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EVOLUTION IN HEALTH AND DISEASE: WORK IN PROGRESS

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ABSTRACT

This article surveys progress in Darwinian medicine since 1991. Evolutionary thinking has been providing an increasing flow of fresh ideas into medical science, ideas that would not be suggested by other perspectives. Recent contributions have shed new light on the evolution of virulence, of antibiotic resistance, of oocytic atresia, of menopause, of the timing of the expression of genetic disease, of links between mate choice and disease resistance, and of genomic conflict between mother and fetus over resource provisioning. An important consequence of changes from the environment of evolutionary adaptedness concerns reproductive cancers; the incidence of reproductive cancers may be linked to changes in the frequency of menstruation in postindustrial societies. Other intriguing developments include some unanticipated and undesirable consequences of good hygiene, hope from an unexpected quarter for progress on nerve and muscle regeneration, evolutionary interpretations of mental disease, and insights from functional genomics into the nature of tradeoffs. The application of evolutionary thinking to problems in medical research and practice has thus yielded an abundant and growing harvest of insights. Some are well founded, others remain speculative. The field is moving from an initial phase dominated by speculation and hypothesis formation into a more rigorous phase of experimental testing of explicit alternatives. Currently the most promising areas, those in which experimental rigor can be applied efficiently, include experimental evolution and functional genomics. The pioneers can be proud of what they have set in motion.

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WILLIAMS AND NESSE (1991) proclaimed the dawn of Darwinian medicine ten years ago in this journal, though parts of the field had been around for some time. The evolution of antibiotic resistance is a classical problem. The use of evolutionary technology to produce attenuated live vaccines by serial passage had long been in use (cf. Ebert 1998). Medawar (1952) and Williams (1957) had used the theory of natural selection to explain senescence four decades earlier, and the evolution of virulence had been under discussion for at least a decade (e.g., Ewald 1980). Nevertheless, their paper gave the field a significant push, rapidly reinforced by two books (Ewald 1994; Nesse and Williams 1994) and further papers in *The Quarterly Review of Biology* (Haig 1993; Profet 1993; Strassmann 1996). By 1997, when a group of 56 experts gathered in Switzerland to survey the field, this literature already contained more than 1200 items (Stearns 1998). Other books soon followed (e.g., Trevathan et al. 1999). Ten years on, where do things stand? Is the evolutionary perspective paying off? What is fair to expect of it, and what is fair to demand of it?

The recent state of the field is summarized below, in Stearns (1998), and in Trevathan et al. (1999). There has been a significant input of fresh ideas. More of them have implications for medical research than for clinical practice, but some important ideas do have clinical applications. The evolutionary perspective is no panacea, and like any perspective, some of the ideas it proposes do not work out, but it has contributed important and useful ideas that would not be suggested by other theories, and that flow of ideas is increasing, not decreasing.

The two kinds of ideas that evolutionary thinking contributes to medical science reflect the two major branches of evolutionary biology. The first kind, representing the adaptationist perspective, focuses on the consequences of natural selection, on adaptations in humans and their pathogens, and on rapid and dynamic responses to human interventions. The second, representing the historical perspective, focuses on the evolutionary histories of humans and their pathogens, on the reconstruction of those histories, and on the consequences of particular histories for health and disease. The historical perspective includes maladaptive legacies that selection has not been able to overcome, such as the crossing of the respiratory and digestive systems in our throats, which can cause death by choking, and the exit of the birth canal through, rather than in front of, the pubis, which can cause death in childbirth (see also Nesse and Williams 1994; Williams 1997). Both the adaptationist and the historical perspectives have contributed important insights, and both are illustrated below.

Some evolutionary insights into medical science benefit individual patients and are of interest to clinicians. Others concern populations and are of interest to epidemiologists and public health managers. Some interventions at the individual level do not imply costs at the population level. Others, such as drug therapy for individual humans that causes the evolution of antibiotic resistance in the pathogen population that in turn leads, with a brief delay, to future problems for individual humans, do imply such costs. Medical science has long recognized that there are occasional conflicts between the best treatment for the population and the best treatment for the individual, but some evolutionary insights place special emphasis on such conflicts for two reasons. One is the focus of evolutionary thought on populations and population processes. The other is the existence of formal tools within evolutionary biology for the analysis of conflicts between levels of selection, including but not limited to conflicts between populations and individuals. We discuss several such cases below.

EVOLUTIONARY IDEAS IN MEDICAL RESEARCH AND PRACTICE

We now consider major ideas contributed to medicine from the evolutionary perspective (see Table 1 for an overview). We do not attempt an exhaustive review. In many cases the recent state of play is summarized in Stearns (1998), in Trevathan et al. (1999), or in review papers cited therein or traceable therefrom. Before we begin, a brief conceptual clarification may help. It is a platitude to note that causes must be clearly distinguished from consequences. Of course they must; everyone knows that. What everyone may not see immediately in an unfamiliar field is that during the development phase of a concept, it is not always

clear what is the cause and what is the consequence. For example, mainstream evolutionary theory explains aging as a by-product of selection for reproductive performance (including survival to reproduce) early in life, but some alternatives see aging as an adaptation in itself. Mainstream evolutionary theory explains the host symptoms described by the term virulence as a by-product of selection for improved reproductive performance in pathogens, but some alternatives see virulence as a pathogen adaptation in itself. As we work through the major ideas, bear this distinction in mind. It is an implicit assumption of each explanation that the thing being explained has a cause worthy of investigation and is not itself simply the by-product of some other more essential process where the action really is. That assumption can and should be questioned.

THE EVOLUTION OF VIRULENCE: THE HISTORICAL DIMENSION

Two major evolutionary mechanisms are believed to have shaped the level of virulence expressed by pathogens. The first is characterized by competition for hosts and is strongly linked to epidemiology of the disease. Here the mode of transmission plays a key role. Diseases that are vertically transmitted, from host to offspring, should evolve lower levels of virulence than diseases that are horizontally transmitted from host to unrelated host. If the symptoms we recognize as virulence alter the transmission probability of the pathogen, then the pathogen will be selected to increase the intensity of those symptoms if by doing so it can increase its lifetime transmission probability. However, it will also be selected to decrease that intensity if the reduction can increase its lifetime transmission probability. The outcome depends on how symptoms affect transmission probability. The second mechanism is characterized by competition of parasites or pathogens within the host. Virulence will be selected to increase if several pathogen genotypes infect the host at once. The resulting intrahost competition rewards the pathogen genotype that exploits the host most thoroughly and most quickly before the other competing pathogens can rob it of resources. The level of virulence that is expected to evolve depends on the conditions that influence the relative importance of competition within and between hosts (i.e., the type and frequency of multiple infections and the costs imposed by early host death induced by pathogens). Epidemiological conditions in which between-host competition is important, can lead to low virulence—e.g., in case of vertical transmitted parasites (Bull et al. 1991; Herre 1993)—while experimental exclusion of between-host transmission dynamics leads to high virulence (e.g., serial passage experiments; Ebert 1998). These cases are extremes along a continuum; vertical transmission largely excludes within-host competition, and serial passage experiments exclude between-host selection. The majority of diseases evolve under a mixture of less extreme conditions. This is where research is most needed, for it is not clear that interpolating between the extremes will help us to predict the evolution of virulence for most diseases. The ecological conditions that allow some pathogens to increase and others to decrease in virulence are far from being understood (e.g., Day et al. 2001).

The evolution of virulence also has an historical dimension that can be explored with the methods of molecular phylogenetics. Such analyses reveal that virulent organisms are "constructed" through the stepwise accumulation of virulence determinants. The stepwise pattern suggests that each step represented an adaptive advance for the pathogen; the overall pattern is compelling evidence that virulence, or something closely tied to virulence, is adaptive (Marc Lipsitch, personal communication). We are still far from understanding the selective benefits of virulence in these cases, however.

For example, to elucidate the evolution of pathogenic *Escherichia coli* strains, Reid et al. (2000) sequenced seven housekeeping genes to build a phylogenetic tree and trace the history of the acquisition of virulence genes. The rate of synonymous substitution for *E. coli* and *Salmonella enterica* (4.7×10^{-9} per site per year) suggested that the radiation of clones began about 9 million years ago and the virulent pathogen responsible for epidemics of food poisoning, *E. coli* O157:H7, branched off from a common ancestor of *E. coli* K-12 as long as 4.5 million years ago. Moreover, old lineages of *E. coli* acquired the same virulence factors

TABLE 1

Some ideas used in the study of health and noninfectious disease. The borders between categories are not hard. Note that some traits have been given several different explanations and therefore occur in several categories. The table is not comprehensive.

	Type of Idea	Examples
Understanding human nature		
1.1	Phenotype adaptive, without obvious disadvantages or with very minor fitness costs	— Incest avoidance behavior — Mate choice in relation to pathogen resistance
1.2	Phenotypes whose evolution is thought to be constrained by connections to other characters (tradeoff, antagonistic pleiotropy)	— Tradeoff between fitness traits early and late in life explains senescence — Tradeoff between offspring fitness and number (e.g., twins have lower survival probability) — Menstruation as a protection against pathogens — Neonatal jaundice
1.3	Characters which evolved under different conditions, were previously adaptive, and now appear to be maladaptive	
1.3.1	Phenotype was adaptive long ago; e.g., in the stone age or earlier	— Human teeth are not adapted to deal with carbon-hydrate rich food; caries became a problem — Drug abuse as hijacking of adaptive pleasure mechanisms; previously such drugs were not available, or only available in small amounts
1.3.2	Phenotype was adaptive until recently (e.g., preindustrialism), but causes health problems under modern conditions (e.g., altered behavior and nutrition, improved medical care and hygiene, environmental pollution)	— Childhood asthma as a consequence of reduced parasitic worm burdens — Sudden infant death syndrome — Infant crying and colic as a consequence of the unnatural separation of the waking infant from the sleeping mother — Cardiovascular diseases as a consequence of reduced levels of exercise and dietary fiber and high levels of dietary fat
1.4	Phenotypes which are adaptive, but whose adaptive value is difficult to understand; misguided (short-sighted) medical treatment may obscure the adaptive value even further	— Otitis media — Childbirth problems — Morning sickness as hypersensitivity to embryo-damaging toxins
Genetic polymorphisms and conflicts		
2.1	Genetic polymorphisms	
2.1.1	Alleles with apparent adaptive value have not yet gone to fixation	— Ability of adults to digest lactose is polymorphic and varies with the historical dependence on fresh milk.

in parallel, including a pathogenicity island involved in intestinal adhesion, a plasmid-borne haemolysin, and phage-encoded Shiga toxins. The parallel evolution suggests that selection has favored an ordered acquisition of genes that progressively built up the molecular mechanisms that increase virulence.

Closer studies of polymorphisms within virulence factor genes have revealed the presence of increased rates of nonsynonymous substitution (amino acid altering mutations) in surface-exposed and secreted proteins, implying the influence of diversifying selection on polymorphism (Donnenberg and Whittam 2001). Virulence genes had levels of nonsynonymous change five to ten times greater than housekeeping genes. Such increased diversity may help the individual organism to escape host immune response, or a variant to spread in a host population made up of individuals that previously acquired immunity to other variants. There has also been recombination within virulence factor genes; mobile genetic elements containing virulence factors may thereby be introduced into established pathogens to increase diversity. *E. coli* is thus a rapidly evolving species capable of generating new pathogenic variants that can escape host protective mechanisms and produce new disease syndromes.

TABLE 1
Continued

Type of Idea	Examples
	— Resistance to the HIV virus exists, but selection has not been long and intense enough to increase its frequency
	— Duffy blood group polymorphism and malaria
2.1.2 Deleterious dominant mutations which may arise spontaneously or have a delayed age of onset of the disease	— Chromosomal disorders; e.g., Down syndrome
	— Cancer
	— Huntington's chorea
	— Myotonic dystrophy
	— Neurofibromatosis
2.1.3 Deleterious recessive mutations in selection-mutation balance (selection is too weak to remove rare recessive deleterious mutations); for some of these disorders it has been suggested that they are advantageous under certain conditions	— Phenylketonuria
	— Tay-Sachs disease
	— Alpha antitrypsin deficiency
	— Cystic fibrosis
	— Beta thalassemia
	— Bloom's syndrome
	— Congenital nephrosis
	— Hemophilia A (X linked)
	— Color blindness (X linked)
	— Albinism
	— Sickle-cell anemia
2.1.4 Characters influenced by heterozygote advantage	— Sickle-cell anemia where malaria is endemic
	— MHC polymorphisms and disease resistance
2.1.5 Inbreeding depression	— Reduced offspring performance due to marriage among relatives
2.1.6 Multifactorial genetic diseases	— Cleft lip and/or palate
	— Congenital heart disease
	— Coronary heart disease
	— Schizophrenia
	— Diabetes mellitus
2.2 Genetic conflicts	
2.2.1 Mother-child conflict and genomic imprinting	— Problems of pregnancy and childbirth
2.2.2 Selection arena: selection occurs within an entity that is a unit of selection in its own right at a higher level.	— Atresia as an adaptation to eliminate mutationally damaged mitochondria
	— Menopause may be causally liked to atresia

Similar evidence—a high rate of nonsynonymous substitutions in genes associated with virulence—was found in a molecular phylogenetic analysis of influenza A viruses (Bush et al. 1999). It was found that the branch of the tree with the highest proportion of nonsynonymous substitutions was the one that always took the virus from epidemic to epidemic, presumably because it had the greatest selective advantage. With such information one could predict which flu strain in this year's flu season will cause next year's epidemic, and vaccination policy could be adjusted accordingly. (See Note Added in Proof, p 430.)

Thus both the adaptive and the historical branches of evolutionary thought shed considerable light on the evolution of virulence.

THE EVOLUTION OF ANTIBIOTIC RESISTANCE

Antibiotic Induction of Elevated Mutation Rates in Pathogens

That pathogenic bacteria rapidly evolve resistance to antibiotics is well established, and mechanisms for the evolution of resistance are known. They include horizontal acquisition of resistance genes carried by plasmids or transposons, recombination of foreign DNA into the chromosome, and mutations in chromosomal loci. A new direction to the story began when Moxon et al. (1994) suggested that bacteria have two different sets of genes: housekeeping genes used for basic metabolism and structure that mutate at low frequency, and highly mutable *contingency genes* important for adaptation

to changing environments. In addition, many bacterial populations contain some cells with a *mutator phenotype*. Their mutation rate is from 10 to 10,000 times that of normal cells, often because they have a defective methyl-directed mismatch repair system. Contingency loci and mutator phenotypes allow a bacterial lineage to rapidly accumulate many alleles, some of which can evade stressful environmental factors such as host defenses or antibiotics. Both resistance and virulence would evolve faster because of the large sample of mutations. It has therefore been suggested that contingency genes and mutator phenotypes have a selective advantage for pathogens living in humans that are being treated for infectious disease.

Recently Martinez and Baquero (2000) reviewed the ideas and evidence and suggested that the mutation rate of bacteria challenged with antibiotics is elevated in human hosts. They conclude, "We must assume that the mutation rate determined under conventional laboratory conditions probably differs greatly from that in vivo at the site of infection. In such a way, more than a single mutation rate, bacterial populations may have multiple different mutation rates. The time has arrived to face this complexity" (Martinez and Baquero 2000:1776).

Opinion on this point is, however, currently divided. Giraud et al. (2001) monitored bacterial mutation rates during the experimental colonization of mouse guts. They found that "[a] high mutation rate was initially beneficial because it allowed faster adaptation, but this benefit disappeared once adaptation was achieved. Mutator bacteria accumulated mutations that, although neutral in the mouse gut, are often deleterious in secondary environments. Consistently, the competitiveness of mutator bacteria is reduced during transmission to and recolonization of similar hosts" (Giraud et al. 2001:2606).

This example nicely illustrates the elaboration of a potentially important hypothesis, its initial support, and its subsequent refinement through experimental tests. Evolutionary biology can be an experimental science, and experimental evolution of the sort used here is a possible, a powerful—and an increasingly popular—technique (Ebert 1998).

THE CONCEPT OF A SELECTION ARENA

A selection arena is a selection process that occurs inside an entity, such as a reproductive female, that is a unit of selection in its own right at a higher level. It has characteristics suggesting that it is an adaptation of the higher level (in this case, the individual human; Stearns 1987). Examples include the selection of zygotes in the mammalian reproductive tract through selective abortion, where the zygotes are the lower level and the females that contain them are the higher level. Selection on variation in reproductive performance of the organisms—the higher level—has shaped the internal selection process at the lower level.

ATRESIA, A SELECTION ARENA FOR MITOCHONDRIA, AND MENOPAUSE

Total oocyte loss occurs by the end of the reproductive life span in humans and in at least two other primates, rhesus macaques and bonobos (Finch and Sapolsky 1999). The process starts, however, much earlier. By the third month of pregnancy, ovaries have developed in the female human embryo. They contain about 7 million oocytes. By birth that number has been reduced to about a million and by the onset of menses to less than 500. This process of oocyte destruction is called oocytic atresia and is found in most mammals.

Jansen and de Boer (1998) and Krakauer and Mira (1999) suggest that atresia is selective and has an evolutionary explanation. Their explanation rests on the assumption of asexual reproduction by mitochondria. If mitochondria reproduce asexually and pass regularly through population bottlenecks, they cannot avoid accumulating deleterious mutations (Muller 1964). Eventually all mitochondria will be damaged. The problem can be avoided, however, if the mitochondria with the deleterious mutations can be segregated and discarded, as Chao et al. (1997) have shown in a phage model system. This would happen if a small number of mitochondria were introduced into each of many oocytes. The oocytes with defective mitochondria would advertise that fact in their biochemical profile, giving the maternal tissue a signal that could be used to decide from which oocytes nourishment should be withdrawn.

The Krakauer-Mira hypothesis makes at least three predictions: (1) If ovaries are sampled from 3 month embryos to birth, the percentage of defective mitochondria should decline towards birth. This has not been confirmed for humans, but Perez et al. (2000) did establish that the state of the mitochondrial genome could, in at least one experimental model, affect the probability of oocyte destruction. Even if such a correlation were demonstrated, it would not prove that the reason for the destruction of the oocytes is the presence of damaged mitochondria. The signals inducing their destruction could simply be correlated with the presence of damaged mitochondria. (2) The number of mitochondria allocated to a new oocyte should be small, ideally just one. The number of mitochondria with which an oocyte starts life is not yet known precisely, but Jansen and de Boer's (1998) review of all published microphotographs of primordial oocytes suggests that the number is less than 10. Steuerwald et al.'s (2000) PCR estimate of the number of mitochondrial genomes in mature oocytes uses a method that could usefully be applied to primordial oocytes. If the number of mitochondria that enter a primordial oocyte were large, the mechanism would not work because most oocytes would get some defective mitochondria, the biochemical signals given off by the oocytes with defective mitochondria would not be distinctive, and oocytes could not be eliminated selectively. Estimating the number of mitochondria with which an oocyte starts life has thus become an important issue. (3) The signal that initiates the process of apoptosis to destroy an oocyte selectively should have a functional relationship to mitochondrial performance; it should reliably indicate the presence of damaged mitochondria. The nature of such a signal is not yet known. Its nature has become a research priority.

Is there a link between atresia and menopause? Menopause has several adaptive evolutionary explanations (reviews in Gosden et al. 1998; Leidy 1999). One is the grandmother hypothesis, which states that after a certain age reproduction has become so risky for a female that she can increase her lifetime reproductive success by stopping her own reproduction and helping her daughters to raise their offspring. One problem with such explanations is this: reproduction becomes riskier with age because the intensity of selection on older females is low. There is thus correspondingly little selection pressure to shape other potential adaptations, such as menopause, in older females. Such explanations are in logical conflict with the evolution of senescence. Gosden et al. (1998) suggest a nonadaptive explanation. They see menopause as a by-product of atresia. Its variability in age of onset results from random variations in the number of oocytes destroyed prior to birth. Slight variations in proportion of destroyed oocytes translate into differences of tens or hundreds of oocytes available at birth and into differences of years in onset of menopause. The rate of oocyte atresia does correlate with the age of "menopause" in mice (Tilly, personal communication). Under this view menopause occurs when the female runs out of oocytes to ovulate, and the number of oocytes with which she is born is determined by atresia. Atresia in turn exists to screen out oocytes with damaged mitochondria—or other types of damage. This explanation neatly connects processes at the beginning and the end of life, but it has not yet been rigorously tested against alternatives.

EARLY SELECTION AGAINST DEFECTIVE EMBRYOS

A selection arena has a greater advantage when the defective gametes, zygotes, or offspring that are discarded are eliminated early in development before the parents have invested much in them (Kozlowski and Stearns 1989). If selection is done late in development, its advantage can be quite small or nothing at all. This logic may explain the rapid early development of the human embryo (Hastings 2000); most organ systems function within 10 weeks of conception. If the embryo lacks, for example, an essential liver enzyme, then the earlier it dies the better. There is selection for rapid differentiation and development of organ systems so that they can be tested as soon as possible to see if they will work later in life. If they will not work, the embryo is discarded and a sibling is rapidly conceived. The next idea is a corollary.

Reproductively active human females in archaic societies normally produce children at

intervals of around 4 years. If death of the fetus or young infant (less than around 3 years of age) occurs, then the mother reenters estrus and produces another child. Such reproductive compensation creates selection on the age of expression of genetic diseases; "infant mortality may evolve when the early death of one infant makes more likely the creation or survival of a close relative" (Hamilton 1966:12). Genetic diseases that significantly reduce the reproductive success of offspring will be selected to become ever more virulent, killing at ever decreasing ages, to allow the mother to reenter estrus and conceive a (hopefully unaffected) sibling. The same effect changes predictions of mutation/selection balance: for any given mutation rate, syndromes which kill early in life may reach much higher frequencies than those killing at later ages (Hastings 2000). These intriguing ideas have not yet been tested against alternatives.

Note here the potential for maternal-fetal conflict; there should be a range of fetal defectiveness that would make abortion adaptive for the mother but not for the fetus. That range would be determined in part by the relationship between fetal defectiveness and the reproductive fitness realized by embryos that survived birth and adolescence. If the defects in the fetus were such that the resulting offspring had low fitness, there would be little selection to reduce the abortion rate. Only if the correlation between defectiveness as fetus and fitness as a reproductive adult were zero or negative would a maternal-fetal conflict reduce the abortion rate.

MATE CHOICE AND DISEASE RESISTANCE

The role of mate choice in human reproductive biology developed for years along two independent tracks before merging recently. One track was evolutionary; the other was medical. The evolutionary track began with Hamilton and Zuk's (1982) suggestion that birds might select their mates on the basis of honest signals that indicate resistance to parasites and pathogens with a genetic basis. If mates vary in genetic quality, and that variation can be detected in the phenotype, then it pays to select a high-quality mate. The experimental confirmation of this hypothesis in stalk-eyed flies revealed quite unexpected underlying mechanisms (Wilkinson et al. 1998). Wedekind (1994) extended the idea to other levels of selection: mother or ova could select sperm haplotypes before, during, and after zygote formation; selection after mating could favor heterozygous zygotes. Here again we encounter selection arenas. As we will see next, one of them operates in humans.

While the evolutionary ideas were being developed, Ober and her colleagues were carrying out a long-term medical study of the Hutterite communities in South Dakota. The Hutterites, who moved to North America from Switzerland in the 19th century, are a small endogamous community that has become relatively inbred. They are notable for their large sibships, communal lifestyle, and the limited number of 5-locus HLA haplotypes known for all 411 Hutterite couples. Some Hutterite women suffer from recurrent spontaneous abortions. Ober et al. (1992) discovered that women whose husbands had similar HLA loci were more likely to suffer spontaneous abortions than women who had married men with different HLA alleles. They then examined mate choice (Ober et al. 1997) to see whether potential mates avoided partners with similar immune genes. Significantly fewer matches of HLA haplotypes between spouses were observed than would be expected at random. Among couples who did match, where a mistake was made, the matched haplotype was inherited from the mother 29 times and from the father 50 times. Thus Hutterites avoid mating with partners with the same HLA haplotype, and the evidence suggests that it is primarily the females who are making the choice.

These studies confirm that humans can detect variation in MHC haplotypes among potential mates and use that information to choose mates, as do both inbred and seminatural populations of mice, where the signals appear to be olfactory (Yamazaki et al. 1990). In outbred human populations, factors other than MHC haplotype—such as social status, income, and ethnicity—are probably usually more important.

MOLECULAR DETECTIVE WORK: INSIGHTS FROM PHYLOGENETICS

The methods of molecular systematics give us new insights into the structure of pathogen populations. The results shed light on many

issues, including the potential for rapid horizontal transfer of antibiotic resistance and the identification of the individuals responsible for the spread of very rapidly evolving diseases. Many of these methods rely on the construction of phylogenetic trees from which inferences are drawn; the standard criteria for judging the reliability of such trees naturally apply. The reliability of phylogenetic trees is a particularly important issue when they are used as evidence in trials for crimes that carry the death penalty.

Are Bacterial Populations Really Clones or Do They Have Sex?

The answer to this question is critical for understanding the spread of resistance genes and the potential of pathogen populations to respond quickly to host evolution and to vaccines and other epidemiological measures. Molecular phylogenies have now shown that the degree of clonality varies among species: some, like *E. coli* and *Salmonella*, are highly clonal, whereas others, like *Neisseria gonorrhoeae* and *Bacillus subtilis*, are effectively panmictic. Even the most clonal bacteria, like *E. coli*, are chimeras containing chromosomal genes and portions of genes of different ancestries. Thus pathogenic bacteria vary in their potential for horizontal genetic transfer; some behave practically like outcrossing sexual eukaryotes, and all manage some exchange of information (see Levin et al. 1999 for a review).

Molecular Forensics

When pathogens have clonal structure, that structure produces patterns of relatedness that are useful for tracking the genetic origin of epidemics (e.g., when and where did AIDS enter the human population; cf. Hillis 2000) and for forensic studies, such as tracing the person who infected others with HIV (Hillis and Huelsenbeck 1994).

Does Alzheimer's Disease Have Prehuman Precursors?

Finch and Sapolsky (1999) argue as follows. All long-lived mammals express Alzheimer's-like neuropathological changes as they age, and estrogens provide some protection from such pathologies. The strength of selection on the age of onset of such pathologies varies from species to species; the timing of the reproductive aging with associated estrogen loss is governed by selection on the reproductive schedule. Primates with early and more frequent reproduction are predicted to have earlier onset of neuropathological changes because of their reproductive decline; this is confirmed in rhesus macaques. What changes in human biochemistry might be protecting us from the earlier onset of neuropathologies experienced by our primate ancestors? Finch and Sapolsky suggest that the mechanism involves new human alleles at the apoE locus. The apoE gene (and the apoB gene) codes for the protein segment of the low-density lipoproteins implicated in cholesterol metabolism, heart disease, and Alzheimer's. Human populations contain 3 variants: apoE 2, 3, and 4. Nonhuman primates have only one apoE allele; it resembles apoE4. Human populations with high frequencies of apoE4 have high frequencies of heart disease and increased levels of total plasma cholesterol. Those with the other alleles are at lower risk for both heart disease and Alzheimer's.

Thus their argument is essentially a phylogenetic correlation: the speciation event that divides humans from nonhuman primates is associated with longer life in humans, later onset of neuropathologies, and the existence of new alleles—apoE 2 and 3—whose frequency is correlated with lower incidence of debilitating late-life diseases. Whether there is a causal connection remains to be established.

INTRAGENOMIC CONFLICT: EVOLUTIONARY CAUSES, MEDICAL CONSEQUENCES

Parent-Offspring Conflicts in Pregnancy

This idea is not new, but it is so striking that we have to mention it. Here evolutionary thinking has brought exciting insights to an important human medical problem. David Haig (1993, 1998) saw that the interests of father and mother could diverge over the rate at which the embryo should extract resources from the mother. Because the father could have children by other females, he should want his offspring to extract more resources from this female than she is prepared to give. She, on the other hand, should want to reserve more resources for future offspring, perhaps fathered by other males, than he would.

Haig and Graham (1991) connected this evolutionary conflict of interest to genetic imprinting. Genetic imprinting occurs in the germ line of the parents; it only happens to a very few genes. The imprinted genes are methylated so that they are not expressed in the offspring early in development. In mice and in humans several of the genes that are imprinted are the genes that control embryonic growth. Experiments in mice have shown that if the father's imprinted growth-related genes are deactivated in the embryo, the embryo grows more slowly and is born at a smaller size. If the mother's imprinted growth-related genes are deactivated, the embryo grows more rapidly and is born at a larger size. The genes that are imprinted in the paternal and maternal germ lines are not the same; they have opposite effects on growth. The genes imprinted in the paternal germ line would, if expressed, decrease growth rate. Those imprinted in the maternal germ line would, if expressed, increase growth rate. The state of the system thus appears to have been determined by an evolutionary history of parental conflict over the allocation of resources, a history recorded in the molecular genetic control of embryonic growth.

MENSTRUATION FREQUENCY AND REPRODUCTIVE CANCERS

Short (1976) noted that in preindustrial societies women spent a much greater proportion of their lives pregnant or lactating than they now do. As a result they went through fewer menstrual cycles per lifetime than do women who use contraception. Because they cycled less often, their breasts, ovaries, and uteri went through fewer episodes of differentiation and de-differentiation and were therefore exposed to fewer opportunities for mistakes in genetic control over the cell cycle to occur and accumulate. Under this hypothesis, the probability of breast, ovarian, and uterine cancer rises as the number of menstrual cycles per lifetime increases. Women in preindustrial societies with traditional reproductive patterns should be at lower risk, and women in postindustrial societies using types of contraception that do not block the menstrual cycle should be at higher risk. Strassmann (1997) confirmed the difference in menstrual patterns between the two types of society and reemphasized its implications for reproductive cancers. This attracted the attention of the popular press (Gladwell 2000). Clinical trials of a contraceptive pill that only allows menstruation four times a year are said to be in progress. If the results demonstrate a decreased frequency of reproductive cancers with lower frequency of menstruation, then this will become one of the few cases in which a concrete benefit has resulted from the idea that our bodies are not adapted to our current lifestyle.

EVO-DEVO: HOPE FOR NERVE AND LIMB REGENERATION

Evo-Devo, the label now given to evolutionary developmental genetics, studies the evolution of major developmental control genes first identified and sequenced in fruit flies, worms, and mice (Carroll et al. 2001). Comparisons of the DNA sequences among these model systems revealed that genes that shared sequence homology also shared function to an astounding degree. The genes that initiate brain, eye, and heart formation in fruit flies are homologous to genes that do the same in mammals. Their products are so similar that when a transgenic mouse gene is expressed in a developing fruit fly, it induces formation of ectopic eyes. Thus the Evo-Devo strategy is a modern application of the classic use of model systems reinforced by the surprisingly deep conservation of developmental function and DNA sequence.

Recently rapid progress has been made in the study of limb development (Carroll 1995) and nerve growth (Hirth and Reichert 1999). We are still a long way from being able to use gene therapy to cause a severed forelimb to regenerate a functional hand or a severed spinal cord to reconnect well enough to restore function. However, never before have we had such good reason to think that such treatments should in principle be possible. If they are to be realized, we will have to trace where in phylogenetic history the ability to regenerate limbs and nerves was lost, and for what reason. To do so we will need more efficient approaches to the comparative study of developmental control genes in an explicit phylogenetic context. Then we will have to develop the new model systems so identified, models that span the critical losses of function.

THE EVOLUTIONARY BIOLOGY OF MENTAL DISEASE AND DRUG ABUSE

Nesse has reviewed these ideas recently; we refer you to him for details. His insight into drug abuse (Nesse and Berridge 1997) is that addictive drugs hijack pathways that evolved to increase fitness but are intrinsically vulnerable

to novel drugs with chemical structures that mimic signals that in the past promised a fitness gain. Thus susceptibility to addictive drugs is a nonadaptive by-product of physiological mechanisms evolved for other reasons. Nesse (2000) also suggests that depression can be adaptive if it causes avoidance of risky or dangerous situations in which the goal could not be obtained. In such cases, where it is better to lay low and do nothing, depression could inhibit types of activity that lower fitness. This is a plausible explanation for a moderate level of depression but not for the types of serious depression associated with suicide. If a selection process had repeatedly encountered the problem of deep depression, one would expect countermeasures to have evolved to prevent mood from worsening too far in a dangerous direction.

CHANGES FROM THE ENVIRONMENT OF EVOLUTIONARY ADAPTEDNESS

Many hypotheses in evolutionary medicine posit a past environment that differed markedly from the present one, assert that in that environment selection shaped human physiology or behavior in a markedly different way, then conclude that the problem under analysis results from modern deviations from an ancestral lifestyle. That lifestyle may be characterized as preindustrial, as preagricultural, as Stone Age hunter-and-gatherer, or as something earlier than that. In fact we rarely have enough information on past environments and past lifestyles to make a strong assertion about the environment of evolutionary adaptedness. Our current evolutionary state has been integrated over a long succession of past environments that have left their traces on us like a moving average weighted toward the recent. Nevertheless such hypotheses are interesting and worth further exploration, even if, at the moment, few of them have withstood experimental tests and exposure to plausible alternatives. Among such hypotheses are the ideas discussed in Flaxman and Sherman (2000) and Trevathan et al. (1999), listed in Table 1, sections 1.3 and 1.4.

THE HYGIENE HYPOTHESIS: THE UNINTENDED CONSEQUENCES OF CLEANLINESS

Bjorksten (1999) suggested that children of families that use antibiotics and vaccinations have more allergies than children of families that avoid them. The implication is that children exposed to a dirty environment and to a variety of pathogens early in life will develop fewer allergies. Weinstock has generalized (Sewell et al. 2000, unpublished abstract) this idea as follows. He thinks that autoimmune disease is expressed when an immune system that has evolved to deal with multiple invaders finds itself unable to adapt to a more sterile environment. In the environment of evolutionary adaptedness, humans may never have experienced life without helminthic diseases. Apparently worms generally tend to reduce our immune response, probably to their own benefit. Because worms were a normal part of our environment, our immune system may have evolved under conditions where it had to overexpress immune function to counter the effect of the worms. In the absence of worms, our immune system may be in a permanent state of overexpression, leading to various forms of autoimmune diseases. The therapy suggested for these diseases, among which are found some syndromes for which there is not yet any other effective therapy, is a modest dietary supplement of helminth surface proteins. Here evolutionary thinking suggests a concrete benefit to the individual, not to the population, from a quite unexpected source. The idea is intriguing and plausible but not yet particularly well tested. It deserves test.

WHAT IS AN ADAPTATION?

Some evolutionary biologists occasionally indulge in adaptive just-so stories without rigorous consideration of nonadaptive alternatives. That practice elicited a reforming response (Gould and Lewontin 1979) so influential that it inhibited discussion of adaptation for a decade. Since then a counterreformation, led by Rose and Lauder (1996), has again made discussion of adaptation acceptable in polite company. In that spirit we offer here four relevant criteria with which we can evaluate the claims of adaptation that characterize many ideas in evolutionary medicine. They are arranged in rough order of rigor and reliability, and they are all variations on a single theme: *to be accepted as an adaptation, a trait state or a change in trait state must be shown to increase the reproductive success of the organisms that carry it.*

THE SELECTION CRITERION

Natural selection on a trait is the correlation between variation in the trait and variation in reproductive success. A response to selection occurs when some of the variation in the trait is heritable. If you observe the process and

document heritable changes in the trait that result from the correlation of trait state with reproductive success, then the *change* in the trait is an adaptation (cf. Grant 1986; Reznick et al. 1990; Stearns et al. 2000). This best and strongest criterion—selection and the response to selection both observed—is fulfilled in studies of antibiotic resistance and in serial passage experiments on virulence.

THE PERTURBATION CRITERION

This criterion accepts as an adaptation the state of a trait whose optimal state has been predicted by a model and tested by experiments. The experiments use mutations, phenocopies, transgenesis, hormones, surgical manipulation, or some other method to perturb the phenotype from the optimal state and, with appropriate controls, to demonstrate that the fitness of the perturbed phenotypes is lower than the fitness of the optimal state (cf. Sinervo and Basolo 1996). This criterion has been met in studies of clutch size in birds (Daan et al. 1990) and body size in lizards (Sinervo 1990). For both ethical and practical reasons it is not often applied to human subjects.

THE FUNCTIONAL CRITERION

Williams (1966) and Curio (1973) define an adaptation as a change in a phenotype that occurs in response to a specific environmental signal with a clear functional relationship to that signal resulting in improved growth, survival, or reproduction. Under other circumstances, where it imposes a cost, it is not expressed. Examples include induced responses to pathogens, parasites, and predators (e.g., Minchella 1985; Lively 1986). Such claims of adaptation are more convincing when they survive tests against at least two alternatives: (a) the host response is an adaptation of the pathogen, not of the host; (b) the host response is pathological, a reflection of damage done to the host by the pathogen.

THE DESIGN CRITERION

Paley (1836), Williams (1966), and Lauder (1996) recognize an adaptation by its complexity and by its resemblance to something that an engineer might design. Lauder suggests that to meet this criterion, some questions should be answered, including: (1) Has the performance of the trait in the fulfillment of that function been compared in experiments with alternative states of the trait? (2) Is the state claimed to be an adaptation repeatedly associated in phylogeny with the kind of natural selection needed to produce that adaptation? (3) Is the state of the trait a by-product of selection on other traits? (4) Has the trait been analyzed as a component part of the organism, or might the analysis be confused by an inappropriate abstraction of a piece of the organism from the larger whole in which it is naturally embedded (cf. Gould and Lewontin 1979)?

There is no problem in principle with claims of adaptation. Problems only arise when such claims are not supported by convincing evidence, when plausible alternatives have not been examined and rejected.

OUTLOOK

Some areas of evolutionary medicine show great promise but have as yet no results. One of the most promising is the detailed study of host-pathogen coevolution made possible by genomics. Another is the analysis of tradeoffs as whole-genome conflicts over gene expression.

THE GENOMICS OF HOST-PATHOGEN COEVOLUTION

Genomics refers to the study of all, or most, of the genes in the genome at once. It has been made possible by two technological developments: the rapid, cheap DNA sequencing that has yielded the complete DNA sequences of an increasing number of species, including our own, and the development of microarrays to measure the expression of many genes at once. During the last 5 years, the sequences of about 30 microbial genomes were completed; within the next 2 to 4 years, we should have the sequences of more than 100 microbial genomes (Fraser et al. 2000). A truly comparative functional genomics will then be possible.

In contrast to single-species studies, the comparative analysis of multiple genomes will provide substantially more information on the physiology and evolution of microbial species and markedly increase our ability to assign putative function to genes. Pollock et al. (2000) suggest that it is now feasible to accelerate the collection of genome-scale data sets by sampling DNA segments prior to cloning and sequencing, then reconstructing the complete sequences computationally. This approach benefits from the automated protocols developed by the genome projects. The number of taxa sampled could soon increase to thousands for targeted genomic regions. Sequence diver-

sity at this level will dramatically improve both the quality of phylogenetic inference and the power of comparative evolutionary studies. In particular, it will be possible to estimate site-specific substitution probabilities and changes in evolutionary patterns, including adaptive bursts and changes in selective constraints. Such estimates could then be used to predict the effects of protein structure and function on sequence evolution and to address questions about the evolutionary origin of virulence and resistance.

THE FUNCTIONAL GENOMICS OF TRADEOFFS

One problem that functional genomics may help to solve is that of the nature and causes of tradeoffs and pleiotropy. A tradeoff occurs when an evolutionary change in one trait that increases fitness is connected to an evolutionary change in another trait that decreases fitness. Pleiotropy occurs when one gene affects two or more traits, and antagonistic pleiotropy is present when the action of the gene on one trait improves fitness, whereas its action on another trait decreases fitness or is otherwise detrimental. What connects traits in such a fashion? And if we understood the connection, could it be manipulated to reduce the implicit costs?

To see the relevance to medicine, consider the following questions: Why do hosts not resist more different kinds of pathogens? Why do pathogens not infect more different kinds of hosts? Why do we not live longer? What causes aging? Such questions arise in every analysis of trait evolution. To answer them we must understand what limits the simultaneous evolution of two or more traits. The answers are usually couched in terms of tradeoffs and pleiotropy.

Tradeoffs and pleiotropy are currently inferred as correlated responses to selection or as correlations among relatives in breeding experiments. Their causes are hidden in black boxes that need to be opened so that we can unpack their mechanisms to understand what limits trait evolution.

Those limits are a particularly pressing problem in life history evolution and the evolution of aging. In life history evolution, the impact of age and size-specific changes in extrinsic mortality rates on the allocation of resources to reproduction, growth, and maintenance is well understood. If extrinsic mortality rates change in a given manner, then we expect a reallocation among growth, reproduction, and maintenance in a specific way, given tradeoffs among those functions with a certain form. The tradeoffs are often assumed, not measured. And the theory predicts neither their existence nor their shape.

The evolutionary theory of aging suggests that early-life fitness components, such as development time and early fecundity, connect to late-life fitness components, such as late-life fecundity and late-life intrinsic mortality rates, through antagonistic pleiotropy. The genes thought to have such effects should improve fitness through their impact on early-life traits that make a major contribution to fitness while eroding performance through their impact on late-life traits that make little or no contribution to fitness. It has proven difficult to find such genes, although correlated responses to selection consistent with (but not necessarily demonstrative of) antagonistic pleiotropy are common (Stearns and Partridge 2001). Thus the idea of antagonistic pleiotropy might be correct, but we appear to have been looking for it in the wrong place or in the wrong way.

How could functional genomics help to solve these evolutionary problems? We can define tradeoffs and antagonistic pleiotropy as conflicts among whole-organism functions over whole-genome patterns of gene expression (Stearns, submitted). For example, consider the case of reproduction and pathogen attack. One pattern of whole-genome expression characterizes the response to reproduction, another the response to pathogen attack. If the organism were not reproducing, it could defend itself better against pathogen attack, and if it were not under pathogen attack, it could reproduce better. For example, some genes that should be up-regulated for reproduction should be down-regulated for pathogen attack. When the organism is both reproducing and under pathogen attack, the deviation of its gene expressions from those appropriate to reproduction measures how much it trades off reproductive performance for pathogen resistance. Thus the classical tradeoff between reproduction and survival can be described as conflicts over whole-genome patterns of gene expression.

These ideas can be applied using gene chips and other microarrays to study any tradeoffs of interest to medical science, both in humans and in model systems. Such tradeoffs include the classical ones between reproduction, maintenance, and survival, but one could use the same approach, given a sufficiently detailed

genealogy, to explore, for example, the hypothesized association between mental disease and creativity. Perhaps the most exciting application would be the study of virulence and resistance in a model system where both the pathogen and the host genomes had been completely sequenced and expression microarrays were available for both.

NOTE ADDED IN PROOF

Virulence also evolves in reaction to the imperfect vaccination of human populations. Vaccines that reduce pathogen growth rate or toxicity protect hosts; they also reduce selection against virulent pathogens. Therefore pathogen populations that have evolved in the presence of vaccines produce more severe disease in unvaccinated individuals, reducing the benefits of vaccination to the population to the point where overall mortality rates may even increase. The policy implications for vaccines that are not expected to provide full immunity, such as candidate vaccines against malaria, need deep thought and broad discussion (Gandon et al. 2001).

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