiquitous and has large populations. It has been introduced in Hawaii and other parts of the world, and is a thriving invasive species. It is a member of the most highly speciose genus of frogs, including more than 600 species. All of the extant species, including *E. coqui*, are direct developers. Most have external fertilization, but *E. coqui* and a few other species, have internal fertilization via cloacal apposition by a male to the female. Following either mode of fertilization, female *Eleutherodactylus* deposit the clutch of fertilized eggs on land, and development ensues through metamorphosis, so that juveniles 'hatch', and the aquatic larval phase characteristic of most frogs is bypassed. Thus in the evolution of the genus, the ancestral life cycle – characterized by hatching as a larva (tadpole) that has a free-living, feeding, period before it metamorphoses into an aquatic or terrestrial adult with a very different morphology and ecological niche – is highly modified. In the evolutionarily derived direct-developing life cycle (which has developed in many lineages, including echinoderms and arthropods as well as vertebrates), eggs typically

are large, and the embryos usually are dependent on yolk as their nutrient material until they hatch fully metamorphosed.

The question arose in the fertile mind of James Hanken, an evolutionary developmental biologist who works on amphibians, whether direct development imparted any specific modifications to developmental pathways that might be concomitant to bypassing the larval period. He and his colleagues have found that there are several novel features of *E. coqui* embryonic development – in particular, many larval features are lost (mouthparts, cartilages, muscles), and nearly all of development occurs before hatching, as indicated above. In fact, bone, rather than cartilage, is the dominant tissue type, the paired limbs develop nearly simultaneously, an egg tooth develops apparently to facilitate hatching from the egg membrane. In addition, the tail of the unhatched tadpole is expanded and appressed to the egg membrane wall, apparently to serve as the principal embryonic respiratory organ, apparently effecting gaseous exchange with the environment.

Limb development in *E. coqui* illustrates a number of modifications, and at the same time, the analysis of the phenomenon is an illustration of integrative biology, as the genetics of limb development are employed to assess changes correlated with life history and ecology. Limb development *in E. coqui* is a mosaic of conserved and novel features. Those conserved are the patterns of *Hox* and *Shh* expression, and *distal-less (dll)* in the zone of polarizing activity, as well as many aspects of the general gross pattern of development at the cellular level. Novel are the fact that limb outgrowth is slow, so that the limbs develop nearly simultaneously; limb development is completed before hatching, rather than being a post-hatching event; and at the cellular level, the apical ectodermal ridge, typical of vertebrate limb development, is not present; the zone of proliferating growth (ZPA) also typical of limb development, is reduced, and its induction properties cease earlier than in other vertebrates examined, and the limb can continue to grow and differentiate after removal of the distal ectoderm (Elinson, 1994; Richardson *et al.*, 1998; summarized in Hanken *et al.*, 2001). These are significant timing differences in aspects of limb development relative to the several frog species with the ancestral life history pattern that have been studied to date.

The main point of this examination of limb development in a species that has evolved direct development, a derived mode of reproduction, is that cellular and molecular aspects of development are both conserved and novel. One emphasis of this research for developmental and evolutionary biology is that it is important to consider that the concept of the limb as a uniform developmental module must be modified to allow for the effects of evolutionary change in other factors of development of the animal (Hanken *et al.*, 2001). The influence of ecology on development and life history strategy remains to be evaluated fully for this system.

The effect of parasites on limb development

In the last decade or so, many multi-legged frogs have been found to occur in populations in which they had not been observed previously. The phenomenon has been reported in many parts of the world, particularly in disjunct areas of the United States. It occurs primarily in frogs, but also has been reported in salamander species, including some that cohabit the same

ponds as affected frogs. The limbs may be three, or up to 8 or 9, in number, almost always the hind limbs. The replication is usually proximal rather than distal in the limb, so that entire limbs from the femur to the toes develop, though sometimes the 'split' is at the tibial region. In the early 1990's, two young investigators, one an ecologist who had been monitoring amphibians in local sites in northern California, the other a developmental biologist/evolutionist, determined to investigate the phenomenon of the sudden appearance of high proportions of multi-legged animals. They quickly found that the frogs with multiple limbs typically were infested with large numbers of trematode cercaria, mostly embedded in the pelvic region (Sessions and Ruth, 1990). That situation has been reported in several species throughout the western United States (Johnson *et al*., 2001, 2002).

The developmental biologist of the team devised experiments to see whether the multiple limb anomaly could be simulated by inserting small glass beads the size of the cercaria in developing joint regions in tadpoles near metamorphosis, especially the pelvis. He found that the beads did induce multiple limbs. The experimenter concluded that the beads, and the parasites, exert a mechanical effect at the site of the outgrowth of the cellular condensation that will form the bones and muscles, causing a replication (Sessions and Ruth, 1990). The action of the cercaria themselves now has been further delineated (Stopper *et al*., 2002).

The interaction of ecology with development in this case seems to indicate that new phenomena are occurring. The presence of the trematodes, and their use of frogs as hosts, in the ponds is an old one. Why, then, are anomalies in the frogs suddenly being found in large numbers in ponds in which they had not occurred previously? Are the trematodes more numerous? If so, why? Are the frogs more sensitive to the cercaria? If so, why?? A new parasitic disease in amphibians may be emerging (Johnson *et al*., 2003). At the same time, increased numbers of the same kind of anomaly are being reported in other species of frogs whose tadpoles inhabit ponds that do *not* have trematode parasites. Acid rain and increased retinoic acid have been implicated in inducing the anomaly, as well as the parasites (summarized by Sessions *et al*., 1999). Again, the question arises why there are ecological and physiological changes that result in developmental anomalies. The evolutionary implications in terms of reduced reproductive capacity of multi-legged adults and the population involved, and consequently potential extinction, are profound.

The effect of predation on hatching time and success

The Red-eyed Tree Frog, *Agalychnis callidryas*, a colorful hylid frog featured on many calendars, postcards, and notecards, inhabits the forests of lower Central America. It lays its eggs, which are externally fertilized by the attendant male, on the leaves of trees that overhang ponds and streams, and the embryos develop into tadpoles, hatch, and then drop into the water, where they feed and metamorphose. The eggs are moderately large, about 2.2 mm diameter, and approximately 40 are laid. A young researcher, Karen Warkentin, is studying their life history in order to explore niche shift theory. She has found that, as occurs in many frogs, the embryos that hatch late relative to others survive aquatic predators better than do early hatchers. The late ones are bigger, swim faster, and have more complete development of the feeding and digestive apparatus. She discovered that there is a 'plasticity' to the hatching period; the embryos can survive if they hatch at 5

days after fertilization and laying, but they often hatch at 6-7-8 days following greater development, and later hatchers have increased survivorship in the water (Warkentin, 1995, 1999a). But Warkentin also discovered that eggs and embryos are subject to terrestrial/aerial predation, and she found, to her surprise, that predation *induces* earlier hatching during the plastic period. This seems to be an ecological/evolutionary trade-off between egg predation, when most or all of the clutch would be eaten, and aquatic predation, when *not* all of the tadpoles would be eaten, even among early hatchers. She found that early hatchers feed earlier, and they change several aspects of the developmental sequence, such as losing their external gills early, and having smaller tails (Warkentin, 1999b). Many animals are predators on the eggs and tadpoles of the red-eyed tree frogs. Aquatic predators of the tadpoles include a diversity of crustaceans and fishes; aerial predators of the eggs include snakes, wasps, and a water-borne fungus (Warkentin, 1999b).

When snakes prey on clutches of eggs/embryos before they are competent to hatch, nearly all of the eggs are consumed. Data for many clutches show high, nearly complete decimation of the clutch (Warkentin, 1995, 1999a). However, when the snakes attack clutches with embryos capable of hatching, hatching ensues, and most of the clutch escapes into the water. This response occurs at any time during the plastic period, and survivorship is enhanced. Warkentin and her students are now determining the factors that induce the hatching response. She and her students record the vibration signals (frequency and amplitude) of rain (no hatching) and snake movement (especially the main predator). Playback experiments show that the embryos sense and distinguish the vibrations of snake movement, and commence hatching. This work is in progress, and it includes a diversity of environmental variables (Warkentin, pers. comm.).

Similarly, wasp predation is most massive when the eggs are attacked before the embryos are competent to hatch. However, they are not as efficient predators as are the snakes---they remove the eggs or embryos one at a time, so that often more of the clutch is left uneaten. If wasps pierce an egg with an embryo not competent to hatch, they consume some yolk and often extract all or part of the embryo. However, wasp predation induces hatching during the plastic period. If the embryo is able to hatch, it usually escapes into the water below its place on its leaf, but occasionally the wasp is swift enough to catch the embryo. Only if the embryo escapes does the wasp then pierce a nearby egg in the clutch. In contrast to the snake predation, however, the embryos that are near the egg being attacked are the only ones that will hatch, leaving most of the clutch to continue development. Few eggs are consumed by wasps, several are induced to hatch, persistence by the wasp at a clutch increases the hatch rate, but not at the rate that snake predators effect. This suggests that the embryos at 5-8 days post-fertilization are capable of sensing the nature of the predator, and responding accordingly (Warkentin, 2000a). Warkentin is now investigating embryonic development, especially that of the neurosensory system, in order to assess how young embryos sense predator type and make responses that appear to be ‘decisions’ whether or not to hatch (Warkentin, pers. comm.). The situation for a fungus attack is similar; the fungus destroys the clutch if it attacks the eggs before the embryos are competent to hatch, but during the embryos’ plastic stage, a fungus infection induces hatching, and the differences are highly significant (Warkentin *et al*., 2001). Warkentin (2000b, 2002; Warkentin and Wassersug, 2001) has recently begun to

explore the relationships of oxygen availability, external gill loss, and hatching time, thereby adding an additional physiological-developmental-ecological dimension to her research program.

Warkentin and her group are assessing the kinds of signals---vibrations, water-borne chemicals, oxygen levels, etc.--- that might induce the hatching response, and she is also examining the development of the embryos and their sensory reception and integration systems to see how early the response can be effected, and what its pathways are. This is truly integrative research, examining development, morphology, physiology, ecology, life history strategies, and evolution, with a strong theoretical base to guide the analysis. It will provide empirical and theoretical information about the development of behaviors, predator-prey system function, a new way of considering phenotypic plasticity and reaction norms, and on the interaction of multiple ecological components on development, physiology, behavior, and the evolution of complex biological interactions.

The effect of UVB on hatching success

The example of the integrative approach that I will develop most fully herein is that of research on global climatic variables as they affect multiple species in diverse habitats. It considers the integrative biology of molecular biology (DNA repair), development (developmental rate, presence or absence of anomalies, hatching success), ecology (oviposition sites), climatic variables (decreased ozone and increased UV-B irradiation), behavior (e. g., activity level of tadpoles, egg-wrapping behavior of salamander larvae) and the implications of the decline of populations of amphibians (which has been reported around the world) (Blaustein and Wake, 1995) for evolutionary and conservation biology. Andrew Blaustein and his colleagues have studied these interactions in the field and in the lab for more than a decade, and for all of the species of frogs and salamanders that are endemic to the Cascade Mountains of Oregon in the western United States.

Blaustein and his group first concentrated on the effects of ultraviolet-beta (UV-B) on egg hatching and survival rates among the species of frogs and salamanders of the Cascades. They did both field and lab experiments (see Blaustein *et al*., 1998 for a summary). Their field experiments took place at natural oviposition sites, and used an experimental design in which fixed numbers of eggs/embryos from naturally laid clutches were placed in same-sized containers. One-third of the containers were shielded with UV-B blocking filters, one-third with an acetate filter that transmitted UV-B, and the remainder lacked filters. The containers were placed in a randomized block design, and the experiments were ended when the embryos either hatched or died, as they were monitored daily. The experiments were repeated at a number of sites at different elevations, and site differences were controlled for (Blaustein *et al*., 1997). They found that there were no differences in Pacific Tree Frog (*Hyla regilla)* or Red-legged Frog (*Rana aurora)* hatching success for the three treatments. However, hatching success of the Western Toad (*Bufo boreas),* the Cascades Frog (*Rama cascadae),* and the Northwestern Salamander (*Ambystoma gracile)* all had significantly greater hatching success under treatments that blocked UV-B radiation compared to those that had the eggs exposed (Blaustein *et al*., 1998). Further, UV-B can cause deformities--more than 90% of the Long-toed Salamander (*A. macrodactylum)* embryos had edema, lateral flexure of the tail, etc., under the UV-B transmitting situations (Blaustein *et al*., 1997).

Similar experiments document developmental responses to UV-B in several amphibian species (Hays *et al*., 1996). In a recent experiment, Belden and Blaustein (2002a, b) tested the predictability of effects of increases in UV-B. They submitted *Rana aurora* tadpoles, currently immune to UV-B, to increased dosages of UV-B, and found that a slight increase caused significantly reduced hatching success. They conjecture that climatic changes that continue to produce increasing amounts of UV-B will soon cause the declines of species now resistant to UV-B.

Blaustein and his group have found that the endemic frog and salamander species vary in their levels of photolyase, an enzyme that is responsible for DNA repair. The species that are resistant to UV-B in general have much higher levels of photolyase (e. g., *Hyla regilla),* and those that are vulnerable (e. g., *Bufo boreas)* have low levels of the enzyme. Levels of the DNA repair enzyme in the various species are significantly correlated with their resistance to UV-B exposure, and with certain behaviors (Blaustein *et al*., 1996, 1999, 2001b).

Over evolutionary time, species in the Cascades have evolved a number of defenses that apparently deal with UV-B radiation, among other factors (summarized in Blaustein and Belden, 2003). They are molecular (e. g., photolyase level), physiological, and behavioral. For example, the species of salamanders and frogs with low levels of photolyase preferentially lay their eggs in shade, while *Hyla regilla,* which has high levels of the enzyme, often lays its eggs in water under bright, unfiltered, sunlight. In a correlated study, Marco *et al*. (2001) found that European newts (*Triturus cristatus),* that wrap their eggs in leaves and grass fronds, effect greater hatching success under UV-B exposure. In order to test ways that behaviors might have evolved, Belden *et al.* (2003) examined survival, the hormonal stress response, and UV-B avoidance in Cascades Frog (*Rana cascadae)* tadpoles. They found that the tadpoles apparently do not perceive UV-B, or react with a stress response to its presence. However, UV-B exposure significantly reduces survival. Therefore the lack of perception and the lack of hormonal response indicate that the species' preference for laying its eggs in shade may be light-mediated, but likely not UV-B mediated. The authors suggest that, consequently, as UV-B increases, the frogs would appear to lack mechanisms for compensating directly for it, and their capacity for modifying their molecular biology, physiology, and behavior as has occurred over evolutionary time is likely to be outstripped by environmental change, thus causing significant population declines and ultimately the extinction of the species.

Blaustein and his group have also considered whether or not there might be synergistic effects among climatic and other variables in causing anomalies, illness, and decreased survival in the species that they have studied. They have found that UV-B radiation interacts with a pathogenic fungus (*Saprolegnia),* often present in the field, so that the mortality of the tadpoles is greater when exposed to both variables than it is with exposure to either UV-B or the fungus alone. The data were obtained from experiments on three species of frogs subjected to the UV-B experimental design in ponds that included the fungus (Kiesecker and Blaustein, 1995). They also found that a combined effect of UV-B, nitrates from fertilizer runoff, and low pH (acidification) significantly reduced Cascades frog survival and activity levels, but the tadpoles were not affected by any of the three factors alone (Blaustein and Hatch, 2000). These results indicate that multiple

stressors have a synergistic effect. Blaustein *et al*. (2003) reviewed the effects of UV, the synergistic effects of other components (e. g., such contaminants as pesticides, heavy metals, acidification, and fertilizers) with UV, and such effects as endocrine disruption that the contaminants mediate. They note that the effects are not only at the population and species levels, but also at community levels. There is a generally increased susceptibility to disease that is emerging, and environmental stressors are implicated. In fact, Kiesecker *et al*. (2004) recently presented evidence for a correlation of amphibian population declines with emerging diseases, and strongly recommended that global climate change and environment stressors be investigated as part of concern about new diseases.

Blaustein and his colleagues, and others, have examined their data regarding the issue of declining amphibian populations (Blaustein *et al*., 1994), global biodiversity loss, and the effects of climate change in order to assess whether there might be patterns that suggest causality. Blaustein *et al*. (2001a) document that climate changes may be influencing the breeding patterns of a number of amphibians, especially in terms of earlier breeding with increased temperature. Pounds *et al*. (1999) and Pounds (2001) illustrate that global warming is causing a rise in the level at which cloud forest begins in Costa Rica, so that cloud forest amphibians are losing their breeding sites and the timing of breeding is being affected---and some species appear to have been lost from the fauna. It is clear that causes of amphibian population declines are complex, and the interactors that appear to contribute to declines vary in different locations in the world, that different species respond differently to the same potential causative agents, and that even populations of the same species can respond differently. In addition, effects of causal agents may occur either at fixed stages during the life cycle, or at all stages (Blaustein *et al*., 2003; Kiesecker *et al*., 2001). Blaustein and Kiesecker (2002) make the point that though some generalizations can be made about amphibian population declines, the generalizations must take into account the context-dependent (abiotic and biotic factors that act together) dynamics of the ecological systems in which the species studied occur (as Wake [1991] had earlier suggested). Only then can lessons about biological complexity result in important pragmatic ventures such as conservation measures designed to protect species and habitats.

Obviously, many factors recently have been demonstrated to be implicated in the ecology and physiology of population declines, particularly of amphibians, but also of many other organisms. These factors are largely anthropogenic, as illustrated by the following list:

- •Habitat modification by humans (houses, farms, roads, dams, etc.),
- •Pollution of water, land, and air,
- •Secondary effects, such as increased UVB, acid rain,
- •Global climate change,
- •Introduced predators, competitors,
- •Exploitation for food or other resources,
- •New, or newly virulent, pathogens (chytrid fungi, algae, arboroviruses, etc.),
- •Many other causes, and the effects of causes are often synergistic (list from D. B. Wake, pers. comm.).

Such anthropogenic effects can affect any and all phases of the life cycle of the organisms that respond to them, including the molecular biology

and biochemistry of their physiological processes, their development from molecular and cell-level responses to whole-animal and whole-population effects, their reproductive capacity, and many other aspects of the components of life. It appears that many anthropogenic effects outstrip an organism's or a population of organisms' capacity to adapt, physiologically and over evolutionary time, to the changes in its environmental regime, so that decline and ultimately extinction are the consequence, rather than the evolution of the population in response to the selection pressure. We must learn our lessons immediately, and take steps to respond responsibly to global change and the issues of environmental degradation, population declines, emerging diseases and developmental anomalies, etc. An integrative approach is necessary at all steps in this consideration, from observation and recognition, to experimentation, to analysis, to appropriate responses.

Conclusions

The several cases presented as examples of research on questions of the complexity of the relationship of ecology to developmental biology and developmental biology to evolutionary biology, and their interactions, allow several conclusions to be drawn about the effectiveness of an integrative approach to such issues. They include:

1. An integrative and hierarchical approach to questions of interactions yields new and different insights than those obtained by focusing on single levels of problems.
2. The tools and techniques of many 'branches' of biology can be successfully integrated.
3. The impact of humans on the ecology of the planet has more dimensions than we normally consider.
4. We have a responsibility to conduct our research with both scientific and societal information and responses in mind.
5. We must train our students to be integrative in approach and attitude, so that they can contribute effectively to 'the new biology' and to society.

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Sciences for Health and Well-Being

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Abstract

A scientific basis for *preventive health and health care* is the acknowledged position of most enlightened societies, however modulated by local belief and culture. Science has been slow, however, to underpin the quest for *well-being* with science, partly because it and its contributors are less measurable. The rapid progression now of the social and neuro-behavioural sciences, compared with the more clinical and biomedical sciences, has great prospects for advances in this area. *Uncertainty and shifting paradigms* characterise science and ought to inform policy and individual choice arising from it. This requires constant review of how uncertain, unpredictable or unchangeable a notional "fact" is; and, for health and well-being, requires a thorough *risk benefit analysis* with its cost considerations and management strategy, at the individual, family, community, national and international levels. Is there, ultimately, a *science of happiness*, a significant ingredient of well-being?

More than this, a *new science and technology coalition* of biomedical, food and nutritional, psychosocial, behavioural and environmental sciences is required for how we live, work, play and relate to environments so that they may be resilient and sustainable. This is a growing challenge in science for health and well-being.

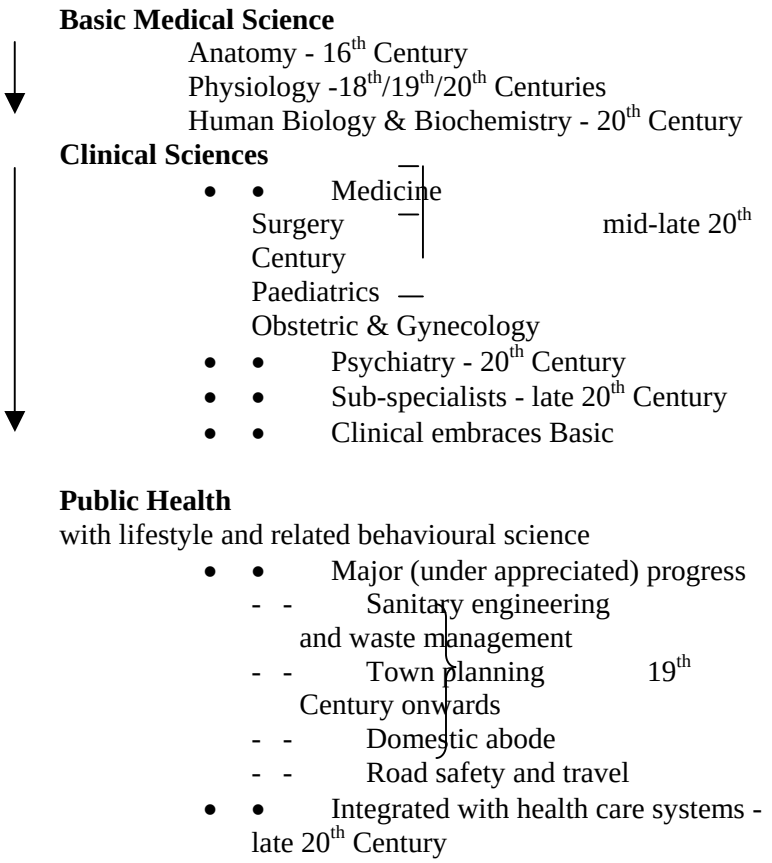
The challenge requires the life sciences to develop partnerships well beyond their own ranks – with geographers, mathematicians, physicists, astronomers, economists and more. It is also likely to require greater acknowledgement of a host of methods, not only through hypothesis – testing, by which knowledge has been and can be derived.

Keywords: Well-being, Wellness, Science, Technology, Risk Assessment, Resilience, Human Security, Family, Global Burden of Disease, Ageing, Biodiversity, Food Variety, Physical Activity, Happiness, Smile, Music, Dance, Locality, Region

Historical Perspective

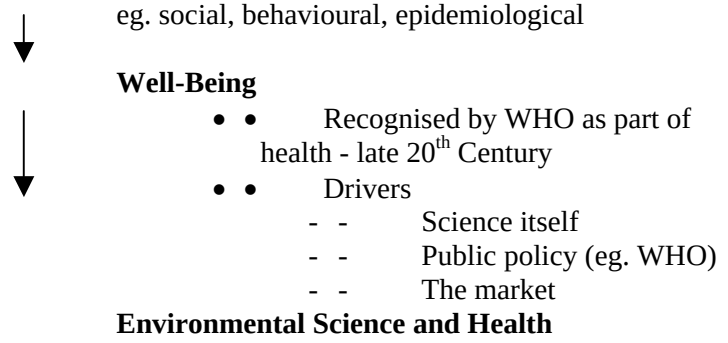
Whilst the concept of well-being is acknowledged by WHO (World Health Organization) to be intrinsic to health and to have physical, mental and social dimensions, in the minds of many it is a more explicit recognition that health can be very much more than the absence of disease or disability. To this extent well-being can represent a favourable collective state of body, mind or social situation, even when any one of these may be dysfunctional. Such an appreciation requires an integrative approach, which has, only in recent times, been of interest or amenable to scientific enquiry. The progress of Science for Health and Well-Being has proceeded from basic or fundamental science, progressively to the clinical sciences and public health science (Figure 1), and now, one might say, to an emergent "Well-Being Science".

Figure 1. The Historical Course of Science for Health & Well-Being



It could be argued that the notion of well-being (or wellness) science has also been facilitated by a Health Orientation of the Environmental Sciences (Figure 2). Increasingly UN agencies and governments seek this science.

Figure 2.



Preventive Health and Health Care systems

Not only has the increased understanding of disease causality enabled a more preventive approach to health, but so has the increased sophistication and sensitivity of health care systems allowed them to incorporate prevention. Both the science of prevention and of care are gathering a momentum which may together allow increasingly superior health achievements. Science plays its part in this field through

- Identification of the problem
- Definition of the situation
- Measurement of the relevant variables
- Evaluation of the indicators
- Recommendations about the findings
- Basis for change and betterment of communities

Provision of a *science and technology*, which are usually closely linked, also provide a partnership for prevention and care. The example of *diabetes*, which is affecting people around the world at rapidly increasing and disturbingly high rates (up to 15-20% or more of adult populations) can be addressed by maternal and child health programmes (for precursor settings), safe environments (for physical activity), food technology (for low glycaemic index foods), early detection with glucose sensors and follow-on risk factor interventions, and affordable low risk drug design and manufacture. A multitude of sciences and technologies are emerging to control this new epidemic. It serves as a prototype for prevention of other emerging health problems.

Well-Being Science

Well-being is not simply our present intrinsic state, but whether and how we are affected by the circumstances around us.
For example, *resilience* (and whatever is its socio-biological basis) can allow for well-being (8). There is evidence that, innately, we have the ability to bounce back in times of loss, with the possible exceptions of the death of a child or life-partner ("soul-mate", spouse) (8).

Again, if we know that we can overcome a sense of loss, we may be ready to let go of something which makes us unhappy (10) which is a way to enhance our well-being. This does, however, beg the question of how coupled

happiness is to well-being; probably happiness contributes to well-being, but well-being may not require happiness. Observations and considerations of this kind are amenable to further scientific enquiry which may allow us to institute techniques for the advancement of well-being (and health).

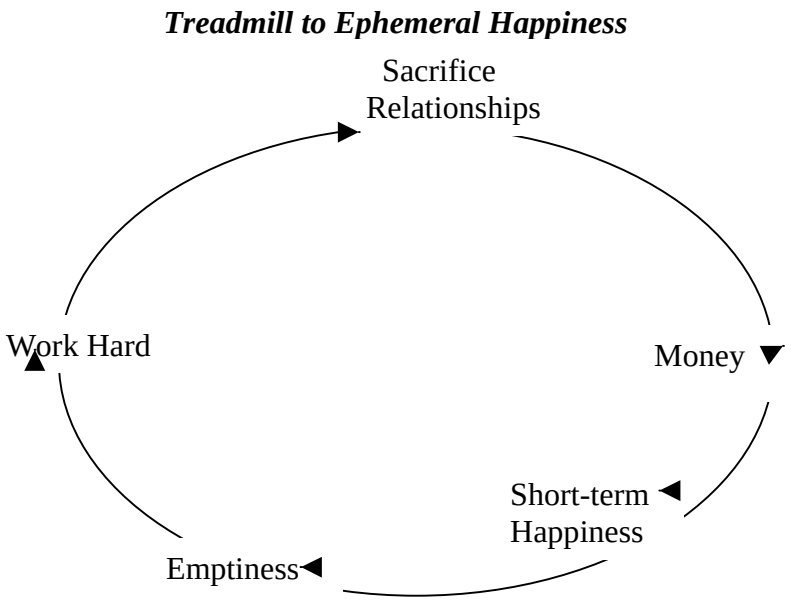
Well-being will also presumably require a sense of *security* at the personal, household and societal levels, constituting the broader situation of "Human Security". Thus, the growing literature on Human Security (18) is relevant to the Well-Being Scientist.

Well-being is also likely to be dependent on *belief and culture*. For example, great emphasis is placed on reducing *expectations* as an approach to happiness in Buddhist societies; material prosperity is often understood not to be the basis of happiness, but reflect various combinations of endeavour and "luck". In a 15 year study by Tim Kasser of the Quest for Happiness, materialism had a high price, and "success" short term benefits. However, going from "poverty to enough" (in the case of the USA in 2003, US$35000 p.a.!) allowed material well-being and a measure of happiness, and more than this no predictable difference (13). There may be a "treadmill to emphemeral happiness" (Figure 3), epitomized by Daniel Gilbert's notion of "miswanting" (10).

In general, there does seem to be a problem of "miswanting", of over-estimating the happiness that "things" might bring (10). Instead, social interaction and relationships seem to provide more lasting happiness. In Well-Being Science we may wish to consider *Happiness* in its own right, since it is a potentially measurable and highly regarded human characteristic or phenomenon. It may be underpinned by

- Sense of personal security
- Related to expectations
- Connectedness
- Recognition
- Task or project completion
- Problem solving
- Conflict resolution

Figure 3. Social interaction and relationships provide lasting happiness, whereas a material quest may not



In reality, there are many *things to measure* in Well-Being Science. This became particularly apparent in programmes to promote and enhance wellness for individuals. An example of such a programme is WLOP (The Wellness On-Line Programme) created by Omar, Kouris-Blazos, Savige and Wahlqvist (www.healthyeatingclub.org). The 6 dimensions currently explored in most wellness programmes are:

- Social
- Occupational
- Spiritual
- Physical (Diet & Physical activity)
- Intellectual
- Emotional

A much-vexed issue in the field of well-being and wellness is "stress" which can play a physiological role, or lead to unease, "unwellness".

Analyzing the hormonal response to stress, by gender, demonstrates the interplay between environmental and human biological factors in wellness. A preparedness for "Fight or Flight" is more apparent in men than women in stressful circumstance and relates to testosterone and catecholamine responses; whereas oxytocin release in women under similar circumstances encourages solace to be sought amongst friends (27).

This social or situational response to stress and its mechanisms leads us to consider the *family unit* as a basis for well-being and health advantage (Figure 4).

Figure 4. The Family, Health and Well-Being (31)

- Married men live much longer than single men (Australian Bureau of Statistics)

- Long-married couples most value the creation of a life together
  - less materialistic
  - fight fairly
  - an over-riding commitment to the commitment
  - inter-dependence as strength, not a weakness

Some health states are regarded as a feature of irreducible uncertainty about pathogenetic mechanisms and risk factors or, at least, the substantial distance yet to go with scientific enquiry. Hence, the notion of "*luck"* about health. This is a salutary reminder that *risk analysis* may get better and better with more science, and that some luck is explicable comes from the observations of Richard Wiseman (31)

"*Lucky people are more relaxed and open to see what is there rather than just what they are looking for."*

Richard Wiseman.
The Lucky Factor. Random House.

Even in *risk assessment* (7) more analytical work is required as pointed out by Jack A. Chenbach (5) once it is perceived to be either "intuitive and emotional" or "logical and analytical" or, maybe, both. One method of risk assessment may be no more or less valid than another if only the mechanism of intuitive assessment, for example, is understood. Can intuition be a more rapid integrative method for compiling and responding to a broad array of information? At least we know that measurement makes a difference to how we think about risk, as provided by a World Bank report on cancer in women (Figure 5).

Figure 5.
Measurement makes a difference to how we think about risk
eg. Cancer in women

(1)(1) Mortality greater for lung cancer, but breast cancer is the greater fear
(2)(2) Rape and domestic violence reduce DALYS (Disability Adjusted Life Years)
in women more than all forms of cancer combined.

World Bank 2003

The *indices of well-being* or wellness are mostly *self-rating scales* (linear and non-linear, uni- or multi-scalar or multidimensional). *Facial expression* and other *body language* may be amenable to accurate and precise measurement with technological advances. One of the most interesting possibilities is the *smile*, although it can be extraordinarily complex as the painting of Mona Lisa by Leonardo de Vinci illustrates. A *smile* may mean a variety of things:

- - Happiness
- - Pleasure
- - Bemusement
- - Satisfaction
- - Contentment

Each of them is relevant to well-being or wellness.

The *detection and reporting* of many well-being variables will depend on, inter alia:

1. 1. A vocabulary
2. 2. The ability to measure the concept
3. 3. The acceptance of the concept by society-at-large
4. 4. Commercialization and Economic Relevance

Well-Being and Fitness (or Disability) in its various forms (mental, physical, sexual, nutritional, social) (25) has been the subject of valuable enquiry in regard to successful ageing. In general, it can be said that activity rather than indolence is conducive to health and well-being. The most striking example is the impact of *regular physical activity* on deferral of disability by up-to 15-20 years (9) and the progressive decline in disability rates with age in Europe in recent years (15). Almost certainly, *dietary variety* (supported by *biodiversity*) is a potent factor, through nutritional fitness, in conferring survival advantage with reduced morbidity as well (11,30). Other important determinants of well-being would appear to be *music* (and *dance* as it combines social activity, physical activity and music) (3,14,21,23,24,26,29)**,** *locality for abode* (and the images we carry of it for reference in times of stress) and *workplace* (increasing the prospects of happiness at work).

Estimates of *the Global Burden of Disease (GBD)* as DALYS (Disability Adjusted Life Years) or HALE (Healthy Life Expectancy) are now available and used by the WHO (World Health Organization) to assess and compare international health status and trends. It can be seen in Figure 6, that we are undergoing a remarkable shift in the burden of disease and its causes. Despite food shortage and abject poverty for at least 1 billion of the world's 6.5 billion population, cardiovascular diseases (affecting heart, brain and other organs) are coming to dominate the global burden of disease, along with mood disorders (principally depression) and road traffic accidents. Certain infectious diseases are again on the rise (tuberculosis, diarrhoea and HIV/AIDS) and a prelude to increasing difficulty with new pathogens and inadequate nutritional resistance to infection. Conflict and war also feature in the emerging top 10 leading causes of Disease Burden.

Figure 6. Disease burden for 10 leading causes
Change in rank 1990-2020

Estimates 1990			Projection 2020		
Rank	Cause	% Total	Rank	Cause	% Total
1	Lower respiratory infections	8.2	1	Ischaemic heart disease	5.9
2	Diarrhoeal diseases	7.2	2	Unipolar depressive disorders	5.7
3	Perinatal diseases	6.7	3	Road traffic accident	5.1
4	Unipolar depressive disorder	3.7	4	Cerebrovascular disease	4.4
5	Ischaemic heart disease	3.4	5	Chronic obs pulmonary disease	4.2
6	Cerebrovascular disease	2.8	6	Lower respiratory infections	3.1
7	Tuberculosis	2.8	7	Tuberculosis	3.0
8	Measles	2.7	8	In war	3.0
9	Road traffic accident	2.5	9	Diarrhoeal disease	2.7
10	Congenital abnormalities	2.4	10	HIV/AIDS	2.6

Both *mood* (mental well-being) and *cognition* (especially its decline with age), which are CNS (Central Nervous System) phenomena, may be more amenable to favourable living environments and lifestyles (social activity, food and exercise) than heretofore thought. In the case of food there are several possibilities:

- Fats which increase apoE4 gene expression, and thereby Alzheimer's risk
- Fish and n-3 fatty acids could protect against depression
- Phytochemicals (like anthocyanins) may protect against neuro-degeneration (and stimulate regeneration)
- Smell and memory
- Food memory in the amygdala

We had previously not thought it possible that neuroprotection could be provided and *neuroregeneration* stimulated by food factors, but recent work on phytochemicals, like anthocyanins from berries and green leafy vegetables, shows this is possible (12).

Several years ago, Ono showed that *food memory* (and its connection with other memory phenomenon in the amydala and rhinencephalon were amenable to subtle dietary manipulation (20).

A difficulty in the pursuit of Well-Being Science is how normative or medicalizing it is as, in turn, this may affect the attention it receives, the resources allocated to it, and how accessible advances in it are to the public-at-large. For example, opportunities for pharmacological management of Well-Being seem to require diagnoses like "Social Phobia" and "Hypoactive Sexual Desire Disorder (HSSD)" and their availability for commercialization, with "pharmacotherapeutic solutions".

Requirements for a New Era in Science for Health and Well-Being

These may be summarized as:

- A clear understanding of what we seek
- A new science and technology coalition
  - within the life sciences
  - beyond the life sciences
- Knowledge partnerships between
  - the Sciences (hypothesis testing) and
  - other traditional knowledge systems
  - new knowledge systems

The partnerships for the life sciences would include at least these:

(i) Within the Life Sciences:

- Biomedical
- Food and nutritional
- Agricultural and horticultural
- Neuro-behavioural
- Psycho-social
- Environmental

It would be helpful to regard these as relevant "To Live, Work and Play".

(ii) Beyond the life sciences:

- Geographers
  - *for sense of location*
- Mathematicians
  - *integrative measurement*
- Physicists
  - *models*
- Astronomers & Cosmologists
  - *seeing beyond ourselves*
- Economists
  - *affordability*
  - *economical health development*

(iii) More generally, *Critical Community Partnerships* would be with:

- *Literate women* as the gatekeepers, brokers, family and societal teachers, basic technologists (food, textiles, shelter, communication) and much more.
- *Children* as partners for their creativity, interests (environment and cosmos), questioning, open-ness.

(iv) *Knowledge Partnerships* will be required between:

- The Sciences (hypothesis testing)
- Other traditional knowledge systems
- New knowledge systems

It is worth *noting that "Sciences for Health and Well-Being"*, at its best:

- Is itself a New Paradigm
- Seeks and espouses
  - Excellence
  - Equity
  - Ethics (6)
- Has regard for Peace rather than Conflict (1,17,19)

Strategies

How can Science and Technology contribute to the reduction and alleviation of the global burden of disease (GBD), now subject to better measurement and advance health and welling being? A promising approach would be through community and inter-community development using alliances within and beyond science.

Inter-Community Nodal (Locality) Approaches

- can provide for a sense of ownership, responsibility and pride
- would allow focus
- encourage replication
- encourage national and international support
- break down barriers
- promote trade
- facilitate small business and local entrepreneurship
- enlarge the world economy and increase investment
- internationalize locality, giving it new opportunities

- draw on telecommunication and information transfer with substantial benefit (eg. www.village-life.com; Honey Bee)
- are a "bottom-up" response to "top-down" trends and empower communities
- identify critical points for intervention

These critical points for intervention may be ones:

- To Unblock (28)
  This is the approach taken up by the Gates Foundation to accelerate human development. Its limitations are that it may not take sufficient account of context, culture, or synergy.

- Where Blocks need to be circumvented
  This is a pragmatic approach and may not always be available. When it is available it may be faster, more affordable and appropriated by communities who want to "get on with it" and solve the problem by going around it or getting rid of it.

- Which allow Catalytic or Multiplicative Intervention
  It is sometimes possible to unleash change and lead to advance by meeting a deeply felt need, or using a populist approach. Music may be a good example (3,14,21,23,24,26,29). Rapid dissemination or propagation of benefit may then be seen.

- Seeking and being alert to *new paradigms* can provide opportunities for overcoming intractable problems and obstacles. It is sometimes worth taking stock of how amenable one is to new ways of thinking, which may be radical at the time, but soon become sensible and established (perhaps needing another challenge or paradigm shift).

In Conclusion

By analytically, constructively and systemically addressing the key health issues confronting all peoples, the sciences can collectively, better than separately, advance health and well-being.

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SUSTAINABLE DEVELOPMENT, SCIENTIFIC DEVELOPMENT AND THE DEVELOPMENT OF PEASANT FARMERS

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A plenary lecture given at the IUBS General Assembly on 18 January 2004, in Cairo, Egypt.

In reviewing the role of small peasant farmers in the dynamics of sustainable development, we must first of all consider their paradoxical situation at the beginning of the 21st Century

For my part, I would draw attention to eight paradoxes in the agricultural world, which make it difficult either to grasp the distinctive features of the peasant population or to turn it into a front line force for sustainable development.

The first paradox is the success of agriculture in the North in its race to modernise.

The second paradox is the urbanisation of agriculture.

The third paradox is the separateness, even the marginalisation of the agricultural world, in developed societies as in developing societies.

The fourth paradox is the small peasant farmer himself, who remains a mythical figure with which agriculture likes to be identified, even though he has been driven out of agriculture.

As to the agrarian paradox, it has to do with the emergence of very powerful agricultural concerns, in the very places where agrarian societies have disappeared.

The sixth paradox is the appeal to nature combined with the increasingly violent shift away from the natural order.

The seventh and eighth paradoxes are the agricultural sector's relationship to the state and the way in which the sector is represented.

An overall view of these paradoxes, all linked to the question of scientific development as applied to agriculture and to the competitive situation in which small peasant farmers find themselves world-wide, sheds light on the potentially complex and ambiguous relationship between scientific development and the future of farming populations.

The paradox of success

The modernisation of French agriculture, begun immediately after the end of the Second World War, achieved a degree of success to which it is difficult to find any parallel in our history.

Whereas a farmer had difficulty in providing for the needs of his family barely a century ago, he can now feed more than 100 of his fellow men[1[1]]. Equally, while France's food sector was in deficit at the end of the Second World War, France has now become a world leader in agriculture and agri-food, so much so that disposing of its surpluses is now a structural problem.

Such is the scale of this problem that it appears, paradoxically, to be paralysing the agricultural sector. There is clearly no question of continuing on the same track: "feeding the world" (having fed France and Europe) would be the ruination of peasant farming in the countries of the South, which is contrary to the wishes of the public and is now forbidden under the rules of international trade. On the other hand, French society is exhibiting new expectations (in terms of food quality, protection of the environment, balanced development of territory, production of non-financial public goods, etc), which open up new horizons for farmers but which the latter have difficulty in accepting, possibly because they are too far removed from the ones that have beckoned for half a century.

Unable to pursue a strategy that has hitherto enjoyed unprecedented success, farmers have run out of options. They seem to be victims of their own success – not the least of the paradoxes in a sector that, in modernising its practice, sought to free itself from material and cultural shackles it had inherited from the past.

The paradox of urban agriculture

If there is one point that nobody appears to dispute it is that the rural world is the ideal location – perhaps to the exclusion of all others – for agricultural production.

However, a far from negligible part of agricultural activity is carried out in an urban setting. It may be that former rural areas have been absorbed by the city with the spread of the urban fringe or that producers have themselves set up in urban areas, like poultry or pig farmers, who are attracted by port areas, where they can find feed and outlets.

The "urbanisation" of agriculture, though not immense, is a significant phenomenon. At the end of the 20th century, agricultural production involved 4 % of the workforce in urban areas compared with 8.5 % in the countryside. It is also a widespread phenomenon, found in other industrialised countries and in emerging countries such as China and Brazil.

As agriculture shifts increasingly to the urban environment, it loses ground in the countryside. Outside isolated rural areas, agricultural production has become a residual productive activity in the French countryside. At the end of the nineties, 8.5% of the rural workforce were employed in agriculture as opposed to 25 % in industry and 45 % in services. Only in two departments, Gers and Lozère, is the agricultural workforce still larger than the one employed in industry.

[1[1]] Paul Bairoch estimates that, between 1950 and 1995, agricultural productivity (net productivity of agricul-tural activity) increased eightfold in the developed countries as a whole, "i.e. 1.6 times more than in the fifteen decades prior to the Second World War and therefore at least as much as, if not more than it did over the 8-9 millennia between the "invention" of agriculture and the Second World War."

French agriculture is therefore developing in towns and diminishing in the countryside; a genuine paradox at a time when the agricultural sector seeks to assume the stewardship and so maintain the vitality of our countryside!

The paradox of separateness

French farmers are only too willing to draw attention to the special features of their profession. Agriculture is indeed distinguished from other economic activities in a great many respects, and notably by its close links with the living world, the climate and the environment. The fact is that in a modern society like France, where the social body has been replaced with a series of juxtaposed communities, the claim to be a separate case has paradoxically become all too commonplace. Farmers are admittedly unlike any other minority social group: they alone have gone from being the overall majority to being one minority among others within less than a hundred years. However, in a context where all or most minorities in French society are demanding special consideration and status, the claims of farmers are all but lost in the general chorus, which in turn produces an uncomfortable sense of "not being recognised".

The claim by the agricultural profession is all the more paradoxical in that the move towards modernisation launched in the sixties was prompted by a desire to make farming a profession like any other and thereby achieve parity with other walks of life. This view involves an element of ambiguity, even a contradiction, which is by no means irrelevant to the problems now being experienced by the agricultural world.

The paradox of the peasant farmer

In the process of modernising, French agricultural producers have abandoned the status of "peasant" – an inherited condition, most often one that was simply endured – to become farmers and thereby accede to a profession that carries with it a genuine professional status. As a result, farming has become like any other activity and the farmer has become one professional among others: another link in the agri-food chain.

As a result of this trend, many farmers are now experiencing a sense of nostalgia for their old identity and, although firmly committed to modernity in most cases, they are paradoxically reclaiming the title of peasant.

At the same time, a number of them – often the same ones – see themselves as heads of enterprises, responsible for the means of production and for ensuring its sustainability.

Given this state of affairs, it is not surprising that, according to surveys, the public have a rather wrong-headed, black-and-white notion of the French agricultural scene, comprising the large-scale cereal growers of La Beauce on the one hand and the small sheep farmers of Larzac on the other.

The agrarian paradox

The modernisation of French agriculture has led to a drastic fall in the agricultural population and a radical restructuring of the social fabric, woven around rural life over thousands of years. So France, like most of the

developed countries, has paradoxically become a major agricultural producer by ceasing to be an agrarian society.

Some countries attempt to resolve this contradiction by trying to increase the productivity of their agricultural sectors without completely restructuring their rural societies. They include India with its “green revolution” plan and more recently China and Vietnam. Although they have enjoyed some success, with an notable improvement in their food situation despite very high population growth, many peasant farmers have been very hard hit by these transformations. At the end of the twentieth century, for example, there were more than 100 million "casual farm workers" in China and the number of Indian peasants living below the poverty line continues to increase.

We are therefore a long way from a solution to the agrarian paradox; the problem is particularly serious given that half the world’s population lives in a rural environment and that agriculture (the main activity in rural areas) is increasingly falling prey to a form of globalisation essentially based on the principle of comparative advantages.

The paradox of nature

Relations between the world of agriculture and the natural or living world are among the principal paradoxes – or ambiguities – in this area of activity.

With the extensive urbanisation of our society, farmers have set themselves up as the guardians of nature and upholders of a relationship with the environment characteristic of the old agrarian society. But this relationship has not been spared by the rapid modernisation of our agriculture. Pollution of groundwater, degradation of soils and disappearance of hedgerows vividly demonstrate that the “eternal order of the fields” is no more. For half a century, farmers have been engaged in another kind of relationship with nature: one that is modern and utilitarian, in which nature is treated as something outside human concern. Unwilling to admit as much, they have maintained a smokescreen of ambiguity, which public opinion is gradually discovering and which it strongly condemns..

The ambiguities and contradictions are just as great in regard to the living world. The link with the living world – notably through livestock and plants – is one of the special features farmers strongly emphasise . The fact is, however, that modernisation has broken this link: domestic animals have become mere factors in the pursuit of profit, weighed, numbered and methodically processed. Their conception (by artificial insemination) and their death (in industrial abattoirs) have acquired an abstract, secret quality for the vast majority of consumers. To a lesser degree, plant production is going the same way: improvements in seeds (not to mention GMOs) and increasing recourse to irrigation and synthetic fertilisers are making crop cycles more and more artificial. The agricultural world has difficulty in coming to terms with these changes (especially as they are at once sought after and denounced), and they produce a sense of disquiet, which public relations campaigns fail to allay.

The paradox of the State

Agriculture is, by its nature, a matter of national concern, notably because food sovereignty – i.e. the capacity of a given political entity to guarantee

food autonomy through production or trade – is the basis of political sovereignty. Hence the importance of agricultural policy, whether national or European.

Farmers, however, have a sense of the autonomy of their calling, which they often find difficult to reconcile with government intervention. The result is an ambiguous – and in a sense paradoxical – relationship, prompting farmers to demand more intervention when the state is not involved and greater discretion when it is too closely involved.

The paradox of disproportionate political representation

Whereas farmers – in the strict sense of the word – represent a small part of the electorate (about 3%), they still have considerable weight in French political life. The procession of politicians – President of the Republic in the lead – through the agriculture fair is evidence of this fact, as is the importance attributed by France to agricultural issues in multilateral and European negotiations.

This paradox can be explained objectively and rationally. In the first place there is the weight of the agri-food sector (of which agriculture is the cornerstone) in our economy and foreign trade. There is also the fact that agricultural activity meets such strategic and essential objectives as the provision of food and land use. Lastly, there are dozens of parliamentary constituencies where farmers, retired agricultural workers and their families are of crucial electoral importance.

These are relevant points but they fail to explain fully why the world of agriculture is over-represented politically. Other explanations need to be sought. These would probably include some sense of debt – a mixture of guilt and nostalgia – on the part of our urban society towards a profession that was once the once the basis of our economy and has now been reduced almost to nothing in the name of modernity and progress.

By forcing the pace of modernisation**,** French agriculture has become one of the most efficient in the world. Such a position has not, however, been won without adverse effects, either on the agricultural world or on French society. Long masked by the success enjoyed by agriculture, the problems stored up are now manifesting themselves in the form of a malaise, which, as time goes by, threatens to grow into a major crisis. In order to remedy this state of affairs, serious analysis and collective review are necessary. These eight paradoxes call for a fundamental debate that has been put off for far too long.

TOWARDS AN INTEGRATIVE BIOLOGY (TAIB) SYMPOSIA In 2003

Three Symposia were held within the framework of the IUBS TAIB Programme in 2003.
The first symposium focused on **"Stress Proteins in Natural Environment and Populations"** was held during the "1st International Congress on Stress Responses in Biology and Medicine" held in September, 2003 at Quebec , Canada.

The 2nd symposium focusing on "**The Biology of Learning and Education**" was convened by Professor Motonori Hoshi, on 25-27 November, 2003, at Shonan Village, Hayama, Japan.
Its rationale stems from the premise that human beings are facing various kinds of problems in education, and that so far, most efforts towards solving them have been undertaken from a purely pedagogical perspective. Although the traditional pedagogical approach is important, it is apparent that a more biological approach must be incorporated into the discussion on the problem of education. Learning and education are closely related to the processes of brain development and function. Recent progress in brain science, developmental cognitive neurosciences, neuroethology and informatics should be reflected in the improvement of learning and teaching systems. This symposium discussed the learning process in various biological systems, from procaryotes to vertebrates, from the viewpoints of evolutionary biology and comparative neuroethology. Secondly, it will focus on the biological mechanisms of learning in the human brain from the viewpoints of brain science and developmental cognitive neurosciences. Thirdly, it will investigate possible applications of informatics and information technology to learning and educational systems. And finally, it will try to establish a new and more comprehensive concept of education and find a way to construct life-time systems for better learning and education.

The 3rd TAIB Symposium, focusing on "**Biological Significance of Stochastic Events**" was held on 28-30 November, 2003, at Shonan Village, Hayama, Japan. Participants examined the biological significance of stochastic events that are well systematized in a one level higher integron. Such phenomena can be studied using the one molecule observation technology developed mainly by Toshio Yanagida (e.g. myosin movement). Similar mechanisms seem to work in various biological systems, from cells to ecosystems.

Stress Proteins in Natural Environment and Populations

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Studies on the cellular response to conditions of physical/chemical stress have played a very significant role in diverse areas of Biology, ranging from studies on protein structure, protein-folding, regulation of gene activity, cell survival, to issues in immunology, developmental biology, ecology and evolution and of course, in view of their wide implications, stress proteins and their biology has found considerable application in agriculture and health sciences as well. The current scientific interest in the cellular response to stress was initiated about four decades ago when specific puffs in polytene nuclei of *Drosophila* larvae were seen to be induced following brief exposure to elevated temperature; later observations correlated these changes in puffing (gene) activity with synthesis of a new set of proteins, the heat shock proteins, in the stressed cells.

A large proportion of studies on stress proteins and their genes has centered round their structure, expression and function. Most of these experimental studies have remained confined to a few model systems maintained under constant laboratory conditions. The mass of such laboratory studies has to some extent masked the role of the stress proteins in the context of evolution and ecology. Considering the remarkable conservation of the stress responses in all living organisms, it is obvious that the genetic programs that govern the stress responses have played very significant roles in adaptation of the different organisms to the varying environmental conditions and consequently their evolution. It is necessary therefore, that those working on the structure, function and regulation of stress proteins interact with those interested in ecological and evolutionary extensions of stress response and stress proteins. With a view to promote such an interaction, the IUBS sponsored a special symposium on “Stress Proteins in Natural Environment and Populations” during the “1st International Congress on Stress Responses in Biology and Medicine” held at Quebec, Canada in September 2003. The following four papers are based on the presentations made at this symposium. As these papers reveal, integration and complementation of data obtained with laboratory experimentation with those in nature provide deep insights into the biological significance of one of the most highly conserved cellular response and which has shaped the evolution and diversification of living systems so profoundly.

How and Why the Stress Response Evolves

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A major unresolved question in evolutionary biology is: how does small-scale genetic and phenotypic variation among members of a population arise and culminate in large-scale genomic and phenomic variations among species and higher taxa? In terms of the heat-shock response, this question becomes: how does small-scale variation in the threshold, magnitude, and kinetics of heat-shock protein expression arise within a population, how is this variation encoded at the level of the nucleotide, and how does this variation give rise to the dramatic large-scale variation in threshold, magnitude, and kinetics of heat-shock protein expression among different kinds of organisms?

First, although the heat-shock response is among the most ancient and highly-conserved features of living things, it does vary within and among populations, species, and higher taxa. Feder and Hofmann (Feder 1999; Feder and Hofmann 1999) have characterized the major axes of variation. Typically the variation is "countergradient". In terms of microenvironmental temperature, for example, the thermal threshold for heat-shock protein (Hsp) expression is correlated with the temperatures that organisms normally encounter, and the magnitude of Hsp expression is correlated with the variability of the thermal environment.

The genetic encoding of this variation might reside at several locations in the genome:

- *Instability of the proteome upstream of the heat-shock response*: The heat-shock response can initiate when heat or other stresses cause pre-existing proteins to lose their native conformation. If evolution affects the sequences of the genes encoding these pre-existing proteins such that these proteins resist stress-induced deformation or denaturation at species-appropriate temperatures, then one outcome could be countergradient variation in threshold temperature.
- *Variation in the hsp coding sequence*: Non-synonymous change in the coding sequence of *hsp* genes could affect the structure and function of Hsps, and thereby the cell's requirement for Hsps. For example, lesser levels of an Hsp might be required if it is highly efficient in deterring protein aggregation and/or promoting protein refolding after stress.
- *Variation in* hsp *gene copy number*: Gene duplication is a major feature of molecular evolution in general and of many *hsp* genes. In opposition, duplicated *hsp* genes can become pseudogenes. Variation in the difference of birth and death rates for *hsp* genes can in principle generate variation in Hsp levels and specialization in the function of Hsps.
- *Variation in* cis*-regulatory elements*: The nucleotide sequence of the proximal promoter and untranslated regions can affect the magnitude of *hsp* gene expression. Although additional *cis*-regulatory elements (e.g., enhancers) have seldom been studied in the context of heat shock, they presumably could have an impact on *hsp* gene expression. Variation in any such sequence could result in evolutionary change in Hsp levels.

- *Variation in* trans-*regulatory factors*: The various transcription factors whose binding helps activate the heat-shock response could themselves evolve in their trans-activation domains or in their own regulatory domains, both *cis* and *trans*. Similarly, any or all of the components of the kinase-based signaling pathways that co-regulate the heat-shock response could evolve, as could any of the proteins that down-regulate or terminate an ongoing heat-shock response.
- *Variation in co-chaperones and co-factors*: Many heat-shock proteins do not function as monomers or as homo-oligomers, but as components of a chaperone machinery comprising many proteins. Even if the principal chaperones themselves do not evolve, the heat-shock response could vary via co-chaperones, nucleotide exchange factors, and other interacting proteins.
- *Variation in chaperone targets*: The number and sequence of the sites at which Hsps recognize and interact with their client proteins could themselves be targets of evolutionary change.

These mechanisms are obviously not mutually exclusive. The allocation of regulatory and evolutionary control among these mechanisms is largely unknown, although evidence is abundant that each could contribute to the overall magnitude, threshold, and/or kinetics of the heat-shock response (and their variation among species). Ascertaining this allocation is largely a challenge for the future.

To address this challenge, my laboratory has begun to investigate many of the foregoing mechanisms in the Hsp70 family of *Drosophila*. The background for this investigation is that *hsp70* family genes are pleiotropic (i.e., they have many phenotypes) and that these phenotypes can conflict. One phenotype, inducible tolerance of stress, benefits from massive expression of Hsp70; hence natural selection for inducible thermotolerance should increase Hsp70 levels. Other phenotypes include roles in the regulation of cell cycle, apoptosis, transcription factors, cell signaling, and membrane conductance. These roles necessitate stringent regulation of Hsp70 levels, and unregulated, massive, or prolonged expression of Hsp70 can be harmful. In the absence of a need for inducible thermotolerance, natural selection should favor low and carefully regulated expression of Hsp70 protein. Thus, within and among natural and laboratory populations of *D. melanogaster*, a pattern is evident: Where *Drosophila* undergo extreme stress, against which Hsp70 is protective, Hsp70 achieves high levels upon heat-shock. Where *Drosophila* undergo mild hyperthermia chronically (which ordinarily induces low levels of Hsp70) but seldom experiences the extreme stress against which Hsp70 is protective, Hsp70 achieves much lower levels upon heat shock. Krebs and Feder (1997) posited that this pattern is an outcome of an evolutionary trade-off of the conflicting functions of Hsp70. Since then, this hypothesis received much support from multiple laboratories.

What specific nucleotides have undergone evolutionary modification to result in these patterns? The *hsp70* genes have undergone both duplication (increasing to as many as 7 copies in some strains of *Drosophila virilis* [see accompanying report of Michael Evgen'ev in this issue]) and degeneration. In the *Drosophila melanogaster* populations that vary in Hsp70 protein levels (see above), however, the copy number is fixed at 5 (Feder and Hofmann 1999; Bettencourt and Feder 2001). Thus, copy number variation is not necessary for variation in protein level in this species. These 5 genes

are in two clusters (*hsp70Aa* and *Ab* at locus 87A7, and *hsp70Ba*, *Bb* and *Bc* at locus 87C1). In the *Drosophila melanogaster* populations that vary in Hsp70 protein levels, *hsp70* coding sequence is remarkably conserved. For example, *hsp70Aa* and *Ab*, which have been distinct genes for >100 million years, exhibit no fixed differences in nucleotide sequence (Bettencourt and Feder 2002). These findings suggest that genetic explanations for variation in Hsp70 protein levels in *D. melanogaster* lie outside the number and sequence of *hsp70* coding regions.

In the non-coding sequence of the *hsp70* genes of *D. melanogaster*, we have discovered abundant variation among populations. The untranslated regions, which encode hsp70 mRNA stability, harbor numerous polymorphisms. These polymorphisms are shared both among *hsp70* clustermates and between *hsp70* clusters, which greatly complicates the discovery of the functional significance of these polymorphisms, if any. The proximal promoters also exhibit numerous polymorphisms, the functional significance of many of those is still undefined. Some polymorphisms in the proximal promoter, by contrast, are striking in their recurrence, environmental correlates, and phenotypes. The background for this finding is that the spacing of heat-shock response elements (HSEs) in the *hsp70* proximal promoter is crucial for full-strength expression of these genes. We have discovered numerous instances of mobile genetic elements (i.e., transposable elements or transposons) that have inserted between HSEs, putatively disrupting the spacing of the HSEs. Findings include: (1) the multiple insertions of tranposable elements are independent events; (2) transposon-bearing alleles segregate in natural populations; (3) frequencies of these alleles vary along climatic gradients; (4) when transposons are present, they reduce *hsp70* transcription and Hsp70 protein levels, and affect thermotolerance and fitness; and (5) the reduction in *hsp70* transcription is due to alterations in the spacing of heat-shock response elements in the proximal promoter rather than an explicit regulatory function of the transposon. We suggest that transposons create variation in Hsp70 expression on which natural selection acts (Michalak et al. 2001; Zatsepina et al. 2001; Bettencourt et al. 2002; Lerman et al. 2003).

Finally, we have surveyed sequence variation in *D. melanogaster* and a close relative (*D. simulans*) for 6 genes encoding proteins with which Hsp70 interacts: HSF, HSBP1, CHIP, Hop, Hip, and the *Drosophila* DnaJ homolog. *HSF*, *HSBP1*, *CHIP*, and *HOP* display a low level of sequence divergence. While the first 4 diverge little among species, the DnaJ homolog-endcoding and *HIP* genes of *D. melanogaster* differ from their *D. simulans* homologs by significant excesses of amino acid replacements and deletions. Numerous sites in these latter two genes may be undergoing positive selection (Bettencourt 2001).

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Functional Genomics of Stress Responses in Cyanobacteria

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Introduction

Environment is fast becoming increasingly stressful. Studies on responses of microbes and plants to environmental stressors, such as salinity drought, heat etc, form a thrust area of contemporary biological research. Compared to microbes, the plants are rather difficult to study and the bulk of information that we have today on stress responses of plants to abiotic stresses generally arose from studies on microbial systems, such as *Escherichia coli* or yeast (Serrano and Gaxiola, 1994). However, such heterotrophs do not have much in common with the photoautotrophic plants. Apart from very different habits, these microbes do not harbor vital processes like photosynthesis or nitrogen fixation. Plants, therefore, display a much higher sensitivity to environmental stresses. In this context cyanobacteria, which combine the prokaryotic structure and function with desirable plant activities like photosynthesis and nitrogen fixation, have emerged as a suitable study system (Apte, 2001).

Cyanobacteria, formerly called the blue-green algae, are a group of photosynthetic prokaryotes, which evolved nearly 3 billion years ago (Brock, 1973) and have survived practically every natural and man-made stress on this planet. Today, they are ubiquitous in distribution and are found from fresh water bodies to arid deserts, from frigid lakes of the Antarctic to hot springs and from agricultural habitats to oceans (Fogg et al, 1973). Many cyanobacteria fix atmospheric nitrogen and are naturally abundant natives of tropical paddy fields. As biofertilizers of nitrogen they are known to contribute appreciably to the nitrogen economy of rice cultivation in China, India and South East Asia (Venkataraman, 1979). Cyanobacteria exhibit a close phylogenetic relationship with plant chloroplasts, which are believed to have evolved through endosymbiosis of “cynobacteria-like cells” in eukaryotic cells. Therefore, cyanobacteria are regarded as most appropriate model systems for studying plant responses to stress (Joset et al, 1996, Apte, 2001). Our laboratory has been studying the cellular and molecular basis of the responses of nitrogen-fixing *Anabaena* strains to major agricultural stresses (reviewed in Apte, 2001). This article presents highlights of our recent findings, using a functional genomics approach, on adaptation and tolerance of cyanobacteria to nutrient deficiency (carbon, nitrogen and potassium) and high temperature.

Response of Anabaena *strains to carbon and nitrogen deficiency*

The filamentous heterocystous cyanobacteria, such as *Anabaena* and *Nostoc*, are photoautrophic diazotrophs capable of both carbon and nitrogen fixation. This self-sufficiency distinguishes cyanobacteria from heterotrophs, which operate elaborate genetic and biochemical mechanisms to face carbon starvation (Hengge-Aronis, 1993). Deficiency of combined nitrogen, however, induces *Anabaena* filaments to differentiate and develop specialized cells called heterocysts, wherein the conversion of atmospheric N_2 to NH_3 is carried out in a micro-aerophilic environment using solar energy trapped in photosynthesis (Stewart, 1980). Differentiation of heterocysts is accompanied by a large number of structural and functional

alterations (Haselkorn, 1992; Wolk, 1991). A major event in heterocyst development is the rearrangement of two *nif* operons encoding the nitrogen fixation genes (Golden et al, 1985). Our recent data show that carbon starvation interferes with the rearrangement and expression of *nif* genes resulting in the loss of nitrogen fixation and cessation of diazotrophic growth (Apte, unpublished results). No major changes in gene expression or protein synthesis are, however, observed in carbon-starved *Anabaena*, in sharp contrast to *E. coli* (Apte et al, 1998).

Response of *Anabaena* strains to potassium deficiency

An interesting recent finding of our laboratory relates to the profound effects of potassium starvation on the growth and metabolism of *Anabaena* strains. Cells subjected to K^+-deficiency show pleiotropic defects, such as loss of turgor, photosynthesis, expression and activity of nitrogenase and inhibition of growth (Alahari and Apte, 1998). The impairment caused by K^+ depletion is *bacteriostatic* and growth resumes within hours of K^+ repletion. Detailed analysis of cyanobacterial responses to potassium starvation has revealed the existence of a novel potassium deficiency-induced stimulon (PDI-stimulon), comprising genes whose expression is induced by K^+-deficiency and further enhanced by osmotic stress (Alahari and Apte, 2004). Addition of potassium to K^+-deficient *Anabaena* cultures switches-off the expression of genes of the PDI-stimulon. Based on their regulation by potassium, the proteins encoded by the genes of this stimulon have been termed as potassium deficiency proteins (PDPs). Our results show that PDI-stimulons are not unique to *Anabaena* and may be widespread among bacteria (Alahari and Apte, 2004).

Identity of the PDPs from *Anabaena*

Using proteomic and other approaches, we have identified several of the PDPs in *Anabaena*. Prominent among these are several genes/proteins/pigments, which constitute the two photosystems (PS-I and PS-II), enzymes involved in scavenging of free radicals, and major cellular chaperones (Apte, unpublished results). The kinetics of the proteomic changes observed suggest that in response to K^+-deficiency, cells enhance their capabilities to trap and generate additional photosynthetic energy, and synthesize chaperones to maintain homeostasis against the prevalent protein denaturation. Long-term K^+ deficiency, however, results in carbon and nitrogen starvation and depletes levels of all the proteins (Alahari and Apte, 1998). Western blotting and immunodetection also identified some of the proteins regulated under K^+-deficiency. Prominent among the up-regulated genes/proteins are the GroEL and KdpB proteins while the dinitrogenase reductase (NifH) is down-regulated or not synthesized The reduced levels of NifH protein explain the loss of nitrogenase activity and resultant N starvation under K^+-deficiency (Alahari and Apte, 1998). *De novo* synthesis of the KdpB, a major constituent of the bacterial membrane-bound potassium-dependent ATPase (KdpATPase), supports the view that cells attempt to scavenge trace levels of K^+ that may be available as contaminant in the medium (Alahari et al, 2001).

Regulation of PDI stimulon in *Anabaena*

The other interesting question relates to how the potassium deficiency is sensed in *Anabaena* and results in the expression of multiple genes of the PDI stimulon. The observed regulation of PDI stimulon is strongly reminiscent of the regulation of bacterial *kdp* operons, which are up-regulated by the dual signals of K^+ starvation and hyperosmotic stress and repressed by K^+ supplementation (Altendorf and Epstein, 1993). In *E. coli*, a two component signal transduction system consisting of a 900 amino acid long membrane-bound sensor kinase (KdpD) and a cytosolic transcriptional factor (KdpE) regulates the *kdp* operon. We have cloned and characterized the *kdp* operon in *Anabaena* to reveal several interesting novel features (GenBank Accession No. AF213466). The most striking of them is the absence of *kdpE* gene and occurrence of a short/truncated version of *kdpD* that can code only for a 400 amino acid long KdpD protein. These unusual features make the regulation of *kdp* operon in *Anabaena* somewhat enigmatic. The truncated KdpD of *Anabaena* shows properties similar to the N-terminal half of *E. coli* KdpD. A chimeric fusion protein AnacoliKdpD, constructed by joining the *Anabaena* KdpD with the transmembrane domains and C-terminal half of the *E. coli* KdpD, performs all the expected functions of the wild-type KdpD of *E. coli* (Ballal et al, 2002). These data suggest that the *Anabaena* KdpD may work with a, as yet unknown, partner to facilitate *kdp* expression. Search for the "missing partner" continues as also the exploration into the possibility that this novel KdpD protein (and its allies) may regulate the PDP-stimulon in *Anabaena*.

Response of *Anabaena* strains to temperature upshift

Exposure of *Anabaena* cells to high temperature induces a heat-shock response typical of other bacteria in terms of the number and molecular mass of the heat-shock proteins (HSPs) synthesized. One of the most abundant cyanobacterial HSP is the GroEL or Hsp60 (Bhagwat and Apte, !989). Antisera raised against the GroEL of *E. coli* or *Bacillus subtilis* identify at least two GroEL-like polypeptides of 58kDa and 60kDa in *Anabaena* (Apte et al, 1998). These are expressed under a variety of stress conditions in addition to heat-shock and appear to be differentially regulated by heat and osmotic stresses. Pre-exposure to mild heat-shock has been found to improve the osmotolerance of *Anabaena* cells (Rajaram and Apte, unpublished results). The GroEL proteins, therefore appear to function more like general stress proteins and contribute to multiple stress tolerance in *Anabaena*.

We have cloned the major *groE* operon from *Anabaena* (GenBank Accession No. AF324500) and shown it to encode a bi-cistronic transcript corresponding to the *groES* and *groEL* genes. This operon has been found to be strongly heat-inducible, as is the 58kDa immuno-crossreactive GroEL polypeptide (Rajaram et al, 2001). The nucleotide sequence analysis of the corresponding promoter displays a CIRCE element encompassing the –10 region, typical of *B. subtilis*. The operon is, therefore, likely to be negatively regulated by an HrcA-like repressor (Mogk et al, 1997). Upstream of CIRCE element are also found several direct and indirect repeats which may serve as the *cis*-regulatory elements responsible for expression of this gene under other stresses, but this remains to be tested (Rajaram et al, 2001). Recently, we have also cloned a second *groEL*-like gene, *cpn60*, from *Anabaena* (GenBank Accession No. AY 328922). This is likely to encode the 60kDa

GroEL polypeptide. Evaluation of relative contribution of the two *groEL* genes to general stress tolerance in *Anabaena* is in progress.

Compared to *E. coli*, *Anabaena* exhibits much superior thermotolerance in terms of the continuous long-term heat stress that it can endure and in terms of its revival from prolonged heat stress (Rajaram and Apte, 2003). This ability seems to be consequent to continuous synthesis, long stability and accumulation of HSPs, particularly GroEL, in heat-stressed *Anabaena* cells. Equally important are the contributions of the GroEL and other heat-shock proteins in cross-protection against other environmental stresses. Regulation of heat-shock response by stresses other then heat is an interesting area of current investigation.

Our recent data emphasize the role of potassium in regulating stress-responsive gene expression and that of major cellular chaperones like GroES/EL in mediating the general stress tolerance of cyanobacteria. While the role of heat-shock proteins in general stress tolerance of bacteria has been known, the regulation of a variety of cyanobacterial stress responses by K^+ is a novelty. The pleiotropic effects of potassium deficiency are reminiscent of the stationary phase-induced stress responses in bacteria subjected to carbon starvation and need to be explored further. A functional genomic and proteomic analysis in future would elucidate the (a) precise role(s) played by K^+ in regulation of PDI-stimulon and stress responses (b) signal transduction linked to sensing K^+ deficiency and evoking PDP expression, and (c) molecular protection conferred by the GroEL like chaperones in cells exposed to stressful environments.

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Thermal Tolerance and Stress Proteins in the Crustacean *Artemia* from Different Habitats

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Artemia, the brine shrimp, is an animal extremophile in the sense that it lives, and often thrives, in environments in which few (or no) other animals exist. The impressive biological success of *Artemia* depends in part on massive amounts of a small heat shock protein (p26) synthesized during embryonic development. Unlike the commonly observed stress response, p26 synthesis is not induced by stress, *per se*, but is strictly programmed into early development. These p26-loaded gastrula embryos are then able to pre-meditate, so to speak, the variety of stresses they will encounter later, based on their evolutionary history. These stresses include severe dehydration, high levels of UV and other forms of radiation and anoxia for periods of years while fully hydrated and at physiological temperatures. Albeit scanty, data from populations ranging from the high plateau of Tibet to hypersaline ponds in the tropics indicate that the amount of p26 made during early development is related to the temperature of the habitat of that particular *Artemia* population. Access to the massive literature on *Artemia*, including that relevant to this brief paper, is available in the recent book by Abatzopoulos *et al.* (2002).

The stress response and the Vietnam experiment

Current research on the stress response is aimed chiefly at understanding the cellular and molecular basis of heat shock protein structure and function. In contrast, the evolutionary and ecological aspects have not been given much attention until recently. I believe that *Artemia* can be used to great advantage in that regard (Clegg and Trotman 2002) an opinion that motivated the present studies, a summary of which is presented here.

Most experimental research on *Artemia* has used *A. Franciscans*, which occurs naturally, and primarily in the Great Salt Lake, Utah, and in salterns located in the San Francisco Bay (SFB), although many other populations exist, some of them due to human intervention. For example, since 1987 *A. franciscana* has been grown on a seasonal basis during the dry season in southern Vietnam, a location at which *Artemia* does not occur naturally. Ambient temperatures are much higher in these ponds compared to the SFB salterns (perhaps by 20 ^{o}C, on average, in the dry season). Thus, it seemed worthwhile to compare embryos from the SFB with those produced in Vietnam to determine whether the latter had acquired enhanced resistance to higher temperatures and, if so, to evaluate how rapidly that adaptation took place. In what I have called the "Vietnam experiment", we compared the thermal tolerance and stress proteins of embryos produced by sequential inoculation of SFB encysted embryos in the Vietnam culture ponds (V) during the years 1996, 1997 and 1998. The results showed that a single growing season (and probably much less) was enough to significantly increase the thermal tolerance of the V embryos over those of the SFB inoculum (Clegg *et al.* 2000 and 2001). Using western immunoblotting we also showed in those studies that this increased thermal tolerance was

reflected in increased levels of p26 (as well as artemin and Hsp70). The ability of *A. franciscana* (SFB) to adapt rapidly to the much higher environmental temperatures in southern Vietnam has now been well-documented. We conclude that this adaptation involves genetic selection because adults raised in the laboratory at about 22 ^{o}C from Vietnam-grown embryos retained a higher level of thermal resistance compared to adults raised in the laboratory from SFB embryos, both raised at the same time and under the same conditions (Clegg *et al.* 2001). These findings from the field and laboratory support the view that *A. franciscana* is extremely versatile in its ability to adapt to diverse thermal environments (Clegg and Trotman 2002).

Artemia tibetiana – high altitude brine shrimp

Encysted embryos of *A. tibetiana* were originally collected by Professor Mianping Zheng**,** National Academy of Geological Sciences, PR China, from hypersaline Lagkor Lake located on the high plateau of Tibet (84^{o} E, 32^{o} 03' N) at close to 4,500 m elevation where the average yearly water temperature is 1-2 ^{o}C. We obtained some of these embryos and found that their thermal lability was striking, with over 50 % being killed during a 40 min transition from 22.0 to 50.0^{o}C. In contrast, those same conditions killed only a few of the *A. franciscana* embryos (Clegg *et al*. 2001). Western immunoblotting revealed that *A. tibetiana* embryos contained a very low level of p26, only about 10 % of that in *A. franciscana* (V) embryos. Interpretation of these results is not straightforward, and is hampered by the lack of detailed information about the habitat of *A. tibetiana*, especially the thermal conditions prevailing during and prior to the time when the embryos were collected. Nevertheless, it seems clear that the much poorer thermal performance of *A. tibetiana* cysts in the laboratory is related directly to the much colder environment existing at the height of about 4,500 m where they were produced. One can reasonably suppose that *tibetiana* escapes the metabolic cost of making large amounts of p26 for similar reasons. Such an interpretation further supports the conclusion from the Vietnam experiment that p26 plays an important role in the adaptation of *A. franciscana* (SFP) to the much hotter growth ponds in Vietnam where water temperatures can approach 40^{o}C on a daily basis.

Artemia genes in human cells

Interestingly, although p26 appears to be vital to the protection of the encysted embryo, this protein is not present (and has not been induced in spite of efforts to do so) in any other stage of the life cycle. If p26 is such a good stress protein/molecular chaperone, why would not other stages take advantage of that as well? One of many peculiarities of *Artemia* biology is the absence of cell division and DNA synthesis during the development of encysted embryos (gastrulae) into nauplius larvae, at which point both processes resume, while p26 is broken down. Thus, it seemed possible that some sort of incompatibility existed between p26 and cell division/DNA synthesis, thus accounting for the lack of p26 appearance in stages other than the encysted embryo. A plausible way to examine that curious situation arose when transfections of the p26 gene into a human cell line (293H) were carried out in the laboratory of Tom MacRae at Dalhousie University: the transfected cells made DNA and underwent cell division very much like the controls (MacRae and Clegg, unpublished results). Did this result disprove the “incompatibility hypothesis?” Not really, since we found that the level of

p26 expression in the 293H cells was very low (~0.1% of total cell protein) and that the p26 being produced was only about a third of the molecular mass of the p26 produced in *Artemia* embryos. Thus, these studies are not conclusive since the p26 might not be functioning in the 293H transfects as it does in encysted embryos: we have yet to understand this curious situation.

Closing comments

The diversity and abundance of *Artemia* habitats world-wide, and the well-known biology of some of its species provide unique opportunities for the study of adaptation to stressful environments, in nature as well as in the laboratory. I believe that *Artemia* represents an extremely valuable model system for use in the emerging area of *evolutionary and ecological functional genomics*, described so well in the recent perspective by Feder and Mitchell-Olds (2003).

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Evolution of Thermotolerance and Heat-Shock Response in the *virilis* Species Group of *Drosophila*

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All organisms studied so far respond to an elevated external temperature and other stress factors by the synthesis of a certain set of proteins, the so-called heat shock proteins (HSP). Under physiological conditions these proteins and their constitutively expressed cognates play essential roles in modulating protein-protein interactions (Georgpoulos *et al.*,1996). Members of HSPs, often called chaperones participate in the folding, assembly, and translocation of many cellular proteins. Hsp70 family has been implicated in playing a key role in the cellular response to heat shock by maintaining the native state and proper folding of cellular proteins under physiological stress and by facilitating the restoration of certain cellular functions in higher eukaryotes. In recent decades we have performed a systematic broad-scale analysis of the heat shock response in various organisms differing by the temperature of their ecological niches. This analysis includes species of unicellular parasite Leishmania, different species of silk worms and lizards, and several mammalian species (Ulmasov *et al.*, 1992). I was shown for all of These organisms that species that have adapted to high temperature conditions are able to synthetize HSPs and many other cellular proteins at significantly higher temperatures in comparison with closely related forms from moderate climatic zones. Moreover, desert organisms are typically characterized by a significantly higher cellular content of HSP70-like proteins at normal physiological temperature when compared with related species from Central and Northern parts of Europe (Ulmasov *et al.*, 1999). To gain further insight into the evolution of heat shock systems in close species inhabiting strikingly different ecological niches, we have studied the peculiarities of heat shock response at molecular level in the related *Drosophila* species with different average temperatures of environment. For our analysis we have chosen members of the *virilis* group of *Drosophila*, which comprises 12 species divisible into two phylads, viz., *virilis* and *montana* (Patterson and Stone 1952). According to many investigators (Patterson and Stone 1952; Nurminsky et al. 1996), *Drosophila virilis* is the most primitive species of the *virilis* phylad and is probably ancestral to it if not to the entire *virilis* group. Its distribution, which extends across Eurasia, is primarily below 40° N latitude. *Drosophila lummei*, considered the closest relative of *D. virilis*, occurs from just above 40° to just above 65° N latitude and from Sweden east to the Pacific coast of Asia. The most parsimonious interpretation of this information is that *D. lummei* descended from *D. virilis* or a common ancestor approximately 5 MYA (Nurminsky et al. 1996) and invaded higher latitudes than its ancestor. *D. virilis* and *D. lummei* can be crossed under experimental conditions and produce partially fertile progeny. Therefore, this model system, contrary to the other models studied so far, enables conventional genetic analysis to investigate genetic basis of the interspecific differences in the observed

thermotolerance. Besides, we used two other species of the *virilis* group (more xeric *D. novamexicana* and more mesic *D. texana*) in the investigation of thermotolerance. It was shown that although both *D. virilis* and *D. lummei* synthesized heat-shock proteins after temperature elevation, synthesis was typically greater and less sensitive to inhibition at high temperatures in *D. virilis* than in *D. lummei.* For example, *D. lummei* cells synthesize much less protein after 40° C and 40.5° C heat shock than do *D. virilis* cells. After 40.5° C heat shock, *D. lummei* cells synthesize some Hsps, with the Hsp70 family most prominent, whereas *D. virilis* cells synthesize comparatively large amounts of all major *Drosophila* Hsps. Typically little or no protein synthesis was detectable in *D. lummei* cells after heat shock at still higher temperatures at which Hsp synthesis was near-maximal in *D. virilis*. In terms of Hsp70 synthesis, the more xeric *D. novamexicana* likewise showed greater synthesis overall and less sensitivity to inhibition by high temperatures than the more mesic *D. texana* and *D. lummei*. For those species in which multiple geographical strains were examined, the thermal sensitivity of heat-shock protein synthesis bore no clear relationship to the geographic origin of the strains. The thermal sensitivity of hsp70 mRNA transcription in these species in general resembled that for Hsp70 protein. Thus, *D. lummei* synthesized no *hsp70* mRNA during heat shock at 40-41° C, whereas *D. virilis* strains did so in abundance. Furthermore, the investigation of HSF activation in these species using mobility shift assays has demonstrated that *D. virilis* underwent HSF trimerization at temperatures $\geq$ 32°C, whereas *D. lummei* exhibited activation at temperatures $\geq$ 31° C. This difference, although small, is again consistent with the environmental regimes of these species. In the cold, *D. lummei* is far more tolerant than *D. virilis*, and even undergoes a diapause, which is absent in all other *virilis* group species (Lumme 1982). By contrast as shown by us, *D. virilis* displays greater basal and inducible high temperature tolerance than does *D. lummei*.. This is not surprising since in *Drosophila*, the Hsp70 superfamily of molecular chaperones (Feder and Krebs 1998) is a key molecular mechanism underlying high temperature tolerance. Corresponding to their differing temperature tolerances, *D. virilis* expresses both more diverse electromorphs of Hsp70 family members and several-fold more Hsp70-family protein and mRNA than does *D. lummei*, especially at high temperatures (Garbuz *et al.* 2003). Interspecific hybrids are intermediate to the parental species in basal thermotolerance and inducible thermotolerance, and resemble *D. virilis* in Hsp concentrations after intense heat shock and Hsp70 protein electromorphs. At the next step we have examined the genetic basis for these inter- and intra-specific differences in thermotolerance and Hsp70-family proteins. Southern blot analysis has demonstrated that hsp70 copy number varies both within *D. lummei* and *D. virilis* and between these species. The detailed organization of the *hsp70* genes in *D. virilis* strain 160 (Japan) and *D. lummei* strain 200 (Moscow) emerges from sub-cloning and sequencing of overlapping lambda clones isolated from the corresponding genomic libraries. *D. virilis* Strain 160 carries six copies in tandem orientation approximately 3kb apart (*hsp70b - g*), and a seventh copy (*hsp70a*) in inverse orientation. By contrast, *D. lummei* strain 200 has a pair of copies (*hsp70a* and *hsp70b*) in inverse orientation as in *D. virilis*, and two more copies (*hsp70c* and *hsp70d*) in direct orientation with 5.5kb spacing. In both species, the *hsp70a-hsp70b* intergenic region is approximately 0.8kb. It was shown that the lambda clones yielding these data all hybridize to the 29C region of Chromosome II, which we previously identified as the *hsp70* locus of *D. virilis* (Evgen'ev *et al.*, 1978).

From the foregoing data, we conclude that multiple tandem duplications of *hsp70* genes accompanied evolution of the *D. virilis* group, and that changes in *hsp70* copy number accompanied changes in thermotolerance both among *D. virilis* populations and between *D. virilis* and *D. lummei*. Multiple lines of evidence are all consistent with this conclusion: *in situ* hybridization indicating that the *hsp70* locus itself is singular, chromosome puff size corresponding to putative *hsp70* gene copy numbers, genomic Southern hybridizations and quantitative densitometry of restriction fragment polymorphisms, and the coding and flanking sequences of the genes themselves. In order to further prove this supposition it will be necessary to investigate additional species and geographical strains of the *virilis* group and obtain transgenic strains of *D. lummei* carrying extra copies of *hsp70* genes (work in progress).

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TAIB SYMPOSIUM ON "THE BIOLOGY OF LEARNING"

ABSTRACTS

Impact of Biology of Learning on Education

Masao Ito, RIKEN Brain Science Institute

Biology of learning will contribute to improvement of education through acquirement of knowledge about learning mechanisms of the brain. My talk will address the following five topics emerged from recent brain research:
1) Synaptic plasticity as a memory process
2) Critical periods for learning
3) Columnar structure of the cerebral cortex
4) Developmental disorders of the brain
5) Formation of an internal model

Evolution of Learning Mechanisms: General Processes and Modularity

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The surprising or unexpected omission of an appetitive reinforcer has at least two consequences: An allocentric effect according to which the organism updates knowledge about the environment, and an egocentric effect that allows the organism to learn about its own emotional reaction to the change. This egocentric effect (traditionally called frustration) is correlated to activation of the hypothalamic-pituitary-adrenal axis, can be modulated by anxiolytics, and is expressed in terms of behavioral changes that have an emotional component (e.g., agonistic behavior). Furthermore, whereas all vertebrates exhibit allocentric effects (e.g., extinction of acquired behavior), only mammals exhibit the egocentric effect described previously. An understanding of these species differences requires a redefinition of the concept of "mechanism." It is argued that there are at least four levels of mechanistic causation: psychological (e.g., S-S associations), neurobiological (e.g., network architecture in specific brain sites), neurochemical (e.g., synaptic plasticity), and cell-molecular (e.g., secondary messenger systems). A levels view of learning mechanisms suggests that modularity is most useful to understand the parcellation of brain function at the neurobiological level, but synaptic and cell-molecular processes appear to be shared by a wide variety of species. It is hypothesized that the notion of general learning processes captures the conservative nature of synaptic and cell-molecular processes. Based on these ideas, the hypothesis is advanced that frustrative mechanisms evolved in early mammals from those underlying fear conditioning, possibly by gene duplication and co-option. These processes allowed for the evolution of a new modular system dealing with surprising nonreward in emotional, egocentric terms.

Classical Conditioning of the Withdrawal Response in the Earthworm

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I believe that the earthworm offers some of the most promising material for investigation of learning mechanisms. For last 10 years, we have studied the mechanism of the neural networks of this tiny animal from the electrophysiological method, several imaging techniques, and also observation of its behavior. In this lecture, I will demonstrate the interesting findings about the learning and memory of the earthworm.

Habituation to the tactile or scratch stimuli to the body wall. When tactile stimulus is applied to the anterior body part, the earthworm quickly shrinks its body (withdrawal reflex). When repetitive tactile stimuli are applied, the withdrawal reflex will be gradually disappeared (habituation). We found the mechanisms of this withdrawal reflex and its habituation in the ventral nerve cord (VNC) of the earthworm. The semi-intact preparation with the body wall and VNC was prepared, and the tactile stimuli were applied to the body wall. We observed that nitric monoxide (NO) was released from the VNC according to the stimuli, and NO decreased the action potential generation at giant fibers. This result indicates that NO-cGMP signal transduction is concerned in the habituation mechanism.

Classical conditioning of the earthworm. When the pairing of vibration (conditioned stimulus, CS) and light (unconditioned stimulus, US) is repetitively applied to the earthworm, vibration can induce the withdrawal reflex (classical conditioning). We studied the characteristics of this classical conditioning by changing the inter-training intervals with a protein synthesis inhibitor. The earthworm that the inhibitor was injected did not show the conditioning. The inhibition of conditioning was also observed when the animal was incubated at 4 C just after the paring of CS and US. We also developed the visualization technique by a fluorescent dye, FM1-43. This dye labeled the pre-synaptic terminals as activity-dependent manner. In only conditioned animals, bright fluorescent spots were observed in the neruopil between 2^{nd} and 3^{rd} lateral nerves. This region is a candidate of the synaptic plasticity for the classical conditioning of the earthworm.

Cognitive Behavior and its Development in Honeybees

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Aged worker honeybees can learn foraged flowers by shape, color, odor, time of day, and their place. They also discriminate the nest mate and non-nest mate by matching the sensory inputs from intruders to the memorized, rewritable template in the brain. On the other hand, they spend the immature stages in dark and constant temperature condition. The environment is well protected from the outside and foods are provisioned by nurse bees, so that no effort is necessary to escape from enemies, and to find the foods by themselves. As a result, their sensory system is kept undeveloped in whole larval stages. In this situation, when and how do they develop their sophisticated learning and memorizing capability? We used associative learning of proboscis extension reflex (PER) as a merkmal to answer this question.

The learning capability was developed during 5 to 9 days from emergence in workers, while it was accomplished during 2 to 5 days in drones, reflecting a difference of the onset day of flight performances. If the workers were reared individually in a confined condition where odor and other social stimuli from the nest environment are deprived, most of the stimuli-limited bees could not develop the learning ability on day 9 or even later. From a series of experiments subjecting the bees to various length and timings of the limited condition, the period important to acquire the learning ability was from day 2 to 6. However, even in the bees once acquired the ability in hive condition, they lost it by exposing to the limited condition for the following up to 15 days. This means that the sensory inputs are essential for both "acquirement" and "maintenance" of the learning capability.

To localize the brain regions concerning the learning process, we evaluated the volume of subcompartments of mushroom body and other neuropils of the brain using stimuli-limited 9-day-old bees (L) which could not learn, and the stimuli-exposed control bees (C) which could learn. Newly emerged bees (N) and pollen foragers (P) were also subjected. Histological preparations were made using "osmium-azure dichrome stain" method.

In the -lobe were all 9 to 10% -lobe, stimuli-limited group L, volumes of pedunculus, significantly smaller than in C. The calyx was 5 % smaller in L than in C, but it was not statistically significant. All neuropiles of the mushroom body were smallest in N. On the other hand, there was no significant difference between those in F and C, which supports the idea that the learning capability matures through various stimuli experienced within the hive before performing the flight. Volume of the Kenyon cell somal region was 10% smaller in L than in C. This volume of the newly emerged group N was 15% larger than in limited group L. If it reflects the reduced number of the cells, it might show that the stimuli-limited condition caused the apoptosis.

Molecular Basis Underlying Social Behaviors of Honeybees

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The honeybee *Apis mellifera* L. is a social insect, and colony members perform various exquisite communications to maintain colony activities. For example, workers inform the other foragers of information related to both the distance and direction of a food source using dance language. Very little is known, however, regarding the molecular basis of the highly advanced social behaviors.

Mushroom bodies (MBs) are insect brain structures that are important for memory, learning, and higher-order sensory processing for different sensory modalities. Honeybee MB function is believed to be closely associated with their social behaviors for the following reasons: 1) Honeybee MBs are more prominent compared with those of other insects. 2) Honeybee MBs have a high degree of structural plasticity and the volume of the neuropil varies according to the division of labor.

To clarify the molecular basis underlying the honeybee MB neural function, we performed differential display analysis and identified 36 genes preferentially expressed in the honeybee MBs. Five of them encoded components of the Ca^{2+}-signaling pathway, which is involved in neural plasticity. Based on these findings, we proposed that the Ca^{2+}-signaling function and thus the synaptic plasticity of the MB neurons are enhanced in the honeybee brain. We also identified a novel gene, termed *Mblk-1*, encoded a transcription factor with two DNA-binding motifs. Its structure is conserved among the animal kingdom. To estimate *in vivo* function of Mblk-1, we identified its nematode orthologue, *mbr-1* (*Mblk-1 related factor-1*) and revealed the following two points: 1) *mbr-1* is selectively expressed in restricted sets of interneurons, involved in sensory processing, like insect MB intrinsic neurons. 2) *mbr-1* is required for proper neural circuit formation and behavioral plasticity. Thus the *in vivo* function of *Mblk-1/mbr-1* seems to be conserved between the honeybee and nematode.

Most of the identified cDNA clones corresponded to 3'-untranslated regions, and identification of proteins encoded by these genes is now in progress. The MB-preferential genes would be promising candidates for genes involved in neural/ behavioral plasticity and associated with honeybee social behaviors.

Song Learning in Bengalese Finches: From Genes to Society

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No Abstract? ?

"Brain-Science and Education" Based on Functional Brain Imaging

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From the viewpoint of biology, learning and education can be defined as the processes of forming neuronal connections in response to external environmental stimuli, and of controlling or adding appropriate stimuli, respectively. Learning and education can thus be studied as a new field of natural science with the entire human life span as its subject, thus including various social issues such as the fetal environment, childcare, general and special education, second language acquisition, rehabilitation, dementia prevention, and nurturing generosity and warm heartedness.
Noninvasive imaging of higher-order brain functions in humans that have recently been developed, such as optical topography (OT), functional magnetic resonance imaging (fMRI), and magneto-encephalography (MEG), will clarify the brain's developmental processes, and will provide various evidences as the basis for learning and educational sciences.
The new comprehensive field centered on developing the brain, and which we might call brain science and education, will also require higher levels of trans-disciplinarity. People from many disciplines need to work together closely to develop this new field that transcends the borders between the different disciplines by bridging and fusing. The science of learning and education could become one of the most important fields in the 21st century for the development of human security and well-being.

Interactive Teaching and Learning

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The spectrum of the teaching methods varies from traditional lectures to distant learning through net services in Finnish universities. The aim of the present series of studies has been to develop better individual and group interaction in learning situations to help both the teachers and students.
Audience participation is the key to any successful presentation. Interactive Presenter TM developed by us helps the teachers to meet the needs of the audience. It is a system of software and individual handsets communicating with the teacher's laptop computer. The teacher guides the learning process with multiple choice questions. The interactive audience feed back system makes each lecture unique in a local infrared network established in the lecture hall. System uses multimedia technology and provides problem based learning and communication. An audience up to one thousand can be identified individually. Ten background factors can be pre-programmed and combined (Boolean operators) with up to 99 questions during a session. The system means also a new possibility to collect data and study learning. At the University of Kuopio, Interactive Presenter TM has been used for the medical students during part of the physiology and pharmacology lectures. Also revision tests before the final exam have been run with this system. Students have been eager to use Interactive Presenter TM and their judgement for revision test has been positive and it has correlated with the outcome of final examination.
Interactive Presenter at http://www.ballotbox.fi

Neural Bases of Language Processing and Language Learning

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Among various cognitive processes, language is allegedly claimed to be uniquely human. Linguistics, as one of the general cognitive theories, has been clarifying what kind of computational operations is in fact unique in human and how the brain performs these operations. The questions we are now facing are when and where these computations are implemented in the human brain and how these operations are acquired as the brain develops. In this talk, I will review electrophysiological and brain-imaging studies on semantic, morphological, and syntactic processing in the Japanese language, based on the findings from our laboratory and will suggest their implications for language learning.

Among the various characteristics of human language, hierarchical organization of sentence structures and the existence of the displaced element from its original position are two of the most important properties. As for the first, we investigated psychophysiological correlates of combinatorial operations in syntactic processing using multichannel EEG system. We found a correlation between the hierarchical positions of the projections in the structure and the peak latencies of the ERP components: the higher the hierarchical positions were, the later the peak latencies appeared. This result, exhibiting that brain potentials reflect internally represented hierarchical structures of language, suggests that syntactic processing is carried out in a ‘bottom-up’ manner.

In relation to the second property, we tested sentences with canonical and non-canonical scrambled word order and explored spatiotemporal characteristics of displacement properties using magnetoencephalography, or MEG, and multichannel EEG system. The main activities were found in the left prefrontal cortex and temporo-parietal cortex in the processing of unintegrated noun phrases in the scrambled sentences, suggesting that these areas are responsible for the memory storage for syntactic processing. The novel finding was the activity in the left anterior temporal cortex at an early latency period, irrespective of the sentence type, suggesting that this area is participated in the computation of syntactic relations.

Finally, the critical examinations on the study of adult learning of phrase structure rules in artificial language will be presented. It will be shown that event-related brain potentials technique has a great potential to measure the proficiency of language learning and its physiological basis. It will be suggested that the universal theory of grammatical knowledge could possibly be a good guideline to delineate the fine-grained brain mechanisms of language learning processes.

Recovery of Brain Function

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Restoration of the central nervous system (CNS) functions lost in neurological disorders, including Parkinson, Huntington's and Alzheimer diseases, multiple sclerosis, and brain injuries has been one of the biggest aims to many neurologists. A fact that CNS normally has a very limited capacity for regeneration has made it difficult. However, recent progress of CNS stem cell research started from their identification in adult brain a decade ago has offered possibilities for cell therapy strategies to repair lost function. In animal models, indeed, both exogenous and endogenous neural precursors can replace lost neurons and restore function. Here, I introduce biology of neural stem cells and discuss its application to brain repair with problems which should be overcome.

TAIB SYMPOSIUM ON
"THE BIOLOGICAL SIGNIFICANCE OF STOCHASTIC EVENTS"

ABSTRACTS

Single Molecule Nano-Bioscience

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Biomolecules assemble to form molecular machines such as molecular motors, cell signal processors, DNA transcription processors and protein synthesizers to fulfill their functions. Their collaboration allows the activity of biological systems. The reactions and behaviors of molecular machines vary flexibly while responding to their surroundings. This flexibility is essential for biological organisms. The underlying mechanism of molecular machines is not as simple as that expected from analogy with man-made machines. Since molecular machines are only nanometers in size and has a flexible structure, it is very prone to thermal agitation. Furthermore, the input energy level is not much difference from average thermal energy, k_BT. Molecular machines can thus operate under the strong influence of this thermal noise, with a high efficiency of energy conversion. They would not overcome thermal noise but effectively use it for their functions. This is in sharp contrast to man-made machines that operate at energies much higher than the thermal noise. In recent years, the single molecule detection (SMD) and nano-technologies have rapidly been expanding to include a wide range of life science. The dynamic properties of biomolecules and the unique operations of molecular machines, which were previously hidden in averaged ensemble measurements, have now been unveiled. The aim of our

research is to approach the engineering principle of adaptive biological system by uncovering the unique operation of biological molecular machines. I survey our SMD experiments designed to investigate molecular motors, enzyme reactions, protein dynamics and cell signaling, and discuss the mechanism of biological molecular machines.

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Connecting Disparate Description Levels ---Lesson from Physics

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There are many levels of description in biology: genome, transcriptome, proteome, metabolome, organella, cell, tissue, *etc*. The current biology is dominated by 'genocentrism' whose foremost aim is to discover the genomic mechanism underlying every biological phenomenon.

There may be two responses to the current biology by mathematically-oriented researchers outside it: What are the following bullets:

( ) to supply critical views; ( ) to contribute technically. Both are important.

( ) Until 30-50 years ago physics believed that everything would be clearly understood, if Atomism was developed sufficiently, but at last realized that this was wrong. The current bology seems about 30-50 years behind the current physics, if we regard Genocentrism as a counterpart of Atomism. The fate of Genocentrism would be similar to that of Geocentrism.

To overcome Atomism or ultra-reductionism, we had to realize that there were many universal features in the world that were more or less materials-free; needless to say, they are atomism-free. A general methodology (consciously phenomenological approach) has emerged that can deal with universal features: (1) development of 'micro-free' (not excessively reductionistic) models, and (2) extraction of universal or global features from microscopic descriptions.

This leads us to ( ) above. Within the current biology framework, it is important to extract long-time and/or large-space scale features from detailed microscopic descriptions. As a straightforward application of the general methodology a systematic approach to extracting long-time features from stochastic reaction systems including disparate time scales will be outlined.

Minority Control, Amplification of Fluctuations, and Dynamic Consolidation in a Biological System

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No Abstract? ?

Effect of Phenotypic Fluctuation on Evolution and Adaptive Response of Unicellular Organisms

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Living organisms are composed of many different types of molecules, such as proteins, RNAs, phospholipids, and so on. Because these molecules are synthesized and decomposed by chemical processes occurring at finite temperatures, the molecules are inevitably affected by thermal fluctuations. Even when experimental conditions are well controlled, most cellular variables, such as biological function of proteins and expression of genes, will vary from cell to cell to some extent, i.e., there will inevitably be fluctuations. The relevance of molecular fluctuations has been previously investigated with regard to the enzymatic function of protein, a molecular machine, and a macromolecular system. Recently, fluctuations in gene expression have been extensively investigated, whereas their relevance on biological phenomena at higher hierarchies, such as adaptive responses of cells, has not yet reached a satisfactory understanding.

In this paper, we address the following two questions, 1) do biological systems with larger fluctuations show higher rates of their evolutions? 2) How do unicellular organisms, probably using fluctuation in gene expression, rearrange their gene networks in adaptive response to irregularly changing environments? To answer the first question, we conducted an in vitro experimental evolution where a mutant of green fluorescence protein (GFP) was subjected to ten cycles of random mutagenesis and selection based on the fluorescence expressed by single host E. coli cell. The evolution rate was defined as the fluorescence change between the parental clone and the selected clone at each generation and was analyzed in terms of fluctuation–dissipation theorem in statistical physics, which claims that the response of a variable to the change in its associated force or parameter is proportional to the variance of the variable. In our experiment, the response of a variable is the evolution rate in the fluorescence per cell, and the parameter and its change are gene sequence and the mutation rate at each generation, respectively. The variance of the variable is defined as the variance in the fluorescence among the clone cells of an identical genetic background in a single environment. After flow cytometry analysis on the distribution in the fluorescence per cell for the parental clone at each generation, we plotted the evolution rate with the fluorescence variance at each generation and found the positive correlation. The positive correlation suggests that the cell or gene rendering a larger fluctuation of the fluorescence shows a larger evolution rate, i.e., positive correlation between fluctuation and molecular evolution rate.

To address the second question, we embedded a gene network composed only of mutually inhibitory operons into E. coli. There are three states of gene expression (multiple attractors) due to the nonlinearity of the embedded network. We found that the host E. coli cells, when faced with the depletion

of some nutrients, selected the attractor that produced an encoded enzyme that compensated for the nutrient depletion. The adaptive response by attractor selection was confirmed to be not mediated through molecular signal transduction to recognize the nutrient depletion and transfer the information to DNA, but it is theoretically proposed to be carried out by the cooperation of fluctuation in gene expression and a cell activity or vigor. As the adaptive response by attractor selection to environmental change does not require preprogrammed molecular machineries for signal transduction, it may be intrinsic in nonlinear network and may work even when unicellular organisms face with environmental changes they have met before.

Chance and Necessity in Evolution: 30 Years after Monod

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The role of chance is a major issue in evolution. Two strong statements were made about the role of chance in evolution: the first one by Jacques Monod in his book "*Le Hasard et la Nécessité*" (*Chance and Necessity*, 1970), and the second one by Motoo Kimura twenty years ago, in "*The Neutral Theory of Molecular Evolution*" (1983). Both authors are in essential agreement. The conclusion by Kimura, that the Darwinian "survival of the fittest" should be replaced by the "survival of the luckiest" could be taken as a summary of his position.
The two stands of Monod and Kimura will be presented and discussed. Recent results on the structural and evolutionary genomics of vertebrates obtained in our laboratory lead, however, to a conclusion which is very different from those of Monod and Kimura.

Somatic Evolution of Cancer

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Cancer progression is somatic evolution. Stem cells in skin or intestinal epithelia keep dividing. After many years, some cells accumulate multiple mutations of key genes, finally giving rise to cells that proliferate without being checked by immune system, causing cancer. The risk of cancer depends on the reproductive rate of cells of intermediate mutants (somatic selection), mutation rate, and the population size.

[1] Chromosomal instability (CIN) is a defining characteristic of most human cancers. Mutation of CIN genes increases the probability that whole chromosomes or large fractions of chromosomes are gained or lost during cell division. The consequence of CIN is an imbalance in the number of chromosomes per cell (aneuploidy) and an enhanced rate of loss of heterozygosity (LOH). We develop a mathematical framework for studying the effect of CIN on the somatic evolution of cancer. Specifically, we calculate the conditions for CIN to initiate the process of colorectal tumorigenesis prior to the inactivation of tumor suppressor genes.
[2] Many epithelial tissues are separated into many compartments. Also there is a clear separation of stem cells that keep dividing and differentiated cells that will be discarded after a finite number of cell division. We examine the role of tissue architecture in reducing the risk of cancer initiation. We also discuss their effect to the age-specificity of cancer incidence.

To formulate these problems, we derived new formulas for the fixation of the second or third mutation without fixation of the intermediate mutants (stochastic tunnels).
This work was done in collaboration with M.A. Nowak, F. Michor (Harvard Univ.), S.A. Frank (UC IRvine), B. Volgelstein, C. Lengauer (John Hopkins).

Pattern Formation Driven by Random Cell Movement and Cell-Cell Adhesion

Atsushi Mochizuki
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One of the clearest examples of self-organization in developmental and morphogenetic processes is spontaneous pattern formation by movements of cells. It is generally considered that differential adhesion is the mechanism responsible for the phenomena. In this talk, I develop a spatial structured model of continuous Markovian transition for pattern formation by cell movement based on differential adhesion.
In teleost fish retina, cone photoreceptor cells are arranged in two-dimensional regular patterns, called cone mosaics. Mosaic pattern differs between species -- two typical patterns are "row mosaic" and "square mosaic", exemplified by the cone mosaics in zebrafish and in medaka, respectively. In these retinas, four subtypes of cone cells, which are maximally sensitive to different wavelength of light, appear in quasi-periodic patterns. The pattern formation mechanism has not been known, yet. However, it is strongly suggested that cell rearrangement after cell division is responsible for the pattern formation.
We have developed some mathematical models to examine how cone mosaics are formed. I introduce cell rearrangement model for the pattern formation process. In the model, we assume that the movement of differentiated cells is functional, and that the movement rate is modified by cell adhesion. The observed mosaic patterns are derived by computer simulation only when the magnitudes of cell adhesion between cell types are chosen appropriately. We determined the conditions of cell adhesion for generating the patterns. The same model can produce both row and square mosaic patterns. In determining whether row mosaic (zebrafish pattern) or square mosaic (medaka pattern) is to be formed, magnitudes of cell adhesion are very important. We also developed theoretical analysis for the parameter ranges to generate the row mosaic and the square mosaic with highest probability rather than alternative defective patterns. The analysis leads to the conditions of cell-cell adhesion that are predictions testable by some experimental methods.

Random-Walkers Active in the Genetic Recombination

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Genetic recombination is a fundamental process rearranging genetic material. Much progress in understanding the process stems from experiments of homologous recombination in *E. coli*, where many enzymes have been found to be involved. Enzymes similar to some of them are now identified in higher eukaryotes, suggesting that the similar mechanism should work in meiotic crossing-over.

The frequency of homologous recombination in E. coli system was believed to be linear with respect to the homology length. However, reexamining Singer et al's data [1], we rather found there the third-power dependence, which was successfully explained by our random-walk model [2]. There, a branch point- an intermediate - is assumed to walk randomly over the homologous region, and to be annihilated if it reaches either end of the homology. The dependence observed in the mammalian gene targeting system can be also explained by this third-power dependence although it was originally thought to be exponential.

A kind of the size effect yields the third-power dependence, which appears if the homology length is small enough to be felt by a branch point during its random walk. If the length is larger than a value characteristic of the system, we recover the linear dependence coming from the probability of the initial production of a branch point in the homologous region. This transition from the third-power to linear dependence does not depend on any fitting parameter.

Once a bit of sequence divergence is introduced in the homologous region, the homologous recombination becomes much less frequent if the methyl-directed mismatch repair system is active. The quantitative observation of this rapid drop-off phenomenon [3] was also explained well in the framework of the random-walk model [4]. Once we introduce one diverged base-pair, assuming a branch point to fall down there, we on average have a random-walker in a halved homologous region. The frequency becomes one-eighths of that for the intact region, according to the third-power dependence. Some other observations have been also analyzed in terms of our random-walk model [5-7].

Let us turn to much larger length-scale phenomenon. In the meiotic crossing-over, one crossover point appears to interfere with occurrence of another in the neighborhood. This genetic interference was clearly observed in *Drosophila* [8] and *Neurospora* [9,10]. The plots of the coincidence (density correlation of crossover points) against the genetic distance (distance measured in terms of the expected number of crossover points) are very similar between these two organisms. Though we are far from total understanding of its details, some premeiotic contact points between intact duplexes prime the homologous recombination [11]. Assuming them to walk randomly, we explained the similarity without fitting parameters [12].

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Persistence and Fluctuation of Lateral Dimorphism in Fishes

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Two morphological types ("righty" and "lefty") has been discovered in several fish species, and referred as a typical example of antisymmetry. It is suggested that 1) this dimorphism (called laterality) is inheritable in a Mendelian one-locus-two-allele fashion with lefties dominating, 2) frequencies of laterality in each species fluctuates around 0.5, 3) predators mainly exploit prey of the opposite laterality, i.e., lefties and righties of predators prey on righties and lefties, respectively, of the prey population (defined as "crossed predation", and the antonym "parallel predation" means the predation within the same laterality). We hypothesized that the crossed predation drives the alternation of survival and reproductive advantages between the two morphological types, leading to the frequency-dependent selection that maintains the dimorphism. To investigate this, we constructed mathematical models of population and gene frequency dynamics of 1-prey-1-predator systems and 3-trophic-level systems. Mathematical analysis and computer simulations incorporating demographic stochasticity well explained the behavior of laterality frequency in the nature, unless the crossed predation dominated over the parallel one. When only one of the morphological types exists in a species, the other type can invade at certain parameter values, suggesting that the dimorphism would be maintained in all interacting species.

Robustness of a Feedback Control Rule with Switching Fisheries in Process and Measurement Errors

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Many fisheries have alternative target stocks and selectively exploit the one with the highest expected income. Although target switching is very common in practice, few attempts have thus far been made to study target switching and management rule of multispecies fisheries. In addition, feedback control in fishing effort to each fish species is known as a robust management rule for uncertainty in stock assessment and population dynamics. In this paper, we investigated the potential effects of target switching and feedback control on the yield and sustainability of fish stocks by equilibrium analysis and stochastic simulation.

The stochastic simulation revealed that well-planned target switching increases yields and simultaneously decreases the risk of the stocks collapsing. Target switching decreases fishing pressure on the less-abundant stock and helps the declined stock to recover. Therefore, the minimum stock level is increased by switching. As switching keeps both stocks at productive levels, the total yield is increased by switching. Target switching is effective, especially when the catchability increases with the depletion of a stock population. Total yield under the target switching depends on the magnitude of measurement errors and process errors on fish stock dynamics. When a stock deviates from the optimal level by environmental fluctuation, effort is allocated against the deviation by target switching. Thus, all stock tends to be kept at constant level by target switching. We examined the vulnerability of switching to stock assessment errors. If the stock assessment is very uncertain, then little or no switching is recommended. Target switching can

have substantial effects on fisheries. Therefore, we must investigate the mechanisms of switching and incorporate switching into management plans. Next we consider a case that total fishing effort is controlled by the current stock abundance. Such feedback control (switching fisheries with feedback control, SFFC) has a strong stabilizing effect on stock abundance. However, feedback control of fishing effort or yield of each species (Independent feedback control, IFC) is usually considered in fisheries management. We investigated total yield and minimum stock abundance under SFFC and IFC. SFFC is much more robust than IFC.
This work is cooperated by Toshio Katsukawa and Norio Kimura.

Noise-Induced Sensitization of Human Brain

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The beneficial role of noise in biological systems has been extensively studied over the past decade. One of the fundamental phenomena considered in this respect is that of stochastic resonance (SR) [1], which is generally defined as the optimization of responses of a nonlinear system to a weak input signal caused by external noise. For instance, in neural systems SR has been shown to optimize sensitivity of sensory neurons [2] which may further lead to the enhancement of perception and/or behavior in animals [3] and humans [4].
As a natural extension of these studies, it has recently been demonstrated that externally added noise can enhance input-output coherence within the human brain [5, 6]. The improved responses were observed at the level of the brain stem (the medullary neuronal networks) where the neurological noise can help trigger changes in heart rate in response to small changes in blood pressure [5], as well as at the level of the cortices including the visual areas where visual noise can enhance behavioral output [6] and responses in the brain waves to weak visual signals. These results indicate that the brain can also work as a stochastic resonator.
In my talk, I first summarize such experimental observations on SR in the human brain. This will be followed by an introduction to our clinical finding showing that the noise-enhanced responses of the brain can also be used to "treat" neurological patients with autonomic failure [7]. Finally, I discuss about a possible mechanism for this noiseinduced sensitization of human brain based on the e®ects of externally added noise on a nonlinear dynamical network.

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Obituary

Francesco di Castri (1930-2005)

UNESC0 and IUBS are saddened to announce the death on 6 July in Nimes (France) of Francesco di Castri, the first Secretary of the International Coordinating Council for the MAB Programme (from 1971 to 1984) and the founding Director of UNESCO's Division of Ecological Sciences. After returning to academic life in 1984, he rejoined UNESCO as Coordinator of Environmental Programmes during the period of the UN Conference on Environment and Development in Rio. Francesco di Castri played a creative and leading role in many international activities on the environment, at both non-governmental and governmental levels. He was a man of vision and rigour. He has left his mark and will be missed and remembered by many.

Francesco di Castri was born in Noale (Venice, Italy)) on 4 August, 1930. Following studies at the Universities of Milan (Italy) and Montreal (Canada), he obtained a Doctorate in Animal Sciences at the University of Santiago (Chile) in 1958 and continued his studies in ecological sciences at the University of Padua in Italy from 1958 to 1960.

In the 1960s, Chile was home for Francesco di Castri, and he maintained close links with that country, and with the hispanophone world, throughout his life. From 1961 to 1969, he was Professor of Ecological Sciences and Director of the Institute of Animal Production at the University of Santiago, and then the founding Director of the Institute of Ecology at the Austral University of Valdivia. During his time in Chile, he combined research on the ecology of soil organisms with comparative ecological studies on the structure and functioning on Mediterranean-climate ecosystems in Chile and California within the framework of the International Biological Programme (IBP).

It was during this period that di Castri became associated with UNESCO. He directed the first UNESCO Latin American Regional Course on Soil Biology in Santiago in 1965. He then contributed substantively to several international symposia organized within the Natural Resources Research Programme, such as those on the ecology of the sub-arctic zones (Helsinki, 1966) and methods of study in soil ecology (Paris, 1967).

In 1971, he was appointed by UNESCO as the Secretary of the International Coordinating Council for the Programme on Man and the Biosphere (MAB), later taking on the functions of founding director of the Division of Ecological Sciences on its creation in 1974. From November 1971 to January 1984, he shaped and directed what came to be considered as one of UNESCO's principal contributions in promoting international cooperation on environmental issues. He tended MAB through its phases of international and regional planning and the launching of a first generation of field activities. With Michel Batisse, he nurtured the birth and development of the biosphere reserve concept and the designation of the early biosphere reserves. He made possible the implementation of the objectives of an almost wildly ambitious undertaking – the aims no less than:

- to develop the basis within the natural and social sciences for the rational use and conservation of the resources of the biosphere;
- to improve the global relationship between man and the environment;
- to predict the consequences of today's actions on tomorrow's world and thereby to increase man's ability to manage efficiently the natural resources of the biosphere".

To borrow a phrase from his fellow countryman, Valerio Giacomini, "this was a challenge that borders on the limits of the possible".

In the late 1970s and 1980s, the Division of Ecological Sciences provided the Secretariat of the "natural part" of the World Heritage Convention. Under di Castri's directorship, this Convention began its operations, laying the foundation of what is today one of UNESCO's most successful and

best known legal instruments.

In 1981, di Castri was centrally involved in planning and convening a scientific conference, organized jointly by UNESCO and ICSU, aimed at taking stock of what had been achieved over the first ten years of MAB. Shortcomings as well as accomplishments were identified. Associated with the conference was the prototype of a 36-poster exhibit 'Ecology in Action' that was later produced in multiple copies in a score of languages, and widely considered as the one of the most significant projects to be carried out within the framework of MAB.

A conclusion of this stocktaking exercise was that the MAB Programme had achieved a fair measure of success, reflected in quantitative terms such as the number of countries, field projects and individual scientists taking part, and the important multiplier effect of the resources invested by UNESCO in the programme. More important were more qualitative measures of success, such as the actual results of testing, at field level, of new approaches to problem-oriented research on complex problems of land use and resource management. But what was implicit, not explicit in the assessment, was the role of di Castri in promoting a certain type of international cooperation – one that recognizes the universality of science but also the diversity of cultures and the geopolitical realities of the day. Whatever achievements the MAB Programme can claim owe not a little to the charisma, innovation and drive of its founding director, Francesco di Castri.

In the early 1980s, while still with UNESCO, Francesco di Castri undertook a review for the French Government of the state of ecology in France. Involvement in that process was one of the factors that contributed to his returning to academic life in early 1984, when he left UNESCO to become Director of Research within the French National Institute for Scientific Research (CNRS). There, he headed the prestigious ecological research institute in Montpellier, and orchestrated its transformation into what became the Centre for Functional and Evolutionary Ecology (CEFE).

Francesco di Castri returned to UNESCO in 1990, when he was invited by the Director-General to be the founding Coordinator of the Bureau for Coordination of Environmental Programmes, responsible for taking in hand the Organization's contributions to the UN Conference on Environment and Development (UNCED) in Rio and its immediate follow-up. He remained in this post until his retirement from UNESCO in December 1992, when he returned to CNRS-CEFE as Emeritus Director. From 1993 to 1998 he chaired the UNESCO Committee on the Follow-up to UNCED.

During his career, Francesco di Castri was gainfully employed in three main ways: as a university professor in Chile, as a promoter of international scientific cooperation within a United Nations institution based in Paris, and as a director of a national research institute in France. Over a period of four-and-a-half decades, he was closely involved in several of the principal international scientific programmes on the environment, from the IBP and MAB to the planning of the International Geosphere-Biosphere Programme (IGBP) in the mid-1980s and the launching of DIVERSITAS in the early 1990s. He was among the scientists invited to take part in the planning of the UN Conference on the Human Environment in Stockholm in 1972, and played a key role in mobilizing the participation of environmental scientists from developing countries.

Over this period, he was closely involved in the work of the international scientific community, particularly through the ICSU family (now, the International Council for Science). Among other honorary positions, he was a long-time member of the Executive Committee of the International Union of Biological Sciences (IUBS). He was elected IUBS Secretary General in 1988 at the IUBS General Assembly in Canberra, then as IUBS President at the following General Assembly in Amsterdam in 1991 till 1994 and served on the IUBS Board as immediate Past President from 1994 to 1997. In September 1994, he chaired the IUBS UNESCO Conference "Biodiversity, Science and Development towards a new partnership". He was the first Vice President and one of the founding fathers of the Scientific Committee on Problems of the Environment (SCOPE) from 1969 to 1972, then again a member of the Executive Committee of SCOPE from 1985 to 1995, and President of SCOPE from 1988 to 1992. During the 80' and 90', he also led several projects of SCOPE, and most recently the project on Environment in the Global Information Society.

Following his return to CEFE-Montpellier in 1993, he became increasingly involved in the rethinking and revamping of teaching and learning programmes at graduate and post-graduate levels, in such fields as land use planning and resource management. His research interests turned towards better

understanding linkages between ecology and economic globalization. Increased contacts with the industrial-production sector reflected his long-held conviction that scientists and environmentalists should seek to understand the motivations and workings of business, as a means of bringing influence to bear on those whose actions have such an enormous impact on the environment. Cooperation with groups such as the Total Foundation was reflected in assessments of the interfaces between tourism, information and biodiversity, among others. Work with local communities included that with the population of Easter Island on taking advantage of modern communication technologies in exploring options for their future. These activities by Francesco di Castri were yet another demonstration of his career-long concern to make science contribute to sustainable development in a practical manner. Otherwise, he was often an outspoken critic of misrepresentations of the concept of sustainable development by scientists and policy-makers alike.

Francesco di Castri was a prolific writer, with over 20 books to his name as author, coauthor, or coeditor. He also authored or coauthored over 350 scientific and popular articles, monographs and book chapters. Subjects addressed included quantitative soil biology, information theory, structure of animal communities from the tropics to Antarctica, convergence of Mediterranean ecosystems, biogeography and biodiversity, strategies and constraints for natural resources development, organization of international interdisciplinary research on environmental issues, increasing the credibility of ecological research, biological complexity, effects of globalization on the environment and society. Among honours and distinctions, he was awarded the Doctor of Philosophy, honoris causa, by the University of Kuopio (Finland) in 1983. He was a member of the Academies of Sciences of Italy and Russia, and Commander of Order of the Italian Republic.

Throughout much of his career, di Castri was centrally involved in bringing together scientists from different disciplines, of both the natural and social sciences, to conduct problem oriented research on resource management and man-environment interactions. Underpinning this work was his conviction that the diversity of natural and socio-economic situations that exist in the world provides a basis and not an obstacle for meaningful scientific cooperation, and that shared responsibilities and respect for different cultures should be the cornerstones of international cooperation. He felt strongly that promotion of innovative responses to new and complex problems implies acceptance of the possibility of failure. He had a lifelong interest in testing new ways of cementing the participation of scientists, planners and local people in particular field projects, and of popularizing and diffusing information on the environment. He eschewed dogma and rhetoric in favor of concrete actions.

As an individual, di Castri was a realistic optimist. An optimist in that he subscribed to the idea that ecology is a science associated with opportunity and hope rather than with doom and despair. He was not one of those who preach that environmental doomsday is nigh. Rather, he emphasized the role of science in contributing to positive goals, such as putting resource use on a more sustainable basis. A realist in that he had a very acute sense of what is possible and what is not.

Francesco di Castri was a four-language polyglot, esteemed by those in government and those in science, worldwide. He was a noble, complex and highly erudite person, in the tradition of his native city. He mixed passions for the cinema, football and linguistics similarly to his near contemporary Umberto Eco. He was a skilled and subtle debater, with a fine sense of irony and metaphor… highly appreciated by those who were not in his line of fire. He was an exceptional motivator of those around him, a man of imagination and foresight, of courage and rigour. He left his mark. He will be missed and remembered by many.