Issue Number 27

INTERFACE:

GENES AND THE ENVIRONMENT

CENTER FOR ENVIRONMENTAL GENETICS UNIVERSITY OF CINCINNATI WINTER/SPRING 2004

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The Superfund Basic Research Science Program in Cincinnati

Historical Background

Industrialization has brought us many benefits, including more efficient fuels, versatile plastics, and chemicals that make our lives easier or healthier. It's difficult for most of us to imagine heating our homes with wood, using cloth diapers, or living without detergents. Unfortunately, the process of producing all these wonders of the industrialized world can leave us with undesirable, sometimes toxic, by-products. Manufacturers should be responsible for taking care of hazardous wastes that they produce, but—prior to 1980—it was often legally difficult to get a company to spend large amounts of money to clean up a contaminated waste site. On occasion, an industrial complex would become so polluted that it was more cost-efficient for the company to abandon a site than to pay for cleanup.

IN THIS ISSUE

In order to avoid having a country dotted with abandoned sites leaking hazardous waste, the U.S. Congress passed the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA). This act enabled the U.S. Environmental Protection Agency (EPA) to respond to hazardous spills, force companies to clean up their waste, and establish a system to clean up abandoned sites. Companies—especially those that produce chemicals or petroleum—were taxed, and this money was set up in a trust as a Superfund. The money in this trust has been used to clean up sites that pose the most imminent and substantial threats to human health and the environment.

Initiation of the Superfund Basic Research Program

CERCLA, as the name states, has been designed to respond to chemical spills, obtain compensation from corporations that spill toxicants, and find ways to clean up abandoned hazardous toxic dump waste sites. Some contaminated sites can be cleaned up with short-term work, whereas other sites are so polluted or complex that they are listed on the EPA National Priority List (NPL) of sites that need work. It is very difficult and costly to remove all the contaminated soil or water from a site, treat it to remove hazardous waste, and restore the area to a usable condition. However, remediation of contaminated sites is impossible without understanding the complex chemical mixtures in these wastes, the microbes, the plants, the environment, and the interaction of each of these on

the others. This includes: [a] how the chemicals are bound to the soil, or biomatrix; [b] how the chemicals are altered by microbes while in the soil; [c] how substances are taken up by bacteria, fungi, plants, or animals; [d] how they are metabolized in the human body; and [e] what effects these substances and/or metabolites can have inside the cell. In order to gain this understanding, the National Institute of Environmental Health Science (NIEHS)-sponsored Superfund Basic Research Program (SBRP) was formed as part of the Superfund Amendments and Reauthorization Act (SARA) of 1986. The goal of the SBRP is to set up multidisciplinary programs at academic institutes to address the various health and environmental issues that arise from the complex nature of hazardous waste sites. Although most research sponsored by the National Institutes of Health uses only biomedical approaches to answer questions on health, the SBRP is specifically designed to form interdisciplinary groups of scientists from a combination of fields such as engineering, toxicology, molecular biology, epidemiology, and ecology. By focusing on a common theme, researchers from various disciplines can come together with related projects; preliminary data from one project can influence the experiments in another project. Experts in areas such as plant phytoremediation can offer insights to the soil expert, and the molecular biologist can add additional expertise to the interpretation of experimental results.

The NIEHS/EPA Superfund program is also concerned with understanding the impact that the hazards might have on human health and the environment, the developing of new technologies to assess and remediate sites, and the prevention of additional health and environmental problems. Lower cleanup costs and improvements in human and ecological health risk assessment are also goals of this program. In addition to basic research, the university-based programs train scientists on Superfund issues, implement community outreach programs, and transfer research technology to industry, community leaders, and government officials.

The SBRP at Cincinnati

The University of Cincinnati SBRP was initially funded in 1988, under the guidance of Roy E. Albert and Jack Loper. Currently, the UC program is under the leadership of Paul Bishop, the Associate Dean for Research and Herman Schneider Professor in the Department of Civil & Environmental Engineering. The UC program wishes to understand the effects that mixtures of different chemicals and metals can have on health. Specific contaminants under study include metals such as arsenic and chromium, and polycyclic aromatic hydrocarbons (PAHs) such as benzo[*a*]pyrene. These metals and hydrocarbons are among the most dangerous substances in hazardous waste. A better understanding of the co-mutagenicity or toxicity of these substances will lead to more accurate health risk analyses of people exposed to Superfund sites, which are often contaminated with several different pollutants.

The UC SBRP includes faculty from Environmental Health, Molecular Genetics, Biological Sciences, Chemistry, and Civil Environmental & Engineering. The projects complement and support one other. For example, more than 30,000 sites in the U.S. are contaminated with PAHs, which are formed during the incomplete burning of coal, oil, gas, or other organic substances. PAHs are compounds that have two or more fused carbon rings; their chemical properties often make them difficult to degrade. Many PAHs are carcinogenic. It is possible to excavate and treat soil to remove the PAHs, but this is very expensive. Consequently, ***Paul Bishop***, ***Jodi Shann***, ***Brian Kinkle***, and ***Dan Oerther*** are using plants and microbial colonies to degrade or remove contaminants from the soil without removing the soil from the site. The process of using plants and microbial colonies to degrade or transport contaminants is called *bioremediation*. The labs of these four scientists are also trying to understand how other aspects of the soils—such as co-contamination with metals—changes the properties of bioremediation.

Individual Projects

Bishop investigates the mechanisms of degradation used by bacterial colonies that grow around soil particles. Bacterial colonies grow, add extracellular material such as proteins or polysaccharides, and develop into complex ecosystems

called *biofilms*. These colonies were originally believed to be homogeneous throughout. However, Bishop's work has led to a new understanding of the structure and function of microbial biofilms. His lab has shown that biofilms may have many layers. Some layers rely on oxygen, while others use processes that are anoxic or anaerobic. Biofilms may contain different combinations of metabolic processes. This heterogeneity can result from the different contaminants in the environment and has an impact on the availability of nutrients and how well the biofilm can degrade toxicants.

Most of the microorganisms in these complex ecosystems cannot be cultured easily in the laboratory. ***Kinkle*** and ***Oerther*** study the microbes in their natural environment, to gain a better understanding of their role in contaminant transformation and degradation. Their labs are combining molecular techniques with classical microbiology in order to "read" the molecular signatures of microbial populations. Monitoring the changes in gene expression with changes in contaminants will give us new insight into how microbes degrade these contaminants. This project integrates basic genetics, biochemistry, physiology, and ecology to investigate the *in situ* structure and activity of microbial communities involved in transformation of PAHs and metals. ***Shann*** takes the research of the contaminants one step closer to the real world by looking at ecological restoration. Her interest is *phytoremediation*, or the way plants are able to alter metal speciation and PAHs or remove them from the soil. There is growing evidence that a variety of contaminated sites may be viable candidates for ecological restoration. Ecological restoration implies recovery of ecosystem structure and function—which is not necessarily the same as site remediation. On the contrary, UC SBRP research demonstrates that ecological restoration can be achieved on sites where high levels of contamination remain. Her future research will look at the bioavailability of contaminants on a site where the levels of pollutants remain high, but the ecosystem appears to have recovered.

Assessing exposure routes and the risks associated with exposure is the realm of the biomedical projects in the UC SBRP. ***Kathleen Dixon*** is studying the effects that co-exposure to arsenic and PAHs have on DNA repair. Faulty DNA repair can lead to cancer, but it is not currently known how arsenic causes cancer in humans. ***Alvaro Puga*** adds to this approach by looking at the exposure of cultured cells to metals and PAHs combined. He has found that these toxicants can disrupt the expression of many detoxification genes by blocking the chromatin remodeling processes that accompany changes in gene expression following exposure to types of PAHs. Similarly, he has shown that exposure to an antioxidant protects cells from the toxic effects of arsenic. He has also developed gene-expression signatures of cultured cells exposed to various combinations of these toxicants. When considered with the work of other UC SBRP scientists, this information will be used to evaluate the effects of soils from local Superfund sites, and to analyze the effects of these soils at the cellular level. Changes in gene expression in human cell culture can also be compared with the changes in metabolism studied by ***Bishop***, ***Kinkle***, and ***Oerther***. This illustrates how discoveries on one project can help advance other projects within an SBRP.

James Stringer is developing mice that can be used to detect very low levels of mutation**;** if successful, his mice could be used as sentinels on contaminated soils containing known microbial communities and plants capable of soil remediation. This approach will enable the UC SBRP to study the effects of transporting or degrading toxicants with regard to the capacity of the toxicants to produce low levels of mutations.

Glenn Talaska is determining how PAHs bind to DNA. This DNA adduct formation can lead to mutations, and these mutations can result in cancer. Metals can alter the way this binding occurs, but the mechanisms by which this happens are not known. ***Talaska*** is looking forward to using the mice developed by ***Stringer*** to detect very rare mutation events.

Individual Usage Cores

The **Analytical Core**, under the direction of ***Joseph Caruso***, uses mass spectrometry to evaluate the levels of different toxicants in samples from

the UC SBRP investigators. Contaminants change form as they are degraded. In metals, these different forms are called *species*, or valency states. The Analytical Core can measure the amounts of the different species of a metal such as arsenic, and chart how the metal changes as it is processed (in the microbe, plant or animal cell). This gives the scientists a better understanding of the metabolic processes involved in degrading contaminants.

All of this science requires the help of good statistics to validate it, which is why the UC SBRP has a **Biostatistics Core**, directed by ***Mario Medvedovic***. His expertise includes novel analysis of microarray data. Microarrays report changes in gene expression, and give data for thousands of genes under each experimental condition**;** microarrays require sophisticated statistical methods to handle so much data. ***Medvedovic*** also helps with experimental design on projects evaluating rare mutations. Different statistical approaches are needed for detecting such rare events.

In addition to basic science, the SBRP interacts with government, industry, and the public on Superfund issues. The UC SBRP is very excited about the new leadership of ***Joyce Martin*** in the **Outreach Core**. She is also Director of the Environmental Policy Center, and served as Chief Counsel to the Governor of Indiana prior to her appointment at the University of Cincinnati. Although she has been Director of the UC SBRP Outreach Core for less than a year, she has implemented several projects that will have a positive impact on the health and well-being of the residents of Cincinnati.

One project is a collaboration with Hamilton County's Urban Land Assembly Program (ULAP) and the City of Cincinnati's Strategic Program for Urban Redevelopment (SPUR). UC SBRP is developing a sophisticated database of area *brownfields*. Brownfields are abandoned, idled, or underused industrial or commercial properties where expansion or redevelopment is complicated by known or potential releases of hazardous substances or petroleum. These sites can be as small as an abandoned gas station, or the size of a manufacturing plant**;** there are many such sites in and near Cincinnati. Companies tend to shy away from purchasing a property that is suspected of harboring pollutants, because they don't want to be responsible for the cost of cleanup. These neglected properties become unsightly, bring down the values of the properties around them, and can become home to disease-carrying rats or mosquitoes. A database of available sites will enable the city and county to assess the properties, apply for funds to clean up the sites, and aid developers in finding properties to buy and develop. Revitalization of the neighborhoods will be aided by the cleanup and sale of these properties, and new businesses will replace urban blight.

Looking toward the Future

Future goals of the UC SBRP include the development of better risk assessment, in collaboration with Toxicology Excellence for Risk Assessment (TERA). Basic research will continue on the mechanisms underlying the toxicity or carcinogenicity of mixtures of PAHs and metals. Bioremediation and ecological restoration of lands contaminated with these hazardous substances will be attempted, using the data collected by UC SBRP scientists. It is the hope of these researchers that a safer, healthier environment will be possible—thanks in part to their contributions to the study of the bioremediation and health hazards of complex mixtures.

Websites of interest:

University of Cincinnati Superfund Basic Research Program**:** http://eh.uc.edu/superfund/

NIEHS/EPA Superfund Basic Research Program**:**
http://www-apps.niehs.nih.gov/sbrp/index.cfm

Environmental Protection Agency Superfund**:**
http://www.epa.gov/superfund/index.htm

National Priorities List**:**
http://www.epa.gov/superfund/sites/npl/npl.htm

Toxicology Excellence for Risk Assessment**:**
http://www.tera.org

—***Contributed by Elizabeth Kopras***

Meanness doesn't happen overnight

web-cytes

For anyone who wants to know the status of projects to sequence the various organisms' genomes, each project scheduled or the actual completion date is listed at http://www.intlgenome.org . If you wish to scan MEDLINE for germane articles, enter a sentence or paragraph (or your latest idea), sit back, and wait for abstracts to be downloaded via email to you within 5-10 minutes: invention.swmed.edu/etblast/index.shtml . For beginning biology students, you can run simple genetics experiments at intro.bio.umb.edu/VGL/index.htm .

A pharmacogenetics knowledge base (PharmGKB) is being built by Stanford University as part of a research consortium. You can look up a specific gene (more than 1300 in the database so far) to see how well a particular drug works. Check out http://www.pharmgkb.org .

Students who need a refresher course on metabolic pathways in the human and 13 bacteria, to find out information about molecular weight, chemical structures and reactions as well as the protein and DNA sequences of the enzymes involved—check out biocyc.org . For a more "cartoon version" of these key processes in biochemistry, genetics, cell biology or physiology, see science.nhmccd.edu/biol/ap1int.htm . Another site that maps life's biochemical reactions and traces connections between signaling pathways is www.reactome.org . For images of human anatomy, there is a *teaching archive* for every level from high school to medical school, with more than 3600 photos/videos/animations, at www.healcentral.org .

The concept of disorder/chaos, to understand the second law of thermodynamics, can be learned at www.entropysite.com . Anything weird you ever wanted to know about, and get a scientific explanation for that phenomenon? Send in questions to Karl Kruszelnicki's site from Sydney Down Under, www.abc.net.au/science/k2 .

Some might know that CHD stands for congenital heart disease (or is it congestive heart disease?) and that RDS stands for respiratory distress syndrome. Check out this site that has more than 200,000 entries, telling you what any acronym in science or medicine stands for: lethargy.swmed.edu/argh/ARGH.asp .

For questions you might have about astronomy, you can ask at a web site of mainly astronomy graduate students: curious.astro.cornell.edu/index.php . We've had many articles in ***Interface*** about "human origins." For everything you've ever wanted to know about human evolution but were afraid to ask, see www.talkorigins.org/faqs/homs .

This May-June was Cincinnati's **invasion of the 17-year cicadas**. These bugs also emerged from the ground in other parts of the U.S. (**Figure 1**). There are cicada parties, recipes, songs, drawings, and scientific information at: insects.ummz.lsa.umich.edu/fauna/michigan_cicadas/Periodical/index.html , collections2.eeb.uconn.edu/collections/cicadacentral , and www.urhome.umd.edu/newsdesk/scitech/cicadas.cfm .

Fig 1. Red=highest counts per square yard; green=cicada infestation also present but less severe.

A site where one can learn more about bioluminescence, and even has a link for those who want to order their very own bag of dinoflagellates (which are one-celled beasties that GLOW) http://www.lifesci.ucsb.edu/~biolum/ .

Genomes are now being sequenced at the rate of almost one per week, and comparing the more-than-150 genomes now sequenced might be difficult. If you wish to contrast different species, check out http://maine.ebi.ac.uk:8000/services/cogent .

BEST TYPO 2004 "Adobe Photochop"

How to Define a "Gene"?

A gene is defined as a segment of DNA responsible for a gene product that alters the phenotype (trait). Consequently, the hundreds of 21-nucleotide miRNA and siRNA fragments scattered throughout the human genome are indeed "genes," since their products are capable of altering the trait of the person by silencing the expression of one or another gene. A gene comprises an allele on each chromosome of the chromosome pair, one allele from the mother and one allele from the father.

Any enhancer or other control element affecting the expression of a particular gene should be considered as part of that gene (or allele)—even if it resides (in *cis*) a large distance away. One can define "cis" as "describing a determinant that is adjacent, on the same chromosome, to the gene it regulates." There are two recent dramatic examples of how far away**!** An enhancer of the mouse sonic hedgehog gene (*Shh*) lies within intron 5 of the limb bud region-1 gene (*Lmbr1*), ~1,000,000 bases (1 Mb) from the target *Shh* gene [*Hum Mol Genet* 2003; **12:** 1725] and is designated the zone of polarizing activity (ZPA) regulatory sequence (ZRS). An enhancer of the forkhead box L2 gene (*FOXL2*) lies within introns 6, 11 and/or 12 of the mitochondrial ribosomal protein-22 gene (*MRPS22*), more than 170 kb away [*Genomics* 2004; **83:** 757].

Thus, clearly, one gene may reside within another, one gene may overlap another, and/or one gene may be located on the opposite strand within or overlapping the other. Also, a single-nucleotide polymorphism (SNP) within a gene may be overridden by the effects of an enhancer or other control element that resides thousands or millions of bases from the gene that it governs.

A PICTURE IS WORTH A THOUSAND WORDS
but it uses up three thousand times the memory

Evidence of Increases in Atmospheric Oxygen

It is commonly believed that there was a sudden increase in atmospheric oxygen concentrations between 2 and 3 billion years ago (BYA), which led to mitochondria being captured by the early eukaryotes. Contrary to previous estimates of 2.8 and 2.5 BYA, several lines of geological and geochemical evidence [*Nature* 2004; **427:** 117] now point to the rise in atmospheric oxygen occurring more recently, by ~2.32 BYA.

The High Jump at the World Olympics

Athletes competing in Athens will no doubt soar *more than 7 feet*, while vying for the world record in the high jump. This human feat is nothing, however, when compared with the froghopper (spittle bug). For their size and mass, froghoppers outperform all other insects [*Nature* 2003; **424:** 509]. The force exerted is 414 times their body weight and is therefore much higher than in other jumpers such as fleas, click beetles or locusts. Being 6 mm long and jumping maximally 700 mm, the froghopper would be equivalent to a 6-foot-tall human jumping 700 feet high. Now *that* would be something worth watching at the World Olympics in Greece this summer**!**

Biotechnology, ...

What follows is a synopsis of some interesting things that happened during the first 6 months of 2004, with regard to genetically-modified (**GM**) plants, biotechology, and related topics, provided chronologically**:**

Jan 2004 The leading article of issue #25 of ***Interface*** described the development of transgenic fish at the University of Cincinnati, for use in monitoring aquatic pollution. In issue #26 under **Q and A**, we acknowledged the commercial development of GloFish™ at the National University of Singapore. Texas-based Yorktown Technologies began selling transgenic zebrafish to pet stores around the U.S., but the Fish and Game Commission has now banned the sale of these GM fish in the state of California [*Nat Genet* 2004; **36:** 15].

Climate change is expected to cause rising ocean levels in coastal rice-growing regions world-wide. Scientists have taken a salinity-resistance gene (isolated from a coastal-growing mangrove tree) and gnetically engineered a new salt-resistant rice variety—designed to stand up to global warming [*Science* 2004; **303:** 308].

Feb 2004 A French company launched a new gene expression test kit that can identify mystery meat in, for example, a restaurant. "*FoodExpert-ID*" contains 88,000 probes from 33 different vertebrates (including cow, goose, duck, ostrich, eel, frog and cat) on an Affymetrix DNA chip. This information can be useful to people with allergies, as well as to restaurants who want to steer clear of mad cow disease [*Science* 2004; **303:** 1761].

Mar 2004 The British government gave a "qualified approval" for the commercial planting of GM maize on British soil. The U.K. government opposed commercial planting of GM sugar beets and oilseed rape, however, because "trials had shown that growing those crops resulted in fewer weeds and insect species in the fields—possibly undermining biodiversity." [*Science* 2004; **303:** 1590].

Voters in wine-growing Mendocino County (California, 130 km north of San Francisco) approved by a 56-44% margin to "ban the planting of all GM crops." Agricultural corporations are considering a legal challenge to the county ban [*Nature* 2004; **428:** 107].

Saying that free access to all scientific journals would cause bankruptcy, more than 40 biomedical societies have banded together to oppose the demands for them to provide immediate and unrestricted access to all the scientific literature they publish. The pros and cons of this ongoing saga are presented at *Nature* 2004; **428:** 356.

Apr 2004 Despite the go-ahead for British farmers to plant GM maize (see above, March), the German company Bayer CropScience said in a statement that "the continued uncertainty about where and when the crop could be planted has made the variety economically nonviable," because it would probably be several years before they could sell the product and, by then, the crop would likely have lagged behind more recently developed varieties with more desirable characteristics [*Science* 2004; **304:** 203].

Western Australia just announced a 4-year moratorium on all GM food crops [*Nature* 2004; **428:** 594].

The California Rice Commission agreed to let Ventra Bioscience of Sacramento grow GM rice in a remote area near San Diego— "far from commercial rice farms in northern California." This GM rice produces *lactoferrin* (can be used to treat anemia) and *lysozyme* (can be used to fight diarrhea). Still, some are concerned about cross-pollination with food crops via migratory birds or through the water supply [*Nature* 2004; **428:** 591].

May 2004 The European Commission agreed to license the sale of fresh or canned transgenic *Bt11* maize (corn), which contains a bacterial gene that protects the crop against pests. This is the first GM product to be approved for sale in Europe since 1998 [*Nature* 2004; **429:** 335].

In a major victory for agricultural biotechnology, Canada's supreme court ruled that a patent on a GM seed extends to the cells and genes in the resulting plant offspring [*Nature* 2004; **429:** 330].

Europe's biotech firms are on thin ice. Although the number of listed biotech firms in Europe continues to increase, 43% of publicly traded companies have less than 2 years' worth of cash [*Nature* 2004; **429:** 235].

Monsanto feared stiff opposition from wheat farmers ("Wheat is a sacred crop"), and therefore decided to halt plans to commercialize a GM wheat variety designed to be resistant to a popular herbicide [*Science* 2004; **304:** 939].

June 2004 Syngenta International (Basel, Switzerland) released 48,000 mutant strains of *Arabidopsis* (the tiny mustard whose plant genome was the first to be sequenced) with no fees or strings attached. Realizing that the collection had outlived its usefulness, Syngenta decided to give it away rather than destroy it [*Science* 2004; **304:** 1426].

Pilot Project Recipients 2004

The CEG Pilot Projects have been awarded!

CONGRATULATIONS

El Mustapha Bahassi, PhD, Department of Cell Biology, Neurobiology and Anatomy. ***"Regulation of BRCA2 function by CHK2 in response to irradiation"***

William Hardie, MD, Division of Pulmonary Biology, Children's Hospital. ***"Mechanisms of protection from nickel-induced lung injury by transforming growth factor-α (TGFA) expression"***

Gary E. Shull, PhD. Department of Molecular Genetics. ***"Effects of perturbations in calcium homeostasis on tumorigenesis"***

Howard Shertzer, PhD. Department of Environmental Health. ***"Mouse Cyp1 gene expression and lung cancer"***

Dorothy Supp, PhD. Department of Surgery. ***"Mutations in the melanocortin-1 receptor gene increase the risk for skin cancer by disrupting DNA repair pathways"***

Craig Tomlinson, PhD. Department of Environmental Health ***"Gene expression profiles to predict disease"***

Nancy Steinberg Warren, MS. Genetic Counseling Program, UC and Children's Hospital Medical Center. ***"Changing the face of the genetic counseling profession"***

Jianhua Zhang, PhD. Cell Biology, Neurobiology and Anatomy ***"An endonuclease in breast cancer development"***

Ethical, Legal and Social Issues, ...

Tidbits from the first 6 months of 2004**:**

Mar 2004 The prosperity of nations varies enormously. If one ranks countries by their per capita incomes, those in the top 10% are on average ~30 times richer than those in the bottom 10%. The richest country is more than 100 times more prosperous that the poorest. Hibbs and Olsson [*PNAS* 2004; **101:** 3715] attempt to explain—via geography, biogeography, politics and culture—this large variation in the wealth of nations.

Apr 2004 A coalition of advocacy groups called on the U.S. Congress to pass the Genetic Information Nondiscrimination Act (GINA). This bill, first introduced in Oct 03, is based mostly on the credible evidence that large numbers of people do not trust doctors and are reluctant to undergo genetic testing, even if this might improve the management of their own health, because they have concerns that insurance companies will use such information to deny coverage [*Nat Genet* 2004; **36:** 429]. To our knowledge, this bill remains hung up in committee.

Jun 2004 Elsevier launched the new *Journal of Men's Health and Gender*, in an attempt to emphasize than men have as many or more health problems than women. Life expectancy of men is 7 years shorter than that of women. Prostate cancer, which kills ~30,000 people a year, is almost as big a threat to men as breast cancer (at ~40,000 deaths per year) is to women [*Science* 2004; **304:** 1441].

The Science Journal Editors' Working Group on Human Genetic Variation Research made a plea to geneticists, clinicians, epidemiologists, anthropologists and historians—calling for equal, consistent, and informative descriptions of geographic or ethnic groups of people. Just to say "Caucasian," for example, is not enough [*Nat Genet* 2004; **36:** 541].

Tis'true sad words were writ by pen,---four little words, "It might have been"
Still sadder words, it seems to me are--- "As we speak so shall it be"

Evolutionarily Speaking,.....

What follows is a synopsis of some of the more interesting things that have happened during the first 6 months of 2004 in the area of ***evolution*-related** news, provided chronologically**:**

Jan 2004 The field of *olfaction* (ability to smell) has recently exploded with knowledge from the Human Genome Project. The superfamily of ~1000 olfactory receptor (*OR*) genes (37% functional genes, 63% pseudogenes) is located in multiple clusters on all but two human chromosomes. Rodents have a much larger percentage of functional *OR* genes, because their survival depends on smell much more than humans' survival. It has been suggested [*PloS Biol* 2004; **2:** E5] that deterioration of the olfactory repertoire—in the human, apes, Old World monkeys and one New World monkey (howler monkey)—has occurred concomitantly with the acquisition of full *trichromatic* (red-yellow-blue) color vision in primates.

Feb 2004 In a microarray study that included ~45,000 probes representing most human, gorilla and chimpanzee genes [*PNAS* 2004; **101:** 2957], examining neuronal gene expression in primate brains, it was found that the *anterior cingulate cortex* (which regulates cognitive changes—perception, mental states and consciousness) of the chimpanzee is more like that of the human than of the gorilla.

Mar 2004 Comparing the human and chimp genes encoding proteases [*Nature* 2004; **428:** 242], all seem almost identical except for one subset—specifically found in the immune system. If these immune-system proteases diverged rapidly after humans and chimps became separate species, this could help clarify differences between the two species in such maladies as AIDS or Alzheimer disease.

Evolution of the genus *Homo* is associated with decreased jaw muscles (used for breaking down food). Gorillas and extinct forerunners of *Homo* (*e.g. Paranthropus* and *Australopithecus*) have strong jaw muscles and an active myosin heavy chain gene (*MYH*), whereas a mutation exists in the human gene that prevents accumulation of the MYH16 protein. It is postulated that this mutation, by decreasing jaw muscle size, might have allowed enlargement of the cranium and therefore increased brain size [*Nature* 2004; **428:** 415].

Apr 2004 When did humans first control fire? Previous theories had suggested maybe 250,000 years ago, but recent archeological evidence of hearths and burned flints in northern Israel now pushes the earliest credible evidence back to almost 800,000 years ago [*Science* 2004; **304:** 725].

Globins are heme-containing "globular" proteins that bind and transport atmospheric oxygen**;** hemoglobin is essential in humans and other mammals in that it carries oxygen necessary to all cells of the body. With the advent of genomes being sequenced, it has been found that globin-coupled sensors in *Eukarya* and *Eubacteria* descended from an ancient globin-like progenitor called protoglobin (Pgb). With the discovery of two Pgb's in *Archaebacteria* [*PNAS* 2004; **101:** 6675], it is now postulated that these archaebacterial globins are the original ancestors of contemporary hemoglobins.

May 2004 More than 99% of all animal species are members of the *Bilateria* lineage, *i.e.* having bilateral symmetry (right side more or less the same as the left side). Bilateral symmetry—the "key" evolutionary transition ~543 million years ago from stationary or drifting planktonic animals to active swimmers and burrowers—was achieved by the intersection of the anterior-posterior (A-P) axis and the dorsal-ventral (D-V) axis. Homeobox (*Hox*) genes function in A-P axis formation and decapentaplegic (*Dpp*) genes function in D-V axis formation. One important outlier group is the phylum *Cnidaria* (sea anemones, corals, hydras and jellyfishes). It has now been shown [*Science* 2004; **304:** 1335] that during development, the sea anemone uses homologous *Hox* and *Dpp* genes, indicating that bilateral symmetry arose before the evolutionary split of *Cnidaria* and *Bilateria*.

Mitochondria, sacs of enzymes that use oxygen to generate energy, are present in the cells of all *Eukarya* (animals, plants, fungi, algae and yeast). *Eubacteria* and *Archaebacteria* are single-celled organisms that lack mitochondria and other specialized organelles (*e.g.* nuclei). Eukaryotes gained their mitochondria between 1.5 and 2.2 billion years ago (at the time that oxygen levels on our planet were increasing). *Giardia*, an intestinal parasite that causes diarrhea, was thought to represent a eukaryote that somehow failed to capture mitochondria. As a fallout from genome sequencing studies [*Nature* 2003; **426:** 172 & 2004; **429:** 236], it now turns out that Giardia ***does*** have mitochondria—in the form of tiny sacs called *mitosomes*. It just took electron microscopy and sophisticated immunohistochemistry to find them.

The ~6 billion humans on this planet have ~6 million *common* (≥10%) single-nucleotide polymorphism (SNPs), 11 million *polymorphic* (≥1%) SNPs, and hundreds of millions of *rare* (<1%) SNPs. One of the strongest arguments in support of the Chimpanzee Genome Project was always that the chimp

sequence would allow us to know which single-nucleotide polymorphism (SNP) represents the "original" base. Well, surprise, surprise**:** it now appears likely that chimps also have their own supply of common, polymorphic and rare SNPs [*Nature* 2004; **429:** 382 & 353]. What next? Compare human and chimp with gorilla at each nucleotide position?

Microcephaly (MCPH) is a neurodevelopmental disorder leading to a decreased cerebral cortex volume and a brain volume comparable to that of early hominids. Mutations in the *ASPM* gene (abnormal spindle-like protein microcephaly-associated in the fruit fly) are the most common cause of MCPH. Looking at the complete *ASPM* gene from chimpanzee, gorilla, orangutan and rhesus macaque by transformation-associated recombination (TAR) cloning in yeast [*PloS Biol* 2004; **2:** 0653], it was shown that evolutionary selection of specific segments of the *ASPM* gene was strongly related to differences in cerebral cortex size.

June 2004 The earliest unequivocal bilaterian forms in the fossil record had been from the Lower Cambrian boundary, now dated at 543 ± 1 million years before the present (see above, May article). Ten phosphatized specimens of a <180-mm long animal showing clear bilaterian features have now been recovered from an archeological dig in China that dates 40-55 million years before the Cambrian [*Science* 2004; **305:** 218]. Whoops**!** The new date of origin of *Bilateria* would now seem to be closer to 600 million years ago.

Cloning Animals

Feb 2004 Cloning by nuclear transplantation has been successfully carried out in various mammals, including mice. These animal clonings have all been done with embryonic stem cells or other dividing (mitotic) cells capable of differentiation. Jaenisch and coworkers [*Nature* 2004; **428:** 44] generated fertile mouse clones by transferring the nuclei of post-mitotic olfactory sensory neurons into oocytes. These data indicate that the genome of a post-mitotic terminally differentiated neuron can re-enter the cell cycle and be reprogrammed to a state of totipotency after nuclear transfer. They also found that the pattern of odorant receptor gene expression and organization of odorant receptor genes in cloned mice was indistinguishable from wild-type animals—indicating that irreversible changes to the DNA of olfactory neurons do not accompany receptor gene choice.

Apr 2004 It is well established that mammals do not undergo *parthenogenesis* (the means of producing offspring solely from maternal germ cells). Male-female reproduction is necessary because of parent-specific epigenetic modification of the genome during gametogenesis—leading to unequal expression of imprinted genes from the maternal and paternal alleles. By deleting in the oocyte donor cell the differentially methylated domain (DMD) that blocks access to enhancers of *Igf2* and deleting the *H19* gene, Kono et al. [*Nature* 2004; **428:** 860] showed that these deletions mimicked the absence of paternal *H19* activity and enabled *Igf2* expression, leading to viable adults. Thus, a viable parthogenetic mouse was made, from two sets of the maternal genome, one derived from non-growing and one from fully grown oocytes. One female offspring (named ***Kaguya***, for a fairy-tale girl found in a bamboo shoot) developed to adulthood with the ability to reproduce offspring. These experiments suggest that paternal imprinting prevents parthenogenesis, ensuring that the paternal contribution is obligatory for the descendant. Microarray analysis of more than 11,000 genes showed the expression levels of Kaguya to be very similar to that of wild-type mice. It seems amazing, however, that changing the expression of just two imprinted genes on the X chromosome can have such a ripple effect on the rest of the genome**!**

Jun 2004 CYP17A1 catalyzes both 17α-hydroxylase and 17,20-lyase activities on the pathway to synthesis of dehydroepiandrosterone (DHEA) and the sex steroids (testosterone and estrogen) but not glucocorticoids and mineralocorticoids. It was postulated [*Mol Cell Biol* 2004; **24:** 5383] that *Cyp17a1(-/-)* knockout mice would have defective sex steroid synthesis and defective brain DHEA production**;** however, *Cyp17a1(-/-)* mice died at embryonic day 7 (E7) prior to gastrulation whereas *Cyp17a1(+/-)* heterozygotes were phenotypically and reproductively normal. A rise in CYP17A1 enzyme activity occurred in wild-type embryos at E6-E7, but *Cyp17a1(-/-)* embryos could not be rescued by treating the pregnant female with DHEA or 17-hydroxypregnenolone. These data suggest that *Cyp17a1* has unknown critical life functions during early embryonic mouse development.

If you try to fail, and succeed, which have you done?

Human Variation, Disease, Migration and Evolution, ...

Tidbits from the first 6 months of 2004**:**

Jan 2004 An association between markers of atherosclerosis (*e.g.* thickness in carotid artery wall) and promoter variants of the gene *ALOX5* encoding 5-lipoxygenase was reported [*N Engl J Med* 2004; **350:** 29]. ALOX5 is an enzyme that generates inflammatory leukotrienes, and atherosclerosis is likely to be a type of inflammation.

The *ABCA1* gene encoding the ATP-binding cassette-A1 transporter has a central role in lipid homeostasis. *ABCA1* haplotypes were associated with both early- and late-onset Alzheimer disease, providing support for a link between this disease and cholesterol metabolism [*Hum Mutat* 2004; **23:** 358].

Among three populations consisting of 207 Chinese, 858 French, and 395 Spanish—64 intragenic SNPs (single-nucleotide polymorphisms) in 35 candidate genes for cardiovascular disease were compared. Strong ethnic differences suggest how difficult it will be to obtain answers to medically meaningful questions [*Genomics* 2004; **83:** 559].

Feb 2004 Persistent developmental stuttering (PDS) usually begins between age 2 and 5, with no apparent brain damage or other known cause**;** ~1% of the population suffers from this condition**!** Neurogenic (acquired) stuttering occurs after a defined incident (*e.g.* stroke, intracerebral damage or head trauma). PDS is not inherited in a simple fashion and its etiology is unknown—although it must represent the combination of genetic predisposition and environment [*PloS Biol* 2004; **2:** 0159].

Huntington disease (Woody Guthrie's disease) is the fatal dominant neurodegenerative disorder caused by a 3-base stutter in the *huntingtin* (*HD* coding region). Why do people with the same number of repeats succumb at different ages? Same mutation, different phenotype is called *genocopy*. In a study of the *HD* gene in 443 Venezuelans [*PNAS* 2004; **101:** 3498], it was calculated that 72% of the variation in age of onset is caused by differences in the mutation itself, whereas 40% of the remainder appears to be determined by modifier genes and 60% by environmental effects such as diet, sanitation, or exposure to pollutants.

Mar 2004 Variants of *CYP46A1*, encoding cholesterol 24-hydroxylase in the brain, appear to contribute to variability in β-amyloid metabolism and risk for Alzheimer disease [*Hum Genet* 2004; **114:** 581].

Focusing on the psychotomimetic effects of three drugs linked to symptoms of schizophrenia—amphetamine, LSD and PCP, which target the dopaminergic, serotonergic and glutamatergic neurotransmitter pathways, respectively—[*Science* 2003; **302:** 1412], it was suggested that the 32-kDa dopamine and cyclic adenosine monophosphate-regulated phosphoprotein DARPP32 represent a point of convergence. Mice lacking DARPP32 are resistant to these drugs' behavioral effects. Gerber and Tonegawa [*N Engl J Med* 2004; **350:** 1047] have proposed important interesting further experiments to prove or disprove this hypothesis.

Glutathione *S*-transferases (GSTs) are important in detoxification. There is a *GSTM3* polymorphism that appears to contribute to the clinical severity of cystic fibrosis [*Pharmacogenetics* 2004; **14:** 295], which could have prognostic and therapeutic significance.

A genome-wide linkage study of coronary artery disease versus premature-onset myocardial infarction [*Am J Hum Genet* 2004; **74:** 262] suggests that different genes may underlie the two phenotypes. The study included 1,613 individuals of European descent who had a mean age of 44.4 years when they first presented with either disease.

In a herculean study of haplotype blocks (ranging from ~5 to >100 kb in length) and resequencing 100 candidate genes for inflammation, lipid metabolism and blood pressure regulation in an African population and a European population [*Am J Hum Genet* 2004; **74:** 610], the conclusion was that the haplotype architecture across the human genome "is more complex than previously suggested." Such studies have important implications for candidate gene and genome-wide association studies.

Apr 2004 The goal of the HapMap Project has been to decrease the number of SNPs from ~11 million to a more reasonable number (~500,000 SNPs?) by identifying "haplotype tag SNPs" (htSNPs) that represent large stretches of DNA in all people. Midway through this 3-year $100 million project, there was a meeting to reconnoiter [*Science* 2004; **304:** 671]. It was agreed that extra SNPs may indeed be needed and that—if some complex diseases are caused by combinations of rare SNPs—the HapMap Project (having chosen 5% frequency as the cut-off) will not detect those.

Who says human genetics is ever simple? Mutations in one gene (*FLNB*) encoding filamin B [*Nat Genet* 2004; **36:** 323], cause four distinct

disorders of human skeletal development**!**

In issues #6, 12 and 21 of ***Interface*** we have discussed the role of genes and the environment in affecting the risk for asthma. Studying almost 900 asthma versus non-asthma patients from Finland and Quebec [*Science* 2004; **304:** 300], the *GPRA* gene encoding an orphan G-protein-coupled receptor for asthma susceptibility has been identified as a risk factor for the disease.

Patients having the *HLA B*5701* genotype are at higher risk for severe hypersensitivity to abacavir, a drug used to treat AIDS patients. Using Monte Carlo simulations [*Pharmacogenetics* 2004; **14:** 335], the cost-effectiveness ratio was calculated to be "22,811 euros saved per hypersensitivity reaction avoided."

May 2004 Most patients with non-small-cell lung cancer have no response, whereas ~10% show a rapid positive response to the tyrosine kinase inhibitor gefitinib [*N Engl J Med* 2004; **350:** 2129]. Somatic mutations in the tyrosine kinase domain of the epidermal growth factor receptor gene (*EGFR*) were found in tumors of eight of nine patients with gefitinib-responsive lung cancer, compared with none of the seven patients having no response to gefitinib. These mutations were found in ~1/4 of tumors from Japan and less than 5% in American Caucasians [*Science* 2004; **304:** 1497]. These findings are good news—for a small group of lung cancer patients.

Fibroblast growth factor-20 (FGF20) is expressed preferentially within the substantia nigra of the brain. Looking at three SNPs in the *FGF20* gene and a haplotype [*Am J Hum Genet* 2004; **74:** 1121], FGF20 was determined to be a risk factor for Parkinson disease.

The Genetic Association Database (GAD) is an archive of published genetic association studies that currently provides a comprehensive public web-based repository of molecular, clinical, and study parameters for more than 5,000 publications [*Nat Genet* 2004; **36:** 431]. This is reminiscent of the Human Phenome Project proposal described in ***Interface*** issue #25.

Jun 2004 Cell culture studies had suggested a potential role for antimony in decreasing toxicity caused by arsenic. An epidemiological study of well-water arsenic and health outcomes in the people of Bangladesh [*Environ Health Perspect* 2004; **112:** 809], however, did not support the cell culture conclusions.

Patients with a mutation in the HMG-CoA-reductase gene (*HMGCR*) show significantly smaller decreases in their serum cholesterol, following treatment with pravastatin [*JAMA* 2004; **291:** 2821].

Glutathione *S*-transferases (GSTs) are important in detoxification, and 30% to 50% of individuals in some populations lack the *GSTM1* or *GSTT1* gene. In nonsmoking females, the null *GSTM1* genotype was associated with susceptibility to senile cataracts [*BBRC* 2004; **319:** 1287].

The efficacy of growth hormone, to treat short stature in children, varies widely. A genetic variant—deletion of exon 3 of the *GHR* gene—was associated with ~2 times more growth acceleration following GH therapy compared with the patients having the consensus *GHR* gene [*Nat Genet* 2004; **36:** 720].

Gene-Environment Interactions

Tidbits of interest.

Jan 2004 A fertilized egg is potentially immortal**:** fusion of an egg and a sperm produces not only a new individual, but also (in theory) an endless series of generations. Three groups have now shown that embryonic stem (ES) cells can give rise to spermatocytes and oocytes, and injecting these spermatocytes into oocytes led to development of viable early blastocysts [*Science* 2003; **300:** 1251; *PNAS* 2003; **100:** 11457; *Nature* 2004; **427:** 148]. It might be possible to use these germ cells to generate ES cells that produce diseased tissues (*e.g.* diabetes, arthritis).

The first draft of the **honeybee genome** has been deposited—the information from which should be valuable for both agricultural research and evolutionary genomics [*Nature* 2004; **427:** 188].

Farmed salmon have higher levels of polychlorinated biphenyls (PCBs) and other organochlorine chemicals than do wild-caught salmon [*Science* 2004; **303:** 226]. The source appears to be their food.

Systems biology is a new term describing interactions of signaling pathways within the cell. The current version of the "Worm Interactome Map" contains 5,500 interactions [*Science* 2004; **303:** 540]. Integrating these protein-protein interaction networks with phenome and transcriptome data sets should lead to numerous biological hypotheses in the nematode *Caenorhabditis elegans*.

Female *Anopheles* mosquitoes, important in spreading malaria, locate their human hosts primarily by olfaction (smell). Chemicals in human sweat, 4-methylphenol and 2-methylphenol, were shown to interact with female-specific members of the mosquito's olfactory receptor superfamily [*Nature*

2004; **427:** 212]. This work might lead to new possibilities in designing insect traps and repellants.

Have you been catching fish that are no longer depressed? Fluoxetine (the antidepressent Prozac(R)) appears to be getting added to the sewer system in North Texas at concentrations high enough to be detected in the tissues of bluegill fish [*Environ Health Perspect* 2004; **112:** A25].

Feb 2004 Excessive nitric oxide (NO) and zinc have been linked to stroke and some neurodegenerative diseases. Now it appears [*Neuron* 2004; **41:** 351] that NO triggers zinc-mediated inhibition of mitochondrial function via increases in reactive oxygen species (ROS). ROS then reacts with NO to activate the p38 MAP kinase, which alters potassium channel function and causes progressive cell death.

Fruit flies with symmetrical and asymmetrical brains were compared in tests for short-term (3 h) versus long-term (4 da) memory [*Nature* 2004; **427:** 605]. Brain asymmetry appears to be required for generating or retrieving long-term memory.

In several past issues of ***Interface*** we have discussed RNA interference (RNAi) as a form of inhibition of gene expression. Now comes a study [*Nature* 2004; **427:** 645] showing that a short interfering RNA (siRNA), ERI-1, in yeast negatively regulates RNAi, *i.e.* two negatives lead to up-regulation of the downstream gene**!**

A population of junco birds left their mountain habitat and set up home at the University of California San Diego campus in 1983 [*Evolution* 2004; **58:** 166]. In just the past 20 years, the males now show 22% less white plumage in their tails than their mountain counterparts—showing how rapid evolution can occur.

Microarray Bioinformatics is a new book illustrating how the "easy" pre-genomic era (one man–one gene approach) has now become much more complex [*Nat Genet* 2004; **36:** 109]. Four key areas where biologists most need help include**:** data extraction, data normalization, organization and selection of genes that are differentially expressed, and maximizing the statistical power of the analysis.

Mar 2004 The growing consensus is that Neandertals lived between 150,000 and 30,000 years ago (*Homo neadertalensis* coexisting with *Homo sapiens* in Europe, parts of Asia and the Middle East) and then died out. A new amino acid test of four Neandertal and five *Homo sapiens* pieces of bone [*PloS Biol* 2004; **2:** 285] supported previous studies that it was unlikely the two human species ever interbred.

One of the earliest signs of type-2 diabetes is insulin resistance in muscle. An 80% increase in levels of intracellular fatty acids and 30% decrease in mitochondrial ATP production was found [*N Engl J Med* 2004; **350:** 664], suggesting mitochondrial dysfunction as a possible culprit in the disease.

As if two sexes don't provide enough problems, the Arizona *Pogonomyrmex* ant is the first organism to have evolved more than two sexes [*Science* 2004; **303:** 1464]. The queen must mate with a male of its own genetic strain to produce other queens and needs sperm from males of a different strain to produce workers.

The global cancer epidemic caused by environmental asbestos is a story of monumental failure to protect the public's health, as recently reviewed [*Environ Health Perspect* 2004; **112:** 285].

Increased cardiovascular fitness through exercise can affect improvements in the plasticity of the aging human brain and may serve to decrease both biological and cognitive senescence in humans [*PNAS* 2004; **101:** 3316].

Apr 2004 There is a new connection developing, between obesity and neurobiology [*Science* 2004; **304:** 63]. In the hypothalamus, leptin activates proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) neurons and blocks the activity of the neuropeptide Y (NPY) and agouti-related protein (AgRP) neurons. These data suggest that the mechanisms underlying leptin's neurobiological role in the central nervous system are similar to those that link learning and memory to the phenomenon of long-term potentiation in the hippocampus.

How high can a tree grow? The tallest known tree (a redwood in northern California) is 112.7 m. Calculating "leaf water stress," water-transport constraints, and gravity [*Nature* 2004; **428:** 851], it was concluded that a maximum tree height of **122-130 m** (405-430 ft) would be maximal on this planet.

Polychlorinated biphenyls (PCBs) may be associated with neuropsychological deficits related to effects of thyroid hormone (T3H) signaling. PCBs can interfere with T3H signaling in the fetal rat brain by direct action on the fetus rather than by producing maternal hypothyroidism [*Environ Health Perspect* 2004; **112:** 516].

With birth-control pills, it was found decades ago that the effects on 100,000 women for 1 year were very different from the effects on 5,000 women for 20 years. Similarly, with antidepressant and antipsychotic medications in children [*N Engl J Med* 2004; **350:** 1489], upbeat reports from short-term

studies are very different from disturbing results from long-term studies of children—whose brains are still developing.

May 2004 On a presidential Italian estate, genetic analysis suggests that the pine trees dying there might have been infected with an American fungus, perhaps imported by U.S. troops during World War II [*Mycol Res* 2004; **108:** 468].

There are long and short versions of the caspase-12 gene (*CASP12*), the long version associated with African origin [*Nature* 2004; **429:** 75 & 35]. These differences may be related to a patient's inherent predisposition to being prone or resistant to infection, as well as to developing arthritis and other chronic inflammatory diseases.

The monoamine oxidase-A gene (*MAOA*) has been linked to aggression. Mice lacking MAOA are more aggressive. Now, a DNA sequence of 30 bp, repeated 3 to 5 times, has been studied in ~600 primates including humans [*Science* 2004; **304:** 818]. Fewer repeats mean less MAOA enzyme and more chance to exhibit violence. This allele arose after the split of New World (South American) and Old World (African and Asian) monkeys, but before apes and Old World monkeys diverged ~25 million years ago. Both Old World monkeys and humans carry both alleles, which could play a role in how "sensitive" or aggressive one appears to be.

Grape skin can be blue, red or white. A retrotransposon insertion in the Myb-related gene *VvmybA1* appears to be the molecular basis of white coloration in all "whiteish-green" grapes of the world [*Science* 2004; **304:** 982].

Polymethoxylated flavones in the peel of citrus fruits not only show anticancer and anti-inflammatory effects but also may help lower cholesterol levels [*J Agric Food Chem* 2004; **52:** 2879].

Laboratory mice were housed outdoors near a major highway and two steel mills [*Science* 2004; **304:** 1008]. Exposure to airborne particulate matter was found to be a principal factor contributing to elevated mutation rates in sentinel mice.

June 2004 In studying epilepsy in mice having one, two or three genes disrupted [*Genes Dev* 2004; **18:** 1397], researchers noticed high death rates in triple knockout mice occurred on Mondays and Thursdays. Turns out that these were the two days of the week that the vacuum cleaner was used to clean the animal room, and this noise set off fatal epileptic seizures**!**

In a large study of 407 children treated either with the popular herbal remedy *Echinacea* or placebo only [*Environ Health Perspect* 2004; **112:** A466], **no benefit** of this over-the-counter medicine to children with upper respiratory infections was found. This latest study confirmed the findings of a similar study 2 years previously.

The same mutated gene that causes an early defect in heart development in mice appears to be directly involved in cardiac dysfunction later in adulthood [*Cell* 2004; **117:** 373]. This is another example of what happens in utero can affect the adult years later.

Horizontal gene transfer (capture of a gene of one species by another) has been described several times in previous issues of ***Interface***. It now appears that horizontally-transferred genes are biased heavily to three categories**:** cell surface, DNA-binding, and pathogenicity-related functions [*Nat Genet* 2004; **36:** 760]—indicating that the transferability of genes seems to depend heavily on their function.

Methicillin-resistant *Staphylococcus aureus* (MRSA) kills about 800 patients a year in Britain alone [*Nature* 2004; **430:** 126]. Vancomycin is commonly used to treated patients with MRSA. Because of horizontal gene transfer, researchers now fear that the bacteria may become resistant to vancomycin and whatever new antibiotic becomes available—leading to increasingly serious epidemics of fatal infections, especially in hospital settings.

"Noise," or random fluctuations in gene expression, represent **stochastic events** that can be quantified in yeast and shown to be yet-another factor affecting gene-environment interactions [*Science* 2004; **304:** 1811].

Only in America

Suggested to the National Forest Service from a visitor to our nation's parks.

"It would be nice to have the Kodak scenic markers so we could identify the photographic sites."

The microwave was invented after a researcher walked by a radar tube and a chocolate bar melted in his pocket.

Observations by a Biologist

Here Is an Example of Convergent Evolution

Animals that look similar, or structures that look similar, are generally considered to be homologous, *i.e.* their similarity has arisen due to their common ancestry. This is by far the most likely explanation and is called *divergent evolution*.

There are some fascinating examples, however, of animals looking similar or structures looking similar, in which their resemblance has come about—not through common ancestry—but through the immediate and urgent need of Mother Nature to assign a particular task to an animal or structure at a crucial point in evolutionary time. This is called *convergent evolution*. For example, the Tasmanian devil is a badger-like animal that feeds on reptiles and small rodents in Tasmania, Australia. Presumably the devils evolved because of the need in this ecological niche for such a predator. The devil is not related to the North American badger, fox or wolf, however**;** the devil is a *marsupial*—more closely related to the kangaroo and opossum.

A molecular example of convergent evolution is the cytochrome P450 (CYP)-like enzyme activity of nitric oxide synthase (NOS) Apparently when a CYP-like activity was needed at a particular subcellular site and no CYP molecule was close enough, Mother Nature tapped an NOS molecule to carry out this function. Careful analysis unequivocally shows no homology between the *CYP* and *NOS* genes [*DNA Cell Biol* 1993; **12:** 1]. Another molecular example is antifreeze glycoprotein (AFGP) in fish [described in issue #11 of ***Interface***]. AFGP originated in Antarctic fish from the recruitment and reiteration of an early portion of a pancreatic trypsinogen gene—to produce 41 repeated segments in tandem. Amazingly, AFGP in Arctic fish is from an independent source [*PNAS* 1997; **94:** 3485]. Again, apparently when an anti-freeze-like activity was needed at a particular moment in these fish (*e.g.* global glaciation), Mother Nature tinkered with a trypsinogen gene in order to build the best molecule to carry out this function. And the process in Antarctica was repeated in the Arctic Ocean, but a different gene was chosen for the starting material**!**

One of the latest fascinating examples of convergent evolution involves the floral organs of the genus *Solanum* from the nightshade family. In some of these species, the *anthers* (pollen-producing organs) are arranged as a cone—which functions like a "pepperpot." In the tomato (*S. lycopersicum*), the anther surfaces are linked by interlocking hairs (*trichromes*) along the edges**;** in the bittersweet (*S. dulcamara*), the anther surfaces are held together by a glue-like secretion. Tomato trichromes are absolutely required for pepperpot formation, since the *dialytic* mutant (lacking trichromes) fails to develop pepperpots. In the bittersweet, in contrast, Glover and coworkers [*Gene* 2004; **331:** 1] showed that trichromes actually prevent pepperpot formation (they did this by introducing a gene from snapdragon, but that's a story for another day).

Here is a cute example of convergent evolution in a floral organ structure in two species within the same genus. The tomato and the bittersweet have chosen different pathways of pepperpot cone development (via different genes) to wind up with the same structure.

Department of Systems Biology

Systems biology is defined as the integration of computer modeling, large-scale data analysis, quantitative biology, and biological experimentation. Lee Hood was the first to set up an Institute for Systems Biology (in Seattle, WA), and the Alliance for Cellular Signaling involves a consortium of universities. Harvard Medical School has now established a full-fledged Department of Systems Biology, the first in the nation to do so. The new Chair, Marc Kirschner, who had campaigned for such a department, plans to recruit about 20 faculty members and will work closely with Harvard's Bauer Center for Genomics Research [*Nature* 2003; **425:** 439].

"Q"uote of the month.....

"Politics is not a bad profession. If you succeed there are many rewards; if you disgrace yourself you can always write a book"

Ronald Reagan, February 6, 1911 - June 5, 2004

Latest in Genetics and Genomics,...

Tidbits from the first 6 months of 2004 with the Human Genome Project (**HGP**), and related genetics/genomics news**:**

Jan 2004 To evaluate *Arabidopsis* (tiny mustard plant) genes, a collection of evolutionarily conserved regions between *Arabidopsis* and rice were studied [*Genome Res* 2004; **14:** 406]—leading to the discovery of an additional 1,931 genes via new "full-length" cDNA sequences. This now raises the number of annotated genes of *Arabidopsis* to ~14,000 and proves, once again, that we are still in the discovery phase and *no eukaryotic genome has truly been completely sequenced yet*. More such proof, that this is the case, follows (repeatedly) below.

Feb 2004 The whole-genome shotgun assembly (WGSA) of the human genome, generated by Celera in 2001, included 27 million sequencing reads that had been organized by end-sequencing 2-kb, 10-kb and 50-kb inserts from clone libraries. With the NCBI Build 34 now available, WGSA analysis shows 97% order-and-orientation agreement, whereas the 3% of sequence out of order was due to "scaffold placement problems" [*PNAS* 2004; **101:** 1916].

Mar 2004 A tiling resolution array, comprising 32,433 overlapping bacterial artificial chromosome (BAC) clones covering the entire human genome, was constructed [*Nat Genet* 2004; **36:** 299]. This study identified minute DNA alterations not previously reported and show the need to move beyond conventional marker-based genome comparison approaches that rely on inference of continuity between interval markers.

Apr 2004 What genomes to sequence next? The list now reported by the National Human Genome Research Institute (NHGRI) includes the South American opossum as a marsupial, four fungi, the red flour beetle (because of its effect as a pest in stored grain and cereal products), and the sea urchin. Already underway is the genome sequencing of the cow, rhesus macaque, three additional roundworms to join the two *Caenorhabditis* species already completed, chicken, a flatworm, and seven additional fruit fly species to join the *Drosophila melanogaster* sequence already completed.

The Brown Norway rat genome (*Rattus norvegicus*) sequence was officially published [*Nature* 2004; **428:** 493].

The DNA sequence and analysis of human chromosome 13, the largest acrocentric human chromosome, consists of 633 genes, 296 pseudogenes, and 105 putative noncoding RNA genes [*Nature* 2004; **428:** 522]. Human chromosome 19, having the highest gene density of all human chromosomes, revealed 1,461 genes and 321 pseudogenes [*Nature* 2004; **428:** 529].

More than 1 billion bp of nonredundant sequence was analyzed from microbial populations collected *en masse* on tangential-flow and impact filters from seawater samples collected from the Sargasso Sea near Bermuda**!** At least 1,800 species were estimated to be in the sample, based on sequence relatedness. More than 1.2 million previously unknown genes were identified, including more than 780 new rhodopsin-like photoreceptors [*Science* 2004; **304:** 66].

The first algal genome (unicellular red alga *Cyanidioschyzon merolae* 10D) complete sequence—of 16.5 Mb and 20 chromosomes—was reported [*Nature* 2004; **428:** 653].

Recombination hot-spots are a ubiquitous feature of the human genome, occurring on average every 200 kb or less, but recombination events appear to occur preferentially outside, rather than within genes [*Science* 2004; **304:** 581].

May 2004 From a diverse collection of 85 domestic dog breeds [*Science* 2004; **304:** 1160], four genetic clusers were identified.

Between human, rat and mouse, there are 481 nongenic segments of DNA—longer than 200 bp—with 100% identity having no insertions or deletions [*Science* 2004; **304:** 1321]. These genetic elements can also be seen in chicken, dog and fish—but not quite 100% identical over hundreds of millions of years of evolution. The functions and evolutionary origins of these sequences are yet to be determined**;** most show no evidence of being transcribed. Such highly conserved nongenic sequences were also described in issue #26 of ***Interface***.

Yet another genomic trick has been uncovered**:** RNA-directed DNA methylation (RdDM). RdDM requires a double-stranded RNA cut into 21- to 26-nucleotide lengths, and, so far, the process seems specific to plants [*Curr Biol* 2004; **14:** 801].

Human chromosome 9 has at least 1,149 genes and 426 pseudogenes [*Nature* 2004; **429:** 369]. The DNA sequence and analysis of human chromosome 10 revealed 1,357 genes and 430 pseudogenes [*Nature* 2004; **429:** 375].

June 2004 DNA from donkeys of 52 countries from across the Old World were analyzed by sequencing 479 bp of the mitochondrial DNA control region [*Science* 2004; **304:** 1781]. The African wild ass appears to be the likely progenitor of the other Asian and Mideastern donkeys. Domestication of the donkey appears mostly likely to have occurred in northeast Africa near present-day Somalia—suggesting a possibly important role during human migrations out of Africa.

Comparing a 10-kb stretch of DNA from two dozen chimpanzees from western Africa with that in two human populations [*PloS Biol* 2004; **2:** 849], it was found that both human populations shared the same six recombination hot-spots, but in the chimp all six hot-spots were missing. This raises questions about the role that hot-spots play in evolution.

The largest database of annotated full-length human cDNA sequences has now been opened for public access [*PloS Biol* 2004; **2:** 856]. This new database—developed over the last 4 years—has built on information from six major cDNA projects, three of which are in Japan. At least 5,155 new gene candidates were found. Analysis of **41,118 cDNA** full-length sequences suggests that as much as 4% of the genome sequence in the NCBI Build 34 assembly may contain misassembled and/or missing regions.

SCIENCE LITE

Arbitrator: A cook that leaves Arby's to work at McDonald's.

Avoidable: Goal of every bullfighter

Bernadette: The act of torching a mortgage

Burglarize: What someone, who breaks-and-enters, sees with

Control: A short, ugly prison inmate

Counterfeiters: Workers who put together kitchen cabinets

Eclipse: What an English barber does for a living

Eyedropper: A clumsy ophthalmologist

Heroes: What a guy in a boat does

Left Bank: What the robber did when his bag was full of loot

Misty: How golfers create divots

Parasites: What you see from the top of the Eiffel Tower

Pharmacist: A helper on the farm

Polarize: What penguins see with

Primate: One way of removing your spouse from in front of the TV set

Relief: What trees do in the spring

Rubberneck: One way you can help your wife to relax

Selfish: What the owner of a seafood store usually does

Sudafed: What he did when he brought litigation against a government official

CEG Members in the News

Scott Belcher received a grant from the Pediatric Brain Tumor Foundation of the U.S. Title: The role of hormones in regulating medulloblastoma cell growth Jan 04 - Dec 05, $100,000.

Dan Nebert was invited to speak at the Session on Transgenic Mice Used in Drug Metabolism Toxicity, during "Discovery Strategies 2004," sponsored by The Jackson Laboratory, Bar Harbor, Maine (May 04). **Nebert** also spoke at the Session on "From Pioneer Times to Latest Advances," 15th International Symposium on Microsomes and Drug Oxidations [Symposium dedicated to Sten Orrenius & Daniel W Nebert], Mainz, Germany (Jul 04) and gave the Keynote Address at the Session on "Polymorphisms and Gene Regulation**:** Toxicological Impact," Tenth International Congress of Toxicology (ICTX), Tampere, Finland (Jul 04).

Mary Beth Genter was co-organizer (with Xinxin Ding, New York State Department of Health) of the symposium "Tissue and Species-Differences in Regulation of Cytochromes P450" at the annual meeting of the Society of Toxicology (Mar 04). She presented a talk entitled "***Olfactory mucosal metabolic enzymes: Modulation of toxic endpoints based on distribution, induction, and age- and species-specific variables."*** She was elected to the Board of Directors for the American Board of Toxicology (Apr 04 - Mar 08).

Alvaro Puga gave a talk at the First International Symposium on Chromatin Structure and Gene Expression Mechanisms as Therapeutic Targets celebrated in Luxembourg (Jan 04) and was an invited speaker at the 15th International Congress on Microsomes and Drug Oxidations celebrated in Mainz, Germany, that honored Dan Nebert (Jul 04).

Glenn Talaska was recently named Vice-Chair of the Biological Exposure Indices (BEI) committee of the American Conference of Governmental Industrial Hygienists (ACGIH).

Tom Doetschman presented the ***"Impact of intestinal microflora on susceptibility to mouse colon cancer"*** at the following meetings/seminars: USC School of Pharmacy, Los Angeles, CA (Jan 04); UCSF Comprehensive Cancer Center, San Francisco, CA (Apr. 04); at the National Congress of Neuroscience, Denizli/Pamukkale University, Turkey (Apr 04); and at Genzyme Pharmaceuticals, Framingham, MA (Jun 04).

Yolanda Sanchez published in the journal Nat Cell Biol (Jan 04) on the ***"DNA damage checkpoint and PKA pathways converge on APC substrates and Cdc20 to regulate mitotic progression."***

Nancy Steinberg Warren received $5,000 from the Ohio Board of Regents Information Literacy grant: ***Integrating genetics into speech-language pathology undergraduate curricula,*** Jean Neils-Strunjas and Charles Kishman, Co PI's. She also received $2,500 from Myriad Genetics: ***Integrating health literacy principles into a genetic counseling brochure***, Andrea Harbison, Co PI. She made presentations entitled ***"Assessing the genetic competency of recent graduates of a college of allied health"*** at the National Coalition of Health Professional Education in Genetics, Bethesda, MD, (Jan 04).

Karen Knudsen was session chair at the Keystone Nuclear Receptor Symposium (Mar 04) and an invited speaker at the American Society of Clinical Oncologists annual meeting (May 04). She was also appointed as associate Editor for Cancer Research.

Congratulations!

Dear Dr Nebert,

May I introduce myself as the publishing person responsible for our journal *Toxicology* here at Elsevier in Amsterdam? I am delighted to let you know that your paper at ICT IX in Brisbane entitled ***"Transcription factors and cancer: an overview"*** which appeared in the December 2002 issue was amongst the 10 top downloaded articles of all articles published in Elsevier journals in the toxicology area in the first half of this year. Please find attached an Email that was sent to interested parties recently, where you can look up the 10 most downloaded articles within the toxicology category.

On behalf of the Editors and the Publisher of Toxicology and of the Proceedings volume, I'd like to extend our sincere congratulations. Thank you for letting us publish this outstanding paper.

With best wishes,

Dr Lulu Stader
Senior Publishing Editor, Elsevier

LETTERS TO THE EDITOR

RESPONSES/COMMENTS TO VARIOUS QUESTIONS

COMMENT **In issue #9 of *Interface*, the unique change of autumn colors seen individually in each tree was described under "Observations by a Biologist." Now comes a microarray study to quantify genes that are expressed in leaves from a single aspen tree during 5 weeks in August and September [*Genome Biol* 2004; 5: R24]. Enzymes involved in photosynthesis are turned off, and enzymes that generate energy by burning fats and sugars are made instead. There were 35 novel genes identified that have never been found anywhere on the planet—except in these autumn leaves!**

Q **In your issue #19 of *Interface* (winter-spring, 2000), you described the launching of the Human Genome Diversity Project (HGDP), but I've not heard much in the news since. Is this program still active?**

A ***Yes, since April 2002 a collection of more than 1,000 DNA samples from 51 populations representing most of the world's genome variation has been made available to nonprofit research laboratories through a collaboration between the HGDP and the Fondation Jean Dausset-C.E.P.H. in Paris. All samples for this resource were collected with proper informed consent, and the privacy of these persons volunteering such samples remains protected. Since 1997, the HGDP has developed and promoted the best legal practices available for studying indigenous populations throughout the world and remains the best source for ethnically diverse human DNA in the world [Nature* 2004; 428: 467*].***

Q **I heard on the radio that one can "buy a book of clones?" What on earth are they talking about?**

A ***You must be referring to Hayashizaki's textbook-sized volume that contains more than 60,000 clones of mouse cDNA sequences [Science* 2003; 302: 217*]. This is a very interesting concept whereby researchers can simply punch out a clone from whatever page, dissolve it in water, amplify it with the polymerase-chain reaction (PCR), and then study it for whatever they want. This has been developed by RIKEN (in Japan) and makes a great contribution to global genomic efforts—as well as making RIKEN more famous!***

COMMENT **Using microarray analysis, changes in mRNA abundance were measured in the brain of adult honeybees, during hive work and foraging [*Science* 2003; 302: 296]..! The researchers showed that changes in gene expression varied with behavior much more than with age. Individual brain mRNA profiles correctly predicted the behavior in 57 out of 60 bees—indicating how robust an association was found between brain gene expression in the individual bee and naturally occurring behavioral plasticity. Unbelievable.**

Q **Is it my imagination, or am I seeing more and more overweight dogs and cats?**

A ***Pet obesity actually does appear to be paralleling very closely the trend in human obesity [Science* 2003; 301: 1665*]. About one-fourth of cats and dogs in the Western world are now too fat. Urbanization and keeping pets indoors no doubt adds to the problem.***

Can humans or pets ever turn this around? When fruit flies fed a restricted diet are switched to a full diet, mortality soars to the level suffered by fully fed flies. Conversely, when the diet of fully fed flies is restricted, mortality decreases within 2 days to the level enjoyed by flies that have experienced a lifelong restricted diet [Science* 2003; 301: 1731*]. This study suggests that perhaps it's never too late for you or for your pet to shape up and live longer!

P.S. **Thank you for the *Interface* Newsletter. Very impressive. (CR, Sweden)**

Thank you for issue 25 of *Interface*. It is an excellent newsletter. It is interesting, informative, succinct, with a light-hearted and user-friendly style. The numerous citations are greatly appreciated. Great Job. Keep up the good work. (MT, Cincinnati).

Interface is supported by NIH grant # ES06096 from the National Institute of Environmental Health Sciences, and is published by the Center for Environmental Genetics, **Marshall Anderson, PhD, Director**

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Kathleen Dixon accepted a position as Chair,
Department of Molecular and Cellular Biology
University of Arizona in Tucson

George Leikauf assumed the position of Interim Deputy Director of the CEG