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INTERFACE:

GENES AND THE ENVIRONMENT

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The "Cough Syrup Disaster" in Panama

Mysterious deaths begin to appear

Hints of something amiss, in this Central American country with a population of 3 million, began in late summer, 2006. One patient presented to the emergency room with nausea and vomiting. Within 2 days, he could not breathe. Then he suffered kidney failure. His arms and legs swelled to twice normal size. He finally died, with IVs and catheters everywhere. The whole downhill illness took less than a week. Then a second patient, and then a third patient, presented to the emergency room and followed the same sequence of events. Some of the patients presented with Guillain-Barré-type symptoms (tingling and paralysis, starting with the feet and moving upward on both sides of the body).

IN THIS ISSUE

By early October, there had been 21 of these mysterious—yet seemingly related—deaths reported. One big worry at the onset was that this was an emerging infectious disease in this tropical country. In fact, the symptoms were not that different from Ebola virus outbreaks in Africa. Most of the patients who died, however, had not been in any known contact with others who had died.

What could be the cause?

The detective work became intense. Health workers fanned out to victims' homes to see if whole families had been infected**;** they had not. Samples of tissue, plasma and blood from the victims were scrutinized for clues in the lab. No apparent clues were found.

When investigators discovered that about half the victims had been taking a high blood pressure medication, which had just recently been introduced in Panama, suspicion immediately turned to that. As a precaution, the health ministry ordered 2 million tablets of the medication Lisinopril off the shelves.

Some victims, however, had not been taking Lisinopril. Moreover, subsequent tests indicated that this drug was not toxic**;** this was not the culprit. As the investigation continued, health workers also noticed that a sugar-free cough syrup was found at the bedside table of many of the victims. In all cases the cough syrup had been dispensed by the social security clinics Caja del Seguro Social (**CSS**).

Samples of this cough syrup were sent quickly by airplane to the Centers for Disease Control and Prevention (**CDC**) in Atlanta, where it was analyzed.

Within 2 days, the chemical analysis of the cough syrup gave everyone the answer to these poisonings.

Using a machine called a *mass spectrometer*, CDC lab workers compared the chemical makeup of the cough medicine with that of ***diethylene glycol*****;** the low-molecular-weight-fragment peaks matched.

Going door-to-door throughout this tiny nation, Panamanian authorities (as quickly as possible) tried to recall all 260,000 bottles of the cough syrup that had been dispensed. Other similar products made in the federal government lab were also recalled. By the middle of October, 2006, it was time for the investigation to begin.

What is diethylene glycol?

Diethylene glycol (**Figure 1**) is a clear sweet syrupy water-soluble liquid, soluble in many organic solvents**;** it is very *hygroscopic* (soaks up water). **DEG** is used as a softener, conditioner, lubricant, and solvent, and is added to cosmetics**;** DEG is not normally used as antifreeze. A solution of DEG and water is commonly used as a *coolant, i.e.* it lowers the freezing-point of the solution, but it also elevates its boiling point, making it especially suitable for hot climates. DEG is also used as a solvent for nitrocellulose resins, dyes, oils, and other organic compounds. It is a *humectant* (moisturizer) for tobacco, glue, cork, and printing ink**;** DEG can be also found in some hydraulic fluids and brake fluid. **DEG is not for human consumption**.

In contrast, **ethylene glycol** (**Figure 1**) is the most common ingredient in antifreeze. As a colorless sweet-tasting hygroscopic nearly odorless syrup, **EG** is relatively nonvolatile and is the simplest member of the glycol family (having only 2 C atoms). Ethylene glycol freezes at "13°C (8.6°F), boils at 197.6°C (387.7°F), and is completely soluble in water, common alcohols and phenol. EG is also used in polyester resins, explosives, brake and shock-absorber fluids, and alkyl-type resins.

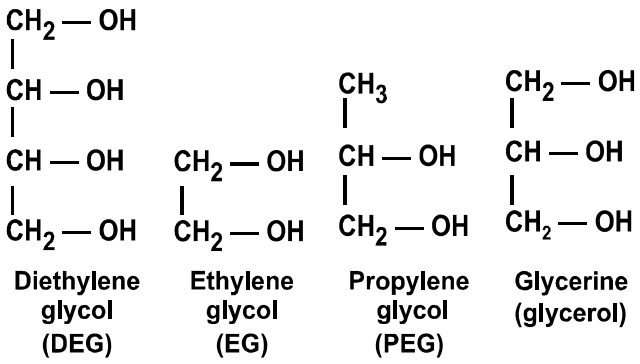

Figure 1. Chemical structures of diethylene glycol, ethylene glycol, propylene glycol and glycerine.

Propylene glycol (propane diol) is also a colorless viscous hygroscopic liquid—used in antifreeze solutions, in hydraulic fluids, and as a solvent (**Figure 1**). **PEG** is used industrially as antifreeze, a solvent stabilizer, and as a preservative in liquid livestock feeds. Pharmaceutically, PEG is used as a vehicle, or solvent, and in some medicinal preparations.

Glycerine (also called glycerol) is a syrupy, sweet, colorless or yellowish liquid (**Figure 1**), obtained from fats and oils as a byproduct of *saponification* (the alkaline hydrolysis of fatty acid esters). Glycerine is used as a solvent, antifreeze, plasticizer, and "non-sugar sweetener". Glycerine is also used in the manufacture of dynamite, cosmetics, liquid soaps, inks, and lubricants. Ironically, although the chemical structures of these four solvents/lubricants/coolants are strikingly similar, glycerine is by far the safest for human consumption whereas the other three are very dangerous.

DEG has been linked to mass deaths in other parts of the world, mostly in poor countries without stringent manufacturing standards or drug regulation. In 2006, for example, out of 64 patients in China with severe liver disease who had received intravenous *armillarisin-A* that contained DEG, 15 died of DEG poisoning**;** armillarisin-A is a derivative of coumarin, used to treat gall-bladder disease. In India in 1998, a locally made cough syrup, later found to be contaminated with DEG, caused 33 children to die of renal failure. An outbreak in Haiti in 1995 and 1996 killed at least 88 children who been given acetaminophen (Tylenol) syrup, meant to treat fevers, which was contaminated with DEG.

Beyond Panama and China, DEG-laced medications, during the 20th century, have caused mass poisonings in Haiti, Bangladesh, Argentina, Nigeria, and twice in India. In the U.S. in 1937, DEG had intentionally been added as a vehicle to medicine, which killed at least 105 people**;** this led to a ban on all future oral use of DEG in the country and a strengthening of the Food and Drug Administration (**FDA**)'s power to regulate drug safety.

How does diethylene glycol cause toxicity?

For more than 50 years it has been known that DEG primarily causes kidney shutdown. Other symptoms include metabolic acidosis (lowering of urinary pH), digestive problems, nervous system impairment (such as tingling and paralysis), and a

high probability of anemia and white blood cell proliferation.

Alcohol-related intoxications—including methanol, DEG, EG and PEG—are due to their metabolites, which can cause metabolic acidosis and cellular dysfunction. Dialysis to remove the unmetabolized alcohol, and possibly the toxic metabolite, can be helpful in treatment. Administration of particular drugs or ethanol to inhibit alcohol dehydrogenase (**ADH**), a critical enzyme in the first step of alcohol metabolism, can be beneficial in treatment of these intoxications. Given the potentially high morbidity and mortality of these poisonings, it is important for the clinician to have a high degree of suspicion for these disorders, so that treatment can be initiated early.

Which metabolite, or metabolites, are the ultimate toxicants in DEG poisoning is not yet clear. Decades ago, oxalate formation was proposed as the cause of DEG-induced kidney toxicity, but this theory has now been proven to be wrong. Metabolism of DEG is known to be carried out principally by an ADH (to form the 1-aldehyde) followed by an aldehyde dehydrogenase (**ALDH**) to form the 2-hydroxy-ethoxyacetic acid. The ALDHs further metabolize the toxic aldehydes**;** thus, ALDHs can be thought of as *detoxication enzymes* in the metabolism of DEG and related alcohols.

The *ADH* gene family has eight genes (***ADH1A***, ***ADH1B***, ***ADH1C***, ***ADH4***, ***ADH5***, ***ADH6***, ***ADH7***, ***ADHFE1***) in the human genome. The *ALDH* gene family has 19 genes (***ALDH1A1***, ***ALDH1A2***, ***ALDH1A3***, ***ALDH1B1***, ***ALDH1L1***, ***ALDH1L2***, ***ALDH2***, ***ALDH3A1***, ***ALDH3A2***, ***ALDH3B1***, ***ALDH3B2***, ***ALDH4A1***, ***ALDH5A1***, ***ALDH6A1***, ***ALDH7A1***, ***ALDH8A1***, ***ALDH9A1***, ***ALDH16A1***, ***ALDH18A1***) in the human genome. The precise ADH and ALDH (or perhaps there are more than one ADHs and ALDHs involved) are not known. Presumably, metabolism to the toxic intermediate occurs in the kidney, but even this is unclear**;** ADH metabolism could occur in the gut or liver, and the most toxic metabolite could still accumulate in the kidney.

There were many more people who had consumed the cough syrup than the number who fell ill or died. Of course, we don't know the exact dose that each person actually swallowed. Many (if not all) of the *ADH* and *ALDH* genes show polymorphisms, meaning that a change in the protein, for example, might be responsible for higher or lower ADH and/or ALDH metabolism. A higher rate of DEG metabolism by ADH combined with a lower rate by ALDH would, of course, lead to potentially greater levels of the toxic intermediate. Thus, this disaster is an example of gene-environment interactions—yet the amount of exposure and epidemiology is not well documented.

Long-term ramifications: social and political problems

According to the latest figures at the end of 2006 from Panama's Health Ministry, 32 people have died from the poisoning, 45 are hospitalized, and eight have been treated and released. Many more suspected cases have yet to be confirmed, officials said. Government officials are now asking everyone who might have used this cough syrup to have a serum creatinine done (to test kidney function). The finger-pointing has just begun, with current officials and former ones trading charges.

By the end of 2006 it was determined that the DEG had come from China. **Taixing Glycerine Factory** bought its DEG from a "glycerine manufacturer" and shipped 46 barrels of **what had been claimed** to be 99.5% pure glycerin to a company in Spain. The material, in turn, was purchased by a small "paper company" in Panama, which then sold the "glycerin" to the Panama Social Security System (**CSS**) laboratories. In Panama, the barrels actually sat unused for more than 2 years, and obviously someone improperly changed the expiration date on the syrup**!**

The counterfeit glycerin had passed through at least three trading companies on three continents, yet not one of them tested the liquid to confirm what was "on the label". Along the way, a certificate falsely attesting to the purity of the shipment was repeatedly altered, eliminating the name of the manufacturer and previous owner. As a result, traders bought the syrup without knowing where it came from, or who made it. With this information, the traders might have discovered that the original "manufacturer" (who had only a 9th grade education) was in fact not even certified to make pharmaceutical ingredients**!**

Over many years the University of Panama had offered to routinely analyze such shipments for the CSS labs, but the latter had steadfastly refused**;** consequently, no one in Panama had checked with mass spectrometry to ensure the purity of the "glycerine"**;** it was simply used to prepare the lethal cough syrup in the federal labs and then dispensed to social security patients.

Addendum (August 2008): Despite a lot of finger-pointing and allegations, no one yet has been charged with any crime. Allegations and law suits are rampant. This is an excellent example of fraud over the internet. The number of 55-gallon barrels that entered Panama from China was **46**. There were **260,000 bottles** of cough syrup made in Panama by the CSS. Analysis by the CDC showed the syrup contained 1% glycerine, 75% sorbitol, and **24% DEG!**. The number of prescriptions given out by the CSS system for this cough syrup was **at least 6,000**. The total number of those with symptoms is **792** (13.2% of those in contact with the syrup). The total number of alleged deaths from DEG-tainted cough syrup is at least **500** (63% of those with symptoms dead within 2 years). Five hundred deaths in a country of 3 million would be equivalent to about 50,000 deaths in a country the size of the U.S. with 300 million.

The number of cases (both deaths and survivors) recognized by the government is currently only **119**. Needless to say, all "trust" and confidence—in clinics sponsored by the social security system of Panama—remain at an all-time low.

—Contributed by Lucia Jorge-Nebert and Dan Nebert

Evolutionarily Speaking.....

What follows is a synopsis of some of the more interesting things that have happened during the last 6 months of 2006 with the Human Genome Project (**HGP**), and evolutionarily-related news, provided chronologically**:**

Jul 2006 A new method is presented, for addressing whether archaic human groups contributed to the modern gene pool (called *ancient admixture*). By studying patterns of variation in contemporary human populations, and using sequence data from the Environmental Genome Project (**EGP**), a study has found strong evidence for ancient admixture (presumably, but not yet certain, from Neanderthals) in both a European and a West African population [*PLoS Genet* 2006; **2:** e105].

Comparing gene-rich clusters with gene-poor deserts along a 4.3-megabase (**Mb**) segment of a mouse chromosome, a study [*J Cell Biol* 2006; **174:** 27] found that deserts are frequently located near the periphery of the nucleus, whereas clusters often occupy the center. Because these patterns are conserved from chickens to primates, it is likely these patterns play a role in positioning the various parts of the chromosomes within the nucleus.

Aug 2006 By studying a dynamic genetic model of human settlement history, coupled with explicit geographical distances of these settlements from East Africa [*Am J Hum Genet* 2006; **79:** 230], the likely origin of modern humans was calculated. Based on 52 human populations analyzed at 783 autosomal microsatellite markers, the best estimates suggest an **initial expansion** of modern-day human occurred **~56,000 years ago** from a small **founding population of ~1,000** effective (breeding) individuals.

Darwin noted that finches in the Galapagos Islands had evolved under the selective pressures of climate and diet. Now a study [*Nature* 2006; **442:** 563] has identified mutations in the bone morphogenetic protein-4 gene (***BMP4***) that are responsible, at least in part, for changes in the shape and size of the beaks of finches.

Sep 2006 What makes humans different from chimpanzees or mice, when we have almost the identical number of protein-coding genes? One study [*Science* 2006; **313:** 1304] found an unknown gene contains a protein-coding domain that is repeated 212 times in humans, 37 repeats in chimps, and only once in mice and rats. Another study [*Nature* 2006; **443:** 167] found a gene (***HAR1A***) that has rapidly evolved in humans and not chimps and is expressed in human brain at 7 to 19 weeks of pregnancy—at a time when neurons are known to be forming and moving throughout the brain.

A new method was developed to infer fertility inheritance from genetic data in human populations [*PLoS Genet* 2006; **2:** e122]. By looking at mitochondrial DNA (*i.e.* inheritance via the mother) from 37 human populations worldwide, the data suggest that, in **hunter-gatherer** populations, individuals belonging to large kin networks might benefit more from stronger social support and thus were more likely to have a larger number of children than **food-producer** populations. **Hmmm**. But, wouldn't the need to have a greater number of children working in the fields—be a reason to have more children, in the food-producer families**?**

Oct 2006 By resequencing a 29,000-base pair (**29-kb**) region of the *MCPH1* gene (implicated in regulating brain size) in 89 individuals, a study [*PNAS* 2006; **103:** 18178] has concluded that two hominid lineages became separated ~1.1 million years ago and remained reproductively isolated until ~37,000 years ago, when a new ("large brain") allele was introduced. If correct, these findings suggest that rare breeding events might have served to introduce the most beneficial form of this gene that contributed to the rapid adaptive evolution of modern humans.

Nov 2006 The **sea urchin genome** sequence has been completed [*Science* 2006; **314:** 938–962]. This is the first full sequence of a *non-chordate deuterostome* (*i.e.* having no neural cord but having both a mouth and anus). The 814-Mb genome offers many new insights about evolution, especially in the areas of developmental biology and systems-biology.

Zonadhesin (**ZAN**) is a male reproductive protein localized in the sperm head. In a study of 47 protein-coding exons among 12 primate species [*Am J Hum Genet* 2006; **79:** 820], it was shown that, by far, the greatest balancing selection and positive selection occurred in the *ZAN* gene in human populations. As described also **in issue #31**, this determination is made by comparing the number of nonsynonymous SNPs (amino-acid-changing) to the number of synonymous SNPs (silent), and **a d_N/d_S ratio** of greater than 1.0 denotes positive selection.

Examining the two sequenced genomes of cultivated Asian rice (*Oryza sativa*), at least **6%** of the genomes were found to be unusually divergent [*PLoS Genet* 2006; **2:** e199]. Looking at ten unlinked DNA segments in 25 *O. sativa* samples from across the world, plus 35 lines from six wild species, the authors conclude that domestication probably was the cause of this **exaggerated diversity:** different haplotypes (pattern of SNPs across one chromosome) developed in response to varying geographical and ecological environments, as the rice migrated across the continents along with humans who were developing increasingly better domesticated varieties.

Dec 2006 A Neanderthal metagenomic library has been developed. Several lines of evidence [*Science* 2006; **314:** 1113] indicate that 65,250 base pairs of hominid sequence so far identified in the library are of Neanderthal origin. Comparing these sequences with chimpanzee and *Homo sapiens* (modern human), the authors calculated that we share a most recent ancestor with Neanderthal ~706,000 years ago, and that the human-Neanderthal split occurred **~370,000 years ago**. Another study analyzing 1 million base pairs of Neanderthal DNA estimates that the human-Neanderthal divergence happened **~500,000 years ago** [*Nature* 2006; **444:** 330]. Neanderthals became extinct an estimated 28,000 years ago. [Curiously, Svante Pääbo (from the Max-Planck Institute in Leipzig, Germany) is coauthor on both of these publications, although all the other coauthors differ between the two reports.]

The total number of cytochrome P450 (***CYP***) **genes** now stands at 6,476—with the addition of *Streptomyces scabies CYP* genes. Among bacterial genomes, the winners for the most *CYP* genes include *Mycobacterium vanbaalenii* with **51** (almost as many as human with **57!**) and *Mycobacterium smegmatis* with 42 [http://drnelson.utmem.edu/CytochromeP450.html]. When **Dan Nebert** at a 1978 meeting suggested that there "probably are hundreds, if not thousands, of P450 genes on this planet" and almost the entire audience laughed, that was a day to remember**!**

Gene-Environment Tidbits of Interest

Jul 2006 The most prevalent theory about homosexuality is that this is a disorder caused by a **genetic predisposition**, plus an **environmental component**. Most likely the latter occurs in utero when the developing fetus might be exposed to abnormal combinations of male versus female hormones. For example, for each additional brother that precedes him (in the womb), boys exhibit an increased ~30% risk of growing up to be gay. For women—their ears, fingers, arms and eyes appear, on average, to be exposed to more fetal testosterone than heterosexual women [*PNAS* 2006; **103:** 10531].

Aug 2006 Exposure of pregnant mothers to airborne polycyclic aromatic hydrocarbons (**PAHs**) in urban polluted air can cause their children to have an

increased risk of *cognitive development* (*i.e.* thinking, perception, learning, attention, problem-solving, reasoning) during childhood [*Environ Health Perspect* 2006; **114:** A487].

Arsenic has long been known to cause oxidative stress. Yet, in comparing the effects of arsenic with those of the classical oxidant *tert*-butylhydroquinone (**BHQ**) [*J Biol Chem* 2006; **281:** 23620], the authors conclude that arsenic activates the NRF2/KEAP1 signaling pathway through a mechanism that is distinct from that by BHQ. It should be noted that the correct name for the "NRF2" gene is nuclear factor (erythroid-derived 2)-like 2 (***NFE2L2***) [http://www.genenames.org].

Sep 2006 Diabetes is defined as a metabolic disease having high blood sugar (hyperglycemia). Type-1 diabetes is generally agreed upon as a "disease characterized by autoimmune pancreatic beta cell destruction". Type-2 diabetes ranges from "predominantly insulin resistance with relative insulin deficiency" to "predominantly an insulin secretory defect with insulin resistance". Type-1 diabetes usually has an early-onset in childhood, type-2 diabetes occurs usually later in life and often with obesity. A beautiful review [*PNAS* 2006; **103:** 12217], based on studies in the non-obese diabetic (**NOD**) mouse, now suggests there is **a *"continuum"*** between type-1, "type-1.5", and type-2 diabetes**:**

	Type-1	Type-2
Age at onset	+	+++
Metabolic stress; environmental factors (e.g. obesity)	+	+++
Genetic predisposition (prevalence in relatives)	+	+++
Insulin secretion failure (ranging from absolute to relative)	+++	+
Beta-cell death and decreased beta-cell mass	+++	+
Islet inflammation (cytokines, chemokines, immune cells)	+++	+
Circulating islet auto-antibodies	+++	+
Insulin resistance	+	+++

Oct 2006 For those of us who have had children, we know that some kids are far more prone to frequent inner ear infections (*otitis media*, **OM**) than others. There is a mutant mouse, called *Junbo*, with hearing loss and chronic OM. A recent study [*PLoS Genet* 2006; **2:** e149] has identified the causal mutation in the ectopic viral integration site-1 gene (*Evi1*). Humans also have the *EVI1* gene, suggesting that this might be an important new pathway in genetic predisposition to clinical OM.

How is arsenic taken up by cells? A recent study in bacteria [*PNAS* 2006; **103:** 15617] has identified a "*metallo-chaperone*" protein **ArsD** that picks up arsenite ions from the cell's cytoplasm and then interacts with an enzyme to activate a pump to send arsenic back out of the cell.

Nov 2006 By high-performance liquid chromatography with mass spectrometry (**HPLC/MS**), it was shown that straight-chain B-type oligomeric procyanidins (**OPCs**) are the principal vasoactive polyphenols in red wine. OPC concentrations are highest in wines from southwestern France and Sardinia [*Nature* 2006; **444:** 566]. Their vasoconstriction effects may account for their anti-atherosclerotic activity.

Dividing 1-year-old mice into three groups, a study gave one group regular food, another high-calorie food, and the third group high-calorie food along with a daily dose of **resveratrol** [*Nature* 2006; **444:** 337]. Resveratrol is found in many plants, including red grapes and therefore also red wine. After 6 months the high-calorie-diet mice were obese, whereas after 1 year the high-calorie-diet-plus resveratrol mice remained healthy despite being overweight. The study suggests that the drug might decouple obesity from its downstream ill-health effects. An obvious follow-up study using obesity-prone mice would be to start from early pregnancy, or even from conception, and then see if resveratrol can prevent obesity from ever occurring.

Dec 2006 Pregnant mice were treated with low, environmentally relevant doses of the estrogenic chemical, *bis*-phenol A (**BPA**) during the middle of the pregnancy—to assess the effect of BPA on the developing ovary in the fetuses. Oocytes from exposed female fetuses showed gross chromosomal aberrations that resulted (as adults) in eggs and embryos with abnormal chromosomal numbers (*aneuploidy*). Interestingly, the untreated **ERβ** knockout mouse *Esr2(-/-)* line showed similar defects to that in BPA-treated mice [*PLoS Genet* 2007; **3:** e5], suggesting that BPA treatment in utero affects ESR2 signaling pathways.

Female and male fruitflies have different fighting styles**:** girls push and shove, whereas boys box. Mutations in the *fruitless* gene (*Fru*) were previously known to cause homosexual males**;** now, a study [*Nat Neurosci* 2006; **9:** 1469] also shows that *Fru* mutant males fight by pushing and shoving, rather than by lunging and boxing like normal males do.

Latest in Genetics and Genomics.....

What follows is a synopsis of some of the more interesting things that have happened during the last 6 months of 2006 with the Human Genome Project (**HGP**), and related genetics/genomics news, provided chronologically**:**

Aug 2006 **Comparative genomics** is the field wherein the genome of one species is aligned with another species to compare how highly conserved is each gene and *intergenic region*. When the human and mouse genomic regions are aligned, about 100,000 regions could not be matched, and the authors suggest that these regions might contain expressed non-coding RNA sequences [*Genome Res* 2006; **16:** 885].

Sep 2006 The black cottonwood tree (*Populus trichocarpa*) has a fast growth rate which makes them ideal for paper, lumber, plywood, and a possible source of biofuels. The **cottonwood** genome has now been **sequenced** and found to contain at least **45,555** likely protein-coding **genes!** It appears that the ancestral poplar genome has been duplicated at least three times: first at the start of all angiosperms (~100-120 million years ago) and most recently at 60-65 million years ago [*Science* 2006; **313:** 1596].

Oct 2006 The **genome of the honeybee** *Apis mellifera* **has been sequenced** and characterized. Compared with other insect genomes, the honeybee genome lacks major transposon families, evolves more slowly and is more similar to vertebrates than to insects in circadian rhythm, RNA-interference, and DNA methylation genes [*Nature* 2006; **443:** 931]. Honeybees (*Hymenoptera*) diverged from *Diptera* (flies) and *Lepidoptera* (moths, butterflies) ~300 million years ago (**MYA**) and the last common ancestor with humans was ~600 MYA. Perhaps the most important aspects of learning about the honeybee genome include the value of this animal in agriculture and that its evolution has culminated in an advanced society of queen, soldier and worker bees. The **10,157 genes** identified so far contain clues about the honeybee's social behavior, physiology and evolution.

Genomes comprise "gene-rich" and "gene-desert" regions. Another comparative genomics study between the human and chimpanzee genomes [*PLoS Genet* 2006; **2:** e171] shows that about half of the expression differences are due to transcripts arising from DNA segments *between* protein-coding genes (*i.e. intergenic regions*, or gene-desert regions).

The Human Gene Nomenclature Committee (**HGNC**) Database (http://www.genenames.org/) was excited to announce that they have surpassed the 24,000-mark in the total number of named human genes. As of Oct 2006, the HGNC data base stands at **24,001 genes**.

Nov 2006 Malignant hyperthermia, which can occur in both humans and pigs, is associated with defects in the ryanodine receptor gene (*RYR1*). This disorder (which has a frequency of ~1 in 40,000 humans) causes a lethal fever when patients receive certain types of anesthesia on the operating table. The trait is inherited as an autosomal dominant (thus, it does not skip generations) but with "variable penetrance"**;** a recent study [*Am J Hum Genet* 2006; **79:** 859] supports the idea that imprinting (an epigenetic phenomenon) is a likely mechanism that makes inheritance of this trait confusing to the geneticist.

The lung disease, cystic fibrosis, occurs when mutations in the *CFTR* gene prevent the membrane channel protein (cystic fibrosis transmembrane conductance regulator; **CFTR**) from folding properly. By partially blocking the expression of the co-chaperone helper protein HSP90 [*Cell* 2006; **127:** 803], the authors show that this helps the mutant protein to form correctly. This approach might also be able to help other diseases that show abnormal protein folding, such as Alzheimer disease.

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

Tidbits from the last half of 2006**:**

Jul 2006 Loss-of-function polymorphisms in the *NAT1* gene were shown to be associated with protection against the risk of newborns having (*spina bifida*) neural tube defects [*Hum Genet* 2006; **120:** 52]. Unfortunately, this study is underpowered (even with *P*-values of 0.008, 0.003) because of insufficient numbers of subjects studied and therefore most likely represents a "false positive" association.

Aug 2006 Gleevec is a cancer drug hailed for its ability to rescue those from dying from leukemia. However, ten patients on this drug with otherwise healthy hearts have now developed heart failure [*Nature Med* 2006; **12:** 908]. This is another good example of **gene-drug interactions:** a person with genetic susceptibility takes a drug at its normally prescribed dose and develops severe or fatal side-effects.

Oct 2006 Age-related macular degeneration (**AMD**) is the most common eye disease causing blindness in the U.S. Some of the responsible genes involve the inflammatory response, but two groups independently have found mutations in **the *HTRA1* gene** to be highly associated with AMD in Chinese and Caucasian populations, respectively [*Science* 2006; **314:** 989 & 992]. HTRA1 is a serine peptidase that might contribute to retinal damage, and also interacts with TGFβ which is involved in new blood vessel growth.

Nov 2006 This month marked the completion of a joint effort between the National Institute of Environmental Health Sciences (**NIEHS**) and **Perlegen Sciences** (San Jose, CA) to collect resequencing and SNP discovery data for 15 commonly used inbred mouse strains. These data are now included on the web site http://www.genenames.org/ (click on "**Useful Links**").

Dec 2006 Using data from 3,839 twin pairs and their family members, it was shown that three nonsynonymous SNPs (nucleotide changes that alter the amino acid) in the *OCA2* gene are strongly associated with having blue eyes [*Am J Hum Genet* 2007; **80:** 241]. Although this might explain as much as three-quarters of the variation in eye color, additional modifier genes are obviously responsible for differences in degree of iris pigmentation.

Obesity appears to have a microbial component, and this could have therapeutic implications. Twelve obese subjects were randomly assigned to either a fat-restricted (FAT-R) diet or a carbohydrate-restricted (CARB-R) diet for one year, and their gut flora was monitored by sequencing 18,348 bacterial 16S rRNA genes [*Nature* 2006; **444:** 1022 & 1009]. Most (70%) of the 4,074 species-level types of rRNA genes were unique to each person, with marked interindividual differences. Obese people before the diet had fewer *Bacteroidetes* and more *Firmicutes*. After the person had lost at least 6% of his body weight on the FAT-R diet or at least 2% on the CARB-R diet, the amount of *Bacteroidetes* had dramatically increased in their gut flora.

Biotechnology....

Tidbits during the last half of 2006, concerning genetically-modified (**GM**) plants, biotechology, and related topics**:**

Jul 2006 One method of genetic engineering—to make mouse models that simulate human disorders—that is gaining in popularity is to insert large chromosomal segments, or an entire mouse chromosome, into another mouse genetic background, thereby making a new mouse line. This topic is reviewed in [*PLoS Genet* 2006; **2:** e86].

Sep 2006 DNA resequencing alone is subject to problems, because one often cannot detect small DNA duplications or deletions. **Microarray-based methods** offer a practical means of carrying out a high-resolution survey of the entire genome, looking for submicroscopic copy-number variants (**CNVs**). Comparing 100 children having *idiopathic* (*i.e.* mechanism completely unknown) mental retardation with normal chromosome analysis [*Am J Hum Genet* 2006; **79:** 500], this study found deletions as small as 178 thousand bases (**178 kb**), duplications as small as1.1 million bases (**1.1 Mb**), and an unexpected mosaic trisomy-9 in another case.

Oct 2006 The **X Prize Foundation** has announced its own latest biotechnology challenge—**offering $10 million** to the first private team to demonstrate they are "able to sequence 100 human genomes in 10 days or less" [*Nature* 2006; **443:** 733].

Nov 2006 DNA sequences that direct the spatial and temporal expression of genes, and defining their function in the intact animal, remain a daunting challenge to those studying vertebrate

genomes. Selecting **167 non-protein-coding (NPC) regions** of these sequences—that are highly conserved among pufferfish, rat, mouse and human—a study has found that 45% of these sequences functioned reproducibly as tissue-specific enhancers of gene expression in the mouse at embryonic day 11.5 [*Nature* 2006; **444:** 499]. Sequence signatures in a subset of these NPC regions were identified to target, for example forebrain expression. This incredible study from a consortium of California labs expands dramatically the catalogue of human gene enhancers that regulate spatial and temporal gene expression.

Dec 2006 Stem cells are increasingly implicated in tumorigenesis. Brain stem embryonic cancers such as *glioblastomas* are particularly resistant to irradiation. One study [*Nature* 2006; **444:** 756] has shown that glioblastoma cells expressing CD133 are resistant to radiation because they are more efficient at inducing the repair of damaged DNA than is the majority of the cancer cells. Another study [*Nature* 2006; **444:** 761] demonstrated that bone morphogenetic proteins (**BMPs**) aid in the differentiation of CD133-containing brain tumor cells.

Using bisulfite DNA sequencing, high-resolution **DNA methylation** profiles of human chromosomes 6, 20 and 22 were reported [*Nat Genet* 2006; **38:** 1378]. They found that **17%** of the 873 genes analyzed are differentially methylated in their upstream regions, and about one-third of these with increased methylation showed decreased transcription. **This type of study is among those that have now opened up the field of EPIGENETICS** by studying **gene methylation**. Epigenetics might explain why one twin of a pair of, say, 58-year-old identical twins develops cancer and the other does not.

Only nature has a right to grieve perpetually, for she only is innocent.

Henry David Thoreau (1817-1862)

Observations by a Biologist

How to Drive Your Competition out of Business

The medium ground finch (*Geospiza fortis*) used to share one of the Galapagos Islands (a small volcanic island, Daphne Major) only with the cactus finch, which uses its pointed beak to eat cactus fruit and pollen. Lacking competition from other finches, the blunt-beaked medium ground finch depended mostly on small seeds that were easier to eat.

A severe drought in 1977 destroyed most of the plants that produced small seeds, such that only those birds with beaks large enough to break open larger hard-to-crack seeds would survive. In just a few generations, surprisingly there was an average **4% increase** in beak size in the medium ground finch [*Science* 2002; **296:** 707].

Then in 1982, the large ground finch (*Geospiza magnirostris*) moved onto the Daphne Major Island and began to compete for the food. At first, the seeds were plentiful for both species of finches.

By 2003, the numbers of large and medium ground finches had swelled to ~350 and ~250, respectively. Then, however, a drought that year resulted in stiff food competition. This led to ~152 medium ground finches dying and ~137 large ground finches dying. And, among the medium ground finches, only about 13% of those having large beaks were able to survive.

Although the beaks of the island's large ground finch did not change in any overt way since the drought, the medium ground finch now seems to be returning to its smaller-beak days because of the selective pressure of a reduced food supply. By 2005, there was an average **5% decrease** in beak size [*Science* 2006; **313:** 224].

This is a very dramatic example of **population numbers** and **gene-environment interactions**. Also, this is evolution occurring in birds before our

very eyes. In this case, however, one selective pressure (paucity of small seeds) led to an increased beak size, and a second selective pressure (decreased food supply) led to a decreased beak size. Perhaps most remarkable is how few generations it has taken for these evolutionary changes to be significantly evident**!** Another interesting point is that one finch species somehow chose to evolve whereas another finch species did not change—during the same adverse conditions.

This finch story is reminiscent of the miniature *Homo sapiens sapiens* (modern man) skeletons found in two caves on different sides of the Island of Palau [*PLoS ONE* 2008; **3:** e1780]. Modern humans apparently had migrated from Southeast Asia to this island between 940 and 2,890 years ago. It appears that, just over the course of 45 to 140 generations, because of severely restricted food supplies, small-sized people were able to survive while larger-sized people could not. These rapid changes certainly cannot be determined by changes in DNA sequence, but rather must be examples of environment-induced **epigenetic changes** such as DNA methylation.

Ethical, Legal and Social Issues....

ELSI tidbits from the last 6 months of 2006**:**

Aug 2006 Kansas voters this month kicked out two conservative members of the State Board of Education, thereby making it possible for the new majority to overturn teaching Intelligent Design in science classes of elementary schools and high schools [*Nature* 2006; **442:** 613].

Sep 2006 In public papers filed in a U.S. district court, deCODE Genetics (based in Iceland) sued four employees of Children's Hospital of Philadelphia (CHOP) for plotting to steal deCODE's most prized assets (their country-wide DNA database crossing three to five generations of thousands of families), thereby "creating a commercial rival". Computer files and data were allegedly stolen from Iceland. CHOP denies these allegations, and a public hearing began on 26 Sept 2006 in an attempt to resolve this lawsuit [*Science* 2006; **314:** 30].

Oct 2006 ***Genetic Savings & Clone***, a Sausalito, California-based company that offered to clone pet cats for the public, is going out of business. It seems that—even though the company recently dropped its fees from $50,000 to $32,000– the price was just too steep to attract sufficient numbers of customers to keep the company afloat [*Science* 2006; **314:** 395].

Nov 2006 Gene names such as *lunatic fringe*, *radical fringe*, *Indian hedgehog* and *sonic hedgehog* have been changed by the Human Gene Nomenclature Committee (**HGNC**). Clearly, if a patient has a disease caused by mutations in such a gene with a whimsical, tongue-in-cheek name, other family members could be offended. Most of these laughable names came from *Drosophila* (fruitfly) genes [*Nature* 2006; **444:** 136].

Dec 2006 An established and unsurprising characteristic of people working with a team (*i.e.* large number of coauthors on a single paper) is that each individual believes that he or she has made a disproportionately large contribution to the group output, so that the summed estimates are far greater than the whole [*J Pers Soc Psychol* 2006; **91:** 857].

Savings&Clone
(you've got to be kidding me)

Best typo - fall 2006
"Webstie" for Website

The Center for Environmental Genetics (CEG) Welcomes Its New Director!

Almost exactly one year to the day, after the 1 Oct 2005 appointment of Professor **Shuk-mei Ho** as Chair of the Department of Environmental Health in the University Cincinnati College of Medicine, Dr. Ho has now accepted the position of **CEG Director**. This follows the resignation of the current CEG Director **Alvaro Puga** in autumn of 2006.

Dr. Ho most recently served as a Professor of Surgery, Cell Biology and Physiology at the University of Massachusetts (**UMass**) Medical Center, where she held numerous positions—including Vice Chair of Research, Director of Urologic Research, and Director of Translational Research in the Department of Surgery. She was also co-Director of the Genitourinary Oncology Program at the UMass Cancer Center. Her own research program emphasizes mechanisms of fetal-based adult disease development. Her laboratory focuses on the significance of hormones and endocrine disruptors in the development of breast, ovary, endometrial, and prostate tumors.

Prior to her work at UMass, Dr. Ho spent 13 years at Tufts University School of Medicine, where she became Professor of Biology and then Associate Dean for Research. Four years prior to her work at Tufts, she had been an Assistant Professor of Biology at Boston University. Dr. Ho completed both her doctoral (Zoology) and undergraduate (Biological Sciences) training at the University of Hong Kong.

Dr. Ho plans to implement the "*systems-biology*" approach to contemporary environmental health research in order to elucidate the functional mechanisms of environmental medicine at the chemical, cellular, organ, whole-body, and population levels—as they are relevant to human health and disease. She is strongly committed to continue building research and teaching programs-of-excellence and to help raise the CEG's, as well as the university's, standings among the top NIEHS Centers and medical schools in the nation.

The new Director also wishes to redirect this ***Interface*** NewsLetter. Each of the last 32 issues of ***Interface*** has focused largely on what the Editor **Dan Nebert** believes are the most intriguing or cutting-edge scientific breakthroughs worldwide, and what is happening month-by-month. Consequently, there has been little news focused on the CEG and Cincinnati investigators. **Shuk-mei Ho** plans to reverse the format and offer almost exclusively internal CEG (and University Cincinnati, Children's Hospital) news and no longer analyze the national and international scientific advances.

Issue #32 is therefore the final issue for Editor **Dan Nebert** and Assistant Editor **Marian Miller**. We thank all those CEG members who have contributed over the past 12 years to the success in making this NewsLetter among the best ever—for any of the NIEHS-funded Centers**!** We also thank all those faithful Readers who have sent us complimentary letters or emails, over the years, telling us how much they have enjoyed each issue!

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LETTERS TO THE EDITOR

RESPONSES/COMMENTS TO VARIOUS QUESTIONS

Q How does the human brain learn? How can short-term and long-term memory be explained in a molecular way that I can understand?

A There were four papers published back-to-back in **Neuron** [Nov 2006; 52: 437–444, 445–459, 461–474 & 475–484] in which a mouse model was used to elucidate the mechanisms surrounding memory. Expression of the **a**ctivity **r**egulated **c**ytoskeletal-associated protein gene (***Arc***) occurs during learning and has long been used as a marker of neuronal activity. This new research shows that ***Arc*(*-/-*) knockout mice** fail to form long-lasting memories. In cell culture studies, the gene was shown to control the appearance and disappearance of receptors for the neurotransmitter α-**a**mino-3-hydroxy-5-**m**ethyl-4-isoxazole**p**ropionic **a**cid (**AMPA**) on neuronal surfaces. This sort of "receptor-trafficking" is known to modify the strength of connections between neurons and this is a fundamental step toward learning and memory. We still have a long way to go, however, to understand these complex complex functions that happen almost every second in our brains.

Comment **Many clinical studies compare a population of "affected" patients with a control (untreated, or never exposed) population, or with the human reference genome, and then try to identify candidate genes associated with the disorder. A "new strategy" was described [*Am J Hum Genet* 2006; 79: 958], based on comparing affected haplotypes (order of mutations on a single chromosome) with closely-matched control sequences from healthy individuals—rather than the human reference genome. Using theory, simulation, and a real data set, they show that this approach is expected to reduce the number of sequence variants by at least a factor of 20. This approach, however, is not really different from the Extreme Discordant Phenotype (EDP) method [*Eur J Pharmacol* 2000; 410: 107]. The robust statistical power of EDP analysis has also been analyzed and proven mathematically [*Pharmacogenet Genomics* 2006; 16: 401]. Thus, rather than studying a group of cigarette smokers versus nonsmokers, for example, one should study a disease caused by relatively small amounts of cigarette-smoking, compared with heavy smokers not having that disease. This is the central principle of the EDP strategy.**