Biology International

N° 45 ((December, 2003)

Editorial

IUBS in Times of Transition

The present issue of *Biology International* coincides with the organisation of the IUBS 28th General Assembly in Cairo, which marks the completion of the First Triennium into the 21st Century. On this occasion, it is important to consider, from a historical perspective, the evolution of the IUBS structure and function in parallel with the development of biological sciences over the 20th Century. Connecting the dots that represent the landmarks in the life of IUBS since its creation in 1919 provides an account of how biology evolved from the stage of natural history 'botanical' and 'zoological' traditions to a stage dominated by analytical and experimental studies and a steady trend towards specialisation, leading to the establishment of a large number of biological disciplines and sub-disciplines. Today, IUBS counts more than eighty different organisations among its members, not to mention many others represented in the large family of ICSU bio-unions.

As a forum bringing together the great diversity of its scientific and national constituencies, IUBS tends to look for unifying themes and common denominators, which explains to a large extent why IUBS programs are international, global, collaborative and interdisciplinary in their nature: the International Biological Programme (IBP) in the Sixties; the Decade of the Tropics Program in the Eighties; the Biological Diversity Program (DIVERSITAS) in the Nineties; the Biological Education Commission, which started in the Seventies and still on-going; and the more recent Program 'Towards An Integrative Biology (TAIB)', are prime examples illustrating how IUBS capitalizes on the excellence, richness and diversity of its constituencies to address major challenges related to the environment, development and society.

The IUBS Conference "Biological Sciences and the Challenges of the 21st Century" held in Naples in 2000 addressed the major advances and breakthroughs in biological knowledge, in particular the emergence of such new domains as Genomics, Proteomics, Regulomics and Biodiversity. The conference also underlined the importance for the 21st Century of 'Integrative Biology' as a prerequisite to address the key questions and fundamental issues of the complexity of biological organisation.

For unforeseeable reasons, the three-year period that followed the Naples Conference, proved to be a 'Period of Transition' with its lot of uncertainties, constraints and limitations. At the economic level, the bursting of the 'economic bubble' - a period of impressive economic growth that marked the Nineties, left behind economic stagnation, if not recession in many parts of the world, with harsh competition among companies, institutions and organisations (including scientific enterprises) in search of quick short-term economic returns. Added to the economic uncertainty, the relentless drive towards globalisation and the problems associated with emerging diseases, contributed to creating a sense of doubt, anxiety and fear among the general public vis-à-vis the capacity of existing science and technology institutions to address these and other societal issues in a timely and responsible manner. Finally, at the political level, the dramatic events of September 11, 2001 in New York and the conflicts that followed, resulted in shifting the world attention away from the 1992's Rio Summit Trilogy of Global Change, Biological Diversity and Sustainable Development, towards the post-September 11 'Global War against Terrorism'.

As with other international non-governmental, non-profit organisations, IUBS is highly sensitive to change, volatility and fluctuations in the international context. While developing in line with the resolutions adopted at Naples General Assembly, the IUBS programs and activities were affected in their magnitude. For IUBS and similar organisations, the need to adapt to the financial constraints has had some positive side-effects: partnership, once a luxury, bas become a prerequisite for work; dialogue with the decision makers, whether in the public or private sector and the public at large is no longer perceived as a nuisance and waste of time but a moral and social obligation; and the human dimensions of the scientific 'problématique' and enterprise have become a foundation for new scientific programs. "Integrative" has become the 'motto' of the science enterprise.

Biology International, which serves as the principal medium for IUBS, reported on the main developments that occurred during the Triennium *(Cf.* Issues n°s 40 - 44). The present issue of *Biology International* 'Featuring Genomics', provides good examples of integrative approaches to biological research, notably with papers on 'Genomics and the Changing Profile of Human Disease' by Bittles; 'Microbial Infection and the Genesis of Chronic Disease' by Whittum-Hudson *et al.:* and 'Integrating Genomic Characters for a Holistic Approach to Understanding Plant Genomes' by Leitch and Bennett. These papers are also relevant to the on-going discussions related to the development of "Science for Health and Well-Being." a new inter-union initiative of ICSU.

In this regard, the Cairo Conference "Biological Sciences, Development and Society" looks exemplary: It focuses on the more applied aspects of modem biological discoveries and their economic, social and ethical implications; adopts integrative approaches to addressing the complexity of biological organisation, and capitalizes on partnerships to meet the challenges of globalisation and sustainable development.

The Conference plenary lectures, symposia and workshops will be directed not only to the community of biologists but to the policy and decision-makers. economic and environmental actors, educators and the public at large. Four symposia will address the important topics of: 'Bioinformatics and the development of biotechnologies and bio-resources'; 'Stress biology from cells to populations and the environment'; 'Integrative biology, complexity and sustainability'; and 'Biological education, ethics and society'. Two workshops will address key issues of biodiversity: "Fostering international access to biodiversity research and conservation" and the "Role of research, training and education in the conservation and sustainable use of biodiversity and the safe application of biotechnology," and a third one will discuss the IUBS contributions to the inter-union initiative "Science for Health and Well-being." At the regional level, the conference will address the current status and prospects of biological sciences in the Arab region; biodiversity in Africa; and collaboration between Spain and Latin America in the domain of molecular systematics. The 1UBS/ASRT Conference in Cairo represents a platform of partnership, combining the excellence and wide range of expertise of its members, with the strength and resources of a large coalition of international organisations, to mention but a few: UNESCO, ICSU, 1UNS, CBD, CIHEAM, ICARDA, IC1PE, IUCN, OECD, RAMSAR. and TWAS.

On the occasion of the Holiday Season, I would like to convey to the IUBS members and partners, my best wishes for happiness and peace for the "New Year.

And to those that will join in the IUBS family reunion in Cairo, see you soon.

Paris, 22 December 2003 Talal Younès, Executive Director, IUBS

Genomics and the Changing Profile of Human Disease

By A.H. Bittles
Centre for Human Genetics, Edith Cowan University, 100 Joondalup Drive
Perth WA 6027, Australia. Phone: +61 8 9400 5623 ; Fax: +61 8 9400 5851
Email: a.bittles@ecu.edu.au

Introduction

Within the last decade, much has been written on the impending impact of the Human Genome Project on human health. A typical perspective was offered in 1995 as part of the submission of the Royal College of Physicians to the Science and Technology Committee of the U.K. House of Commons. In describing the future contribution of genetics to medical practice it was stated that, '*The process has scarcely begun and may not have a major clinical impact for many years; however it represents the change from empirical to rational management of disease and hence its significance can hardly be exaggerated'*. Clearly, any body of knowledge that could effect such a change would be of global importance, and if this and similar predictions prove to be correct, they would match in significance the revolution in medical practice provoked by the anatomical discoveries of the Renaissance period.

The Human Genome Project was initiated in 1991, and by 2001, the first consensus sequence of the human genome was simultaneously published by publicly supported researchers (International Human Genome Sequencing Consortium 2001) and the privately financed Celera Genomics (Venter *et al*. 2001). Access to the growing database on genome structure and function made available through the Human Genome Project has greatly assisted medical researchers, and as a result, by March 2003 over 14,000 single gene disorders affecting both the human nuclear and mitochondrial genomes had been identified (OMIM 2003).

Of itself, the recognition of specific and often very rare mutations is unlikely to change medical practice or to impinge on the everyday lives of the vast majority of the world's population. Data on the contribution of predisposing genes to common diseases is still rudimentary, and there is limited information on non-biological factors that influence the genetic structure of human populations and thereby govern the distribution and transmission of disease mutations. An appreciation of the importance of these topics has, however, gradually been emerging, accompanied by the establishment of two new academic disciplines, Community Genetics and Public Health Genetics. The aim of this article is to briefly review the potential influence and effects of these changes on the future profile of genetic disease in industrialized and developing countries.

The prevalence of genetic disease in industrialized and developing countries

Preliminary evidence of a major change in the profile of human disease in industrialized countries was provided by a record-based study conducted in the U.K. at the Great Ormond Street Hospital for Children, London (Carter 1956). Genetic disorders had been diagnosed in 16.5% of childhood deaths in the hospital in 1914, but by 1954 this figure had risen to 37.5%. Over the same period, deaths described as 'environmental' had decreased from 68.0% of the

total to just 14.5%, reflecting the beneficial preventive effects of vaccination programmes for infectious diseases and the successful introduction of antibiotic therapy. This epidemiological transition was affecting all industrialized countries, and by the 1970s, the growing social and financial burden of childhood genetic disease had already become a source of concern in the U.S.A. (Hall *et al.* 1978).

Current estimates suggest that in the industrialized countries approximately 5% of individuals will exhibit symptoms of genetic disease by young adulthood. However, if congenital anomalies are included the prevalence increases to some 8% of all live births (Baird *et al.* 1988). Comparable data are not separately available for developing countries, but in global terms it has been suggested that at least 7.6 million children per year are born with a severe congenital or genetic disorder (Alwan and Modell 2003).

Because of the continuing importance of infectious disease and nutritional disorders in developing countries, it has been assumed that their burden of genetic disease is relatively unimportant. While it is undoubtedly true that in proportional terms genetic disorders are responsible for a minority of childhood disease diagnosed in developing countries, in many parts of the world up to 40% of the population are carriers of an inherited haemoglobin disorder (Livingstone 1967). Overall, this means that an estimated one in seven of the world's population are carriers of a gene either for thalassaemia or a haemoglobin variant (WHO 2002). In both types of disorder, individuals who have inherited the causative mutations from each parent commonly have severe anaemia, and the vast majority of these people are resident in tropical regions.

Demography, population genetics and genetic disease

The social and demographic structures of populations play very significant roles in the distribution patterns of specific inherited disorders, albeit with marked differences between the industrialized and developing countries. For example, following the onset of the Industrial Revolution in Europe, there was widespread population movement from the countryside into the rapidly expanding towns and cities. These large-scale population changes resulted in the dissolution of historical local, regional and national boundaries, which in turn helped to exert a partial homogenizing effect on national gene pools. Likewise, through time, large-scale migration from Europe to the Americas and Australasia resulted in significant mixing of previously distinct populations (Bittles 2002a).

The situation is very different in most developing countries, where local and regional clan, tribal and ethnic groupings have largely remained intact. Thus in India, Pakistan and Bangladesh, which collectively account for more than 20% of the world's population, marriage continues to be arranged within caste and *biraderi* boundaries that probably date back some 3,000 years. In India alone, there are an estimated 50,000 to 60,000 separate endogamous communities (Gadgil *et al.* 1998). Furthermore, some 25% of the population of 1,050 million are members of the 1,600+ scheduled tribes and castes that exist outside the Hindu caste system (Bhasin *et al.* 1992), and a further 130 million persons are Muslim. In effect, each of these groupings, whether Hindu caste or non-caste, Muslim, Christian, Buddhist, Sikh, Jain or Parsi, form separate breeding pools. The net result is that while disease mutations of ancient origin may be distributed throughout the population, those which have arisen more recently may be restricted or even unique to individual ethnic groups, sub-castes, tribes or clans (Bittles 2002b).

Gene mutations can be rapidly transmitted and increase to high frequency via genetic drift within social, religious and geographical isolates of this type, especially in communities that are numerically small. Due to the restricted nature of their gene pools, there also is a high probability that by chance alone, couples who marry are biological relatives, an extreme example being the remote island of Tristan da Cunha in the South Atlantic, which was colonized in the early 19th century (Roberts 1992). In many developing countries, there also is a strong preference for consanguineous marriage, and so in North and Sub-Saharan Africa, the Middle East, West, Central and South Asia, 20% to over 50% of marital unions are intra-familial, most commonly contracted between first cousins (http://www.consang.net ; Bittles 2001).

Community Genetics and Public Health Genetics

The influence of these various factors and the increasing contribution of genome-based information to health studies have led to the development of new, multidisciplinary approaches to the role of genetics in medicine. Community Genetics starts from a medical genetics/community medicine perspective and seeks to provide guidelines for the establishment and surveillance of programmes to prevent and control the adverse effects of human genetic disorders (Henneman *et al.* 2001). These programmes can variously be run at local, national and regional levels, and they emphasize strengthening the role of primary health care, integrating interventions into reproductive health programmes, and ensuring the feasibility and cost-effectiveness of preventive strategies (Alwan and Modell 1997). As its name suggests, Public Health Genetics derives from a broader public health background and aims to prevent mortality, morbidity and disability of genetic origin by integrating genome-based information into existing public health practice (Khoury *et al.* 2000; Beskow *et al.* 2001). The perceived remit of Public Health Genetics thus encompasses single locus disease genes, polygenic, multifactorial disorders, and pharmacogenomics, i.e. the interaction of genes with therapeutic agents.

Community Genetics has tended to concentrate on providing services to populations where genetic disorders are present at high frequency and on establishing community-specific care programmes. This includes communities in developing countries. By comparison, Public Health Genetics has been more concerned with providing solutions to the growing genetic problems faced by the populations of industrialized countries and calls on the services of a wider range of non-clinical, health-related professionals, including groups such as anthropologists, lawyers and social workers. Both disciplines are dependent on population- and subpopulation-based studies, and they also share a strong emphasis on the need for informed public consultation and the development of rigorous ethical guidelines.

The sharp community-based subdivisions characteristic of most developing countries can effectively delineate the distribution and frequency of specific disorders, ranging from inherited anaemias (de Silva *et al.* 2000), to cancers (Shanmugaratnam *et al.* 1989), and pre-disposition to major infectious diseases (Pitchappan 2002). Although data on regional origins are collected from patients in many of these countries, very limited attention has been paid to genetic differences between ethnic groups or specific communities. Where disorders are community-specific, this type of information is essential if efficient preventive programmes are to be introduced. However, any such information-gathering exercise has to be conducted with due caution and discretion, lest families or even entire communities become inadvertently stigmatized on the grounds that they carry a gene(s) for a particular genetic disorder (Bittles 2003b).

Similar problems stemming from inadequate definitions of ethnic subpopulations have been a major problem in the industrialized countries, with a common tendency to broadly refer to individuals as being of 'Maghrebian' or 'South Asian' origin. By ignoring the very marked genetic subdivisions that exist within these supra-regional categories, disease prevalence surveys may be of little practical relevance. There also has been over-emphasis on the adverse effects of consanguineous marriage, perhaps fuelled by historical suspicions of inbreeding in western countries (Bittles 2003a). This prejudice is commonly accompanied by a failure to recognize the potential effects of community endogamy and the outcomes of random inbreeding on the prevalence of genetic disorders.

Discussion

The changing profile of human disease has been especially apparent in the countries of the Gulf region, where traditional tribal and clan endogamy and high levels of consanguineous marriage have resulted in the accumulation of specific disease mutations within individual communities (Teebi and Farag 1997). In previous generations, the adverse outcomes of these mutations would largely have been obscured by the high rates of infant mortality typical of developing countries. But since development of the oil and petrochemical industries within the region during the mid- to late 20th century and the introduction of high technology health care programmes, a wide range of genetic disorders has increasingly been diagnosed.

The financial and health infrastructure problems associated with genetic diseases have yet to fully emerge in developing countries. However, two examples illustrate their potential scale. In Pakistan 5,000+ infants with β–thalassaemia, an usually severe inherited form of anaemia, are born each year and require regular blood transfusions to survive. The yearly blood requirement of each annual birth cohort of affected children is 90,000 units of blood, with an associated cost per patient for chelation therapy to remove excess iron of US$4,400 (Ahmed *et al.* 2002). This compares with the annual GNP per person in Pakistan of US$1,860 (PRB 2002). In related terms, in Indonesia it has been estimated that the blood transfusion requirement for patients with severe forms of β–thalassaemia is now approaching 1.25 to 1.5 million units per year (WHO 2002). Demands of this nature will be extremely difficult to sustain, especially in developing countries where blood may be infected with a range of viruses and blood banking and testing facilities are limited, hence the central emphasis on disease prevention in Community Genetics programmes.

In fact, a similar if less acute scenario also applies in many industrialized countries, where β–thalassaemia mutations have been maintained in the gene pool of countries such as Italy and Greece as a historical protective response to the selective pressure of the malaria parasite *Plasmodium falciparum*. In Northern Europe, North America and Australasia, α- and β–thalassaemia mutations also may be present at high frequency within migrant communities from regions of the world where malaria was or remains endemic, once again placing major demands on supplies of blood for remedial transfusion.

Besides social and economic considerations, the growing changes in human disease profiles have exposed poor levels of understanding of genetic disorders among many clinicians in major industrialized countries (Baird 2001). This problem is even greater in developing countries, in part because genetic disease is still mistakenly considered to be of limited significance and accordingly, training in genetics is given low priority. As a result, the need for the formal

training of clinicians and non-clinical support staff and for effective public education programmes is all the more pressing in many less affluent countries (Verma and Bijarnia 2002).

Although genetics can be expected to contribute positively to future programmes for the maintenance of human health, and particularly to the prevention of currently intractable age-related disorders, these goals will not be achieved as a matter of course. There are major concerns that the principal targets for genomic research will be chosen primarily on commercial grounds rather than on the basis of need, and the patenting of disease mutations has served to heighten these suspicions. As a current example, the tactics employed in the marketing of BRCA1 mutation testing for breast cancer, the lack of ensured provision for pre-and post-test counselling, and the scale of charges involved all have been subject to strong criticism, but as yet to limited avail. Although these matters have so far principally affected the industrialized world, in developing countries there are worries that medical staff may be encouraged to adopt expensive therapeutic treatments when prevention would provide a more appropriate and low-cost, if less glamorous, alternative (Alwan and Modell 2003).

A recent World Health Organization publication proposed that the health rewards expected to flow from the commercial development of genomics should be equitably distributed between the industrialized and developing countries (WHO 2002). Recent experience would tend to suggest that while such altruistic behaviour would be welcomed by many, the proposal might not receive unreserved support from the companies concerned and their shareholders. In part, difficulties have arisen because of legal discrepancies between the countries and political and trade groupings most immediately concerned. It is also a fact of life that a highly focused and financially robust private company, with good international connections and offering substantial financial returns to potential backers, can act with a determination and dispatch that governmental agencies simply cannot match.

If genome- and proteome-based research is to proceed for the greater good of all, decisions as to the targets and directions of research, its timing, sources and extent of funding, and how outcome benefits can be efficiently and equitably distributed, require informed and non-partisan counsel. Manifestly, this is neither the realm nor the primary concern of commercial enterprises, and in recent years the governments of most industrialized countries have demonstrated a greatly diminished interest in tackling issues of this nature. It is, however, precisely the type of critical area where IUBS, ICSU and other representative international scientific and medical bodies are uniquely placed to act, given their ready access to the requisite cross-disciplinary, trans-national expertise. Far-sighted and principled decision-making on the future course of genomic research across the Biological Sciences is sorely needed. This is surely a task that merits the urgent attention of IUBS.

References

Ahmed, S., Saleem, M., Modell, B. and Petrou, M. 2002. Screening extended families for genetic hemoglobin disorders in Pakistan. *New England Journal of Medicine* **347**: 1162-1168.

Alwan, A. and Modell B. 1997. *Community Control of Genetic and Congenital Disorders*. Alexandria: World Health Organization.

Alwan, A. and Modell B. 2003. Recommendations for introducing genetics services in developing countries. *Nature Reviews Genetics* **4**: 61-68.

Baird, P.A. 2001. Current challenges to appropriate clinical use of new genetic knowledge in different countries. *Community Genetics* **4**: 12-17.

Baird, P.A., Anderson, T.W., Newcombe, H.B. and Lowry R.B. 1988. Genetic disorders in children and young adults: a population study. *American Journal of Human Genetics* **42**: 677-693.

Beskow, L.M., Khoury, M.J., Baker, T.G. and Trasher, J.F. 2001. The integration of genomics into Public Health research, policy and practice in the United States. *Community Genetics* **4**: 2-11.

Bhasin, M.K., Walter, H., and Danker-Hopfe H. 1992. *The Distribution of Genetical, Morphological and Behavioural Traits among the Peoples of Indian Region.* Delhi: Kamli-Raj, pp. 13-35.

Bittles, A.H. 2001. Consanguinity and its relevance to clinical genetics. *Clinical Genetics* **60**: 89-98.

Bittles, A.H. (ed.) 2002a. Community Genetics in Developing Countries. *Community Genetics* **81**: 91-98.

Bittles, A.H. 2002b. Endogamy, consanguinity and community genetics. *Journal of Genetics*, in press.

Bittles, A.H. 2003a. The bases of Western attitudes to consanguineous marriage. *Developmental Medicine and Child Neurology* **45**: 135-138.

Bittles, A.H. 2003b. Consanguineous marriage and childhood health. *Developmental Medicine and Child Neurology* **45**: 571-576.

Carter, C.O. 1956. Changing patterns in the causes of death at the Hospital for Sick Children. *Great Ormond Street Journal* **11**: 65-68.

de Silva, S., Fisher, C.A., Premawardhena, A., Lamabadusuriya, S.P., Peto, T.E.A., Perera, G. *et al.* 2000. Thalassaemia in Sri Lanka: implications for the future health burden of Asian populations. *Lancet* 355: 786-791.

Gadgil, M., Joshi, N.V.C., Manoharan, S., Patil, S. and Prasad, U.V.S. 1998. Peopling of India. In: *The Indian Human Heritage*, Hyderabad: Universities Press, pp. 100-129.

Hall, J.G., Powers, E.K., McIlvaine R.T., and Ean V.H. 1978. The frequency and financial burden of disease in a pediatric hospital. *American Journal of Medical Genetics* **1**: 417-436.

Henneman, L., Langendam, M.W. and ten Kate, L.P. 2001. Community Genetics and its evaluation: a European Science Foundation Workshop. *Community Genetics* **4**: 56-59.

International Consortium on Consanguinity website, http://www.consang.net

International Human Genome Sequencing Consortium 2001. Initial sequencing and analysis of the human genome. *Nature* **409**: 860-921.

Khoury, M.J., Burke, W., Thomson, E.J. (eds.) 2000. *Genetics and Public Health in the 21st Century*. New York: Oxford University Press.

Livingstone, F.A. 1967. *Abnormal Hemoglobins in Human Populations*. Chicago: Aldine.

OMIM 2003. *Mendelian Inheritance in Man*. http://www.ncbi.nlm.nih.gov/Omim/Stats/mimstats.html=20

Pitchappan, R.M. 2002. Castes, migration, immunogenetics and infectious diseases in South India. *Community Genetics* **5**: 157-161.

PRB 2002. *World Population Data Sheet*. Washington DC: Population Reference Bureau.

Roberts, D.F. 1992. The price of isolation. In: A.H. Bittles and D.F. Roberts, eds., *Minority Populations: Genetics, Demography and Health*, London: Macmillan, pp. 35-67.

Science and Technology Committee of the House of Commons 1995. *Human Genetics: the Science and its Consequences.* London: HMSO, p. xxxiii.

Shanmugaratnam, K., Hin-Peng, L. and Day, N.E. 1989. Cancer in migrant populations: a study in Singapore. In: *Ethnic Factors in Health and Disease*, London: Wright, pp. 145-154.

Teebi, A.S. and Farag, T.I. (eds.) 1997. *Genetic Disorders among Arab Populations*. New York: Oxford University Press.

Venter, J.C., Adams, M.D., Myers, E.W., Li, P.W., Mural, R.J., Sutton, G.G. *et al.* 2001. The sequence of the human genome. *Science* **291**: 1304-1351.

Verma, I.C. and Bijarnia, S. 2002. The burden of genetic disorders in India and a framework for community control. *Community Genetics* **5**: 192-196.

WHO 2002. *Genomics and World Health*. Geneva: World Health Organization.

Microbial Infection and the Genesis of Chronic Disease

By Judith A. Whittum-Hudson[1,2], Robert H. Swanborg[2], and Alan P. Hudson[2]

[1]Depts. Internal Medicine and Ophthalmology
Wayne State University School of Medicine
Detroit, Michigan 48201 USA

[2]Dept. Immunology and Microbiology
Wayne State University School of Medicine
Detroit, Michigan 48201 USA

Correspondence: Alan P. Hudson PhD, Dept. Immunology and Microbiology,
Wayne State University School of Medicine, Gordon H. Scott Hall, 540 East Canfield Avenue,
Detroit, Michigan, 48201 USA. Tel: 313-993-6641; Fax: 313-577-1155
Email: ahudson@med.wayne.edu

Introduction

Since the time of Anton van Leeuvenhoek and his invention of the first crude microscope, continuous progress has been made in elucidating the microbial causes of human disease. The result of that progress is our current and quite sophisticated understanding of the agents responsible for cholera, influenza, diphtheria, smallpox and a host of other infectious clinical entities. Indeed, not only have we identified the organisms responsible for many important diseases, but we have also developed a detailed understanding of the molecular mechanisms by which those causative microbes elicit pathology. Based on such knowledge, effective therapeutic modalities have been designed to prevent microbially-based diseases, and to treat them when they occur.

A brief survey of any medical microbiology textbook will demonstrate that the overwhelming majority of infectious diseases that have yielded to efforts of the scientific community are acute in nature. Over the last two decades, however, interest among infectious disease researchers has diversified somewhat. While a few chronic diseases such as tuberculosis and AIDS are known to be caused by infectious agents, the etiologies of many important chronic clinical entities, including rheumatoid arthritis, atherosclerosis, multiple sclerosis, Alzheimer's disease, and others remain to be elucidated. The idea that chronic diseases may involve causation or exacerbation *via* long-term microbial infection is not new, of course. Indeed this is a notion that has remained a relatively constant fixture in the background of both chronic disease and infectious disease research for much of the 20th century. The success of this viewpoint in elucidating the etiologies of chronic diseases has been limited, but demonstration in the 1990's by Marshall and his colleagues that *Helicobacter pylori* is commonly involved in peptic ulcers (e.g., Lee *et al.*, 1993 for review) provided significant new impetus for researchers to investigate infectious involvement in other idiopathic chronic diseases. The result of some of these studies

has been the association of *Nocardia asteroides* with Parkinson's disease (Kohbata and Beaman, 1991), *Chlamydia trachomatis* with chronic reactive arthritis (Villareal *et al*., 2002 for review), human herpes virus 6 with multiple sclerosis (Tejada-Simon *et al.*, 2002), *Chlamydia pneumoniae* with atherosclerosis (reviewed in Mahony and Coombes, 2001), as well as the possible involvement of this latter organism in various neurologic diseases (e.g., Balin *et al.*, 1998; Sriram *et al*., 1998), herpes virus type 1 with sporadic Alzheimer's disease (Itzhaki *et al.*, 1997), and early asymptomatic prenatal infection by *Toxoplasma gondii* with later development of schizophrenia (Yolken *et al.*, 2001). While these and other such associations are controversial, they remain a focus of interest and active investigation.

The tentative associations among idiopathic chronic diseases and diverse bacterial and viral infections raise several important scientific issues. For example, most associations between microbial agents and chronic diseases have been made, at least initially, on the basis of identification of the organism in appropriately chosen diseased tissues but not, or with significantly lower frequency, in congruent tissues of control individuals. If a specific organism is present at relevant anatomic sites in some disease state, is that organism directly responsible for the initiation of pathogenesis? If it is not the initiator of disease, does its presence exacerbate the disease process, or is it simply an innocent bystander that gained access to the relevant site due to a disease process initiated by some other means? If the organism does function as etiologic agent in the disease, or even if it simply acts to exacerbate symptoms or accelerate disease progression, by what mechanism(s) does the organism accomplish its damage? That is, do chronically infecting pathogens manipulate their hosts' responses so as to establish and/or maintain their own longevity, thereby generating disease chronicity? If so, what is the nature of that manipulation? The converse question is also paramount: how does the host respond to chronic bacterial infection, and do some responses contribute meaningfully to disease genesis and/or chronicity? For chronic diseases not of genetic origin, does some specific aspect of host genetic background confer susceptibility to elicitation of disease by a microbial agent? If so, is that susceptibility to all agents, some agents, or only to specific bacteria or viruses? Whether the susceptibility is relevant to one, some, or all agents, is the time of acquisition of the microbe important in disease genesis? That is, must the eliciting bacterium or virus be acquired at a specific point in childhood or at some other period for disease generation to occur?

In this article we discuss current and emerging ideas relating to the possible involvement of bacteria and viruses in chronic disease genesis. In so doing, we discuss what is understood of the established or postulated roles of micro-organisms in the pathogenesis process, and we address the issues raised above and others whose elucidation will be crucial before firm conclusions can be drawn concerning any relationship between an infectious agent and subsequent development of disease. In the following discussion, we generalize from the example of human herpes virus 6 and the bacterium *Chlamydia pneumoniae* and their recently postulated association with multiple sclerosis, rather than simply summarizing available evidence for microbial involvement in a range of idiopathic chronic diseases.

Infectious Agents and Multiple Sclerosis

Multiple sclerosis is a demyelinating disease of the human central nervous system. The disease includes an autoimmune component in which self-reactive lymphocytes target constituents of the myelin sheath, such as myelin basic protein and myelin oligodendrocyte glycoprotein, among others. Multiple sclerosis occurs in two forms, remitting-relapsing and chronic progressive, with the former showing higher prevalence (Paterson and Swanborg, 1988). Most patients with remitting-relapsing disease progress eventually to the latter form, termed

secondary progressive. Despite intensive research over many decades, the etiology of multiple sclerosis remains unknown, but much evidence indicates an infectious involvement in disease genesis. For example, compelling data exist for seasonal variation in the incidence of new cases of multiple sclerosis. Moreover, the disease is most prevalent in high northern latitudes (Kurtzke, 1983). Other evidence for infectious involvement in multiple sclerosis comes from long-term studies in the Faroe Islands which suggest that, about 1940, introduction of an unspecified microbial agent occurred in the islands, and that this agent has since engendered pulses of new multiple sclerosis cases at roughly 13 year intervals (Kurtzke and Hyllested, 1988).

Although a number of studies have claimed to identify genetic components important in the development of the disease, multiple sclerosis is clearly not purely genetically determined (Oksenberg *et al*., 1999; Haines *et al*., 2002). Involvement of a bacterium or virus in multiple sclerosis could explain at least some aspects of the autoimmunity that characterizes the disease. For example, the idea that anti-myelin T cells or auto-antibodies might be elicited *via* molecular mimicry has generated much recent interest (Wucherpfennig and Strominger, 1995; Ufret-Vincenty *et al.*, 1998). Molecular mimicry is a postulated mechanism for generation of an auto-immune response in which auto-reactive T cells or antibodies are induced by microbial antigens but cross-react with self-antigens (see e.g., Lenz *et al*. 2001). Based on epidemiologic data and this mimicry hypothesis, many laboratories have searched for infectious agents in samples from patients with multiple sclerosis. To date, about twenty organisms have been associated with the disease. The screening methods in these studies varied from serology to polymerase chain reaction (PCR), and of critical importance, the quality and numbers of controls examined in these studies varied widely as well. However, none of the organisms so far implicated in multiple sclerosis has gained acceptance as the causal agent. Indeed, virtually all have been discarded due to the lack of confirmatory evidence from independent laboratories.

Recently, two new organisms were added to the list of possible etiologic agents in multiple sclerosis: human herpes virus 6 (HHV-6) and the bacterial pathogen *Chlamydia pneumoniae*. Both have elicited significant interest from investigators in the multiple sclerosis research community, and both have generated controversy concerning their possible role in disease generation (e.g., Vastag, 2001). HHV-6 is a DNA virus first isolated from peripheral blood mononuclear cells (PBMC) of AIDS patients. Isolates also have been obtained from PBMC of children with undifferentiated acute febrile illness, as well as from healthy adults and immunocompromised patients. HHV-6 is ubiquitous, showing a seroprevalence worldwide of nearly 100% (Campadelli-Fiume *et al.*, 1999). *C. pneumoniae* is an obligate intracellular respiratory pathogen first given species status in 1989. The organism causes community-acquired pneumonia and is thought to be primarily a human pathogen (Grayston *et al.*, 1990). Like all *Chlamydiae*, *C. pneumoniae* infects mucosal surfaces, in this case the oral and nasal mucosa, and dissemination of the organism from its site of primary infection has been demonstrated. Interestingly, since its description as a species, this organism has been associated with a surprisingly diverse panel of chronic human diseases in addition to multiple sclerosis (e.g., Balin *et al*., 1998; Mahony and Coomes, 2001; Koskiniemi *et al*., 1996). Epidemiologic studies indicate that *C. pneumoniae*, like HHV-6, is ubiquitous, and that the prevalence of infection increases with increasing age (Leinonen, 1993 for review).

Both HHV-6 and *C. pneumoniae* are reasonable candidates to play some role in multiple sclerosis. The virus is usually acquired during infancy or early childhood, an observation consistent with epidemiologic evidence suggesting that childhood exposure to a pathogen is implicated in the disease (Kurtzke, 1983; Kurtzke and Hyllested, 1988). Moreover, HHV-6 is

neurotropic and can persist or remain latent in many tissues types, including the central nervous system. Like other herpesviruses, HHV-6 can be reactivated by stress or infection with other microbes, and viral infections and stress can trigger clinical exacerbations of multiple sclerosis. In this scenario, HHV-6 acquisition in infancy may initiate a persistent or latent infection of the central nervous system, and at some later time reactivation of the virus leads to damage to oligodendrocytes that ultimately culminates in multiple sclerosis.

C. pneumoniae has been associated with several neurologic conditions, and DNA from the organism has been identified in samples from the central nervous system in a number of disease contexts. Although not normally acquired in early childhood in western nations, acquisition of *C. pneumoniae* usually does begin during the teen years, and this would satisfy in a general sense any requirement for initial infection in some specified age interval. Further and importantly, inflammation is an important component of multiple sclerosis, and this bacterium is well known to be a powerful inducer of the inflammatory response at sites of its residence.

Currently available evidence supporting a role for HHV-6 in development of multiple sclerosis is equivocal (Moore and Wolfson, 2002 and Enbom, 2001 for review). For example, the virus and/or its DNA have been identified in some studies in cerebrospinal fluid and other appropriate clinical samples from patients with multiple sclerosis but not from normal or other controls using various methods, including PCR and immunohistochemistry (e.g., Wilborn *et al.*, 1994; Knox *et al.*, 2000); relatively high anti-HHV-6 antibody levels in some patients *vs.* controls also support the present of active viral infection (Wilborn *et al.*, 1994; Soldan *et al.*, 1997). However, not all searches for the organism or its components have been successful (e.g., Miradola *et al.*, 1999; Taus *et al.*, 2000), and not all studies targeting anti-HHV-6 antibody levels have confirmed high titers in multiple sclerosis patients (e.g., Enbom *et al.*, 1999). To make matters more confusing, a few studies have identified the organism and/or its DNA in relevant samples from both multiple sclerosis patients and various control groups, suggesting that its presence in the central nervous system is not uniquely associated with the disease (e.g., Challoner *et al.*, 1995).

As with HHV-6, evidence concerning a role for *C. pneumoniae* in multiple sclerosis is equivocal at this point. The first report of an association between the organism and multiple sclerosis appeared in a case study in which a patient severely disabled with the disease was demonstrated by PCR and other means to have *C. pneumoniae* in cerebrospinal fluid (Sriram *et al.*, 1998). A larger study was published later by the same group; in that work, the presence of *C. pneumoniae* was examined in cerebrospinal fluid from patients with remitting-relapsing disease, chronic progressive disease, and controls. PCR-positivity among the multiple sclerosis patients was extremely high, and culture-positivity was also significant; most control samples were negative (Sriram *et al.*, 1999).

Several laboratories have reported failure to confirm these observations. For example, in some studies only a small proportion of samples from multiple sclerosis patients were PCR-positive for the organism, although samples from control individuals usually were negative (*e.g.*, Sotgiu *et al.*, 2001; Lay-Schmitt *et al.*, 2000), and in several studies no positive samples at all were identified by any means used (*e.g.*, Boman *et al.*, 2000; Saiz *et al.*, 2001). In parallel with the HHV-6-related studies briefly summarized above, one report identified *C. pneumoniae* DNA in cerebrospinal fluid from 21% of patients with multiple sclerosis and 43% of patients with other neurologic diseases (Gieffers *et al.*, 2001). The authors of this study concluded that the organism is relatively common in cerebrospinal fluid from patients with neurologic disorders, but that it appears not to be specifically associated with multiple sclerosis.

Experimental Problems

The inconsistency among published data regarding the relationship between *C. pneumoniae* or HHV-6 and multiple sclerosis highlights critical technical issues concerning how any association between a micro-organism and a particular disease is established. First, no standard PCR, immuno-histochemical, or other assay system targeting either *C. pneumoniae* or HHV-6 DNA or antigens, or those from any other organism for that matter, in any sample type has been agreed upon among researchers (*e.g.*, Hammerschlag *et al.*, 2000). Each laboratory, of course, develops and uses its own screening system(s), and these can vary widely in sensitivity, specificity, *etc.* from one group to another. Such variation among assay systems makes data comparison among reports virtually impossible. Moreover, regardless of the specific screening systems used, no groups studying HHV-6 in patient samples have assessed those same samples for *C. pneumoniae* or any of the other organisms previously associated with multiple sclerosis, and *vice-versa*. This may be a critical issue, since poly-microbial infection well may be critically important in the context of complex disease elicitation (see *e.g.*, Gérard *et al.*, 2001 and references therein). Moreover, both *C. pneumoniae* and HHV-6 are ubiquitous, and most adults have antibodies to each organism (see Leinonen, 1993; Soldan *et al.*, 1997).

Yet another aspect of how screening is performed contributes to confusion. In some reports for each of the pathogens at issue in multiple sclerosis, little or no information is provided concerning clinical characteristics of the cases studied, including disease duration, disability (EDSS) score, whether patients had remitting-relapsing or chronic progressive disease, and so on. Further, some studies reported analyses of cerebrospinal fluid samples, while others assayed tissue samples from the central nervous system of patients. All these issues equivocate conclusions as to whether *C. pneumoniae* or HHV-6 is related to development or exacerbation of multiple sclerosis. This point becomes critical in view of a recent analysis that identified different categories of neuropathology in multiple sclerosis, as developed next.

Neuropathology in Multiple Sclerosis

Given the variation among multiple sclerosis patients in clinical course, responses to treatment, and other aspects of disease, large-scale comparative neuropathologic studies of patient clinical materials have been surprisingly rare. One report, however, provided an important basis for understanding the heterogeneity in many aspects of the disease. In that study, neuropathology was examined comparatively in materials from a large number of patients with clear diagnoses of multiple sclerosis (Lucchinetti *et al.*, 2000). Materials studied from each patient included tissues with lesions in active stages of demyelination; patients from whom samples were chosen included only those with detailed clinical histories and documented remitting-relapsing or progressive disease. This comprehensive examination revealed four distinct patterns of demyelination. Patterns I and II are consistent with demyelination *via* autoimmune-mediated mechanisms, while patterns III and IV are more consistent with demyelination *via* toxin-mediated mechanisms. Among the samples studied, pattern II was the most common, followed by III, I, and IV in decreasing order of prevalence. Within any given patient, the pattern of neuropathology was consistent, and regardless of pattern, all patients from whom samples were obtained developed clinically evident multiple sclerosis.

These neuropathologic observations clearly suggest that multiple sclerosis may have a more diverse etiology than originally thought, *i.e.*, clinically evident multiple sclerosis may be a common end point for several different starting points. Development of the disease in this

scenario would depend on exposure to a specific initiator at an appropriate time or age, with the genesis of subsequent neuropathology a function of genetic background of the patient. For example, autoimmune-derived demyelination of patterns I or II might develop in a subset of patients subsequent to infection at some appropriate age or time with HHV-6, *C. pneumoniae*, or some other organism *via* molecular mimicry; demyelination of pattern III or IV in other patients might derive more directly from such infection(s) *via* virally-elicited or bacterial toxin-induced oligodendrocyte death. Pathogen-specific T cell-mediated killing of such cells may occur in some cases as well.

The critical point is that, if multiple sclerosis does have a diverse set of initiating factors including one or more micro-organisms, and if the pattern of neuropathogenesis culminating in disease derives from time- or age-sensitive exposure to those factors in the context of the genetic background of the individual patient, then one would not expect cerebrospinal fluid or central nervous system tissue from any set of randomly chosen multiple sclerosis patients to show universal, or even necessarily a high proportion of positivity for any one initiating factor, including a specific micro-organism. Indeed, there is no *a priori* reason to assume that a virus, bacterium, or other initiating factor(s) should endure in the central nervous system past the initiation phase and throughout the disease course in every patient. Rather, one would expect precisely what has been reported for *C. pneumoniae*, HHV-6, and other organisms in relation to multiple sclerosis: variable positive/negative results depending on the distribution of samples among the four patterns of neuropathology, the stage and clinical characteristics of disease in each patient from which samples were obtained, what the specific initiating factor (bacterium, virus, or other) was, and so on. Results would also vary with the sensitivity and/or specificity of the assay system(s) used for screening, as well as various other technical factors relating to the analyses.

Koch's Postulates

If, as the observations outlined here suggest, multiple sclerosis is a disease state that represents a common clinical endpoint for differing initiating factors and pathogenic mechanisms, then Koch's postulates, one of the most successfully employed paradigms of modern infectious disease research, may not be applicable in this context. Specifically, if multiple sclerosis is a consequence of the acquisition of one of several different micro-organisms or toxins during a special window of susceptibility, and if disease genesis is dependent on some aspect(s) of genetic background of the individual acquiring that particular organism or toxin at that time, then the classic notion of a single specific causative agent cannot be applied to this disease. Instead, a new, far more complex paradigm is required. We note in passing that observations from other human diseases have already undermined to some extent the general utility of Koch's postulates. For example, in the case of chronic inflammatory (reactive) arthritis caused by *Chlamydia trachomatis*, the organism can only rarely be cultured from affected joints. It is reasonably well-accepted, however, that this bacterium is viable and metabolically active in those joints, but that it exists at affected sites in an unusual biological state which does not allow completion of its standard developmental cycle to produce new infectious organisms (Inman *et al.*, 2000 for review). Moreover, an essentially identical inflammatory reactive arthritis is caused by infection with enteric pathogens such as *Salmonella*, and these organisms appear not to be viable at all in the joints of affected patients (Villareal *et al.*, 2002).

The Analysis of Causation

In this article, we have developed ideas concerning microbial involvement in the causation and/or exacerbation of chronic diseases using available data for multiple sclerosis as an example. However, this complex scenario for the origin of such diseases can be applied equally well to other idiopathic chronic clinical entities. For example, as the case for multiple sclerosis, rheumatoid arthritis is considered to be a heterogeneous disease, and extensive analyses have clearly indicated that it does not develop from straightforward genetic causes; rather, it is multi-factorial both genetically and environmentally (John and Worthington, 2001 for review; MacKay *et al*., 2002). Further, while early-onset (familial) Alzheimer's disease has been demonstrated to result from lesions in one or more of the genes encoding the amyloid precursor protein, presenilin-1, or presenilin-2, the late-onset (sporadic) form of the disease is not genetic in origin (Tanzi and Bertram, 2001 for review). Rather, it, too, appears to be a multi-factorial disease whose development involves several factors, including some genetic loci whose individual contribution(s) to disease genesis remain to be fully elucidated (Tanzi and Bertram, 2001). Importantly, microbial involvement has been implicated in the genesis of both rheumatoid arthritis and sporadic Alzheimer's disease, as well as atherosclerosis and several other chronic diseases, but conflicting data abound concerning such associations in all cases. Moreover, as with multiple sclerosis, various bacteria and viruses have been identified in appropriate samples obtained from patients with a number of different chronic diseases, but assessment of more than a single infectious agent in those sample sets has been extremely rare.

If Koch's postulates can no longer be applied generally to elucidation of microbial involvement in the etiology of multiple sclerosis, then that paradigm is probably not tenable as a foundation strategy to investigate such involvement in rheumatoid arthritis, atherosclerosis, sporadic Alzheimer's or other idiopathic chronic diseases. Rather, the definition of such involvement must be approached in an entirely different manner. For multiple sclerosis, future screening of materials from patients targeting HHV-6, *C. pneumoniae*, or other organisms or toxins must be done on samples for which detailed neuropathologic characteristics are known, and for which extensive clinical histories are available. It also will be important to study patient materials from individuals early in disease development and from those with established disease. Moreover, while it will be difficult to reach agreement among investigators regarding common PCR-based or other screening systems, such general systems would facilitate comparative analyses among laboratories. The same strategies also must be applied to future studies of infectious involvement in other chronic diseases. Further, in all cases, information regarding the patients' genetic background should be obtained, although it is anything but clear at this point which aspects of that background are relevant. This latter subject will constitute a major research focus in the future. Given enough samples studied from well-characterized patients with multiple sclerosis and other chronic clinical entities, and given enough information concerning the clinical and genetic characteristics of those patients and their disease, it should be possible to reach meaningful conclusions as to whether and which organisms are involved in the genesis and/or exacerbation of chronic diseases, and if so, which subset(s) of patient(s) are affected.

Acknowledgments

The writing of this article was supported by NIH grants AR-42541 and AI-44055 (A.P.H.), NS-06985 (R.H.S.), and AI-44493 and AR-48331(J.A.W.-H.), as well as grant RG1073 from the National Multiple Sclerosis Society (R.H.S.).

References Cited

Balin BJ, Gerard HC, Arking EJ, *et al.* (1998). Identification and characterization of *Chlamydia pneumoniae* in the Alzheimer's brain. *Med. Microbiol. Immunol.* **187:**23-42.

Boman J, Roblin PM, Sundstrom P, *et al*. (2000). Failure to detect *Chlamydia pneumoniae* in the central nervous system of patients with MS. *Neurology* **11:**265.

Campadelli-Fiume G, Mirandola P, Menotti L (1999). Human herpesvirus 6: An emerging pathogen. *Emerg. Infect. Dis.* **5:**353-366.

Challoner PB, Smith KT, Parker JD, *et al.* (1995). Plaque-associated expression of human herpesvirus 6 in multiple sclerosis. *Proc .Natl. Acad. Sci.* (USA*)* **92:**7440-7444.

Enbom M (2001). Human herpes virus 6 in the pathogenesis of multiple sclerosis. *APMIS* **109:**401-411.

Enbom M, Wang F-Z, Fredrikson S, *et al.* (1999). Similar humoral and cellular immunological reactivities to human herpesvirus 6 in patients with multiple sclerosis and controls*. Clin. Diag. Lab. Immunol.* **6:**545-549.

Gérard HC, Wang Z, Wang GF, *et al.* (2001). Chromosomal DNA from a variety of bacterial species is present in synovial tissue in patients with various forms of arthritis. *Arthritis Rheum.* **44:**1689-1697.

Gieffers J, Pohl D, Treib J, *et al*. (2001). Presence of *Chlamydia pneumoniae* DNA in cerebrospinal fluid is a common phenomenon in a variety of neurological diseases and not restricted to multiple sclerosis. *Ann. Neurol.* **49:**585-589.

Grayston JT, Campbell LA, Kuo CC, *et al*. (1990). A new respiratory tract pathogen: *Chlamydia pneumoniae* strain TWAR. *J. Infect. Dis.* **161:**618-625.

Haines JL, Bradford Y, Garcia ME,Reed AD, *et al*. (2002). Multiple susceptibility loci for multiple sclerosis. *Hum. Mol. Genet.* **11:**2251-2256.

Hammerschlag MR, Ke Z, Lu FM, *et al.* (2000). Is *Chlamydia pneumoniae* present in brain lesions of patients with multiple sclerosis? *J. Clin. Microbiol.* **38:** 4274-4276.

Inman RD, Whittum-Hudson JA, Schumacher HR, Hudson AP (2000). *Chlamydia*-associated arthritis. *Curr. Opin. Rheumatol.* **12:**254-262.

Itzhaki RF, Lin WR, Shang D, *et al.* (1997). Herpes simplex virus type 1 in brain and risk of Alzheimer's disease. *Lancet* **349:**241-244.

John S, Worthington J (2001). Genetic epidemiology. Approaches to the genetic analysis of rheumatoid arthritis. *Arthritis Res.* **3:**216-220.

Knox KK, Brewer JH, Henry JM, *et al*. (2000). Human herpesvirus 6 and multiple sclerosis: systemic active infections in patients with early disease. *Clin. Infect. Dis.* **31:**894-903.

Kohbata S, Beaman BL (1991). L-dopa-responsive movement disorder caused by *Nocardia asteroids* localized in the brains of mice. *Infect. Immun.* **59:**181-191.

Koskiniemi M, Gencay M, Salonen O, *et al*. (1996) *Chlamydia pneumoniae* associated with central nervous system infections. *Eur. Neurol.* **36:**160-163.

Kurtzke JF (1983). Some epidemiological trends in multiple sclerosis. *Trends Neurosci.* **6:**75-80.

Kurtzke JF, Hyllested K (1988). Validity of the epidemics of multiple sclerosis in the Faroe Islands. *Neuroepidemiology* **7:**190-227.

Layh-Schmitt G, Bendl C, Hildt U, *et al*. (2000). Evidence for infection with *Chlamydia pneumoniae* in a subgroup of patients with multiple sclerosis. *Ann. Neurol.* **47:**652-655.

Lee A, Fox J, Hazell S (1993). Pathogenicity of *Helicobacter pylori* – a perspective. *Infect. Immun.* **61:**1601-1610.

Leinonen M (1993). Pathogenic mechanisms and epidemiology of *Chlamydia pneumoniae*. *Eur. Heart J.* **14**(suppl K)**:**57-61.

Lenz DC, Lu L, Conant SB, *et al*. (2001). A *Chlamydia pneumoniae*-specific peptide induces experimental autoimmune encephalomyelitis in rats. *J. Immunol.* **167:**1803-1808.

Lucchinetti C, Bruck W, Parisi J, *et al*. (2000). Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann. Neurol.* **47:**707-717.

MacKay K, Eyre S, Myerscough A, *et al*. (2002). Whole genome linkage analysis of rheumatoid arthritis susceptibility loci in 252 affected sibling pairs in the United Kingdom. *Arthritis Rheum.* **46:**632-639.

Mahony JB, Coombes BK (2001). *Chlamydia pneumoniae* and atherosclerosis: does the evidence support a causal or contributory role? *FEMS Microbiol. Lett.* **197:**1-9.

Miradola P, Stefan A, Brambilla E, *et al*. (1999). Absence of human herpes virus 6 and 7 from spinal fluid and serum of multiple sclerosis patients. *Neurology* **53:**1367-1368.

Moore FG, Wolfson C (2002). Human herpes virus 6 and multiple sclerosis. *Acta Neurol. Scan.* **106:**63-83.

Oksenberg JR, Barcellos LF, Hauser SL (1999). Genetic aspects of multiple sclerosis. *Semin. Neurol.* **19:**281-288.

Paterson PY, Swanborg RH (1988). Demyelinating diseases of the central and peripheral nervous systems. In: M Samter, DW Talmage, MM Frank, *et al.* (Eds.), Immunological Diseases, Little Brown and Co., Boston, pp. 1877-1916.

Saiz A, Marcos MA, Graus F, *et al*. (2001). No evidence of CNS infection with *Chlamydia pneumoniae* in patients with multiple sclerosis. *J. Neurol.* **248:**617-618.

Soldan SS, Berti R, Salem N, *et al*. (1997). Association of human herpes virus 6 (HHV-6) with multiple sclerosis: increased IgM response to HHV-6 early antigen and detection of serum HHV-6 DNA. *Nature Med.* **3:**1394-1397.

Sotgiu S, Piana A, Pugiatti M, *et al*. (2001). *Chlamydia pneumoniae* in the cerebrospinal fluid of patients with multiple sclerosis and neurological controls. *Mult. Scler.* **7:**371-374.

Sriram S, Mitchell W, Stratton C (1998.) Multiple sclerosis associated with *Chlamydia pneumoniae* infection of the CNS. *Neurology* **50:**571-572.

Sriram S, Stratton CW, Yao S-Y, *et al*. (1999). *Chlamydia pneumoniae* infection of the central nervous system in multiple sclerosis. *Ann. Neurol.* **46:**6-14.

Tanzi RE, Bertram L (2001). New frontiers in Alzheimer's disease genetics. *Neuron* **32:**181-184.

Taus C, Pucci E, Cartechini E, *et al.* (2000). Absence of HHV-6 and HHV-7 in cerebrospinal fluid in relapsing-remitting multiple sclerosis. *Acta Neurol. Scan.* **101:**224-228.

Tejada-Simon MV, Zang YCQ, Hong J, *et al*. (2002). Detection of viral DNA and immune responses to the human herpesvirus 6 101-kilodalton virion protein in patients with multiple sclerosis and in controls. *J. Virol.* **76:**6147-6154.

Yolken RH, Bachmann S, Ruslanova I, *et al*. (2001). Antibodies to *Toxoplasma gondii* in individuals with first-episode schizophrenia. *Clin. Infect. Dis.* **32:**842-844.

Ufret-Vincenty RL, Quigley L, Tresser N, *et al*. (1998). *In vivo* survival of viral antigen-specific T cells that induce experimental autoimmune encephalomyelitis. *J. Expt. Med.* **188:**1725-1738.

Vastag B (2001). Not so fast: research on infectious links to MS questioned. *J. Am. Med. Assoc.* **285:**279-281.

Villareal C, Whittum-Hudson JA, Hudson AP (2002). Persistent *Chlamydiae* and chronic arthritis. *Arthritis Res.* **4:**5-9.

Wilborn F, Schmidt CA, Brinkmann V, *et al*. (1994). A potential role for human herpesvirus 6 in nervous system disease. *J. Neuroimmunol.* **49:**213-214.

Wucherpfennig KW, Strominger JL (1995). Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. *Cell* **80:**695-705.

Integrating genomic characters for a holistic approach to understanding plant genomes

By I.J. Leitch and M.D. Bennett
Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK
Tel: +44 (0)208 332 5329; Fax: +44 (0)208 332 5310; Email: i.leitch@rbgkew.org.uk

1. Introduction

The term 'genome' has been defined as 'the entire nuclear DNA of an organism.' In recent years there has been an upsurge of interest in the genome as scientists have raced to decode the complete DNA sequence of a variety of organisms' genomes. Following on from the major achievements of sequencing the first multicellular organism in 1998 (a nematode - *Caenorhabdites elegans*, The *C. elegans* Sequencing Consortium, 1998), the first insect (a fruit fly - *Drosophila meloanogaster*, Adams *et al.*, 2000) and the first plant (a weed – *Arabidopsis thaliana*, Arabidopsis Genome Initiative, 2000), the human genome sequence was announced in 2001 (International Human Genome Sequencing Consortium, 2001; Venter *et al.*, 2001). Indeed, the number of organisms whose genomes have now been sequenced is over 650, and currently hardly a month goes by without yet another 'complete' genome sequence being published.

It is perhaps not surprising that there are high hopes that these sequence data will contribute significantly to our understanding of many different biological perspectives of the genome. From the human genome sequence alone these range from broad topics such as understanding cancer, addiction, gene expression, and evolutionary genomics, to the more focused aspects such as membrane trafficking, cytoskeleton, cell cycle and circadian clocks (Birney *et al.*, 2001). Sequences of other organisms are also hoped to be highly informative. For example, of the micro-organisms, the recent release of the DNA sequence of the protozoan *Plasmodium falciparum* genome is of great interest, as this is the organism that causes malaria (Gardner *et al.*, 2002). The elucidation of its genome sequence together with that of the mosquito (*Anopheles gambiae*) which transmits it (Holt *et al.*, 2002) is hoped to provide researchers with a powerful tool for dissecting the biology of this complex organism and may speed the discovery of desperately needed drugs and vaccines for malaria. For plants, it is hoped that analysis of the rice (*Oryza sativa*) genome sequence announced in April 2002 (Goff *et al.*, 2002; Yu *et al.*, 2002) will contribute to a better understanding of its genetics, which is essential for designing breeding programmes aimed at increasing yield – an essential target given that rice is the staple food for around half of the world's population.

Although much new and exciting data will undoubtedly come out of these sequencing projects, they are expensive in terms of money and human resources. For example, the cost of the human genome sequencing project alone is estimated to be around U$300 million dollars so far (and some have estimated the final bill to be closer to U$1 billion dollars). Further, there is much more to the genome than just knowing its DNA sequence. Indeed, there are many other aspects of the genome (e.g. control of gene expression, genome organisation, protein: DNA interactions, genome structure) where knowledge is essential if one is to understand how the information contained within the DNA sequence is translated into a fully functional organism.

This paper deals with three aspects of the genome beyond the DNA sequence, namely (i) Chromosomes (ii) Ploidy level and (iii) Genome size, and discusses how knowledge and understanding of these different aspects can contribute towards a more holistic understanding of the genome[1].

2. Beyond the genome sequence

2.1 Chromosomes

The DNA in all eukaryotic organisms is organised into chromosomes by the successive coiling and folding of the DNA together with other components such as proteins. In humans, the ca. 3 billion bases of DNA which constitute the genome are organised into 23 different chromosome types which are found in the unfertilised egg or sperm. When the egg and sperm fuse, each resulting cell has two copies of each chromosome type, one from the mother and one from the father. Thus, in most of the 100 million million cells which constitute the human body, there are 46 chromosomes. Because each of these cells contains two copies of the genome, one from each parent, humans are considered to be diploid.

However, packing DNA into 23 chromosomes is not the only option. In other organisms there is a great variety of different chromosome numbers and sizes. In animals, the number of chromosomes found in each cell ranges from just 2n = 2 (e.g. the primitive Australian ant *Myrmecia pilosula* (Figure 1a, Crosland and Crozier, 1986) to over 440 in the lycaenid butterfly *Lysandra atlantica*, (Figure 1b, de Lesse, 1970). In plants an even greater diversity has been reported. Although no plant has yet been found with 2n = 2, as in the ant, six species have 2n = 4 (Vanzela, Guerra and Luceno, 1996), including the Australian ephemeral *Brachycome dichromsomatica* (Figure 1c). At the other extreme, the highest chromosome number recorded in an organism to date is 2n = ca. 1440, found in the Adder's tongue fern *Ophioglossum reticulatum* L. (Figure 1d, Khandelwal, 1990).

2.2 Ploidy level

A further layer of complexity in understanding the genome is the biological phenomenon known as polyploidy. As already mentioned above, a diploid organism contains two genomes in each nucleus, one from each parent. A polyploid contains more than two genomes in each nucleus. Thus a tetraploid contains four genomes, a hexaploid has six genomes and so on. Polyploidy is considered to have played an important role in the evolution of both animals and plants. In our own distant ancestry, it has been suggested that two polyploidy events have taken place since vertebrates first evolved (Ohno, 1999). In plants, estimates of polyploidy may be as high as 70% in flowering plants (angiosperms) and 95% in ferns (pteridophytes) (e.g. see review by Leitch & Bennett, 1997). So an understanding of how a polyploid nucleus functions and evolves is important for understanding the genome.

2.3 Genome size

[1] The text presented here is an abridged version of a paper presented at the African Renais-Science meeting held in Durban, South Africa, March 2002, the full text of which may be found in Leitch and Bennett (2002).

A final layer of diversity to be discussed is nuclear genome size, a term used to describe the total amount of DNA present in each genome. The genome size of humans is ca. three billion bases (= ca. 3000 Mb) corresponding to ca. 3.2 pg of DNA (N.B. 1 pg = 10^{-12} g; 1 pg = 980 Mb). If the DNA from each genome were unwound, it would stretch for 2 m, but this is by no means large for an organism. For example, the onion (*Allium cepa* L.), with a genome size of 16.8 pg, contains around five times as much DNA in each cell as humans, whereas the nucleus of the single-celled amoeba *Amoeba dubia*, contains 700 pg (Friz, 1968). Indeed, from currently available data, the genome sizes of all organisms that have so far been investigated have been shown to vary over 80,000 fold (Li, 1997).

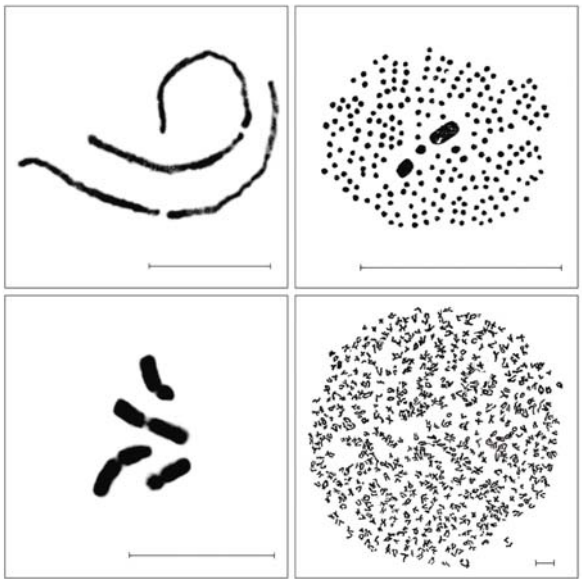


Figure 1: Chromosome number diversity found in animals and plants.
(a) The bulldog ant, *Myrmecia pilosula*, 2n = 4 (from Crosland & Crozier, 1986)

(b) The lycaenid butterfly, *Lysandra atlantica*, 2n = ca. 440 (from de Lesse, 1970)

(c) *Brachycome dichromosomatica*, 2n = 4)

(d) Adder's tongue fern *Ophioglossum reticulatum* 2n = ca. 1440 (from Ninan, 1958). Scale bar = 10 μm.

3. Why is it important to know about chromosomes, ploidy level and genome size?

While the DNA sequence can provide valuable data, knowledge of chromosome numbers, ploidy level and genome size can provide additional information that not only gives further insights into the functioning of the genome, but can also have considerable predictive powers. Below are some examples.

3.1 Importance of knowing about chromosomes

Knowledge of chromosome number and structure is important for understanding some human diseases. For example, extra copies of chromosomes can lead to disorders such as Down's syndrome, where individuals contain an extra copy of chromosome 21. In other cases, chromosome translocations (reciprocal exchange of chromosome material between chromosomes) have been shown to lead to the onset of certain types of cancers. One of the best-characterised cases is found in patients with chronic or acute myelogenous leukemia. This

is caused by a reciprocal translocation between segments at the ends of chromosomes 9 and 22. The location of the break in chromosome 22 determines which type of leukaemia develops.

In plants, knowledge of which chromosomes are present can be useful in, for example, plant breeding programmes designed to incorporate useful genes such as disease resistance from wild (alien) species into cultivated crops. Using a chromosome painting strategy known as genomic *in situ* hybridisation (GISH), the presence, position and size of alien chromosomes, arms or chromosome segments can be determined, and this enables progeny carrying the desired chromosomes or segments to be readily identified (Bennett, 1995; Schwarzacher *et al.*, 1992). For example, saline soils are a major problem particularly in arid and semi-arid regions where high salt concentrations can significantly reduce yield or kill crops. Breeding strategies have been designed to incorporate salt-tolerant characteristics into cultivated wheat (*Triticum aestivum* L.) from one of its wild relatives *Thinopyrum bessarabicum* (Forster, Miller and Law, 1988). GISH provides a fast, sensitive and accurate way of analysing the chromosomes present in the resulting progeny to determine the physical size and number of chromosomes or chromosome segments that have been successfully transferred. Such information can considerably speed up the breeding programme (Bennett, 1995; Schwarzacher *et al.*, 1992).

3.2 Importance of knowing the ploidy level of an organism

Knowing the ploidy level can also be important in plant breeding studies. For example, an important cereal crop in Ethiopia is Teff (*Eragrostis tef*), and yet it is notorious for its low yield, averaging around 1 tonne per hectare. For plant breeders to improve the yield, they need information on its genome structure. However, until recently most of the applied research on Teff was conducted without adequate knowledge of its nuclear genome. Even the ploidy levels of many of the existing cultivars were unknown. The report by Ayele *et al*. (1996) that all major Teff cultivars are tetraploid rather than a mixture of ploidy levels will considerably help plant breeders to make informed decisions when designing crossing experiments.

3.3 Importance of knowing the genome size of an organism

As already noted above (Section 2.3), the genome sizes of organisms so far studied have been shown to vary over 80,000-fold (Li, 1997). Even within angiosperms, the DNA amount has been shown to vary over 1000-fold (Bennett, Bhandol and Leitch, 2000). In trying to understand the significance of this huge variation, comparative studies in angiosperms have played a leading role in showing that the amount of DNA is correlated with a wide range of different characters and that understanding this relationship has considerable predictive powers.

At the cellular level, for example, it has been shown that genome size is correlated with nuclear volume (Figure 2a, Baetcke *et al.*, 1967). Thus, the bigger the genome size or total amount of DNA within the nucleus, the large the minimum nuclear volume. This makes sense as the more DNA present, the larger the nucleus needed to hold it all. Similar studies have shown that DNA amount is correlated with a wide range of characters at the cellular level including pollen volume (Figure 2b, Bennett, 1972), chromosome volume (Figure 2c, Bennett *et al.*, 1983), and duration of meiosis (Figure 2d, Bennett *et al.*, 1981). Clearly, DNA amount correlates closely with many important phenotypic characters.

It is important to note that these relationships are all independent of the DNA sequence of the genome, and from studies such as these it has become clear that the nuclear DNA of an organism influences its phenotype in two ways:

1. By the expression of its genic content (an area where knowing the DNA sequence is important).
2. By the physical effects of its mass and volume which impose absolute limits on the range of phenotypes that can be expressed by genic control. This second effect of the DNA on an organism's phenotype is known as the 'nucleotype' – a term coined by Bennett to define those conditions of the nuclear DNA which affect the phenotype independently of its encoded informational content (Bennett, 1971; Bennett, 1972).

An example of this nucleotypic effect can be seen by comparing *Arabidopsis thaliana*, which has a small genome (ca. 150 Mb) with the large genome of the fritillary (*Fritillaria assyriaca*; ca. 125,000 Mb). With a small genome, *A. thaliana* is an ephemeral, as it can complete its entire life cycle, from seed to plant and back to seed again, in just four weeks. In contrast, *F. assyriaca* has so much DNA that it needs six weeks alone just to undergo meiosis (Bennett, 1977). The nucleotypic effect of the large DNA amount means that whatever genes *F. assyriaca* might contain, there is no way it could behave as an ephemeral, as it simply can not develop quickly enough.

The value of these nucleotypic correlations is that (i) they apply to all species, irrespective of genome size or chromosome number, and (ii) they have considerable predictive powers. For example, the duration of meiosis in rice (*Oryza sativa*) has not yet been measured, but from the nucleotypic regression for DNA amount on duration of meiosis given by Bennett (1977; see Figure 2d) it is predicted that it would take around 18 hours at 20ºC.

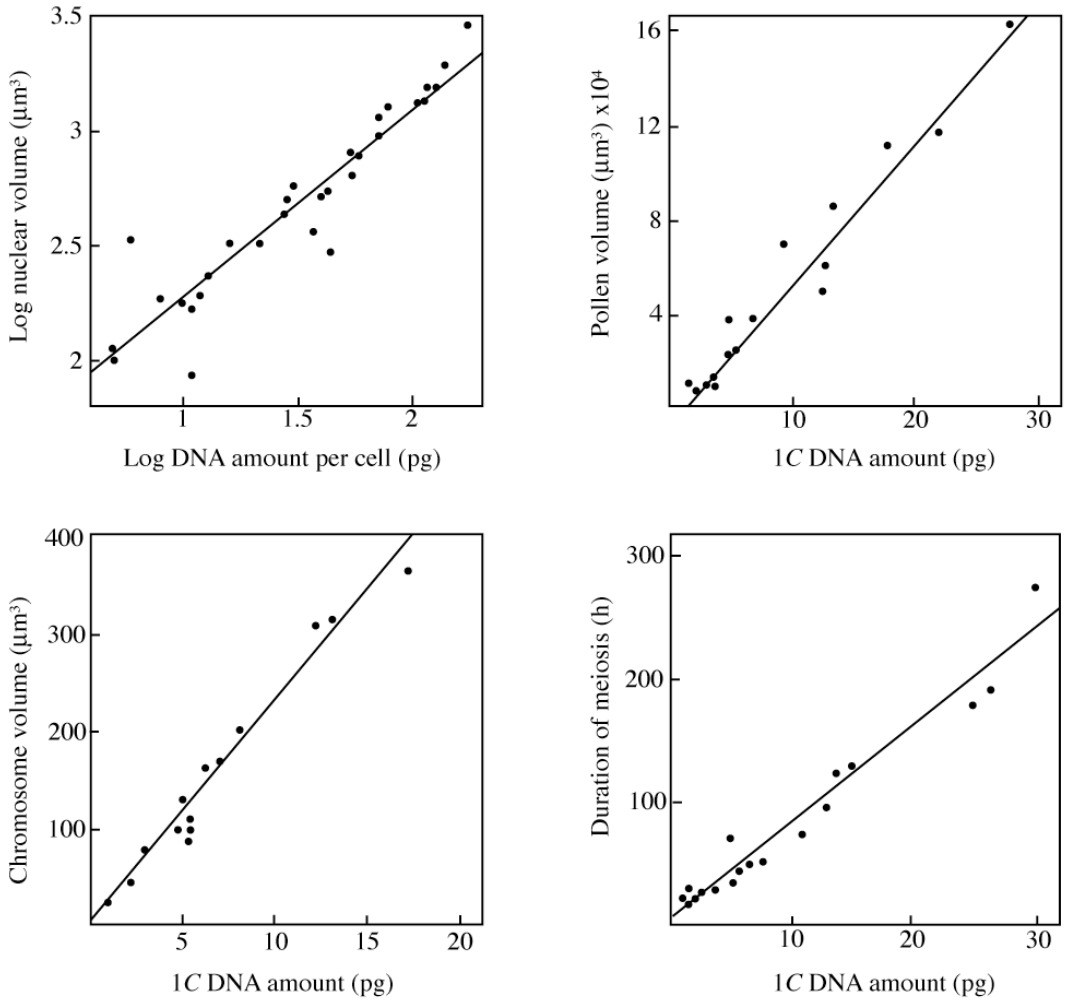
Such predictive powers of genome size can also be applied to areas such as investigating how plants will respond to global warming. A study by Jasienski and Bazzaz (1995) looked at how the growth of seven annual grasses was affected by increased CO_2 concentrations. They found that (i) at elevated concentrations, the growth rate of the grasses was enhanced and that (ii) this enhanced growth was directly correlated with genome size. Plants with larger genomes showed a greater enhancement of growth compared with plants with small genomes.

They concluded that genome size potentially can both influence the responsiveness of a plant species to CO_2 and be affected by CO_2. Such relationships also suggest that there may be significant practical benefits to be gained by manipulating genome size in crops (Bennett, 1998). Another set of studies by Grime (1996) looked at how plants responded to elevated temperatures, another key element of global climate change. The research showed that plants with small genomes showed a greater enhancement of growth than those with larger genomes. Taken together, such studies highlight the complexity of working with biological systems. Perhaps, more importantly, they also stress the need to obtain and incorporate genome size information into computer models and laboratory experiments designed to examine how organisms will respond to the multifaceted environmental changes induced through human activities.

Figure 2: Relationships in angiosperms between DNA amount and different nuclear or cellular characters

(a) nuclear volume in 30 herbaceous species (redrawn from Baetcke *et al.*, 1967);

(b) pollen grain volume at anther dehiscence in 16 species of wind-pollinated grasses (from Bennett, 1972);
(c) total mitotic metaphase volume per cell in 14 species (from Bennett *et al.*, 1983);
(d) duration of meiosis in 18 diploid species grown at 20°C (redrawn from Bennett, 1977).



4. A holistic approach to understanding plant evolution and biodiversity

In isolation, data on chromosomes, ploidy level or genomic size can be useful, as illustrated in the above examples. However, an even greater understanding of the genome can be obtained by combining these data with taxonomic and morphological data. This more holistic approach to understanding plant evolution can be extremely powerful as illustrated below.

4.1 The classification and evolutionary relationships between angiosperms

The Angiosperm Phylogeny Group (APG) is a large international group of researchers who aim to understand the true classification and evolutionary relationships between angiosperms by combining both molecular (e.g. DNA sequence data) and more traditional (e.g. morphological and anatomical data) approaches to provide an accurate name for plants and determine their

nearest relatives. Such studies underpin all scientific research, as without an accurate name for each organism being studied, scientists cannot communicate their results. Further, if a scientist thinks that two species are closely related when they are not or *vice versa*, then the results of research will be mistakenly interpreted.

The first landmark paper from the APG was published in 1993 (Chase *et al.*, 1993) and contained the results of an analysis of DNA sequence data together with 252 non-molecular characters for 499 different angiosperm species. In just five years, further research, combining data from many more species, enabled the relationships of nearly all of the ca. 460 angiosperm families to be published (Angiosperm Phylogeny Group, 1998). This publication attracted considerable interest, as some of the new data challenged more traditionally held views on the relationships between different plants and their families. For example, traditional taxonomy based largely on morphology had always considered the lotus flower (*Nelumbo* - Nelumbonaceae) to be closely related to the waterlily (*Nymphaea* - Nymphaeaceae). Yet the new data from the Angiosperm Phylogeny Group (1998) showed that similarities in appearance were just superficial. Instead, the closest relatives of the lotus were members of the Proteaceae and Platanaceae. Since 1998, the work has continued to progress with ever more genome sequence data being fed into analysis. The emphasis is now on extending the study to look at relationships between the ca. 14,000 angiosperm genera.

The construction of a phylogenetic family tree showing relationships between angiosperm families and genera provides a robust framework on which to hang on other aspects of the genome. Two examples of where this has been done give insights into how this can work.

(a) Evolution of genome size in angiosperms

Genome size, as noted above (Section 3.3), varies over 1000-fold in angiosperms (Bennett, Bhandol and Leitch, 2000). With the availability of a robust phylogenetic tree of the angiosperms, Leitch, Chase and Bennett (1998) superimposed available genome size data to investigate how genome size had evolved since angiosperms first appeared in the fossil record over 165 million years ago (Mya). The results showed that ancestrally angiosperms had small genomes (defined as genome sizes of $\leq$ 3.5 pg). All taxa with large genomes (defined as genomes $\geq$14.0 pg) were found at the tips of the tree within groups that were also ancestrally small.

This is an important finding as it provides one possible factor responsible for the evolutionary success of angiosperms (ca. 250,000 species) compared with their nearest relatives, the gymnosperms (ca. 700 species). Genome sizes in extant gymnosperms vary only 14-fold compared with the 1000-fold variation in angiosperms (Leitch *et al.*, 2001), but their modal genome size of 15.8 pg is about 25 times larger than angiosperms (modal genome size = 0.6 pg). Also, a recent study showed that ancestral gymnosperms were probably characterised by large genomes (Leitch *et al.*, 2001). But why should a small genome size contribute to the success of angiosperms over gymnosperms? It may well be causally related to the nucleotypic effects of genome size discussed above (see Section 3). Having a small genome would enable angiosperms to complete their life cycles (*i.e.* seed to seed) faster than gymnosperms with larger genomes, making it possible for them to adapt more quickly to new environmental conditions as they arose (Leitch, Chase and Bennett, 1998; Rejmanek, 1996).

(b) Chromosome evolution in the peacock irises (*Moraea*; Iridaceae)

Another example of an holistic genomics project is the combination of DNA sequence and chromosome number data to give insights into chromosome evolution in one of the larger South African plant genera, the peacock irises *Moraea* (Goldblatt *et al.*, 2002), comprising ca. 200 species. Until recently, controversies concerning the relationships between *Moraea* species led to uncertainties over the ancestral chromosome number of the genus. As chromosome numbers are highly variable, with n = 4, 5, 6, 7, 8, 9, 10 all being reported (Goldblatt *et al.*, 2002), the direction of chromosome evolution in the genus was disputed.

However, recent collaborative work between researchers at Kirstenbosh, RBG, Kew and Missouri Botanical Gardens has led to the construction of a robust phylogeny of *Moraea* using DNA sequence data. By superimposing chromosome data onto this phylogeny, the ancestral *Moraea* species were shown to have chromosome numbers of n = 10, indicating that this is the ancestral chromosome number for the genus. The derived chromosome number of n = 6, possessed by the majority of species analysed, seems to indicate rapid species diversification after a chromosome number of 6 evolved. As in other plant groups, this could reflect the attainment of a presumable adaptive gene arrangement in six chromosomes (Stebbins, 1950).

5. The future: identifying and filling gaps in the data

The work described above gives some glimpses into the way in which information on different aspects of the genome can be brought together into a more holistic understanding of the genome. It becomes pertinent, therefore, to identify some of the gaps in the data and how future work could contribute to filling them.

5.1 Where are the gaps in plant chromosome numbers?

In angiosperms, only ca. 25% of species have a chromosome count, and yet even these data may be incorrect if the plant has been misnamed. In addition, many of the counts were made for only one individual or population of a species and may thus be unrepresentative for the species. For example, 61 chromosome numbers, varying from 16 to 96, have been recorded for *Cardamine pratensis* (Clapham, Tutin and Warburg, 1952). Clearly, one count may give a very incomplete picture for a species.

It is also dangerous to assume that a count for one species is representative for a genus or family, as work at RBG, Kew on palms showed. Until 1989, the highest chromosome number recorded for the palm family (Arecaceae) was 2n = ca. 200. Then material for a rare rainforest palm (*Voaniola gerardii*) from Madagascar was examined at RBG, Kew. Cytological analysis showed that it had a count of $2n \approx 600$ (Johnson *et al.*, 1989), three times the previous maximum known for a palm.

Such studies illustrate the importance of recognising that the full range of chromosome numbers in angiosperms is currently uncertain and may be several times that already reported. There is clearly a real need to obtain this basic data.

5.2 Where are the gaps in plant ploidy level data?

To estimate the ploidy level of an organism accurately requires data on (i) chromosome number, (ii) chromosome behaviour during meiosis and (iii) knowledge of DNA sequences. However,

rarely is such complete data available. For example, the estimate that 70% of angiosperms are polyploid is based largely on chromosome number alone, and since chromosome numbers are only known for ca. 25% of species (see above), this estimate of polyploidy in angiosperms may be incorrect.

It is also interesting to note that data from the Arabidopsis genome sequencing project (Arabidopsis Genome Initiative, 2000) have shown that over 70% of its genome is duplicated. It has been proposed that these duplications arose by the occurrence of two polyploidization events predicated to have taken place ca. 180 and 112 Mya (Walbot, 2000). Thus *Arabidopsis*, long regarded as a typical diploid, should be considered to be an ancient polyploid. This observation raises questions as to the ploidy level of other organisms that had hitherto been assumed to be diploids based on chromosome number alone. Clearly there is still much fundamental data and understanding needed here.

5.3 Where are the gaps in plant genome size data?

Despite the importance of genome size as a key biodiversity character of biological importance, and the fact that it has been possible to measure DNA amounts since the 1950s, data are only known for just over 4,000 angiosperms, corresponding to just 1.4% of all known species. These were recently compiled into the Plant DNA C-values database (release 1.0, Sept. 2001), which is accessible on the web in recognition of the recommendations of the Convention on Biological Diversity (CBD) to make biodiversity data available (Bennett and Leitch, 2001). This resource is clearly valuable to the research community as evidenced by its use; it currently receives over 1000 hits per month with each enquiry taking on average over 100 DNA amounts.

Yet despite its value, data within the database is very unrepresentative of the world's angiosperm flora for taxonomic groups, geographic regions and plant life forms (Bennett, 1998). At the Angiosperm Genome Size meeting held at RBG, Kew in September 1997, key gaps in genome size data were identified and targets were set to fill them (see Key recommendations of the meeting at http://www.rbgkew.org.uk/cval/conference.html). While some progress has been made in filling these gaps (e.g. Hanson *et al.*, 2001a; Hanson *et al.*, 2001b), a Second Plant Genome Size meeting is planned at RBG, Kew in September 2003 to assess progress and set further targets to improve genome size knowledge.

Such a meeting is timely, as the world currently faces a mass extinction of biodiversity, losing plant species at 10,000 times the normal rate (May, Lawton and Stork, 1995). Within this context it is worth noting that currently it is not yet known whether there is a relationship between genome size and the likelihood of extinction for a species, although there is some reason to expect that such a relationship may exist. Indeed, it has been suggested that slow growing gymnosperm taxa with long life cycles, producing relatively few large seeds are probably at increased risk of extinction (Rejmanek, 1996). These characteristics are often obligately associated with large genomes such as those found in the monocots. As Bennett, Bhandol and Leitch. (2000) suggested, it may well be that large genome sizes identify over-specialised end products of evolutionary lineages with increased chances of extinction. However, until there is sufficient data to determine the true relationship between genome size and extinction rates, we are not in a position to contribute informed data into conservation strategies.

6. Conclusions

Overall, further work is clearly needed to determine, collate and analyse data on chromosome numbers, ploidy level and genome size. These data will be essential if we are to understand the full extent to which these genomic characters impinge on, and interact with the many other facets of the genome to produce a fully functioning organism.

Literature cited

Adams, M.D., Celniker, S.E., Holt, R.A., Evans, C.A., Gocayne, J.D., Amanatides, P.G., Scherer, S.E., Li, P.W., Hoskins, R.A., Galle, R.F., *et al.* 2000. The genome sequence of *Drosophila melanogaster*. *Science*, **287:** 2185-2195.

Angiosperm Phylogeny Group. 1998. An ordinal classification for the families of flowering plants. *Annals of the Missouri Botanical Garden*, **85:** 531-553.

Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature*, **408:** 796-815.

Ayele, M., Dolezel, J., VanDuren, M., Brunner, H. and Zapata Arias, F.J. 1996. Flow cytometric analysis of nuclear genome of the Ethiopian cereal Tef [*Eragrostis tef* (Zucc) Trotter]. *Genetica*, **98:** 211-215.

Baetcke, K.P., Sparrow, A.H., Nauman, C.H. and S.S, S. 1967. The relationship of DNA content to nuclear and chromosome volumes and to radiosensitivity (LD_{50}). *Proceedings of the National Academy of Sciences of the United States of America*, **58:** 533-540.

Bennett, M.D. 1971. The duration of meiosis. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **178:** 277-299.

Bennett, M.D. 1972. Nuclear DNA content and minimum generation time in herbaceous plants. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **181:** 109-135.

Bennett, M.D. 1977. The time and duration of meiosis. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **277:** 201-277.

Bennett, M.D. 1995. The development and use of genomic *in situ* hybridization (GISH) as a new tool in plant biosystematics. In: Brandham, P.E., Bennett, M.D., eds. *Kew Chromosome Conference IV*. Kew: Royal Botanic Gardens, Kew. 167-183.

Bennett, M.D. 1998. Plant genome values: How much do we know? *Proceedings of the National Academy of Sciences of the United States of America*, **95:** 2011-2016.

Bennett, M.D., Bhandol, P. and Leitch, I.J. 2000. Nuclear DNA amounts in angiosperms and their modern uses - 807 new estimates. *Annals of Botany*, **86:** 859-909.

Bennett, M.D., Heslop-Harrison, J.S., Smith, J.B. and Ward, J.P. 1983. DNA density in mitotic and meiotic metaphase chromosomes of plants and animals. *Journal of Cell Science*, **63:** 173-179.

Bennett, M.D. and Leitch, I.J. 2001. Plant DNA C-values database (release 1.0, Sept. 2001). http://www.rbgkew.org.uk/cval/homepage.html

Bennett, M.D., Smith, J.B., Ward, J. and Jenkins, G. 1981. The relationship between nuclear DNA content and centromere volume in higher plants. *Journal of Cell Science*, **47:** 91-115.

Birney, E., Bateman, A., Clamp, M.E. and Hubbard, T.J. 2001. Mining the draft human genome. *Nature*, **409:** 827-828.

Chase, M.W., Soltis, D.E., Olmstead, R.G., Morgan, D., Les, D.H., Mishler, B.D., Duvall, M.R., Price, R.A., Hills, H.G., Qiu, Y.L., *et al.* 1993. Phylogenetics of seed plants - an analysis of nucleotide-sequences from the plastid gene *rbc*L. *Annals of the Missouri Botanical Garden*, **80:** 528-580.

Clapham, A.R., Tutin, T.G. and Warburg, E.F. 1952. *Flora of the British Isles*. Cambridge University Press, Cambridge, UK.

Crosland, M.W.J. and Crozier, R.H. 1986. *Myrmecia pilosula*, an ant with only one pair of chromosomes. *Science*, **231:** 1278-1278.

de Lesse, H. 1970. Les nombres de chromosomes dans le groupe de *Lysandra argester* et leur incidence sur sa taxonomie. *Bulletin de la Societe entomologique de France*, **75:** 64-68.

Forster, B.P., Miller, T.E. and Law, C.N. 1988. Salt tolerance of two Wheat - *Agropyron junceum* disomic addition lines. *Genome*, **30:** 559-564.

Friz, C.T. 1968. Biochemical composition of free-living Amoebae *Chaos chaos, Amoeba dubia* and *Amoeba proteus*. *Comparative Biochemistry and Physiology*, **26:** 81-90.

Gardner, M.J., Hall, N., Fung, E., White, O., Berriman, M., Hyman, R.W., Carlton, J.M., Pain, A., Nelson, K.E., Bowman, S., *et al.* 2002. Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature*, **419:** 498-511.

Goff, S.A., Ricke, D., Lan, T.H., Presting, G., Wang, R.L., Dunn, M., Glazebrook, J., Sessions, A., Oeller, P., Varma, H., *et al.* 2002. A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). *Science*, **296:** 92-100.

Goldblatt, P., Savolainen, V., Porteous, O., Sostaric, I., Powell, M., Reeves, G., Manning, J.C., Barraclough, T.G. and Chase, M.W. 2002. Radiation in the Cape flora and the phylogeny of peacock irises *Moraea* (Iridaceae) based on four plastid DNA regions. *Molecular Phylogenetics and Evolution*, **44:** 341-360.

Grime, J.P. 1996. Testing predictions of the impacts of global change on terrestrial ecosystems. *Aspects of Applied Biology*, **45:** 3-13.

Hanson, L., McMahon, K.A., Johnson, M.A.T. and Bennett, M.D. 2001a. First nuclear DNA C-values for 25 angiosperm families. *Annals of Botany*, **87:** 251-258.

Hanson, L., McMahon, K.A., Johnson, M.A.T. and Bennett, M.D. 2001b. First nuclear DNA C-values for another 25 angiosperm families. *Annals of Botany*, **88:** 851-858.

Holt, R.A., Subramanian, G.M., Halpern, A., Sutton, G.G., Charlab, R., Nusskern, D.R., Wincker, P., Clark, A.G., Ribeiro, J.M.C., Wides, R., *et al.* 2002. The genome sequence of the malaria mosquito *Anopheles gambiae*. *Science*, **298:** 129.

International Human Genome Sequencing Consortium. 2001. Initial sequencing and analysis of the human genome. *Nature*, **409:** 860-921.

Jasienski, M. and Bazzaz, F.A. 1995. Genome size and high CO_2. *Nature*, **376:** 559-560.

Johnson, M.A.T., Kenton, A.Y., Bennett, M.D. and Brandham, P.E. 1989. *Voanioala gerardii* has the highest known chromosome number in the monocotyledons. *Genome*, **32:** 328-333.

Khandelwal, S. 1990. Chromosome evolution in the genus *Ophioglossum* L. *Botanical Journal of the Linnean Society*, **102:** 205-217.

Leitch, I.J. and Bennett, M.D. 1997. Polyploidy in angiosperms. *Trends in Plant Science*, **2:** 470-476.

Leitch, I.J. and Bennett, M.D. 2002. The genome: integrating genomic characters for a holistic approach to understanding plant genomes. In: Baijnath, H., Singh, Y., eds. *Rebirth of Science in Africa - a shared vision for life and environmental sciences.* Pretoria: Umdaus Press. 39-50.

Leitch, I.J., Chase, M.W. and Bennett, M.D. 1998. Phylogenetic analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants. *Annals of Botany*, **82:** 85-94.

Leitch, I.J., Hanson, L., Winfield, M., Parker, J. and Bennett, M.D. 2001. Nuclear DNA C-values complete familial representation in gymnosperms. *Annals of Botany*, **88:** 843-849.

Li, W.-H. 1997. *Molecular Evolution*. Sinauer Associates, Inc., Sunderland, MA.

May, R.M., Lawton, J.H. and Stork, N.e. 1995. Assessing extinction rates. In: Lawton, J.H., May, R.M., eds. *Extinction rates*. Oxford: Oxford University Press. 1-24r.

Ninan, C.A. 1958. Studies on the cytology and phylogeny of the pteridophytes VI. Observations on the Ophioglossaceae. *Cytologia*, **23:** 291-316.

Ohno, S. 1999. Gene duplication and the uniqueness of vertebrate genomes circa 1970-1999. *Seminars in Cell & Developmental Biology*, **10:** 517-522.

Rejmanek, M. 1996. A theory of seed plant invasiveness: the first sketch. *Biological Conservation*, **78:** 171-181.

Schwarzacher, T., Anamthawat-Jonsson, K., Harrison, G.E., Islam, A.K.M.R., Jia, J.Z., King, I.P., Leitch, A.R., Miller, T.E., Reader, S.M., Rogers, W.J., *et al.* 1992. Genomic in situ hybridization to identify alien chromosomes and chromosome segments in wheat. *Theoretical and Applied Genetics*, **84:** 778-786.

Stebbins, G.L. 1950. *Variation and evolution in plants*. Columbia University Press, New York.

The *C. elegans* Sequencing Consortium. 1998. Genome sequence of the nematode *C. elegans*: A platform for investigating biology. *Science*, **282:** 2012-2018.

Vanzela, A.L.L., Guerra, M. and Luceno, M. 1996. *Rhynchospora tenuis* Link (Cyperaceae), a species with the lowest number of holocentric chromosomes. *Cytobios*, **88:** 219-228.

Venter, J.C., Adams, M.D., Myers, E.W., Li, P.W., Mural, R.J., Sutton, G.G., Smith, H.O., Yandell, M., Evans, C.A., Holt, R.A., *et al.* 2001. The sequence of the human genome. *Science*, **291:** 1304-1351.

Walbot, V. 2000. A green chapter in the book of life. *Nature*, **408:** 794-795.
Yu, J., Hu, S.N., Wang, J., Wong, G.K.S., Li, S.G., Liu, B., Deng, Y.J., Dai, L., Zhou, Y., Zhang, X.Q., *et al.* 2002. A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). *Science*, **296:** 79-92.

Integration of transcriptional and translational activities in normal and stressed cells by the noncoding hsr-omega transcripts in *Drosophila*

By Subhash C. Lakhotia

Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University,
Varanasi 221 005, India. Email: lakhotia@bhu.ac.in

Much of the very exciting progress in biology during the past four decades has been propelled by the reductionist belief, commonly known as the "central dogma of molecular biology," that a functional "gene" must produce RNA, which must be translated into a protein (Crick, 1970). Studies using this paradigm have enabled us to move from molecular genetics to genetic engineering and genomics, and now to the post-genomic era.

Success of the "central dogma" led to the common belief that any sequence of DNA or a gene is of relevance only if it has a protein-coding function. Paradoxically, however, almost all eukaryotes have much more DNA than accounted for by the protein coding "genes": e.g., the protein coding DNA sequences account for only ~2% of the human genome (Venter *et al*., 2001). Consequently, the role of the bulk of the genomic DNA in eukaryotes has remained a persisting riddle, and because of the strong belief in the "central dogma," such "non-coding" sequences have often been brushed aside as "selfish" or "junk." In this context, it is still more paradoxical that a large proportion of the "noncoding" genomic DNA is indeed transcribed (Mattick, 2001). Therefore, it appears that the "noncoding genes" are not "junk," but indeed meaningful components of genomes (Lakhotia, 1996, 1999; Erdmann *et al*., 2001; Mattick, 2001; Barciszewski and Erdmann, 2003).

A noncoding gene of *Drosophila melanogaster* being actively studied in the Cytogenetics Laboratory of Banaras Hindu University is the *93D* or the *hsrω* gene. This gene is developmentally active, is induced by a variety of stresses and produces several transcripts, but does not code for any protein (for recent reviews, see Lakhotia, 2001, 2003). The *93D* or the *hsr-omega* (*hsrω*) gene of *Drosophila melanogaster* became an interesting gene more than 3 decades ago in view of its unique inducibility with a brief benzamide treatment. Subsequent studies revealed many unusual features of this gene, a homologue of which is present in all the *Drosophila* species examined. This gene is developmentally active in nearly all cell types of *Drosophila*, is induced by heat shock along with the other heat shock genes, but is singularly induced by a variety of amides, all of which also inhibit general chromosomal transcription. The *hsrω* gene in all species of *Drosophila* has a characteristic architecture with two exons and an intron and a long stretch (>5 to ~15 kb) of tandem repeats on the 3' end of the gene. Like several other noncoding genes, the base sequence of the unique as well as the tandem repeat region of the *hsrω* gene is not conserved in different species. However, in all the *Drosophila* species examined, two primary nucleus-limited transcripts, ~2 kb and >10 kb, respectively, are produced, but none of them carry any significant open-reading frame. The ~2 kb transcript is spliced to generate a 1.2 kb cytoplasmic transcript, which has a translatable ORF of 23-27 amino acids.

The large nucleus-limited >10kb hsrω-n transcript is so far the only known eukaryotic large RNA that shows a speckled distribution in the nucleoplasm (Lakhotia *et al*., 1999; Prasanth *et al*., 2000). In addition to being present at the site of transcription, the hsrω-n transcripts are distributed in the nucleoplasm as many nucleoplasmic speckles close to the chromatin domains. The various nuclear hnRNPs (heterogenous RNA-binding proteins) and some other proteins like Sxl remain bound with the different transcriptionally active chromatin sites and with the nucleoplasmic speckles formed by the hsrω-n transcripts. These speckles, designated as "omega speckles" (Prasanth *et al*., 2000), are distinct from the well-known inter-chromatin granule clusters or IGCs. The hsrω-n transcripts have an essential role in organizing the omega speckles, which serve to dynamically regulate the availability of hnRNPs and related proteins for RNA processing activities at any given time (Lakhotia *et al*., 1999; Prasanth *et al*., 2000; Lakhotia 2001, 2003). Mutants that mis-express the hsrω gene and thus affect the omega speckles have diverse phenotypic consequences (Rajendra *et al*., 2001), presumably because of aberrant processing of various nuclear pre-mRNAs due to altered availability of hnRNPs, etc.

Every cell needs an enormously large variety of transcripts and proteins in variable quantities, and this requirement keeps changing with time. The regulatory strategies at transcriptional level ensure the production of different transcripts required by a cell at any given time. These nascent transcripts are subjected to intricate processing steps (splicing, capping, poly-adenylation, *etc*.) that not only generate the functional mRNAs but also regulate their transport, translatability and half-lives in the cell. One of the very important steps in the post-transcriptional processing of the precursor mRNAs is splicing of the exons (Hastings and Krainer 2001). Since many of the eukaryotic genes are multi-exonic, a versatile regulatory strategy has evolved for cell-type and/or development stage specific alternative splicing of certain transcripts to generate a significantly greater diversity in gene products (Maniatis and Tasic, 2002; Harrison *et al*., 2002; Stamm, 2002; Venables, 2002). In addition to the normal developmental requirements, each cell must also be ready to adapt quickly to unexpected changes in its environment. All these obviously require very elaborate and precise regulatory circuits so that the highly integrated organization displayed by live cells can be maintained and sustained. A large variety of classes of proteins have been identified and shown to be the key players in these diverse regulatory circuits (Neubauer *et al*., 1998). It is also known that these proteins, belonging to two major families, *viz*, the hnRNPs (Dreyfuss *et al*., 2002) and the SR proteins (Graveley, 2000), interact in different combinations to fine-tune the post-transcriptional regulatory circuits (Smith and Valcarcel, 2000).

The unengaged hnRNPs, which are not productively associated with chromatin sites for RNA processing, remain localized at the omega speckles. Under conditions of cellular stress, which inhibit most of the nuclear transcription and RNA processing, the hnRNPs move away from chromatin and get associated with the concomitantly increased levels of hsrω-n RNA (Prasanth *et al*., 2000). The omega speckles provide a storage site for the unengaged hnRNPs and, therefore, modulation of the levels of the hsrω-n transcripts has a pivotal role in regulating the availability of the various hnRNPs for post-transcriptional processing of pre-mRNAs. Since the ratio of hnRNPs and SR proteins at the splice-sites has significant roles in regulating alternative splicing (Smith and Valcarcel, 2000), the hsrω-n transcripts assume a key role in integrating RNA processing activity by regulating the levels of hnRNPs in the active (chromatin associated) and inactive (omega speckles) compartments (Lakhotia 2003).

The hsrω-c, the smaller (~1.2kb) transcript of the *hsrω* gene, is cytoplasmic, generally short-lived and codes for only a short peptide (23-27 amino acids long), which, together with the

1.2kb RNA, is apparently degraded as soon as translated. The purpose of the act of translation of the short ORF in hsrω-c RNA seems to be to monitor the efficiency of cellular translational machinery. Any perturbation in translational activity stabilizes the hsrω-c RNA, resulting in an increase in its level. The increase in hsrω-c RNA level perhaps signals other response/s in the cell.

Thus the *hsrω* gene, although not coding for a typical protein product, contributes to the self-organization of cellular activities through its transcripts (Lakhotia 2003).

The novel function of this noncoding RNA, together with the increasing awareness about other classes of noncoding RNAs, like the Xist in mammals, Rox1 and Rox2 in *Drosophila*, the large variety of micro-RNAs, etc., make it clear that the different noncoding RNAs present in pro- as well as eukaryotes have essential functions rather than merely being products of "junk" or "selfish" DNA sequences (Barciszewski and Erdmann, 2003). Further studies on such DNA sequences will be essential to integrate our understanding of the diverse ways in which the genome functions and maintains the self-organization of biological systems.

References

Barciszewski, J. & Erdmann, V. A. (eds.) 2003. *Noncoding RNAs: Molecular Biology and Molecular Medicine*. Eurekah.Com, Austin, 1-270.

Crick, F. 1970. Central dogma of molecular biology. *Nature*, **227**: 561-563.

Dreyfuss, G., Kim, V. N. & Kataoka, N. 2002. Messenger-RNA binding proteins and the messages they carry. *Nar. Rev. Cell Biol.*, **3**: 195-205.

Erdmann, V. A., Barciszewska, M. Z., Szymanski, M. *et al.* 2001. The non-coding RNAs as riboregulators. *Nucleic Acids Res.*, **29**: 189-193.

Graveley, B. R. 2000. Sorting out the complexity of SR protein functions. *RNA*, **6**: 1197-1211.

Harrison, P. M., Kumar, A., Lang, N., Synder M. & Gerstein, M. 2002. A question of size: the eukaryotic proteome and the problems in defining it. *Nucleic Acids Res.*, **30**: 1083-1090.

Hastings, M. L. & Krainer, A. R. 2001. Pre-mRNA splicing in the new millennium. *Curr. Opin. Cell Biol.*, **13**: 302-309.

Lakhotia, S. C. 1996. RNA polymerase II dependent genes that do not code for protein. *Ind. J. Biochem. Biophys*., **33**: 93-102.

Lakhotia, S. C. 1999. Non-coding RNAs: versatile roles in cell regulation. *Curr. Science*, **77**: 479-480.

Lakhotia, S. C. 2001. Heat shock response – regulation and functions of coding and non-coding heat shock genes in *Drosophila*. *Proc. Indian Natn. Sci. Acad*. (*PINSA*), **B67**: 247-264.

Lakhotia, S. C. 2003. The non-coding, developmentally active and stress inducible *hsrω* gene of *Drosophila melanogaster* integrates post-transcriptional processing of other nuclear transcripts. In *Noncoding RNAs: Molecular Biology and Molecular Medicine* (eds. Barciszewski, J. & Erdmann, V. A.), Eurekah.Com, Austin, pp.203-220.

Lakhotia, S. C., Ray, P., Rajendra, T. K. & Prasanth K. V. 1999. The non-coding transcripts of *hsrω* gene in *Drosophila*: do they regulate trafficking and availability of nuclear RNA-processing factors? *Curr Science*, **77**: 553-563.

Maniatis, T. & Tasic, B. (2002). Alternative pre-mRNA splicing and proteome expansion in metazoans. *Nature* **418**: 236 - 243

Mattick, J. S. 2001. Noncoding RNAs: the architects of eukaryotic complexity. *EMBO Rep.*, **2**: 986-991.

Neubauer, G., King, A., Rappsilber, J., Calvio, C., Watson, M., Ajuh, P., Sleeman, J., Lamond, A. & Mann, M. (1998). Mass spectrometry and EST-database searching allows characterization of the multiprotein spliceosome complex. *Nat. Genet.*, **20**: 46-50

Prasanth, K. V., Rajendra, T. K., Lal, A. K. & Lakhotia S. C. 2000. Omega speckles - a novel class of nuclear speckles containing hnRNPs associated with non-coding hsr-omega RNA in *Drosophila*. *J Cell Sci*, **113**: 3485-3497.

Rajendra, T. K, Prasanth, K. V. & Lakhotia, S. C. 2001. Male sterility associated with over-expression of the non-coding *hsrω* gene in cyst cells of testis of *Drosophila melanogaster*. *J Genetics*, **80**: 97-110.

Smith, C. W. & Valcarcel, J. 2000. Alternative pre-mRNA splicing: the logic of combinatorial control. *Trends Biochem. Sci.*, **25**: 381-388.

Stamm, S. 2002. Signals and their transduction pathways regulating alternative splicing: a new dimension of the human genome. *Hum. Mol. Genet.*, **11**: 2409-2416.

Venables, J. P., 2002. Alternative splicing in the testes. *Curr. Opin. Genet. Dev.*, **12**: 615-619.

Venter, J. C., Adams, M. D., Myers, E. W., Li, P.W., Mural, R. J., Sutton, G. G., Smith, H. O., Yandell, M., Evans, C. A., Holt R. A. *et al*., 2001. The sequence of the human genome. *Science*, **291**: 1304-1351.

Sustainable Agriculture for Developing Countries

A report of the meeting organized by the European Group on Life Sciences (EGLS) held in Brussels, Belgium, at the end of January, 2003

by Alan D.B. Malcolm
Executive Director, Institute of Biology, 20 Queensberry Place, London, SW7 2DZ, UK
Email: a.malcolm@iob.org

You can't really blame developing economies for viewing Europe and the USA's offer of GM technology with a degree of scepticism. Our colonial powers have in the past given them railways and legal systems. We have also forced them to adopt our languages as well as one or other variation of Christianity. The gifts of smallpox and whisky are even more questionable.

Now we promise them that Genetic Manipulation (GM) technology will feed all of them for ever more, while at the same time demonstrating that Europe itself is unconvinced of the risk benefit analysis, and that consequentially they may find their exports to Europe blocked. It is amazing that they are even prepared to go as far as looking the gift horse in the mouth, or travelling to an International Meeting sponsored by the European Union (EU).

It was with these thoughts in mind that the European Group on Life Sciences (EGLS) set up by Pierre Busquin (Commissioner, EU), decided to organise a meeting to explore these issues. 900 scientists and policy makers from all over the globe assembled in Brussels at the end of January, 2003, to discuss "Sustainable Agriculture for Developing Countries."

It should be pointed out at the start that even the base line is capable of different interpretations. Sustainable to a starving population means sustaining the current inhabitants rather than worrying about agriculture 20 years down the line, much less the preservation of species that may be of interest to scientists working in laboratories in rich countries.

Some of the background facts have been so well rehearsed that some audiences may becoming blasé. Yet they bear repeating as was elegantly done in the first presentation by Ismail Serageldin (Egypt), and later by Timothy Reeves.

Serageldin drew the meeting to its senses very quickly. He reminded us that in just 50 years the amount of fertiliser used in world agriculture had risen 23 times and pesticides by 53 times. While a return to "peasant" style agriculture of the type endorsed by the organic movement may have enormous emotional attractions, a quick glance at some hard data returned us to reality. In 1900, 0.85 billion hectares cultivated worldwide supported 1.6 billion people. Today's figure of 1.5 billion hectares could therefore provide food for 2.9 billion people if agronomic methods remained the same. With current estimates of world population at around 5-6 billion, the levels of malnutrition and starvation would be even more appalling than they are already. He made a plea for the various valid issues involved in the discussion of GM technology to be kept separate. He saw these as :

a. Ethics
b. Safety
c. Economic domination and concentration in the hands of a few nations and companies
d. Ownership of Intellectual Property (IP)

e. Proportion of Research and Development funded by the public purse (it was 70% only fifteen years ago, now it is a mere 38%)

The ethical challenge, often phrased as "is it natural?", has always been weak. The answer is an unequivocal "No," but then conventional plant breeding, and use of pharmaceuticals are not exactly "natural" either. Safety is not an absolute, but a relative matter. Starvation is obviously highly dangerous.

Economic domination and ownership of IP is a more tricky matter. Serageldin was the first of several speakers who encouraged private industry not to exploit the technology as fully as their legal position might allow.

He reminded us that the criticism by those opposed to the introduction of new technology: "where are the benefits?" was a very narrow view. The development of yellow rice was unquestionably a benefit if you were unfortunate to live in a country where Vitamin A deficiency was a serious problem.

Florence Wambugu (Kenya) has been one of the more articulate ambassadors on behalf of GM technology in developing countries for several years now.

She reminded us that the world population continued to grow faster than the present increase in agronomic efficiency. This inevitably meant that more land had to be made available for food production. This led inexorably to environmental degradation and loss of biodiversity, stunted economic growth and resulted in a lack of job creation. In fact it is poverty that is the worst destroyer of biodiversity.

Her table showing the output per hectare of ten staple crops in Africa *versus* the rest of the world was little short of alarming. The ratio was 1:3 at best and sometimes was as low as 1:6.

Louise Fresco (FAO Agriculture Department) underlined some of the political and social parameters that need to be in place before acceptance is likely.

First there needs to be an openness, particularly about the ownership of Intellectual Property. The introduction of GMO's needs to be monitored, databases developed and made widely available. Some countries might need help in establishing such monitoring and both contributing to such databases as well as benefiting from them. Many countries would need support to assess and manage risk assessment in the food chain. Amplifying Serageldin's observation, she appealed for an increase in publicly funded resources in this area. Additionally the private sector should provide a higher level of information to developing countries.

She embellished her presentation with two references to Alice in Wonderland: The Cheshire Cat speaking "*Where do you want to go? If you don't know, it doesn't matter which path you take,*" and then Alice "*If you walk long enough, you will always get somewhere.*" It was not clear whether she expected the audience to find these quotations comforting or not.

Peter Hartmann (Nigeria) gave us some clear case histories while reminding us that most of increased production results from increased acreage. 200 million Africans depend on cassava as a staple food. The spread of a mosaic virus using the white fly as a vector was chillingly well documented as it migrated from Uganda in 1990, first to Tanzania, then to Rwanda and Kenya

and most recently to Congo. If a biological solution to this is not found before it reaches Nigeria, tens of millions could starve.

Food security is not only about production. It can also be about reducing post harvest losses. Cereals in the warm moist climate of many African countries provide a comfortable home to *Aspergillus flavus*, a mould that produces the carcinogenic aflatoxin. In Benin and Togo, as many as 99% of children test positive for exposure to it.

Finally, the cow pea is an important source of protein that is being put at risk because of the Maruca pod borer.

GM technology is not only about transferring new characteristics to old crops. It can also be used to develop improved analytical techniques to identify disease. This point was emphasised by Tilahun Yilma (California) using his approach to rinderpest as an example. Hundreds of millions of cattle have been killed by this virus over the centuries. More than 30 African countries are now using an ELISA kit, and diagnosis has improved enormously. Additionally, the use of recombinant DNA to make vaccines has provided a powerful prophylactic agent.

Fish provide an important food supply for many cultures. Farming them is still in its infancy, as we were reminded by Toong Jin Lam (Singapore). There is heavy mortality from larvae to fry stage, some of which is due to infectious agents. Once again, production of recombinant vaccines may have a vital role to play. However, growth rates can also be manipulated. In Tilapia, where the male grows much more rapidly than the female, the sex ratio can be manipulated. Alternatively, transgenic fish can be produced containing active copies of a growth hormone gene.

A huge area (30%) of the world's arable land is too acidic for ideal crop production. Luis Herrer-Estrella (Mexico) described how such soils expose plants to too high levels of aluminum and manganese, and deprive them of necessary levels of phosphorus and magnesium. Transgenic plants (tobacco, papaya, Arabidopsis and alfalfa so far) can be produced that secrete organic acids such as citrate from roots. This chelates the toxic aluminum and thus stimulates plant growth. This is a more feasible approach to the problem than the classical one of shovelling tons of lime on to the soil. Jim Peacock (Australia) pointed out that hyperacidity was an under-estimated agronomic problem, because it was invisible, whereas hypersalinity displayed itself by whiteness in the soil.

Similarly plants can be provided with the phytase gene from *Aspergillus*. When this is secreted into the soil, it hydrolyses phytate (inositol hexaphosphate) and thus greatly increases the availability of free phosphate for the plant's nutritional requirements.

Paulo Arruda (Brazil) reminded us that agriculture produces more than food. In the case of Brazil, 50% of their sugar cane is used to produce ethanol by fermentation of sugar cane. By intercropping soya and sugar cane, they can improve delivery of fixed nitrogen to the sugar. About one quarter of all the gasoline consumed in Brazil has some ethanol added. Apart from reducing the carbon dioxide produced from fossil fuel, it also significantly reduces pollution from carbon monoxide and from sulphur dioxide. They have recently sequenced more than quarter of a million EST's (Expressed Sequence Tags) and this will allow them to select for sugar cane cultivars that are stress (drought, cold, *etc.*) resistant.

The Chinese may have entered this arena comparatively recently, but Huanming Yang showed us elegantly and amusingly how they have caught up. In addition to contributing to sequencing the human genome, they have also led the way in rice, pig and soya gene identification and exploitation.

Jim Peacock (Australia) left us in no doubt that without the use of Bt transgenic cotton, Australian cotton production would be zero. As it is, more than $Aus 1.5bn is produced annually. Switching from chemical pesticides has been hugely beneficial to insects other than *Helicoverpa armigera*. The 60% reduction in the use of chemicals has also clearly improved the purity of local water courses to the satisfaction of local populations. He was at pains to point out that it took about five years before the farmer saw the economic benefit. The likelihood of the transgene escaping by cross fertilisation with wild relatives is highly unlikely, since *Gossypium hirsutum* (the cultivated variety) is tetraploid, whereas the wild strain is diploid. Using two separate Bt genes increased the time for the acquisition of resistance from 10 generations to a more comfortable 200 generations. An economic (and environmental) by-product is that companies specialising in aerial spraying are going out of business in much the same way as the ice salesman of Boston (Mass.) disappeared a hundred years ago with the advent of the domestic refrigerator !

There was an oddly irrelevant criticism by Gilles-Eric Seralini (France), on the grounds that Australia was not a developing country ! Professor Seralini gave the impression of trying to occupy the niches once occupied by Mae Wan Ho and by Jose Bové !

Olivier Hanotte (Kenya) eloquently expounded how animals are used not only for meat, but also to provide milk. He even showed how surplus milk can be used to pay school tuition fees – a fact often forgotten by those used to the welfare state in prosperous countries. The depredation by diseases such as *trypanosomiasis* is therefore serious. The tsetse fly vector is probably responsible for an annual economic loss of some 5bn Euros in sub-Saharan Africa. We don’t yet have the complete genome of the cattle or the fly or the parasite, thus holding up selective breeding of resistant strains, the development of recombinant vaccines and targeted drug production.

East coast fever caused by the parasite *Theileria parva* is yet another example, although the development of a recombinant vaccine against the p67 major sporozoite antigen offers hope.

It is reasonably well known that there are many gene banks world wide storing plant seeds. The likelihood of any irreversible decline in the biodiversity of plants as a consequence of GM technology is therefore vanishingly small. This is not quite so clear in the case of animals. Anne McLaren made an impassioned plea for the creation of what she called a “frozen ark” *i.e.*, frozen blood (or better, DNA) samples. These could be rescued should it ever become clear that a valuable characteristic had been lost in the wild.

Commissioner Poul Nielson (EU), described how the (Bill) Gates Foundation intends to invest $200m in order to stimulate pharmaceutical research for drugs where there may be a clinical need, but no lucrative market.

Philippe Busquin, whose brainchild the meeting was, talked about the 63 projects currently supported by the EU Biotech programme. Capacity building is a key aim, and Framework VI (to be launched soon) has double the amount of money to support mobility of appropriate scientists.

Tim Reeves gave a useful summary in which he emphasised that Biotechnology concerned itself with all the following:

a. Genomics
b. Marker Assisted Breeding
c. DNA Fingerprinting
d. Tissue Culture / Cloning
e. Wide Hybridisation
f. Genetic Engineering

He was clear that until concerns caused by:

a. Budget share of food
b. Population share in agriculture
c. Livelihoods in agriculture
d. Political / Market power by farmers and consumers
e. Willingness to take risks
f. Health *versus* food concerns
g. Environmental *versus* food concerns,

the obvious benefits of:

a. Higher productivity / Lower costs
b. Reduced risks
c. Reduced input
d. Better food
e. Improved biodiversity
f. Sustainable use of natural resources,

would remain theoretical.

He identified that the risks that needed to be spelled out clearly (and tackled) included:

a. Allergens
b. Toxins
c. Cross fertilisation / Gene flow
d. Loss of biodiversity
e. Reduced farmer access

It was important that research remained relevant and in particular addressed:

a. Crops relevant to small farmers
b. Appropriate traits

Key issues that needed to be addressed as a matter of some urgency included:

a. Expand public investment
b. Biosafety
c. IPR – new approaches
d. Trade
e. Informed debate
f. Responsible behaviour – to include the public sector, the private sector as well as advocacy groups.

Incidentally, it is no longer necessary to travel to Brussels to listen to and watch such debates. They are available live by webcam. You can't yet ask questions live and of course you miss the networking, but it does allow the travel budget to go further.

Biology International N° 45 (December. 2003)

ENCYLOPEDIA OF LIFE SUPPORT SYSTEMS (EOLSS)

An integrated knowledge base dedicated to the health, maintenance, and future of the web of life on planet Earth, focusing on sustainable development in ail its myriad aspects front ecological issues to human security.

The World's largest source of knowledge on the subject of sustainable development was officially released by the UNESCO Director General on the 3rd September 2002 during the World Summit on Sustainable Development in Johannesburg, South Africa. With contributions from more than 6000 scholars, this Internet-based archive will be regularly updated and made available free of charge to universities in the least developed countries and disadvantaged individuals worldwide. The EOLSS is a knowledge base that addresses ail the myriad aspects of sustainable development from ecological issues to human security.

The EOLSS is the result of an unprecedented global effort and a decade of planning. Never before has a publication of this kind gone beyond ecological sciences to cover ail aspects of sustainable development. EOLSS is unique in that it comprehensively examines from their origins, the threats facing ail the Systems that support life on Earth—from the climate, the world's oceans, forests, water cycle, and atmosphere to social Systems. It is becoming increasingly apparent that our complex industrial Systems, both organizational and technological, are the main driving force of global environmental destruction, and thus the main threat to the long-term survival of humanity. To build a sustainable society for our children and future generations—the great challenge of our time—we need to fundamentally redesign many of our technologies and social institutions so as to bridge the wide gap between human design and the ecologically sustainable Systems of nature. This means that organizations need to undergo fundamental changes, both in order to adapt to the new business environment and to become ecologically sustainable.

The contributions offer step-by-step explanations on how to apply the abstract or the pure sciences such as mathematics, to assess environmental pollution or to predict food consumption patterns. However, technical solutions alone won't resolve the current ecological crisis. EOLSS therefore covers a diverse range of social issues—from human rights and poverty to psychology and anthropology.

The leading experts who have contributed to this state-of-the-art publication come from diverse fields such as: the natural sciences (like chemistry and biology); social sciences (such as history. economies, law, psychology, etc.); humanities; engineering, and technology. EOLSS also deals with interdisciplinary subjects, like earth and atmospheric sciences, environmental economics as well as the most effective approaches for managing natural resources like renewable and non-renewable energy, biodiversity, and agriculture.

This approach is critical for managing life on Earth. The global water crisis, for example, cannot be resolved by a single discipline. The most experienced civil engineer responsible for constructing dams and mapping the flows of rivers may have little knowledge on tapping groundwater sources, which offer tremendous potential provided that proper safeguards are

taken. EOLSS provides not only the technical information required but also critical analysis on the economies and politics involved in managing such a resource.

"The Encyclopedia of Life Support Systems is different from traditional encyclopedias. It is the result of an unprecedented world-wide effort that has attempted to forge pathways between disciplines in order to address contemporary problems," said UNESCO Director General Koïchiro Matsuura. "A source-book of knowledge that links together our concern for peace, progress, and sustainable development, the EOLSS draws sustenance from the ethics, science and culture of peace. At the same time, it is a forward-looking publication, designed as a global guide to professional practice, education, and heightened social awareness of critical life support issues. In particular, the EOLSS presents perspectives from regions and cultures around the world, and seeks to avoid geographic, racial, cultural, political, gender, age, or religious bias."

"EOLSS has the goal to provide a firm knowledge base for future activities to prolong the lifetime of the human race in a hospitable environment", according to Richard R. Ernst, Nobel Laureate in Chemistry.

Leon M. Lederman, Nobel Laureate in Physics remarked: "The EOLSS is not only appropriate, but it is imaginative and, to my knowledge, unique. Much of what we can write about science, about energy, about our far-ranging knowledge base, can indeed be found in major encyclopedias, but as I understand your vision, never as a central theme; the theme of humanity, embedded in nature and constrained to find ways of maintaining a relationship with nature based upon understanding and respect."

In the words of M.S. Swaminathan, First World Food Prize Winner, "Ecotechnology involving appropriate blends of traditional technologies and the ecological prudence of the past with frontier technologies such as biotechnology, information technology, space technology, new materials, renewable energy technology and management technology, can help us to promote global sustainable development involving harmony between humankind and nature on the one hand and tolerance and love of diversity and pluralism in human societies on the other. We need shifts in technology and public policy. This is a challenging task to \which the Encyclopedia of Life Support Systems should address itself. "

According to Jean-Marie Lehn, Nobel Laureate in Chemistry: "Pursuit of knowledge and truth supersedes present considerations of what nature, life or the world are or should be, for our own vision can only be a narrow one. Ethical evaluation and rules of justice have changed and will change over time and will have to adapt. Law is made for man, not man for law. If it does not fit any more, change it…. Some think that it is being arrogant to try to modify nature; arrogance is to claim that we are perfect as we are! With ail the caution that must be exercised and despite the risks that will be encountered, carefully pondering each step, mankind must and will continue along its path, for we have no right to switch off the lights of the future…. We have to walk the path from the tree of knowledge to the control of destiny."

J.L. Lions, Japan Prize winner in Applied Mathematics said: "EOLSS is concerned with the Life Support Systems.... Each of these Systems is a very complex one. ...we have to think of all these "systems" as closely related "subsystems" of the Planet Earth System. The situation is extremely different in most of life support Systems modeling.... There is not one model, but a hierarchy of models. Examples of these situations will be given throughout the Encyclopedia. … More delicate

are the global problems, involving several goals, with possible conflicts of interest. …Rational decisions will be more and more possible to envision if one will be able to couple the physical modeling to economic and financial models and to human factors…. These delicate and fundamental questions will deserve a lot of attention in the Encyclopedia."

S.P. Kapitza, UNESCO Kalinga Prize Winner said: "The population of our planet and its development over the ages sets the scène for considering ail global problems and it is reasonable to begin their discussion with population growth. ... Thus we are dealing with an interdisciplinary problem in an attempt to describe the total human experience, right from its very beginning. But without this perspective of time it is not possible to objectively assess what is happening today and provide an objective view of the present state of development, the challenge now facing humanity."

Knowledge is dynamic. It grows and evolves according to the needs of human society. In the past, different civilizations categorized knowledge to suit the cultural paradigm of their times.

A key focus of the present time and an area demanding much further investigation, is the relationship between humans and nature. Sciences must be our guide in this endeavor, but history too can teach us important lessons of co-existence with our environment. To date, education and the media have only succeeded in fostering a culture characterized by narrow vested interests intolerance and violence. While we meddle with the natural environment at our peril, and have failed to improve on the best that nature provides, human culture is the fountain of our progress and creativity. There must be a fundamental change in education, creating the desire for environmental protection and respect for human dignity and rights, as the two are mutually empowering. We must build on the best of our culture to engender a new attitude towards the quality and sustainability of life on earth.

In view of the above the EOLSS body of knowledge is inspired by a vision that includes the following paradigm: the sciences should be at the service of humanity as a whole and should contribute to providing everyone with a deeper understanding of nature and society a better quality of life and a sustainable and healthy environment for present and future generations.

The Encyclopedia is designed to be a guide and référence for a wide range of users: from natural and social scientists to engineers, economists, educators, university students and professors, conservationists, entrepreneurs, law and policy-makers. The aim is not merely to provide raw information but to serve as a kind of expert advisor. The various chapters are divided into different levels of specialization to cater to a diverse readership. General readers might turn to the EOLSS for summaries on energy, for example, while university students may focus more on the explanations of the theoretical principles of energy, and policy makers turn to the future perspectives and related recommendations.

"Our best hopes for future peace and global security rely upon strengthened international cooperation to protect the web of life support Systems that we destroy, so ridiculously, day in and day out. We share only one planet. We—and future generations—have nowhere else to go," according to Mostafa K. Tolba, formerly Executive Director of the United Nations Environment Programmed and the editor of ‘Our *Fragile World: Challenges and Opportunities for Sustainable Development'* a two volume publication in about 2300 pages published in 2001 as forerunner to the Encyclopedia. "It is hoped that the encyclopedia will provide the necessary impetus and

knowledge support to enable humanity to choose the right direction to move towards sustainable development."

The EOLSS project is coordinated by the UNESCO-EOLSS Joint Committee and sponsored by Eolss Publishers, which is based in Oxford (United Kingdom). Through the many and diverse consultation exercises around the world, the EOLSS has benefited immensely from the academic, intellectual, and scholarly advice of each and every member of the 1000-strong International Editorial Council, which includes Nobel and UN Kalinga Lauréates, World Food Prize Lauréates and several fellows of academies of science and engineering of countries throughout the world.

Teams of experts will regularly update the various sections of the web-based encyclopedia, making EOLSS a "living library and a site for action rather than just a publication," according to Mustafa El Tayeb, Secretary of the UNESCO-EOLSS Joint Committee. The Inaugural Edition that was released during the World Summit already contains about 25 million words, equivalent to about 50,000 standard pages, and several thousand tables, graphics, boxes, and photographs. Within the next two years, it will mature to its full size of about 70 million words (equivalent to about 150 volumes) through new editions and regular updates as often as every three months.

"Most United Nations projects of this size begin by consulting government representatives. But EOLSS went straight to the scientific communities involved," said Andréas Szôllôsi-Nagy a member of the UNESCO-EOLSS Joint Committee and Director of UNESCO's International Hydrological Programmed. In 1996 thousands of scientists, engineers and policy-makers began meeting just to define the scope of the project, before discussing the details of the contributions. Regional workshops verve held in Washington DC, Tokyo, Moscow, Mexico City, Beijing, Panama, Abu sultan (Egypt), and Kuala Lumpur to develop a list of possible subjects and debate analytical approaches for treating them.

"From the start, we had to be absolutely certain that one school of thought did not dominate the conceptual basis of the encyclopedia," said Szôllôsi-Nagy. "This democratic process guided every step in the encyclopedia's development. With thousands of authors from more than 100 countries the editors have set up a self-regulating mechanism to ensure that the subjects are considered from a variety of cultures and perspectives."

Access to the EOLSS is by subscription, via the website http://www.eolss.net. Subscription rates will vary, depending upon the nature of the applicant. Universities from the UN list of Least Developed Countries will have free access for one year, renewable subject to the submission of annual reports on educational and research activity. Those universities are invited to sign an agreement on the website and submit to the UNESCO for endorsement. Likewise, disadvantaged individuals worldwide registered through charitable organizations will be given free access for one year. Universities from developing countries will also receive an appropriate discount.

EOLSS covers roughly 200 themes, each managed by an internationally recognized expert in the field. Each theme comprises an overview chapter of about 30 pages that is addressed to the general reader. This is followed by five to eight 'topic level chapters', of about 20 pages, intended for university students specializing in the field. Every topic includes another five to eight articles on the latest advances and findings in the subject, as well as indications of future trends.

The themes are organized under the following major subject categories:

1. Earth and Atmospheric Sciences
2. Mathematical Sciences
3. Biological and Medical Sciences
4. Social Sciences and Humanities
5. Physical Sciences. Engineering and Technology Resources
6. Chemical Sciences
7. Water Science and Resources
8. Water Engineering Resources
9. Energy Science and Resources
10. Energy Engineering Resources
11. Environmental and Ecological Sciences and Resources
12. Environmental Engineering Resources
13. Agricultural Sciences and Resources
14. Food and Agricultural Engineering Resource
15. Human Resources Policy and Management
16. Natural Resources Policy and Management
17. Development and Economic Resources
18. Institutional and Infrastructural Resources
19. Technology, Information, and Systems Management Resources
20. Regional Reviews

Knowledge for Sustainable Development: An Insight into the Encyclopedia of Life Support Systems, reviews the themes of EOLSS for the general reader, and is a three-volume printed publication of about 3300 pages. This major publication by more than 150 world experts in their fields was also released by the UNESCO Director General on the 3rd September 2002 during the World Summit on Sustainable Development in Johannesburg, South Africa.

For further information: http://www.eolss.net

OBITUARY

Jànos Salànki

(1929-2003)

Academician Professor Jànos Salànki - an outstanding Hungarian physiologist, author of nearly 260 research papers and books, organizer of invertebrate neurophysiology at Tihany, member of numerous committees and scientific councils, president elect of IUBS for the period 1988-90, recipient of governmental and academic prizes, esteemed colleague and friend, passed away on 29 January 2003, following a serious illness. Professor Salànki initiated and organized modem comparative neurobiological research in Hungary. He was known for his sharp character, sober judgment, brilliant intellect, excellent creative talent and wide interest in both of scientific and social affairs.

Jànos Salànki was born in Debrecen (11 May 1929), and obtained his diploma there, at the Medical University, in 1954. His scientific career started as an "aspirant" (Ph.D. student) with Professor H. Koshtoyants at the Moscow State University and continued at Tîhany since the early 1960s. At Tihany he established a Comparative Physiology group dealing with Invertebrate Neurobiology at the Biological Research Institute of the Hungarian Academy of Sciences, at the shore of Lake Balaton. Here he organized regular courses focusing on current physiological, morphological and biochemical backgrounds of invertebrate neurobiology.

In September 1962 he became Director of the Biological Research Institute (until 1990), where he organized a complex laboratory using physiological, morphological, and biochemical techniques. The problem he proposed to deal with concerned a basic and fascinating question of the Fifties: are behavioral motor patterns genetically determined in the brain or they can be modified by external factors? To answer such general questions, he used the mussel *Anodonta cygnaea* as model animal. Studying the effect of chemical stress on the freshwater mussel, Salànki demonstrated that the duration and both activity and rest can change significantly in response to chemical variation of the environment. This basic observation inspired him to deal with two, basically different questions. The first was to clarify the mechanism underlying the regulation of periodicity, and the second one, to use the phenomenon in testing and monitoring water quality.

The IUBS Scientific Program on Biological Monitoring of the State of the Environment launched in 1982 following Salànki's proposal provided the possibility to widen his activities and to contribute and develop the Biomonitoring program.

For many years he held special courses in Invertebrate Physiology at Loràn d Eötvös University (Budapest), as well as Environmental Science and Neurobiology at Veszprém University. Many of his students already have Ph.D. degrees; half a dozen have D.Sc. and Professor titles. Based on his achievements, he was honored by election to the Academy as a Correspondent Member in 1976 and as an Ordinary Member in 1987.

One should point out Salànki's extremely successful activities in the domain of science development and organization. Founder of the Hungarian Neurophysiological Society, since

1967, he organized a series of international symposia in relation to invertebrate neurobiology at Tihany. Their proceedings were published and edited by him in 10 volumes in English and in 4 volumes in Hungarian.

He played a significant role in the development of ecotoxicology and monitoring of heavy metals in both invertebrates and vertebrates inhabiting Lake Balaton. Although he was a neurophysiologist, he had a keen interest in hydrobiology and aquatic ecology and contributed significantly to these fields.

Since 1988, Salànki acted as President of IUBS for three years, and up to 2002, he chaired the IUBS Interdisciplinary Commission on Biological Monitoring. Professor Salànki chaired the IUBS Hungarian National Committee from 1980 to 2002; acted as President of the Veszprém Academic Committee from 1985 to 1997, and initiated the foundation of the International Society for Invertebrate Neurobiology in 1989 and became its first President. Since 1985, he was the chief editor of *the Acta Biologica Hungarica.* Prof. Salànki was invited to join the Scientific Committee of the International Lake Environment Committee (ILEC). With his governmental assignment, he took part in planning and coordination of Balaton research with the participation of 28 laboratories between 1995-98.

Professor Salànki had deep feelings for his family, who always supported him, and in his last year, took care of him. His wife, Professor Katalin Ròzsa, is a reputed invertebrate physiologist, who worked together with Jànis Salànki at Tihany for more than three decades. His daughters Zsuzsa (linguist) and Katalin (geneticist) are also professionally active. Grandchildren were the joy of Professor Salànki's life.

He left a huge gap behind him…

Peter Birò,
Correspondent Member,
Hungarian Academy of Sciences (HAS)
Balaton Limnological Research Institute (HAS)
8237 Tihany, Hungary

PUBLICATIONS REVIEW

BOTANICAL MEDICINES
The Desk Reference for Major Herbal Supplements

By Dennis J. McKenna, Kenneth Jones & Kerry Hughes. Published by The Haworth Herbal Press, 2002. ISBN 0-7890-1266-9 (1138 pages).

This book provides up-to-date information on thirty four of the most popular dietary supplements used in North America and Europe. Each entry includes the supplement's history and its traditional uses, botanical information, therapeutic applications, and results of recent studies. The book also discusses recommended dosage, safety profiles, side effects, contraindications, drug interactions, safety recommendations during pregnancy and lactation, and other special precautions that users should be aware of.

NEUROTRANSMITTERS IN PLANT LIFE

By Victoria V. Roshchina, Institute of Cell Biophysics, Russian Academy of Sciences, Moscow, Russia. Published by Science Publishers: Enfield, USA, Plymouth, UK. 2001. ISBN 1-57808. (286 pages). Email: Sales@scipub.net.

Neurotransmitters acetylcholine and biogenic amines dopamine, noradrenaline, serotonin and histamine are present not only in animals, but also in plants and microorganisms. This book is a first attempt to consider the role of the substances in plant life and how to use their characteristics for human practice, mainly for medicine and agriculture.
Neurotransmitters may play a universal role as elementary molecular agents of irritation in any living cell. Neurotransmitters participate in the information processes in plant cells and have many functions in plant organisms as a whole-from changes in ion permeability of membranes, energetic and metabolism to complex processes such as a fertilization, motility and at last germination, growth and morphogenesis. They may have a significance in sensory processes of the recognition and reception in the chemical relations between organisms in biocenosis.

OZONE AND PLANT CELL

By Victoria V. Roshchina & Valentina D. Roshchina, Institute of Cell Biophysics, Russian Academy of Sciences, Moscow, Russia. Published by Kluwer Publishing House, 2003. (286 pages). Email: orderdept@wkap.nl

Ozone is a normal constituent of air but this gas becomes dangerous for living organisms when its concentration in the troposphere is too high. Most previous studies of this substance examined it merely in its role as an earth screen for the biosphere or an air pollutant. This book will view its derivatives (active oxygen species), at a molecular and cellular level, as substances that have both positive and negative effects on plant life. Plant cells will be considered as both recipients and sources of ozone, as well as possible biocensors and bioindicators for low and high concentrations of the compound.

THE NEW PANORAMA OF ANIMAL EVOLUTION

Edited by A. Legakis, S. Sfenthourakis, R. Polymeni & M. Thessalou-Legaki. Published by Pensoft, Sofia-Moscow, in collaboration with MAB UNESCO (738 pages).

This volume consists of a collection of 85 papers presented at the 18th International Congress of Zoology, treating the modern developments and trends in zoology and animal science. Several of the authors are prominent international authorities in zoology and ecology. The book is unique in its attempts to review and evaluate the recent stateand the future of animal science, ecology and biodiversity studies.

CALENDAR OF MEETINGS

IUBS-sponsored meetings are indicated in bold-type face
Additional information may be obtained from addresses in ( ) parentheses

2004

BEE RESEARCH
8th IBRA International Conference on Tropical Bees: Management and Diversity
6-10 September, Ribeirao Preto, Brazil
(Contact: IBRA Tropical Conference, 18 North Road, Cardiff CF10 3DT, UK. Fax: +44(0)29 2066 522
Website: www.ibra.org.uk)

BIOLOGY EDUCATION
BioEd 2004: Biological Education, Sustainable Development, Ethics and Citizenship
13-18 October, Rio de Janeiro, Brazil
(Contact: Prof. André Giordan, LDES, Université de Génève, Génève, Switzerland. Tel: +41(0)223799618;
Fax: +41(0)223799828;
Email: giordan@pse.unige.ch)

GLOBAL CHANGE
IOC-SCOR-GLOBEC Symposium on 'Quantitative Ecosystem Indicators for Fisheries Management'
31 March-3 April, Paris, France
(Contact: Philippe Cury,
Email: curypm@uctvms.uct.ac.za)

4th World Fisheries Congress
Reconciling Fisheries with Conservation: The Challenges of Managing Aquatic Ecosystems
2-6 May, Vancouver, Canada
(Contact: http://www.worldfisheries2004.org)

ICES-GLOBEC Symposium on 'The Influence of Climate Change on North Atlantic Fish Stocks'
11-14 May, Bergen, Norway
(Contact: Harald Loeng,
Email: harald@imr.no)

EURESCO LIFE SCIENCES
Bacterial Neural Networks
8-13 May, San Feliu de Guixols, Spain
(Contact: J. Armitage (Oxford)
Email: euresc@esf.org;
Website: http://www.esf.org/euresco)

Neural Mechanisms of Learning
14-19 May, Obernai, France
(Contact: R. Menzel (Berlin)
Email: euresc@esf.org;
Website: http://www.esf.org/euresco)

ECOLOGY
7th INTECOL International Wetlands Conference
25-30 July, Utrecht, The Netherlands
(Contact: Jos Verhoeven, Dept. of Geobiology, Utrecht University, P.O.Box 80084, 3508 TB Utrecht,
The Netherlands. Tel: +31 30 2536851
Website: www.bio.uu.nl/intecol)

GENETICS & POPULATION HEALTH incorporating the 2nd Australasian Thalassaemia Workshop and the 3rd Intl Workshop on Consanguinity, Endogamy and Cultural Diversity
8 - 10 August, Fremantle, Australia
(Contact: Prof. Alan Bittles, Centre for Human Genetics, Edith Cowan University, 100 Joonalup Dr., Joonalup WA 6027, Australia.
Tel. +61 8 6304 5467 Fax +61 8 6304 5851
Email: a.bittles@ecu.edu.au or Dr. Wendy Erber, Dept. of Haematology, PathCentre, Hospital Ave., Nedlands WA 6909, Australia.
Tel. +61 8 9346 2893 Fax +61 8 9346 3848
Email: wendy.erber@health.wa.gov.au
http://www.geneticsandpopulationhealth.com)

ICSU BIO-UNIONS
IUBS Conference "Biological Sciences, Development and Society" & 28th IUBS General Assembly
18-22 January, Cairo, Egypt
(Contact: IUBS Secretariat, 51 Bd de Montmorency, 75016 Paris, France
Tel: +33 (0)1 45 25 00 09 Fax: +33 (0)1 45 25 20 29
Email: secretariat@iubs.org ; http://www.iubs.org)

IMMUNOLOGY
XII International Congress of Immunology & IUIS General Assembly
18-23 July, Montreal, Canada
(Contact: IUIS Central Office, Vienna Academy of Postgraduate Medical Education & Research, Alser Strasse 4, A-1090 Vienna, Austria
Tel: +43 (0)1 405 13 83 13 Fax: +43 (0)1 405 13 83 23
Email: iuis-central-office@medacad.org
Website: http://www.iuisonline.org)

INTEGRATIVE AND COMPARATIVE BIOLOGY
SICB 2004 Annual Meeting
5-9 January, New Orleans, USA
organised by The Society for Integrative and Comparative Biology with the Animal Behavior Society, the American Microscopial Society, The Physiological Ecology Section of the Ecological Society of America, and the Crustacean Society
(Contact: SICB Business Office, 1313 Dolley Madison Blvd., Suite 402, McLean, VA 22101, USA.
Tel. +1 703 790 1745, Fax +1 703 790 2672
Email: sicb@burkinc.com ; www.sicb.com)

PALAEOBOTANY
VII International Palaebotany Congress (IOPC)
21-26 March, Bariloche, Argentina
(Contact: General Coordiantor, N.R. Cuneo
Email: rcuneo@mef.org.arg
Website: http://www.IOPC.org)

PALYNOLOGY
XI International Palynological Congress
4-9 July, Granada, Spain
(Contact: Ana Teresa Romero, 11 IPC Chairwoman
Departamento de Botánica, Facultad de Ciencias
Universidad de Granada, 18071 GRANADA (Spain)
Email: atromero@ugr.es ; http:// www.11ipc.org)

PHOTOBIOLOGY
14th International Congress on Photobiology and 11th Annual Meeting of the Korean Society of Photoscience
10-15 June, Jeju (Cheju), Korea
(Contact: Sang Chul Shim, Dept. of Chemistry, Korea Advanced Institute of Science & Technology, 373-1 Kusung-Dong Yusung-Ku Taejon, Republic of Korea
Email: scshim@sorak.kaist.ac.kr or photos@khu.ac.kr
Website: http://www.photos.or.kr/ICP2004)

PSYCHOLOGY
XXVII International Congress of Psychology & IUPsyS General Assembly
8-3 August, Beijing, China
(Contact: P.L-J. Ritchie, School of Psychology, University of Ottawa, 145 Jean-Jacques Lussier Street, Ottawa ON K1N 6N5, Canada
Tel: +(1 613) 562 5289 Fax: +(1 613) 562 5169
Email: pritchie@uottawa.ca
Website: http:// www.iupsys.org)

TOXICOLOGY
X International Congress of Toxicology (IUTOX)
11-16 July, Ttampere, Finland
(Contact: Prof. H. Tähti, Secretary General, Medical School, 33014 University of Tampere, Finland
Email: hanna.tahti@uta.fi
Website: www.ictx.org)

VERTEBRATE MORPHOLOGY
7th International Congress of Vertebrate Morphology
27 July - 1 August, Boca Raton, Florida
(Contact: Jeanette Wyneken, Dept. of Biology, Florida Atlantic Univ., 777 Glades Road, Boca Raton FL 33431-0991, USA
Email: icvm7@science.fau.edu)

ZOOLOGY
XIX International Congress of Zoology
23-27 August, Beijing, China
(Contact: Organising Committee
Email: icz2004@panda.ioz.ac.cn
Website: http://icz.ioz.ac.cn)

2005

BIOMETEOROLOGY
17th International Congress of Biometerorology
5-9 September, Garmisch-Partenkirchen, Germany
(Contact: Dr. Peter Hoeppe, Institute for Occupational and Environmental Medicine, Ludwig-Maximilians-University, Ziemssenstr. 1, D-80336 Munich, Germany
Email: phoeppe@arbeits.med.uni-muenchen.de
Website: http://www.icb2005.de)

BOTANY
XVI International Botanical Congress
17-23 July, Vienna, Austria
(Contact: Meredith Blackwell, Email: mblackwell@lsu.edu
or Dr. Karin Vetschera, Secretary-General, Institute of Botany, University of Vienna Rennweg 14 A-1030 Vienna, Austria
http://lsb380.plbio.lsu.edu/IABMS/IABMS.home)

ICSU BIO-UNIONS
35th International Congress of Physiological Sciences & IUPS General Assembly
31 March-5 April, San Diego, USA
(Contact: S. Orsoni, IUPS Secretariat, Hôpital de la Pitié-Salpêtrière, 83 bd de l'Hôpital, 75013 Paris, France
Tel: +33 (0)1 42 17 75 37 ; Fax: +33 (0)1 42 17 7575
Email: suorsoni@iinfobiogen.fr
Website: http:// www.iups.org)

BIOLOGY INTERNATIONAL

The News Magazine of the International Union of Biological Sciences

Editorial Board

Free copies of *Biology International* are offered to Ordinary and Scientific Members of the Union. The annual subscription rate for individuals is 45 Euros.

Signed articles express the opinion of the authors and do not necessarily reflect the opinion of the Editors of *Biology International*. Prospective authors should send an outline of the proposed article to the Editors, with a letter explaining why the subject might be of interest to readers.

ISSN 02532069

Biology International

the News Magazine of the

INTERNATIONAL UNION OF BIOLOGICAL SCIENCES

IUBS SECRETARIAT
51, Boulevard Montmorency, 75016 Paris, France.
Tel.: +33 (0)1 45.25.00.09 · Telefax: +33 (0)1 45.25.20.29
Email: secretariat@iubs.org
http://www.iubs.org

The International Union of Biological Sciences is a non-governmental, non-profit organisation, established in 1919. Its objectives are to promote the study of biological sciences, to initiate, facilitate, and co-ordinate research and other scientific activities that require international cooperation, to ensure the discussion and dissemination of the results of cooperative research, to promote the organisation of international conferences and to assist in the publication of their reports.

The membership of the IUBS presently consists of 45 Ordinary Members, adhering through Academies of Science, National Research Councils, national science associations or similar organisations, and 88 Scientific Members, all of which are international scientific associations, societies or commissions in the various biological disciplines.

National Members

ARGENTINA - Consejo Nacional de Investigaciones Científicas y Técnicas
AUSTRALIA - Australian Academy of Science
AUSTRIA - Österreichische Akademie der Wissenschaften

BELGIUM - Royal Academies for Science and the Arts of Belgium
BRAZIL - Conselho Nacional de Desenvolvimento Cientifico e Tecnologico
BULGARIA - Bulgarian Academy of Sciences
CHILE - Sociedad de Biologia de Chile
CHINA – Chinese Association for Science and Technology, Beijing
CHINA - Academia Sinica, Taipei
CUBA - Academia de Ciencias de Cuba
CZECH REPUBLIC - Czech Academy of Sciences
DENMARK - Det Kongelige Danske Videnskabernes Selskab
EGYPT - Academy of Scientific Research and Technology
FINLAND - Delegation of Finnish Academies of Sciences & Letters
FRANCE - Académie des Sciences
GERMANY - Deutsche Forschungsgemeinschaft
HUNGARY - Hungarian Academy of Sciences
INDIA - Indian National Science Academy
IRELAND - Royal Irish Academy
ISRAEL -Academy of Sciences and Humanities
ITALY - Consiglio Nazionale delle Ricerche
JAPAN - Science Council of Japan
KOREA Republic of - Korean Association of Biological Sciences
LEBANON - National Council for Scientific Research
MEXICO - Consejo Nacional de Ciencia y Tecnologia
MONACO - Centre Scientifique de Monaco
NETHERLANDS - Koninklijke Nederlandse Akademie van Wetenschappen
NEW ZEALAND - The Royal Society of New Zealand
NORWAY - Det Norske Videnskaps-Akademi
PHILIPPINES - National Research Council of the Philippines
POLAND - Polish Academy of Sciences
PORTUGAL - Ordem dos Biólogos
ROMANIA - Romanian Academy of Sciences
RUSSIA - Russian Academy of Sciences
SAUDI ARABIA - King Abdul Aziz City for Science & Technology
SLOVAK REPUBLIC - Slovak Academy of Sciences
SOUTH AFRICA - National Research Foundation
SPAIN - Ministerio de Ciencia y Tecnologia
SWEDEN - Kungliga Vetenskapsakademien
SWITZERLAND - Swiss Academy of Sciences
TUNISIA - Association Tunisienne des Sciences Biologiques
UNITED KINGDOM - Institute of Biology
U.S.A. - National Academy of Sciences / National Research Council

VENEZUELA - Ministerio de Ciencia y Tecnología