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The Molecular Signature of Selection Underlying Human Adaptations

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ABSTRACT In the last decade, advances in human population genetics and comparative genomics have resulted in important contributions to our understanding of human genetic diversity and genetic adaptation. For the first time, we are able to reliably detect the signature of natural selection from patterns of DNA polymorphism. Identifying the effects of natural selection in this way provides a crucial piece of evidence needed to support hypotheses of human adaptation. This review provides a detailed description of the theory and analytical approaches used to detect signatures of natural selection in the human genome. We discuss these methods in relation to four classic human traits—skin color, the Duffy blood group, bitter-taste sensation, and lactase persistence. By highlighting these four traits we are able to discuss the ways in which analyses of DNA polymorphism can lead to inferences regarding past histories of selection. Specifically, we can infer the importance of specific regimes of selection (i.e. directional selection, balancing selection, and purifying selection) in the evolution of a trait because these different types of selection leave different patterns of DNA polymorphism. In addition, we demonstrate how these types of data can be used to estimate the time frame in which selection operated on a trait. As the field has advanced, a general issue that has come to the forefront is how specific demographic events in human history, such as population expansions, bottlenecks, and subdivision of populations, have also left a signature across the genome that can interfere with our detection of the footprint of selection at particular genes. Therefore, we discuss this general problem with respect to the four traits reviewed here, and describe the ways in which the signature of selection can be teased from a background signature of demographic history. Finally, we move from a discussion of analyses of selection motivated by a "candidate-gene" approach, in which *a priori* information led to the analysis of specific gene, to discussion of "genome-scanning" approaches that are directed at discovering new genes that have been under positive selection. Such scans can be designed to detect those genes that have been positively selected in our divergence from chimpanzees, as well as those genes that have been under selection as human populations have migrated, differentiated, and adapted to specific geographic environments. We predict that both approaches will be applied in the future, enabling a greater insight into human species-wide adaptations, as well as the specific adaptations of human populations. Yrbk Phys Anthropol 49:89–130, 2006.

A major endeavor of biological anthropology is to identify and understand how humans have adapted by natural selection to their environment over evolutionary history. Human adaptation has occurred at two different temporal levels. At one level, human adaptation has occurred during the six to seven million years of time since humans diverged from chimpanzees and prior to the differentiation of anatomically modern humans. Such adaptations are what we call species-wide adaptations. In more recent evolutionary times, human populations have undergone a process of differentiation and have adapted to different environments as they spread and occupied different regions of the world. Such adaptations are what we call population adaptations.

While our focus in this paper is on human *genetic adaptations*, we do recognize the process of human adaptability. Adaptation of this sort refers to the physiological, biochemical, and behavioral adjustments (i.e. nongenetic adjustments) that human individuals are capable of making in response to changes in their environment (Baker, 1988).

In order to demonstrate genetic adaptation in humans, or in any organism, it is necessary to acquire evidence substantiating that natural selection underlies the evolution of a particular trait (Harrison, 1988). Ascertaining the operation of selection in the evolution of a trait is a rigorous exercise that requires evidence of various types. Strong evidence for the adaptive status of a trait would require the following: evidence of differential fertility or mortality dependent on a particular genetic difference, evidence from in vitro or in vivo studies of functional differences between genotypes that affect reproductive success, and evidence of geographic concordance between the distribution of a genetic trait and some environmental factor that could be a selective force. As a consequence of

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these demands for evidence, G.A. Harrison (1988) pointed out that "evidence for polymorphisms being maintained [in humans] by selection is largely nonexistent." He observed that our strongest evidence of selection and genetic adaptation involves the polymorphisms at *G6PD*, at β-globin, and at the Duffy blood group, most likely maintained due to the selective pressures from *falciparum* malaria and *vivax* malaria.

Over the last decade, advances in comparative genomics, population genetics, and molecular evolutionary theory have begun to provide us with data and methodology that can be used to test hypotheses of natural selection and adaptation. The first advance is our ability to collect DNA sequence data on very large scales. This has allowed both the characterization of entire genomes (e.g., the human and chimpanzee genomes are two among an increasing list of species that are completely sequenced; see Lander et al., 2001; Chimpanzee Sequencing and Analysis Consortium, 2005). It has also become possible to collect DNA sequence data from many individuals within a species, providing information on variation within and between populations. The abundance of new DNA sequence data has motivated the development of novel analytical methods, guided towards testing specific hypotheses concerning the role of natural selection in shaping human genetic variation (Nielsen, 2005). Such developments have made it possible to test for the effects of selection at particular genes, and also to scan the entire genome to discover those genes or genetic regions that were likely targets of selection in our evolutionary history.

However, we have learned that the detection of natural selection from genetic data is not simple. One difficulty that is now widely appreciated is teasing apart the signal of selection from the signal left by the demographic history of our species. Helpful in this regard has been the increasing availability of large genome-wide data sets of human DNA polymorphism that have allowed us to make inferences about our demographic history. It is expected that demographic phenomena such as population expansions, subdivision and bottleneck events will affect variation at all genes. On the other hand, natural selection is expected to have locus-specific effects. An improved understanding of the common pattern of variation across many genes will be necessary so that we can detect those genes having histories of selection.

In this paper, we begin by providing background discussion of the theory and analytical methods that are used to detect natural selection in studies that compare differences in DNA sequences *between* species, as well as studies that analyze variation in DNA sequences *within* species. Next, we review and discuss recent genetic evidence for four classically studied human polymorphic traits hypothesized to have been under natural selection. These polymorphic traits include skin color, the Duffy blood group, bitter-taste sensation, and lactase persistence. We chose these specific traits, rather than any of a number of other traits for which DNA evidence of selection is accruing (Bamshad and Wooding, 2003; Vallender and Lahn, 2004; Sabeti et al., 2006), for several reasons. First, we believe that most biological anthropologists are familiar with hypotheses that have been proposed over the years to explain the evolution of these traits. Second, we believe that there is reasonably good evidence concerning which particular gene or genes contribute to the phenotypic diversity of these traits. Third, we chose these traits because they allow us to discuss how different regimes of selection such as directional selection, balancing selection, diversifying selection, and purifying selection can be detected from patterns of DNA polymorphism. Finally, these examples allow us to discuss the wide array of analytical methodologies that can be successfully applied in the study of natural selection on human genes.

After discussing the signature of selection at specific genes investigated through a so-called "candidate-gene" approach, in which a gene is chosen for further study based on its presumed involvement with a trait, we describe the recent studies directed at searching for positively selected genes across the entire human genome. These studies, referred to as "genome-scanning" analyses, have focused on two different time frames in our evolutionary history. First, genome-scanning studies have been directed at detecting which of our genes have experienced positive selection during the divergence of humans from chimpanzees. Second, the approach has been directed at discerning which genes have been targets of positive selection as human populations have adapted to different environments. Genome-scanning studies have the potential to generate lists of putatively selected genes that can be further studied from a functional perspective. In addition, these genome-wide surveys help answer broad evolutionary questions about our species, including the extent to which selection has been an important process in shaping our genetic variation.

THEORY AND ANALYTICAL METHODS: THE DETECTION OF SELECTION FROM DNA SEQUENCES

Selection and neutrality

One of the challenges faced by evolutionary geneticists is to determine whether the genetic data available to them was shaped by natural selection over its evolutionary history. Rather than detecting selection by observing its ongoing dynamics, population genetic approaches aim to establish whether or not observed patterns of genetic variation would be unlikely in the absence of selection.

In this paper, the main types of data we will be focusing on are DNA sequences (i.e. complete nucleotide sequences). Nucleotide sites that differ among individuals within a species are said to be *polymorphic*, and nucleotide sites that differ among species represent *divergence* and are often referred to as fixed differences. Sites that are polymorphic within a species are known as single nucleotide polymorphisms (SNPs). There are several advantages to using SNP data to study the effects of natural selection on human genes. First, high-throughput genotyping methodologies currently exist that allow large number of SNPs to be surveyed rapidly; indeed, large data sets already exist (Altschuler et al., 2005; Hinds et al., 2005). Second, the molecular theory of evolutionary change at biallelic polymorphisms (which is the case for the majority of SNPs) is relatively mature and allows the implementation of robust tests of neutrality as well as inferences of population parameters. Third, comparative analyses require the establishment of homology between human and outgroup sequences (e.g. the chimpanzee sequence), and this is relatively easily done for DNA sequence data.

The neutral model of molecular evolution postulates that most evolutionary change at the molecular level is a consequence of random genetic drift, and not adaptive evolution. It should be noted, however, that the neutral model does not exclude the role of natural selection: natural selection is assumed to remove deleterious mutations (i.e. purifying selection) and fix the rarely arising advan-

tageous mutation. Thus, under the neutral model, selection can occur, but it contributes little to the observed variation within or the differences between species (Kimura, 1983).

Under the neutral model, specific theoretical predictions can be made regarding the relationship between the rate of mutation and evolutionary parameters. 1) The polymorphism within a species is a function of the mutation rate and population size, following the relationship established by Crow and Kimura (1970): $H = 4N\mu/(1 + 4N\mu)$, where H stands for expected heterozygosity, N is the effective population size, and μ is the per locus mutation rate; 2) The rate at which mutational differences accumulate as two species diverge (i.e. the substitution rate) is the same as the rate at which neutral mutations arise (μ) (Kimura, 1969); 3) The expected frequency of alleles in a sample is a function of the population and sample sizes (Ewens, 1972). Statistical tests designed to detect natural selection take advantage of the relative ease with which these predictions made by the neutral theory can be matched against empirical data.

Notice that the study of natural selection using genetic data is based upon tests of the null hypothesis of neutrality, rather than tests of natural selection. Neutral evolution is an analytically tractable model of evolution, which makes simple predictions about the frequencies of alleles and polymorphisms, the expected proportions of polymorphism, and divergence for different genes or classes of mutation (Kimura, 1983). Thus, the tests employed in the study of natural selection upon genetic variation are more adequately defined as tests of the null hypothesis of neutrality, or *neutrality tests*.

A key aspect of many tests of neutrality is that they in effect test a broader set of assumptions than whether or not genetic drift explains evolutionary change. Among the assumptions are that the population is panmictic, that it is not subdivided into smaller subpopulations, and that it has remained constant in size sufficiently long so that demographic events in the past no longer leave a signature on the genetic data (Bamshad and Wooding, 2003; Sabeti et al., 2006). These conditions characterize a population that is in *equilibrium*. For many neutrality tests the violation of equilibrium assumptions can result in the rejection of the null hypothesis, even in the absence of natural selection. Thus, neutrality tests in effect test the null hypothesis of *neutrality-equilibrium*.

Different types of selection

Different forms of selection shape genetic variation within and between species. *Positive selection* refers to the cases in which a novel DNA variant has a selective advantage over others, and consequently rises in frequency. *Negative* or *purifying selection* refers to the cases in which novel DNA variants have a selective disadvantage with respect to others, and tend to remain at low frequencies or be removed. *Balancing selection* refers to selective regimes that increase genetic variation within a species. Several different biological processes can increase genetic variation. In one case, selection can be *overdominant,* in which heterozygous individuals have a higher fitness compared to homozygotes. In a second case, selection can vary in space, with different environments inhabited by the species displaying distinct selective regimes, thus favoring different alleles. Finally, selection can vary over time, with different alleles being favored at different time intervals, as selective regimes change.

Natural selection is expected to directly affect the genetic variants that alter an individual's survival probability. However, the effects of selection need not be restricted to the causal variant associated with the selective differences among individuals. Consider, for example, those genetic variants present on the same chromosome upon which a favorable mutation has arisen (known as linked variants). As the favorable mutation rises to high frequency within a population, so will the linked variants, even if they have no selective effect. Thus, the consequences of natural selection extend beyond the immediate selected region of the genome, and tests of selection explore this effect in various ways, as will be seen below.

Tests of the neutral-equilibrium model

We have grouped tests developed to discern selection into five main categories (see Table 1 for summary), although other classifications are possible. 1) tests based on analyses of the frequencies at which polymorphisms occur in a series of DNA sequences collected from a population (e.g. Tajima's D, Fu and Li's F, Fay and Wu's H); 2) tests based on contrasts between DNA variation within and between species (the MacDonald-Kreitman test, and the HKA test); 3) tests based on the amount of variation and differentiation within and between populations (e.g. based on differences in F_{ST}); 4) tests based on differences between nonsynonymous and synonymous substitution rates; 5) tests based on the amount of linkage disequilibrium (LD) within a specific genomic region.

Tests based on the frequency of polymorphisms. Under neutrality-equilibrium, genetic variation accumulates in a population as a function of the population size and mutation rate. Neutral theory allows us to make predictions not only about the diversity expected in a sample (often measured by the heterozygosity), but also about the frequency spectrum of polymorphisms, referred to throughout this paper as the *site frequency spectrum* (SFS, Fig. 1). Different forms of selection will impact the frequency spectrum in distinct ways. If a mutation is favored by natural selection (i.e. positive selection) it will rise to high frequency in a population. This will result in an overall decrease in the genetic variation at the selected site, as well as at sites linked to it (Smith and Haigh, 1974). This process is known as a *selective sweep* (see Fig. 7). The mutational process will introduce new mutations after the selective sweep. These novel DNA variants will initially be present at low frequencies. Thus, shortly after a selective sweep, we expect to observe a large proportion of low-frequency variants in a sample. Purifying selection is also expected to result in an increase in the proportion of low-frequency variants. This can be understood as a consequence of the fact that novel mutations that enter the population generally remain at low frequencies, because their deleterious effects make it unlikely that they will reach high frequencies. In contrast, balancing selection will increase the proportion of variants at intermediate frequencies, since this selection regime favors the maintenance of variation of multiple alleles. Various quantitative approaches have been developed to interpret whether the frequency spectrum of a population sample reveals the action of one or another of these forms of selection.

The most widely used test that explores the frequency spectrum was proposed by Tajima (1989). This test is based on the comparison of two measures of the neutral parameter θ, which corresponds to $4N\mu$, the neutral population mutation rate. The parameter θ can be estimated

TABLE 1. A partial list of tests of the neutral-equilibrium model that are commonly used in making inferences regarding whether natural selection may have shaped the pattern and level of nucleotide variation observed within a set of DNA sequences

Tests of neutrality-equilibrium model	Premise of test	Type of DNA sequence data needed	Potential uses in studies of human adaptation	Reference
Tests based on the frequency of sites (SFS)	The frequencies at which nucleotide variants are found within a sample are a function of selection and demographic history. SFS tests examine whether the observed frequencies of variants depart from neutral expectations.			
Tajima's D		Within population polymorphism data	$D < 0$ indicative of positive selection or demographic expansion $D > 1$ indicative of balancing selection or population subdivision	Tajima (1989)
Fay And Wu's H		Same as above, and in addition DNA sequence data from an outgroup species	$H < 0$ indicative of positive selection	Fay and Wu (2000)
Tests that contrast different classes of mutation	These tests compare the amount of variation with a species (polymorphism) to divergence between species, at two classes of sites. If both sets of sites are evolving neutrally, they will have similar ratios of within and between species differences.			
McDonald Kreitman test	The classes of sites that are compared are synonymous and nonsynonymous sites	Both within population polymorphism data AND between species comparative data	Excess nonsynonymous divergence indicates adaptive evolution	McDonald and Kreitman (1991)
HKA test	The classes of sites that are compared are those from a candidate locus and those of a second, putatively neutral locus	Both within population polymorphism data AND between species comparative data for two or more loci	Excess divergence at candidate locus indicates adaptive evolution	Hudson et al. (1987)
Tests based on the amount of within and between population differentiation	Differentiation among populations is a function of both demographic history and natural selection. Selected sites display extreme F_{ST} values			
F_{ST}-based tests		Within population polymorphism data for two or more populations	Unusually high values of F_{ST} at genomic regions compared to usual values seen in the genome may indicate selection	Lewontin and Krakauer (1973)
Tests based on differences between non-synonymous and synonymous substitution rates	The ratio of nonsynonymous to synonymous substitutions differs depending on the selective regime			
dN/dS test		Between species comparative data AND/OR within population polymorphism data	Neutral expectation $dN/dS = 1$ Positive selection $dN/dS > 1$ Purifying selection $dN/dS < 1$	Hughes and Nei (1988)
Linkage Disequilibrium tests	Selection at a specific site may affect linked regions through hitch-hiking effects, typically resulting in decreased variation (in the case of positive selection)			
EHH test and others		Within population polymorphism data	Long haplotypes of low diversity indicative of recent (partial) selective sweep, due to positive selection	Sabeti et al. (2002)

A.

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T...C..T...C.C.T..C...T
T...C..T...C.C.T..C...A
A...C..T...C.C.T..C...A
A...A..T...C.C.T..G...A
A...A..G...C.C.T..G...A
A...A..G...T.A.C..G...A
2   3  2   1 1 1  3   1 ← minor allele count
4   3  4   5 5 5  3   5 ← major allele count
```

B.

Copies of minor variant	Frequency of sites with count
1	0.5
2	0.25
3	0.25

C. Graph of Site frequency spectrum

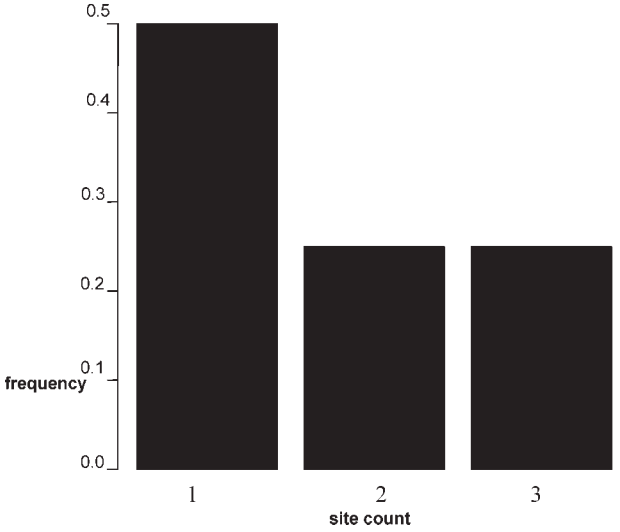

Fig. 1. An example of the site frequency spectrum. **A:** Six hypothetical sequences that are polymorphic at eight sites. Each polymorphic site is a single nucleotide polymorphism (SNP), and the dots represent nonvariable sites (identical in all sequences). Each SNP has two alleles, and the one occurring at a lower frequency is the minor allele. Below the sequences are the number of haplotypes that carry the minor and major allele. For example, at the first SNP there are 2 haplotypes carrying the T variant and 4 carrying an A, so the minor allele count is 2. **B:** The minor allele count of each SNP is counted and recorded, and summarized in the table. **C:** This information can also be presented as a histogram. In this example, most of the SNPs have a minor allele that is found in only a single DNA copy.

from a sample of DNA sequences by each of two measures. First, it can be estimated by the mean number of differences among DNA sequences in a sample (π). Second, it can be estimated based on the number of polymorphic sites (θ_S). Tajima proposed that a statistic (known as Tajima's D), corresponding to the standardized difference between these two measures of diversity, may be used to summarize information on the allele frequency spectrum:

$$D = \frac{\pi - \theta_s}{sd(\pi - \theta_s)}.$$

Under neutrality π and θ_S are expected to be identical to each other, so that the expected value for D is zero. However, when values of D are excessively high or low, we have evidence of deviation from the neutral model. To test whether Tajima's D is significantly different from values expected under the neutral theory, the distribution of the D statistic under the null hypothesis can be generated using simulations.

Interpreting the biological meaning of a significant deviation of Tajima's D from its neutral expectations is challenging, since various forms of selection (or demographic scenarios) are plausible explanations. As described previously, a selective sweep can result in a large proportion of low-frequency variants. This will result in a decrease in the genetic variation measured by the mean number of differences among sequences (π), since the low-frequency variants will contribute little to the mean difference among sequences. However, the measure of diversity based on the number of polymorphic sites (θ_s) will be comparatively higher than that based on the mean number of differences (π) because it is not sensitive to the frequency of polymorphisms. Under purifying selection (i.e. negative selection), most novel variants reduce the fitness of the individual carrying them, rarely rising to high frequencies. Thus, once again we have an excess of low-frequency variants, resulting in a Tajima's $D < 0$. Under balancing selection, on the other hand, selection favors the maintenance of different alleles in the population, resulting in a proportionally higher mean pairwise difference (π) compared to the measure of diversity based on the number of polymorphic sites (θ_s), and thus Tajima's $D > 0$.

Fay and Wu (2000) proposed a different test of the frequency spectrum that offers a solution to the challenge of distinguishing among different selective processes that result in negative values of Tajima's D (i.e. purifying selection or positive selection). These authors explored the fact that when positive selection takes place, it can drive other mutations, found at nearby locations on the chromosome, to high frequencies along with it. High frequencies will even be attained by mutations that are evolutionary derived (i.e. in the case of humans, those mutations that have arisen after our divergence from chimpanzees). Under neutrality, DNA variants attain high frequencies through the vagaries of genetic drift, but this process is expected to be slow, so that derived variants would rarely be found at high frequencies. Fay and Wu (2000) therefore proposed a test based on contrasting two diversity estimates; an estimate based on the nucleotide diversity (π), and a measure of diversity that is sensitive to high-frequency *derived* mutations (θ_H). The statistic that compares these two measures of diversity is known as Fay and Wu's H. Under neutral-equilibrium conditions, H has an expected value of zero. When a recent selective sweep has taken place there will be an excess of high-frequency derived mutations, resulting in a negative value for H (Fay and Wu, 2000). Thus, using the H statistic it becomes possible to distinguish directional selection (in which derived variants are found at high frequencies) from purifying selection (in which an excess of derived variants at high frequencies is not expected). The test based on the H statistic therefore complements the test proposed by Tajima (1989), by offering a method that can distinguish between positive or purifying selection.

Tests that contrast different classes of changes. Neutral theory makes explicit predictions about the polymorphism expected within a species, and the number of substitutions expected between it and another species (i.e. divergence). As noted earlier, both of these quantities are

expected to be proportional to the mutation rate. These predictions are applicable to all classes of mutations, regardless of whether they result in amino acid changes (nonsynonymous mutations) or do not (synonymous mutations).

McDonald and Kreitman (1991) proposed a test of neutrality based on a comparison of the proportion of synonymous to nonsynonymous change within and between species. Under neutrality, the proportion of these two types of change will be the same regardless of whether one examines polymorphism or divergence. Now consider the possible effects of selection. If positive selection is acting, we expect the data to be enriched for nonsynonymous mutations because these result in functional changes. This effect will be more pronounced for the divergence data, due to the fact that the time associated with the divergence between species is greater, and therefore selection has operated over a longer amount of time. Within species, on the other hand, mutations may persist transiently (due to genetic drift) even if they are not favored by selection, and so there will be proportionately fewer nonsynonymous changes, even when positive selection is ongoing. If negative selection (purifying selection) is operating, nonsynonymous mutations will be removed. Again, this effect will be more pronounced in the contrasts between species, since within species the deleterious mutations can transiently be present as polymorphisms, before being removed by selection. Thus, under purifying selection, there will be comparatively fewer nonsynonymous changes between than within species.

Whereas the McDonald–Kreitman test contrasts different sets of sites within a specific gene, the Hudson–Kreitman–Aguadé (HKA) test has extended the theoretical basis of this test to contrast polymorphism and divergence among multiple loci (Hudson et al., 1987). In the HKA test, the assumption is made that under neutrality the ratio of polymorphism to divergence will be the same for two or more genes that accumulate variation under neutrality. In a typical HKA test, a gene of interest is compared to a putatively neutral locus, and differences in the ratio of polymorphism to divergence between these is taken as evidence of selection in the gene of interest. For example, if a gene shows excess divergence with respect to a putatively neutral gene, a plausible interpretation is that positive selection has fixed favorable mutations at this gene along the lineage leading to this species, resulting in a ratio in which DNA polymorphism is low relative to divergence.

Tests based on the amount of differentiation among populations. Natural selection also alters the amount of differentiation between or among populations within a species. For example, consider the case of a positively selected variant that rises to a high frequency within a population. This process is expected to augment differentiation between the population carrying the selected variant and other populations (either because the variant did not arise in these populations, or was not favored by selection). Thus, if a locus shows greater differentiation among populations than expected under neutrality, this may be a consequence of positive selection.

The most commonly used statistical measure of population differentiation was devised by Wright (1951) and is known as the fixation index, or F_{ST}. A test of selection, based on the comparison of observed F_{ST} values with those expected under neutrality, was proposed by Lewontin and Krakauer (1973). The main difficulty of this approach is determining the distribution of F_{ST} values under neutrality. Differentiation among populations is sensitive to a variety of demographic factors (including the rate of drift within populations and the extent of gene flow among them), making it difficult to rule out demographic scenarios that could account for the observed F_{ST} values. Because of this difficulty, tests of neutrality based on population differentiation were largely abandoned. Recently, however, the abundance of genetic data available for various species, coupled with the development of novel analytical methods, has resulted in the development of new versions of the Lewontin–Krakauer test (Goldstein and Chicki, 2002; Beaumont, 2005). One approach is to take advantage of the extremely large numbers of genetic loci for which we have information of population differentiation to create an empirical genome-wide distribution. Thus, rather than statistically testing specific loci, we can use their position relative to this distribution to gain insights about their possible selective histories (see discussion of the Duffy locus later). Another approach is to use computer simulations under realistic demographic scenarios (inferred from multilocus studies) to obtain the distribution of F_{ST} values under neutrality (Beaumont, 2005).

Tests based on differences between nonsynonymous and synonymous substitution rates. While the preceding tests explore some properties of the expected variation within a species, or the contrast between variation within and between species, another class of tests is aimed at comparing sequence data from different species. These tests explore the fact that mutations can be nonsynonymous or synonymous, and that nonsynonymous mutations are much more likely to have an effect on fitness than are synonymous changes. The rates at which these two types of change occur can be expressed by the measures dN (the number of nonsynonymous substitutions per nonsynonymous site; also symbolized as K_a) and dS (the number of synonymous substitutions per synonymous site; also symbolized as K_s).

The ratio dN/dS is expected to have different values under distinct selective regimes. In the case of genes evolving in a strictly neutral manner, where all mutations have identical probabilities of persisting, we expect equal rates for both classes of substitution (i.e. $dN/dS = 1$). In the case of purifying selection, in which nonsynonymous mutations are more frequently removed by selection, most of the changes that persist are expected to be synonymous, resulting in $dN/dS < 1$. In the case of positive selection, nonsynonymous mutations are expected to have been maintained more frequently than those that are synonymous, given their ability to alter protein structure and function, resulting in $dN/dS > 1$. Therefore, a test of deviation from neutrality is the test of the null hypothesis of $dN/dS = 1$.

Various statistical tests of the null hypothesis of neutrality ($dN/dS = 1$) have been developed (Nei and Kumar, 2000). Within a maximum likelihood framework, it is possible to test whether two hypotheses are significantly different by comparing their likelihoods (Yang and Nielsen, 1998). Typically, a likelihood ratio test of dN/dS compares the log likelihood of the data under the null hypothesis ($dN/dS = 1$) to an alternative hypothesis (e.g. dN/dS can assume any value). Because the null hypothesis is a special case of the alternative hypothesis, the models are said to be nested, and twice the difference between the logarithms can be assumed to be chi-squared distributed, with

one degree of freedom. If the difference between the log likelihoods for these two models is significant, we can reject the null hypothesis and infer selection. For tests designed to detect positive selection, the alternative hypothesis is $dN/dS > 1$. This test can be implemented by using computer simulations: data are generated assuming $dN/dS = 1$, and likelihood values for the data are obtained under the null and the alternative models. In this way a null distribution of the test statistic is obtained, and the empirical value can be compared to it (Nielsen et al., 2005a).

Tests based on disequilibrium. Linkage disequilibrium (LD) coefficients measure the extent to which the variants present at different positions in a DNA sequence are correlated. When a novel mutation arises, it does so in the context of a genetic background, and we say it is in complete LD with those variants. Over time, the effects of recombination will shuffle the mutation to different backgrounds breaking down the original association, thus leading to a decay of LD. When a mutation is under positive selection, we assume it will rise in frequency quickly. If the rise in frequency of the favored mutation occurs comparatively faster than the rate of recombination, an extended region around the selected site (including all DNA variants that may be present within this region) will also rise in frequency, creating an extended region of LD.

Different tests have been proposed to asses the extent to which genetic variation in the region surrounding a putatively selected site is indicative of selection (e.g. Slatkin, 2001; Tishkoff et al., 2001). A simple approach is to quantify the extent of diversity in the haplotype bearing the putatively selected variant. Under the assumption that positive selection has taken place, we expect to find lower levels of diversity on the haplotypes carrying the putatively favored variant compared to other haplotypes that do not carry this variant. The nonselected haplotypes are expected to have comparatively more diversity at neighboring sites since recombination and mutation would have operated over larger amounts of time and would have broken down any original association (reducing LD on this haplotype). Recently, this approach has been more formally developed (Sabeti et al., 2002). A test, called the Long Range Haplotype (LRH) test, was designed to detect selection on a haplotype characterized by a set of variants, called the "core haplotype." A set of additional SNPs are surveyed at increasing distances from this core haplotype. The degree of LD between the core haplotype and the SNPs at various distances is measured using the *EHH* (extended haplotype homozygosity) statistic. *EHH* is the probability that two randomly chosen chromosomes sharing the same core haplotype are identical over the region extending from the core haplotype up until the SNP at a distance x (Sabeti et al., 2002). A haplotype that has been positively selected is expected to display high *EHH* values and high frequencies. Haplotypes that reach high frequencies due to genetic drift are likely to have taken longer to attain these frequencies, and so have experienced more recombination as well as mutation. Thus, they will present lower values of *EHH*.

The effect of demographic history on tests of neutrality

Various demographic scenarios can result in the rejection of the null hypothesis of neutrality-equilibrium. Population expansion, for example, can produce an increase in the proportion of low-frequency variants (mirroring the effect of a selective sweep; Simonsen et al., 1995; Nielsen, 2005). A population bottleneck, on the other hand, is expected to cause the preferential loss of low-frequency variants, and thus produce an excess of intermediate-frequency variants (mirroring the pattern seen under balancing selection; Simonsen et al., 1995; Nielsen, 2005). Thus, when tests based on the allele frequency spectrum are employed (e.g. Tajima's D and related methods), significant deviations from neutrality-equilibrium can be attributed to either selection or changes in population size. However, *failure* to reject the null hypothesis can also be the result of a particular demographic history. For example, a gene that is under balancing selection in a population that has recently expanded may show values for Tajima's D that are not significantly different from those of a population in neutrality-equilibrium, because these two processes tend to cancel each other out.

How can the effect of demographic history be accounted for in tests of neutrality? One approach is to carry out a test of neutrality that accounts for the demographic history of the population. For example, the null distribution of Tajima's D can be obtained under the assumption of population expansion, or under more specific (and more complex) demographic scenarios (such as bottlenecks followed by population expansion), rather than assuming that the population is in equilibrium (e.g. Bamshad and Wooding, 2003; Akey et al., 2004). The challenge with these approaches is that some prior information about the demographic scenarios is needed, in order to allow the simulations to be performed.

Another approach that can be used to distinguish between the effects of selection and demographic history is to perform contrasts among loci. For example, because we can assume that all loci in the genome share a common demographic history, those loci with extreme values for a test statistic (such as Tajima's D or F_{ST}), compared to a genome-wide distribution, are likely to be under selection. However, even this interpretation is sensitive to assumptions of demographic history. For example, Nielsen (2001) showed that for groups of populations with low levels of gene flow between them, the variance of values expected for Tajima's D under neutrality is far greater than that expected in a single random mating population. Thus, deciding whether the result for a locus represents an "extreme" value also requires making assumptions about the population's demographic history.

Two classes of tests are robust to demographic factors, implying that a significant result can be attributed to natural selection: those based on dN/dS ratios and the McDonald–Kreitman test (see earlier descriptions). These tests compare two classes of mutations (nonsynonymous and synonymous) within a single locus, and so the effects of demography are expected to be identical for both classes of mutations (Nielsen, 2001). Notice that this differs from the case of tests comparing different loci (e.g. the F_{ST}-based tests, or the HKA test). In these, although all loci share a single demographic history, the genealogical history of each locus is independent (due to segregation) and, therefore, there can be considerable variance among loci.

SIGNATURES OF SELECTION UNDERLYING HUMAN TRAITS

These analytical methods allow us to test the hypothesis that specific human traits have been under natural selection. In what follows, we review and discuss genetic findings with respect to four traits: lactase persistence, skin

color, bitter-taste sensation, and the Duffy blood group. We emphasize the ways in which these methods have been applied to DNA variation in genes associated with these traits, as well as the complexities in interpreting the results of tests of selection. In so far as building strong adaptive cases for traits requires the synthesis of multiple lines of evidence (Harrison, 1988), we have broadened our discussion to cover other critical components of the adaptive arguments. Thus, whenever possible, we include descriptions of the most current understanding of the genetic underpinnings and inheritance of these traits, discussions of functional studies aimed at revealing the link between putative adaptive DNA variants and their phenotypic expression, and in the case of the Duffy blood group, discussion of genetic evidence concerning the evolution of *Plasmodium vivax*, the pathogen proposed to be the selective agent on Duffy. While several of the cases we present have been well studied, it should become clear that many important questions about the role of natural selection upon these traits remain unanswered. We expect many other human traits will be the focus of new studies in the near future. It will be essential to conduct research at various levels, and anthropologists, geneticists, physiologists, and other researchers will play essential roles. Readers might usefully ponder: which pieces of the puzzle of human adaptation will each of these groups best be able to contribute?

LACTASE PERSISTENCE AND MILK CONSUMPTION

The ability to digest the milk sugar lactose depends upon the action of the enzyme lactase-phlorizin hydrolase (lactase). In most mammals, activity of lactase declines after the weaning phase and consequently the capacity to digest lactose is reduced. Decline in lactase production after weaning is also characteristic of most humans throughout the world who are described as being lactase nonpersistent (Swallow and Hollox, 2000), or sometimes as being lactose intolerant or malabsorbers (however, these terms are less accurate and are often misinterpreted as indicating pathology; see Wiley, 2004). In some individuals, however, lactase activity remains high after weaning allowing them to digest lactose into adulthood, a trait known as lactase persistence (or lactose tolerance). Lactase-persistent individuals can consume large quantities of fresh milk without complication, but persons with lactase nonpersistence usually experience some degree of adverse symptoms due to the increased action of intestinal-bacteria in breaking down lactose. These symptoms may include intestinal gas, bloating, severe abdominal pain, and diarrhea (Järvelä, 2005).

It has been known for some time that lactase persistence varies considerably in frequency among human populations (Durham, 1991 and references therein). Lactase persistence has a very high frequency among Northern Europeans and their descendents (>90% in some populations) but declines in frequency as one moves south and west. The trait is largely absent in East Asians, although it is found at intermediate frequencies in some Middle Eastern and North African populations. In most African populations, lactase persistence is at low frequencies. However, the trait is widely variable and shows a complex pattern of distribution. Pastoralists and other groups having milk-drinking cultures, such as the Fulbe (Cameroon), the Wolof (Senegal), the Tussi (Uganda, Congo, Rwanda), and Hima (East Africa) (see Table 5.1 in Durham, 1991 for a more complete list), typically have relatively higher frequencies of lactase persistence than nonpastoralist groups (Simoons, 1978; Durham, 1991; Mulcare et al., 2004).

The unusual geographic distribution of lactase persistence, as well as its association with the cultural habit of consuming milk, has led to the proposal of several selective hypotheses. The first hypothesis explains that milk-drinkers gained a *nutritional benefit* (Simoons, 1969, 1970, 1978; McCracken, 1971; see Holden and Mace, 1997). Thus, individuals that were lactase persistent had a selective advantage over those who were not because lactase persistent individuals were able to hydrolyze and absorb the carbohydrate lactose. Importantly, the advantage was proposed to be greatest in situations or under conditions in which individuals were under nutritional stress and milk became a critical food (Simoons, 1969). A second hypothesis proposed that the most important benefit of drinking milk was not nutritional but was due to the *water and electrolyte content in milk.* This hypothesis is linked to Cook's (1978) proposal that lactase persistence originated in the Arabian Peninsula where the benefit is explained in the cultural context of drinking camel milk on long desert journeys. A third hypothesis proposed that lactase persistence was advantageous because it *improved calcium absorption* (Flatz and Rotthauwe, 1971). Thus, individuals who were lactase persistent were able to drink larger quantities of milk thereby improving their calcium absorption. The advantage is greater in persons living in regions where there is low incidence of ultraviolet light (e.g. northern latitudes), since synthesis of vitamin D (necessary for calcium absorption) is reduced in these areas.

It is outside the scope of this paper to evaluate the support for these various hypotheses (however see Durham, 1991; Holden and Mace, 1997; Mace et al., 2003 for discussions). It is, however, relevant to point out that a recent study by Beja-Pereira et al. (2003) examined nonsynonymous base changes in milk-protein genes of European cattle and found evidence of increased allelic diversity in breeds found in North Central Europe. The increased diversity appears to be specific to milk-protein genes since other genetic systems including mtDNA, microsatellite markers, and protein polymorphisms do not show increased diversity in North Central Europe. Although such evidence does not allow us to distinguish between different hypotheses explaining the benefits of consuming milk, Beja-Pereira et al. (2003) do infer that early Neolithic dairy farmers in North Central Europe were selecting cattle for altered milk protein composition and increased milk yield. Furthermore, the evidence suggests gene-culture coevolution, since the milk-protein diversity is highest in cattle breeds from the same areas of Northern Europe where the allele for lactase persistence is at greatest frequencies.

Genetic basis and inheritance pattern

The genetic basis of lactase persistence is today well supported by evidence (Swallow, 2003). However, until as recently as the early 1970s, it was believed by some researchers to be a substrate influenced trait—that a continued presence of lactose in the gut after weaning could stimulate the activity of lactase. Lactase persistence is now known to have a genetic basis and to be inherited as an autosomal dominant trait. Activity levels of lactase in adults show a trimodal distribution pattern that suggests

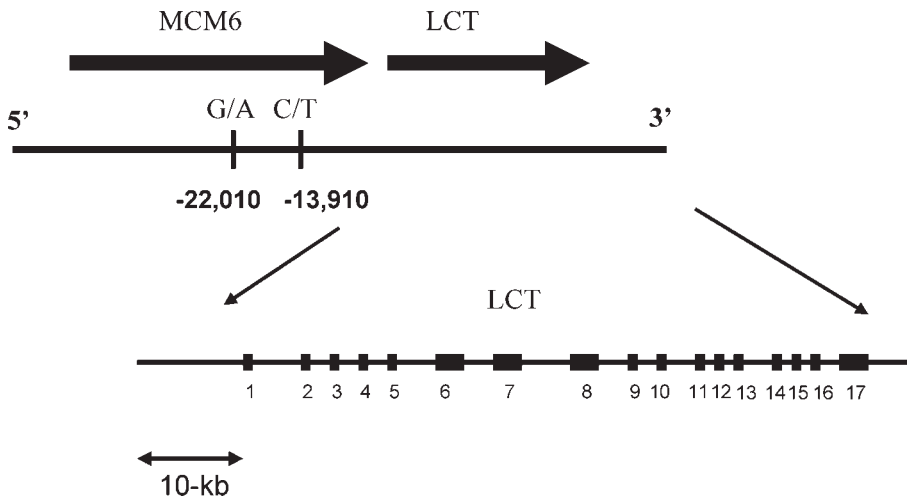

Fig. 2. Schematic representation of the genomic region comprising the lactase gene (*LCT*), and the neighboring minichromosome maintenance type 6 gene (*MCM*). The lactase gene in the close view shows the 17 exons (small exons are not to scale). The derived **T** variant at position −13,910 is the putative causal variant of the lactase persistence trait. The derived **A** variant at position −22,010 is more loosely associated with lactase persistence. Figure adapted from Coelho et al. (2005) and Harvey et al. (1998).

three genotypes: homozygotes for the persistence allele (*LCT***P*/*LCT***P*) having lactase persistence, homozygote recessives (*LCT***R*/*LCT***R*) having lactase nonpersistence, and heterozygotes (*LCT***P*/*LCT***R*) having lactase persistence but with intermediate levels of enzyme activity.

The lactase enzyme is coded by a single gene ~50 kb in length that has 17 exons (Boll et al., 1991). The lactase gene encodes an mRNA transcript 6,274 bp in length and a preprotein of 1,927 amino acids (Swallow, 2003). In 1993, the gene for the lactase enzyme was localized to the chromosomal position 2q21 (Harvey et al., 1993). Expression of *LCT* is restricted to the absorptive cells of the small intestine, and is maximally expressed in the middle part of the jejunum (Swallow, 2003).

The search for the causal mutation of lactase persistence

Within the *LCT* gene, a series of polymorphic nucleotide sites (DNA variants) have been identified by screening in European and European-derived samples (Boll et al., 1991; Lloyd et al., 1992; and Harvey et al., 1995). Within these populations, only three common haplotypes were identified (A, B, and C). In order to study variation worldwide, DNA variants were surveyed in a 60 (kb) region encompassing the gene across eleven Old World populations (Hollox et al., 2001). Four common DNA haplotypes were found, named haplotypes A, B, C, and U (Hollox et al., 2001). In northern Europe, the A haplotype was found at high frequencies (0.86), contrasting markedly with its frequency in southern Europe (0.36) and India (~0.43) where lactase persistence is also less common. In these populations, haplotypes B and C are at much higher frequencies than they are in northern Europe. In Asian and African populations, in which lactase persistence is also rare, haplotype A is found at reduced frequencies (Malay, 0.49; Chinese, 0.47; Japanese 0.37), with particularly low values among Africans (Bantu, 0.10; San; 0.06).

Evidence on several levels points to a strong association between haplotype A and lactase persistence (Harvey et al., 1998). As outlined above, the first piece of evidence is the geographical association between the haplotype and the trait. A second piece of evidence is that individuals determined to be lactase persistent via laboratory assay are found to bear haplotype A, either as homozygotes or heterozygotes (Swallow, 2003). Third, highly expressed mRNA transcripts in the small intestine of lactase persistent individuals were genotyped and found mainly to be haplotype A (Harvey et al., 1998).

Using a combination of linkage disequilibrium studies in families, and association studies (comparing the frequency of nucleotide variants between lactase persistent and nonpersistent individuals), it was proposed that a T variant at position −13910 (with respect to the *LCT* gene; see Fig. 2) might be the causal factor for lactase persistence (Enattah et al., 2002; Poulter et al., 2003). The variant appears to have evolved from an ancestral C nucleotide on an A haplotype background. The A haplotype has been shown to display extensive LD (up to 1 Mb in length) that extends beyond the *LCT* gene (Poulter et al., 2003). With respect to this finding, it is interesting to note that the putative causal variant is not located within the *LCT* gene itself but is located 13,910 nucleotides upstream of the *LCT* initiation codon within intron 13 of the *MCM6* gene (minichromosome maintenance-6 gene; Enattah et al., 2002).

While the −13910*T variant has been found to be in complete association with lactase persistence in several European and European-derived US populations (Enattah et al., 2002), it is absent or extremely rare in most sub-Saharan African populations, including those possessing dairying cultures and with relatively high frequencies of the lactase persistence trait (e.g. Ibibio and Oron, Nigeria; Chewi and Ngoni, Malawi; Wolof, Senegal; Dinka and Nuer, South Sudan; Neur and Anuak (Anywak), Ethiopia) (Mulcare et al., 2004). The only exception was the detection of the −13910*T variant in several populations from Cameroon, West Africa, most notably the Fulbe and Hausa. However, the presence of the variant here may be the result of gene flow from populations outside Africa. Independent molecular markers (e.g. haplogroup IX of the Y-chromosome), that have an origin in Asia, are also found in Cameroon at considerable frequencies, and may indicate back-migrations from Asia to Africa over 4,000 years ago (Cruciani et al., 2002; Mulcare et al., 2004).

The finding that the −13910*T variant is rare or absent in sub-Saharan populations that display high frequencies of the lactase persistence trait has been interpreted in two ways. First, it is possible that the causal variant or variants of lactase persistence has not yet been found. That is, despite the fact that the −13910*T variant is in nearly complete association with lactase persistence in northern Europeans, it is possible that it is not the causal mutation in Northern Europeans. The −13910*T mutation falls

within an extended region of LD (Poulter et al., 2003), making it possible that an undetected variant located within this region, and linked to the 13910*T variant, could be the cause of lactase persistence (Poulter et al., 2003; Bersaglieri et al., 2004). However, recent evidence has favored the view that −13910*T is indeed the causal variant in Northern Europeans. Studies *in vitro* demonstrate that the −13910*T variant functions as a *cis*-element that considerably upregulates the promoter region of the *LCT* gene (with respect to the −13910*C variant) and consequently increases gene transcription (Olds and Sibley, 2003; Troelsen et al., 2003; Lewinsky et al., 2005). Therefore, it is increasingly likely that lactase persistence in sub-Saharan pastoralist groups is caused by a different yet unknown DNA variant within or near the *LCT* gene. If this is confirmed in future studies, then lactase persistence would appear to have evolved independently in European and African populations. Since studies of variation at *LCT* in global population samples (including Africans) have screened for only variants originally discovered in European samples, no new variants in these populations have been detected. Thus, sequencing *LCT* in diverse African samples will be necessary in order to identify new variants, and to determine a possibly distinct causal mutation in Africans.

The pattern of DNA variation at *LCT*: evidence for natural selection

Two main studies have surveyed nucleotide variation in the lactase gene (*LCT*) in diverse human populations (Hollox et al., 2001; Bersaglieri et al., 2004). The first study genotyped a set of SNPs in over 1,000 individuals derived from 13 different world populations (Hollox et al., 2001). The second study genotyped a larger set of SNPs within a larger genomic region surrounding and encompassing the *LCT* gene in 63 different world populations (Bersaglieri et al., 2004). The general pattern of nucleotide variability at *LCT* appears to have been influenced by both historical demographical forces as well as natural selection. For example, when heterozygosity (i.e. the proportional contribution of different haplotypes to a population) was measured at *LCT*, African populations showed considerably greater heterozygosity (0.91, San; 0.87, Bantu) compared to most non-African populations (0.26, northern Europe; 0.75 southern Europe; 0.65, northern India; 0.78, Japanese) (Hollox et al., 2001). This general pattern, higher African diversity compared to non-African diversity, is consistent with the pattern of geographic diversity observed in the majority of genes that have been studied to date (Tishkoff and Verelli, 2003; Tishkoff and Kidd, 2004). The pattern is thought to have been produced by these population's different demographic histories; that is, the relatively older age and larger effective size of African populations compared with non-African populations, and the hypothesis that Eurasian populations experienced bottlenecks as they migrated from Africa (Tishkoff and Verelli, 2003). Among non-African populations, however, Northern Europeans are unique in some respects. They display an exceptionally low level of heterozygosity (0.26), a value that is almost threefold less than values for other non-African populations (Hollox et al., 2001). This marked reduction in heterozygosity in North Europeans is evidently due to the fact that a single haplotype (haplotype A) dominates.

In what follows, we discuss two main aspects of *LCT* polymorphism data that indicate the locus has been strongly affected by natural selection. First, DNA haplotypes discovered at *LCT*, as well as the putative causal DNA variant (−13910*T), show marked differences in their frequencies between different world populations. This pattern leads to very high values of genetic measures of population differentiation (F_{ST} and P_{excess}) at the *LCT* locus, and is a strong indication of local adaptation. Second, variability surrounding the *LCT* gene differs markedly depending on whether or not the gene carries the −13910*T mutation.

Differentiation among populations. The putative causal DNA variant shows marked differences in frequency between populations: this variant was detected in 77% of European Americans, 14% of African Americans, and 0% of East Asians. These frequencies are broadly similar to the frequencies of the lactase persistence phenotype in these populations. The F_{ST} metric of population differentiation is 0.53 for the −13910*T variant, an unusually high value that exceeds 99.9% of F_{ST} values estimated for a genome-wide set of over 28,000 SNPs (Bersaglieri et al., 2004).

To investigate if a putative selective event left a footprint in haplotypic variation, 99 DNA variant sites were genotyped in a large (3.2 Mb) region directly flanking the *LCT* locus (Bersaglieri et al., 2004). DNA variation surrounding a putatively selected variant can be informative about the nature of selection. When a beneficial mutation undergoes strong positive selection it increases very rapidly in frequency. At the onset of selection the beneficial DNA mutation is linked to other DNA variants found on the same haplotype. These linked variants will also rise in frequency along with the beneficial variant but to an extent determined by the recombination process that acts to break down the association between the beneficial mutation and these variants. When selection is very strong, the rate of increase of the beneficial mutation will markedly outpace the rate of recombination and the selected variant will be found in one or few haplotypes, resulting in strong linkage between the beneficial mutation and linked DNA variants (see *Tests based on linkage disequilibrium* in *Theory and Analytical Methods* section). Thus, if the beneficial mutation is found at high frequency in a population that experienced selection, but at low frequency in other populations, which were not selected, linked variants will also show signs of increased population differentiation. Consistent with this expectation, there was an excess of relatively high F_{ST} values for the 99 flanking DNA sites on either side of *LCT* (see Fig. 3A). However, while the F_{ST} values were highest ($\sim$0.53) in the regions nearest the *LCT* locus, F_{ST} values varied considerably over the entire 3.2 Mb region. For example, between two different DNA variants, each having high values of F_{ST} (0.53), three variants were found that had much lower F_{ST} values (0.07, 0.26, 0.21). Furthermore, very low F_{ST} values ($\leq$0.01) were found for $\sim$16 DNA variants near the *LCT* gene, indicating (at least for these variants) little support for population differentiation. However, considerable differences in the F_{ST} values at flanking sites may be expected since the F_{ST} metric is substantially affected by the frequency of the polymorphism before the onset of selection. For example, variants on the selected haplotype that were at high frequency before the onset of selection in all populations will not accumulate as much differentiation between the selected and nonselected populations as will variants originally at low frequencies (since the latter can accrue large frequency differences

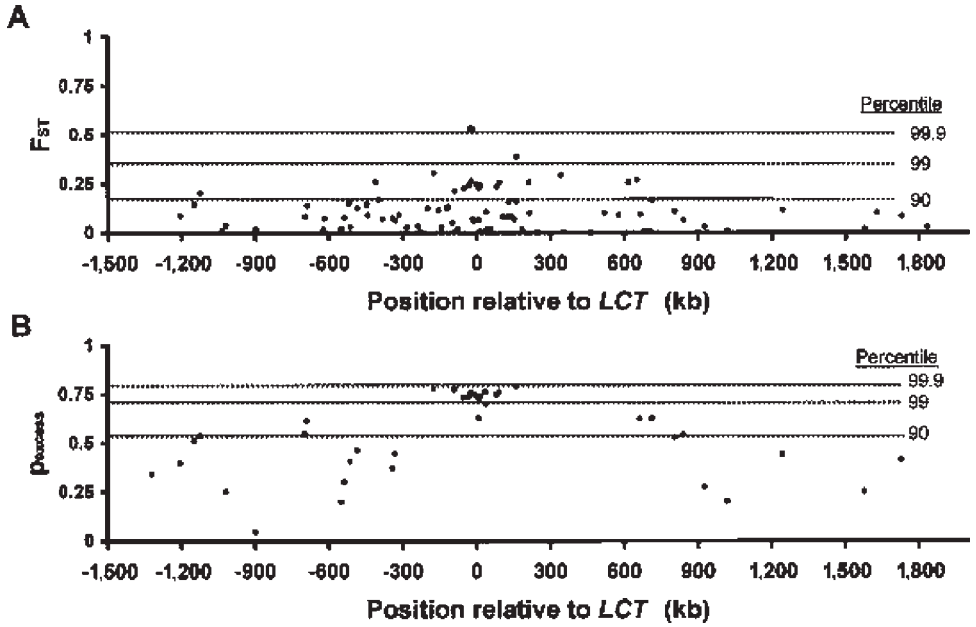

Fig. 3. This figure shows the elevation in (**A**) F_{ST} and (**B**) P_{excess} for multiple SNPs in a 3.2 Mb region surrounding the lactase gene (*LCT*). On the x-axis, the position indicated by 0 indicates the start of transcription of the *LCT* gene. For both F_{ST} and P_{excess}, the 90th, 99th, and 99.9th percentiles obtained for 28,440 and 13,696 SNPs from throughout the genome (respectively) are indicated by dashed lines. (Figure from Bersaglieri et al., 2004 and used by Permission from publisher).

between selected and nonselected populations) (Bersaglieri et al., 2004). Therefore, measuring F_{ST} values at flanking sites has limited power in detecting selection since it is expected that only a subset of DNA variants within a selected haplotype (as seen at *LCT*) will have high F_{ST} values.

To get around this problem, Bersaglieri et al. (2004) quantified interpopulation differentiation using a different metric, known as P_{excess}, which accounts for the original frequency of each variable site on the putatively selected haplotype. The frequencies of variants prior to selection were assumed to be equivalent to their average frequency in all East Asian and African populations sampled (i.e. the populations that apparently did not experience selection). In effect, P_{excess} measures the rise in a variants frequency relative to its original value. When a haplotype is strongly selected, P_{excess} values of flanking DNA variants are expected to be relatively constant over long distances and close to the frequency of the selected haplotype (i.e. haplotype A, 0.77). Determining P_{excess} values for flanking sites has the potential to provide more powerful and reliable evidence of selection than F_{ST} values at flanking sites. Results for *LCT* indicated that P_{excess} values were high and close to the frequency of the selected site (0.77) for a cluster of DNA variants within a region spanning 500 kb around *LCT* (see Fig. 3B; Bersaglieri et al., 2004). Furthermore, P_{excess} was consistently high across all the 99 DNA variants and remained high for about 1.5 Mb surrounding the *LCT* locus. In order to compare the P_{excess} values at *LCT* with average values of this metric genome-wide, P_{excess} was determined for SNPs within 952 different genomic regions. None of these regions were found to have P_{excess} values approaching those for the *LCT* gene, and within each genomic region, P_{excess} values between variants (even those separated by relatively small distances of $\sim$100 kb) showed minimal correlation. This result indicates that the region around the *LCT* gene is very unusual with respect to the rest of the genome, and strongly suggests that a selective sweep occurred in Northern Europe. Under this scenario, the beneficial mutation in the *LCT* gene is hypothesized to have increased in frequency so rapidly that many linked DNA variants in a broad swath around the variant were also dragged up in frequency with it, a process known as *genetic hitch-hiking* (Smith and Haigh, 1974).

The extent of linkage disequilibrium. A second test of selection applied to *LCT* is known as the Long Range Haplotype (LRH) test, described by Sabeti et al (2002) (see *Tests based on linkage disequilibrium* in *Theory and Analytical Methods* section). The test first identifies relatively short haplotypes at a specific locus, called "core haplotypes," that show little or no evidence of recombination. At the *LCT* locus, the core region contained the site at which the putative causal mutation for lactase persistence occurs (−13910C/T) as well as a nearby site at which a slightly less strongly associated mutation occurs (−22018G/A). The method then examines increasingly distant nucleotide variants to determine the decay of LD from each core haplotype. This is measured by determining the probability, at a given distance from the core haplotype, that two randomly chosen chromosomes carrying the same core haplotype are homozygous at all nucleotide variants for the entire interval from the core region to that particular position (Sabeti et al., 2002). For each core haplotype in the population, the relative extended haplotype homozygosity (REHH) is determined. REHH is a comparison of the extended haplotype homozygosity of one haplotype against the extended haplotype homozygosities of other haplotypes in the same sample. A high REHH (>1.0) indicates that a haplotype displays increased homozygosity at greater distances compared with other haplotypes. It also indicates that the haplotype is relatively recent otherwise recombination would have broken down the haplotype with time. For the haplotype containing the putative lactase-persistence DNA variant, REHH was determined to be 13.2, indicating that this haplotype displayed homozygosity over much longer distances (>800 kb) compared with lactase nonpersistent haplotypes (see Fig. 4). Evidence for positive selection is obtained when the haplotype with a high REHH is so common in a population—as it is for haplotype A with a frequency of 0.77—that it could not have risen to such a high frequency without the aid of selection.

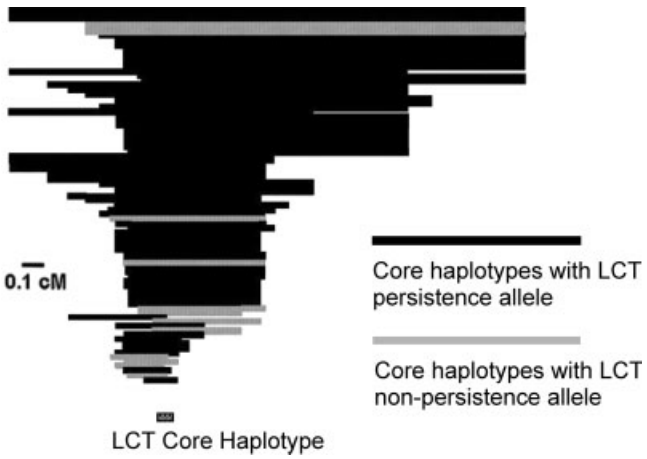

Fig. 4. Long-range extended homozygosity for the core haplotype containing the persistence-associated alleles at *LCT* at various distances from *LCT*. The extent to which the common core haplotypes remains intact is shown for each chromosome in cM. The core region containing the −13910C/T is shown as a stippled black bar, and the *LCT* gene is oriented from left to right. Core haplotypes containing the persistence-associated allele (−13910T) are shown in black, and those containing the nonpersistence allele (−13910C) are shown in gray. Haplotypes are from European-derived US pedigrees. All chromosomes with core haplotypes having a frequency greater than or equal to 5% in this population are depicted. (Adapted from Bersaglieri et al., 2004 and used by permission from publisher).

The statistical significance of the REHH value for the *LCT* lactase persistence haplotype was determined in several ways. First, the value for the persistence haplotype was compared with values of REHH obtained for data generated by 10,000 coalescent simulations, and *P*-values were obtained for the excess of homozygosity observed on the *LCT* persistence haplotype (for more details see Sabeti et al., 2002). Virtually none of the simulated data had REHH values approximating that seen at *LCT* (P-value $<$ 0.0004; see Bersaglieri et al., 2004). Second, the REHH value at *LCT* was compared to genotype data collected from 12 regions in the genome spanning 500 kb each. The REHH values found for these regions (similar to the values found for the simulated data), also did not approximate the value at *LCT*, again indicating that the extended homozygosity at *LCT* is very unusual. In another examination of the effect of positive selection at *LCT*, the REHH value for the lactase persistent haplotype can be compared to values obtained for other genes known to be under selection. Thus, the REHH value at *LCT* (13.2) is found to be relatively much greater than the REHH value determined for the *G6PD* haplotype (REHH = 7.0; see Sabeti et al., 2002) hypothesized to provide resistance to malaria (Tishkoff and Verrelli, 2003).

The time frame of selection at *LCT*

Bersaglieri et al. (2004) suggested that the onset of positive selection on lactase persistence occurred in Europeans subsequent to their differentiation from Asians and Africans. This is inferred from two facts. The first is that the frequency of the putative causal DNA variant (−13910*T) varies widely among different European populations, southern Europeans showing much reduced frequencies compared to northern Europeans and French Basques. The second fact is that this variant is rare or absent in almost all non-European populations (except Algerians and Pakistanis). It should be noted, however, that while positive selection on *LCT* may have occurred relatively recently, the DNA variant associated with lactase persistence may in fact be much older. In fact, the presence of the −13910*T variant in several East Asian populations (e.g. the Daur, China, 0.05; Mongola, China, 0.10; Yakut, Siberia, 0.06; see Bersaglieri et al., 2004), even though at low frequencies, seems to indicate that it predates the differentiation of Europeans and Asians.

The age of onset of selection of the lactase-persistence haplotype was estimated based on the decay of LD in either direction from the *LCT* core region (Bersaglieri et al., 2004). The rationale behind this method lies in the fact that intragenic recombination occurs as a function of time (known as the recombination rate) and will operate to break down long haplotypes with time. Therefore, if we know the recombination rate, as well as the degree to which recombination has taken place, then it is possible to estimate the time that has elapsed since the long *LCT* haplotype was formed. This time is presumed to indicate the age of the onset of selection. Analyses were performed on two different populations: a European-derived (US pedigree) population, and a Scandinavian population. Analyses indicated that the persistence haplotype began to rise in frequency between 2,188 and 20,650 years ago (in the European-derived population) and, more recently, between 1,625 and 3,188 years ago (in the Scandinavian population) (Bersaglieri et al., 2004). A recent analysis used microsatellite diversity within the haplotypes to estimate the onset of selection (Coelho et al., 2005) and found broadly similar dates (i.e. between 7,000 and 12,000 years). Thus, all estimated dates point to selection on lactase persistence in Northern Europe as being very recent. These dates are consistent with the estimated origin of the domestication of cattle (*Bos taurus*) around 9,000–11,000 years ago (Durham, 1991). Furthermore, recent archeological studies analyzing residue on pottery indicate that dairying was widespread in Britain in the Neolithic around 8,000 years ago (Copley et al., 2003, 2005).

The fact that the lactase persistence associated haplotype has achieved such high frequencies in Northern Europe over such a short time indicates that the strength of selection favoring lactase persistence must have been considerable. Based on the estimated time since the initial rise of the haplotype, Bersaglieri et al. (2004) estimated a coefficient of selection (a metric of the selective advantage to the individual) between 0.014 and 0.15 for the European-derived population, and between 0.09 and 0.19 for the Scandinavian population. In other words, individuals who are lactase-persistent are estimated to have had a 1.4–15.0% advantage over nonpersistent individuals in the European-derived population, and a 9.0–19.0% advantage in the Scandinavian population. Note that the selective advantage estimates in Scandinavians range to higher values compared with those of the European-derived population indicating that selection may have been stronger in this population, consistent with the more recent estimates of the onset of selection. Previous estimates of coefficients of selection for lactase persistence, based purely on theoretical considerations, were 1.5 and 3.0% (Bodmer and Cavalli-Sforza, 1976) or 10% (Feldman and Cavalli-Sforza, 1989). The recent estimates (Bersaglieri et al., 2004) indicate that selection for lactase persistence may have been stronger than had previously been thought. In fact, selection for lactase persistence may have been as strong as (or even stronger than) selec-

tion for the sickle-cell gene or for *G6PD* deficiency in malaria-endemic regions where coefficients of selection have been estimated at 5.0–18.0% (sickle-cell) and 2.0–5.0% (*G6PD*) (Bersaglieri et al., 2004).

The origin of lactase persistence

Questions about the origin of lactase persistence must consider whether the distribution of the trait worldwide is due to a single origin, or whether the trait has evolved independently in different populations. In most sub-Saharan populations screened by Mulcare et al. (2004), including populations that exhibit lactase persistence as a phenotypic polymorphism, the proposed causal variant of lactase persistence (i.e. −13910*T) is either absent or so rare that it cannot explain the observed frequency of the lactase persistence trait in these populations. (As noted above, however, the Hausa and Fulbe of Cameroon represent exceptions since the frequency of the −13910*T variant is relatively high in these populations though is believed to be due to gene flow from outside Africa; see Mulcare et al., 2004.) Thus, the rare occurrence, or complete absence of the −13910*T DNA variant in sub-Saharan African populations with lactase persistence seems to indicate that the origins and genetic basis of the trait in sub-Saharan African pastoralist populations is different from that in Europeans. It is possible that lactase persistence evolved independently in these two geographic regions, an explanation that Coelho et al. (2005) recently argued on theoretical grounds to be most plausible. As noted above, a more comprehensive survey of nucleotide diversity in the *LCT* gene of sub-Saharan pastoralist groups will help to answer more definitively the question of possible independent evolution of lactase persistence. Such surveys will help determine the extent to which the −13910*T variant is actually absent in African pastoralist populations. We know that this DNA variant is found at considerable frequencies in the Fulbe and Hausa of Cameroon; could it also be present in other sub-Saharan populations? Furthermore, since most studies have merely genotyped known variants in world populations, more intensive discovery of new variants in Europeans and in sub-Saharan pastoralist populations (through full-sequencing efforts) will help to clarify whether lactase persistence is indeed due to different causal DNA variants in these two geographic populations.

For some time it has been known that lactase persistence is relatively high within certain populations in the Middle East and in North Africa (Cook, 1978; Durham, 1991; Holden and Mace, 1997; Swallow, 2003). Recent genotyping studies in these populations found the −13910*T variant to be at relatively high frequencies. Frequencies in the Middle East are known for Pakistani populations (~0.30) and Bedouin from Israel (0.21) and in North Africa for the Mzab, Algeria (0.22), Amizmiz (0.14) and Tamazighy speakers from the Middle Atlas Mountans, Morocco (0.16) (Bersaglieri et al., 2004, Myles et al., 2005). As in Europeans, the frequency of −13910*T was found to strongly predict the frequency of lactase persistence in these populations (Myles et al., 2005). Based on these results, Myles et al. (2005) suggested that the practice of dairying in North Africa, and the associated presence of the lactase persistence variant −13910*T, are due to past migrations from the Middle East (Myles et al., 2005). Evidence corroborating the hypothesis of a Middle Eastern origin of North African *LCT* derives from several independent sources: 1) recent Y chromosome data indicating demic diffusion into North Africa from Middle Eastern pastoralists (Arredi et al., 2004); 2) linguistic evidence indicating that a proto-Berber language was carried to North Africa by migrating pastoralist from the Nile Valley around 7,000 BC (Blench, 2001); 3) archeological and faunal evidence indicating that early domestication in North Africa centered on sheep and goats introduced from the Middle East; and finally, 4) that the transition to pastoralism in coastal North Africa was sudden, and did not appear to undergo local development over time (Myles et al., 2005).

The geographic origin of the −13910*T mutation is unknown. However, as noted above, the mutation appears likely to have originated in a non-African population after its differentiation from Africans, and prior to the differentiation of European and Asian populations. Recently a specific locale of origin for the mutation has been proposed, though this needs further investigation. Enattah (2005) hypothesized that its origin was in populations in the Ural Mountains (Kaiser, 2004; Enattah, 2005). The study surveyed haplotypic diversity of *LCT* and the distribution of the −13910*T variant in a larger array of populations from the Ural Mountains, Caucasus and West Asia than had been previously surveyed (Kaiser, 2004; Enattah, 2005). From a reconstruction of haplotype evolution, two key haplotypes were identified that were inferred to temporally bracket the origin of the −13910*T mutation. Since these two haplotypes were found at highest frequency in populations in the Ural Mountains, it was concluded that "this geographical region is the most likely focal origin of the most common lactase persistence allele" (Enattah, 2005; 77). It was proposed that demic diffusion later brought the major lactase persistent haplotype westwards towards Europe and southward towards Western Asia and the Middle East.

HUMAN VARIATION IN SKIN COLOR

The geographic distribution of human skin color shows a clear geographic pattern: populations inhabiting areas close to the equator in general have dark skin, whereas populations in higher latitudes have lighter skin (reviewed in Jablonski and Chaplin, 2000). In addition, skin color presents an amount of differentiation among world regions that far exceeds that observed when other genetic variation is surveyed; that is, although only 10% of the total genetic variation within humans lies between major geographic groups (sometimes referred to as "races"), 88% of the differences in skin color are found among world regions (Relethford, 2002).

Evolutionary interpretations of human skin color attempt to explain the transition in skin color from nonhuman primates, as well as the observed geographic diversity in human skin color, by placing them in the context of known physiological consequences associated with differences in skin color. However, an understanding of the genetic basis of skin color and the evolutionary processes that account for change have been hampered by the relative complexity of the phenotype and the multiplicity of environmental factors that can explain variation. Nevertheless, over the last decade there have been advances in our understanding of skin color in several important areas. These include improved understanding of the genetic basis of skin color as well as specific DNA variation underlying phenotypic variation. These developments have enabled analyses aimed at evaluating models of the evolution of skin color variation, including models of natural selection and adaptation.

Adaptive hypotheses for skin color evolution

The difference in skin color between humans and other primates requires that we explain the transition between light skin covered with hair (the common nonhuman primate condition), to a condition of dark skin and hairlessness (under the assumption that dark skin, common in African populations, is the ancestral state for humans). One of the hypotheses advanced to explain this transition is that increased encephalization required efficient cooling mechanisms, since the brain is a heat sensitive organ, and the cooling of core body temperatures would therefore have been evolutionary favored (Falk, 1990). The loss of body hair and an increase in the number of sweat glands could thus have occurred by natural selection, resulting in a hairless condition. Hairlessness, in turn, would have favored the origin of increased pigmentation of the skin, in order to protect it from the effects of ultra-violet radiation. These damaging effects include increased propensity to developing skin cancers and degradation of folate, an important nutrient in human development sensitive to ultra-violet radiation. Given an adaptive explanation for the transition from light to dark skin, two additional questions must be addressed: What explains the subsequent shift to light skin, observed in human populations that migrated to higher latitudes? Why did the dark skin phenotype remain common in Africa, and rare elsewhere? Various adaptive explanations have been proposed to account for these patterns. One hypothesis invokes the importance of vitamin D biosynthesis to human metabolism, especially due to its importance in calcium absorption (Loomis, 1967). Because UV radiation is necessary for the skin to synthesize vitamin D, darkly pigmented populations inhabiting higher latitudes, where UV incidence is lower, would have a decreased ability to synthesize vitamin D and thus absorb calcium. The effects of vitamin D deficiency have a broad range of consequences, including rickets, osteomalacia, and osteoporosis. Thus, in the regions with lower incidence of sunlight, lighter skin would allow the penetration of UV and vitamin D synthesis could take place at normal levels (Loomis, 1967).

Several evolutionary models have proposed explanations for the persistence of populations with dark skin in regions of greater exposure to sunlight. Loomis (1967) argued that vitamin D excess could have toxic consequences, and thus dark skin would offer protection from this effect. However, as summarized by Jablonski and Chaplin (2000), this argument has been disproved by clinical studies. Another hypothesis invokes the protective role that melanin can play in the face of UV-induced damage (e.g. skin cancers and the degradation of nutrients). Although skin cancers have been argued to be of minor importance because these are usually of late onset (Blum, 1961), others have argued that selection could be effective because of the contributions parents and grandparents can make to their offspring's survival (Diamond, 2005). Robins (1991) has argued that sunburns could have been significant selective pressures on Pleistocene hominids for any number of reasons—by reducing foraging efficiency, reducing infant survival, or decreasing effective thermoregulation by transpiration due to sunburn damage to sweat glands. On the other hand, nutrient photolysis is a process that may severely reduce an individual's survival at a young age and consequently their reproductive fitness. Of particular importance is folate, which has been documented to play a key role in the development of the human nervous system, and has been demonstrated to be sensitive to UV radiation (Jablonski, 1999).

A completely distinct hypothesis for the evolution of pigmentation was proposed by Darwin (1871) in *The Descent of Man*. He proposed that sexual selection was the dominant evolutionary force explaining diversity in skin color. In Darwin's view, the skin color of individuals of a population was a trait that was selected for because of a sexual preference. Diamond (1992) has made a similar argument suggesting that skin color and other traits specific to populations likely originally arose through "founder effects," in which the traits were common in the original progenitors of a particular geographical population, and later became enhanced through sexual selection. While citing the importance of sexual selection, Diamond (1992) and others (Aoki, 2002) also acknowledge the role of natural selection due to climate (i.e. UV damage), particularly in selecting for darker skin in equatorial populations of the Old World.

Although selective hypotheses have been in the literature for several decades, it was only recently that the ideal data needed to address them became available. Jablonski and Chaplin (2000) used extensive data on UV incidence in various regions of the world, and explored the correlation between this variable and data collected on skin color (as measured by reflectance of light at varying wavelengths). Skin reflectance was found to correlate strongly with UV radiation levels, as well as latitude. The authors found that a pattern of clinal gradation in skin color seen among indigenous populations can be explained by variation in UV radiation levels. They concluded that variation in skin color represents a compromise between two conflicting physiological requirements: photoprotection and vitamin D synthesis (Jablonski and Chaplin, 2000).

Genetic basis and associated phenotypes

It is only recently that the genetic basis of skin color variation has become better understood. Before the development of direct molecular approaches, the genetics of skin color was addressed using classical genetic tools. These involved the analysis of phenotypic variances in skin color found in admixed and parental populations, which were treated as "parental," "F1" and "backcross" generations and were used to obtain rough estimates of the number of genes of equal effect that would be needed to explain the amount of observed phenotypic variation. Using this approach, Cavalli-Sforza and Bodmer (1971) and Stern (1970) estimated that as few as four genes could underlie skin color (but see Byard and Lees, 1981 for a criticism of the method used by Stern, 1970).

Two decades later, the localization and characterization of a gene directly involved in human pigmentation, the melanocyte stimulating hormone receptor (*MC1R*) was achieved. The cloning of *MC1R* allowed it to be characterized as a seven-pass transmembrane receptor that triggers increases in intracellular cAMP (Mountjoy et al., 1992). In humans the *MC1R* locus is localized to the chromosomal position 16q24 and until recently it was assumed to consist of a single exon. However, a study by Tan et al. (1999) found that an alternative splice variant at the 3′ end of the gene can result in the production of a second exon, but its function is as yet unknown.

An understanding of the relationship between this gene and normal genetic variation in human populations was aided by studies in mice that showed that loss-of-function mutations at the *MC1R* locus result in a change from

black/brown to yellow pigmentation (Robbins et al., 1993). It was established that *MC1R* can be thought of as a switch that determines the relative proportion of pigment that a melanocyte will produce: if the receptor is activated, the dark form of melanin, eumelanin, will be produced. Alternatively, if the receptor is not activated or becomes nonfunctional, more pheomelanin is produced and results in yellow or red pigmentation of hair and skin.

The earlier findings that *MC1R* explains differences between coat color and skin color in a variety of animals opened the door for studies in humans, including population-level surveys aimed at understanding whether variation at this locus explains differences in skin and hair color among human individuals.

Association between *MC1R* and phenotypic variation in humans

In the early 1990s a series of studies of European individuals revealed a strong association between red hair and several loss-of-function mutations in the *MC1R* gene, and heterozygous individuals for these mutations were usually found to have intermediate phenotypes (Valverde et al., 1995). Few individuals carrying these mutations had dark hair or skin, a finding that supported the hypothesis that normal *MC1R* function is necessary for eumelanin production and that loss of function at the *MC1R* gene leads to pheomelanin production. Associations were also found between specific *MC1R* mutations and different forms of skin cancer, including melanoma and nonmelanoma types (Smith et al., 1998). This latter association can be explained as a consequence of the previous finding, since eumelanin offers more protection from UV radiation than does pheomelanin.

These findings pointed, for the first time, to a single locus that explains a substantial portion of variation in human skin color. Having established this association, the next step was to investigate the extent to which *MC1R* variation explains differences in skin color among diverse human populations.

MC1R variation in human populations

To date, the studies that have addressed polymorphism at *MC1R* in human populations from Europe, Africa, and Asia, have all obtained largely concordant results. African populations show relatively little nucleotide variation, while Europeans and Asians show substantially greater amounts of variation (Rana et al., 1999; Harding et al., 2000; John et al., 2003). Harding et al. (2000) found nucleotide diversities (π) of 0.12% and 0.09% among Europeans and Asians, respectively, and only 0.07% among the African samples.

African and non-African populations were also found to be strikingly different in the types of nucleotide polymorphisms they contained. Although most of the polymorphisms found in non-African populations result from nonsynonymous substitutions, polymorphisms found in African populations were almost exclusively due to synonymous changes. In the surveys of variation among African populations by Harding et al. (2000) and John et al. (2003), a total of eight synonymous variants were detected as compared to only three nonsynonymous variants. A strikingly different pattern was observed in Europe where only two synonymous variants were detected as compared to 10 nonsynonymous variants (Harding et al., 2000). Subsequent studies in Europeans have detected additional variants, raising the number of nonsynonymous variants to more than 30 (Makova and Norton, 2005).

Interestingly, the three nonsynonymous variants in African populations were found when populations from South Africa were surveyed (John et al., 2003), whereas none had been found in surveys of populations from central and western Africa (Rana et al., 1999; Harding et al., 2000). This calls attention to the importance of sampling regional variation within Africa, and raises the possibility that selective pressures may be different among regions in this continent.

The human polymorphism data detected in the *MC1R* gene, as well as the difference detected in relative proportions of nonsynonymous and synonymous changes between Africans and non-Africans, have enabled detailed studies aimed at evaluating models of natural selection of skin color evolution. These studies will be discussed next.

Selective hypothesis for the pattern of *MC1R* polymorphism

The pattern of variation at *MC1R* is unusual when compared to that observed at other genetic loci. At the majority of loci, Africans show greater nucleotide variation (π) than Europeans and Asians (Cavalli-Sforza et al., 1994; Tishkoff and Kidd, 2004). Lower variation in Africans, as well as a marked difference in the proportion of synonymous and nonsynonymous polymorphism between these populations, cannot be accounted for by the demographic scenarios invoked to explain the genetic diversity at other loci in these geographic regions. Therefore, two distinct hypotheses have been proposed to account for the results obtained at the *MC1R* locus, both invoking a role for natural selection.

In both of these hypotheses it is proposed that natural selection among Africans is of a purifying nature, removing mutations that result in loss-of-function alleles (Rana et al., 1999; Harding et al., 2000). This mode of selection would result from the decreased fitness of individuals with fair skin (including those who carry any possible loss-of-function mutations) in an environment where ambient sunlight is very intense and could result in damage to tissues or nutrient loss. However, the hypotheses differ in their explanations for the differences in genetic variation observed between Africans and Eurasians. According to the first hypothesis, due to Harding et al. (2000), markedly higher variation in Europeans and Asians would result from a *relaxation of the selective constraint* that operates in Africa; that is, Europeans with loss-of-function mutations would not experience any fitness reduction, because they inhabit an environment where exposure to UV is lower. Thus, populations inhabiting higher latitudes would accumulate variation in the *MC1R* at a neutral rate, and there would be no selection against nonsynonymous substitutions (explaining their abundance in these populations).

The second hypothesis, proposed by Rana et al. (1999), similarly assumes that natural selection among Africans is purifying, but differs in its interpretation of why variation is observed in Europeans. According to these researchers, the loss-of-function mutations, which are associated today with red hair and fair skin in Europeans, also resulted in lighter skin phenotypes when they occurred in individuals originally having dark skin. The selective advantage derived from having lighter skin at high latitudes is explained by an increased ability to synthesize vitamin D, which would have otherwise taken

place at only low rates in individuals with dark skin in regions of reduced UV exposure. Thus, according to this second hypothesis, natural selection favoring the maintenance of loss-of-function mutations (a form of diversifying selection) operates in European populations, explaining the comparatively higher level of polymorphism observed in this region (Rana et al. 1999).

Given that one of these hypotheses proposes that polymorphism in non-Africans accumulates in a neutral manner, whereas the other proposes that polymorphism in these populations is enhanced by diversifying natural selection, tests of the neutrality-equilibrium model can be used to distinguish between these two hypotheses.

Tests of a neutral-equilibrium model applied to *MC1R*

Several different classes of tests have been applied to the *MC1R* data. Their results and interpretations are discussed below.

McDonald–Kreitman test. Under the assumption of neutrality, the ratios of synonymous to nonsynonymous mutations both within species (polymorphism) and between species (divergence) are expected to be similar (McDonald and Kreitman, 1991). When the chimpanzee and the human consensus sequences are compared, there are 10 nonsynonymous substitutions, and 6 synonymous substitutions. In an initial study of polymorphism within Africans, Harding et al. (2000) found 4 synonymous and 0 nonsynonymous polymorphisms. These proportions were found to be significantly different, rejecting the hypothesis of neutrality. The observed pattern can be explained by a process of purifying selection, which removes deleterious nonsynonymous substitutions in Africans. These results were confirmed in a larger sample of Africans by John et al. (2003) who observed 3 nonsynonymous and 8 synonymous polymorphisms. In contrast, when the same analysis is applied to the non-African populations, which carry 10 nonsynonymous and 3 synonymous polymorphisms, no significant difference was observed, thus failing to reject the hypothesis of neutrality. The results of this test are therefore concordant with a model in which purifying natural selection removes nonsynonymous mutations in Africans but allows them to accumulate in a neutral manner in non-Africans.

HKA test. This test compares the ratios of polymorphism to divergence at two or more loci (Hudson et al., 1987). For the African populations, the putatively neutral sites at *MC1R* are the synonymous sites (i.e. those that, if mutated, would not alter the amino acid sequence of the protein). Among non-Africans, under the assumption of relaxed selective constraint, all sites within the *MC1R* coding region were assumed to be neutral and were included in the analysis. The goal is to test whether the patterns of polymorphism and divergence for these two data sets is significantly different from that of a putatively neutral locus sequenced for a similar sample of populations. Harding et al. (2000) used the synonymous variation at the β-globin locus as a reference for neutral variation. The goodness-of-fit tests revealed no significant differences between β-globin variation and either *MC1R* synonymous site variation in Africans or overall variation in non-Africans. These results are therefore inconsistent with a hypothesis of diversifying selection in non-Africans as proposed by Rana et al. (1999), but are consistent with the hypothesis that *MC1R* evolved neutrally in non-Africans (Harding et al., 2000), under a scenario of relaxation of constraint. The results are also consistent with the hypothesis of a selective constraint within Africa, according to which only a subset of synonymous sites are free to evolve neutrally (Harding et al., 2000).

Tests based on the frequency spectrum of mutations. The results from the MacDonald-Kreitman and HKA tests just described, compare polymorphism and divergence data, and may therefore be more sensitive to selection that has occurred over larger timescales. On the other hand, tests based on the frequency spectrum of haplotypes (Ewens-Watterson test) and mutations (Tajima's *D*) use information on variation only within populations, and thus may be more sensitive to recent selection (see *Theory and Analytical Methods* section). When analyzed using Tajima's *D* statistic, *MC1R* showed predominantly negative values for populations in Africa, Asia, and Europe, but all results were nonsignificant (Harding et al., 2000). Similarly, among 23 sampled populations, the Ewens-Watterson test was only significant for a sample of Irish individuals (Harding et al., 2000). Taken in their totality, these results suggested that selection has not played a role in shaping variation in non-African populations, and that "the atypically high ratio of nonsynonymous to silent polymorphism in Europe and Asia reflects relaxed functional constraint, not positive selection" (Harding et al., 2000).

However, a subsequent study by John et al. (2003) of San Bushmen from South Africa obtained significantly negative Tajima's *D*, and a joint analysis of three available African data sets also obtained negative Tajima's *D* and significant Fu and Li's *F* and *F** statistics (also based on the spectrum of mutations; see Fu and Li 1993). The significant values for these tests in African populations indicate an excess of low-frequency variants, and can be interpreted in different ways. One possibility is that the demographic history of these populations explains the results. For example, it is known that either rapid population growth, or the analysis of an admixed sample of individuals (i.e. from many ethnicities, as was the case in the analysis of individuals from many regions within Africa), can both result in a data set with an excess of low-frequency variants (Ptak and Przeworski, 2002). Alternatively, purifying natural selection could explain these results, with most variants being maintained at low frequencies due to selection against deleterious mutations. This interpretation would imply that although selective constraint exists in African populations, it is not complete; that is, some variants can persist in the population, albeit at low frequencies.

The lack of deviation from neutral-equilibrium expectations when European and Asian populations were tested can also be interpreted in different ways. The populations may have experienced a relaxation of selective constraints, resulting in a neutral pattern of variation. Alternatively, these populations may be under diversifying natural selection, but the power of the tests may be insufficient to reject the null hypothesis of neutrality. In addition, it is possible that population expansion in non-Africans has resulted in a deviation from neutral expectations that masks the effects of diversifying natural selection. The challenge of distinguishing among selective and demographic hypotheses at *MC1R* will require further studies, including joint analyses of different loci, which will allow demographic and selective scenarios to be reliably distinguished (Goldstein and Chikhi, 2002).

Alternative interpretations of tests of neutrality at *MC1R*

The strikingly low levels of variation in African populations, coupled with the results of the McDonald–Kreitman test described above, favor the scenario proposed by Harding et al. (2000), according to which this locus was under selective constraint in Africa, and these constraints became relaxed in Europe and Asia. An alternative interpretation, however, was proposed by Rogers et al. (2004). They explained that the evolutionary transition from light skin covered with hair (characteristic of nonhuman primates) to dark skin in early humans may have involved adaptive evolution at the *MC1R* locus. Evidence for this argument comes from the fact that the ratio of nonsynonymous to synonymous substitutions at *MC1R* between humans and chimpanzees is unusually high (0.63) when compared to the genomic background for primates (0.21; see Yang and Nielsen, 1998), indicating that change at this locus was driven by selection in the divergence between these two species. The comparison of human and chimpanzee sequences does not allow us to distinguish whether changes occurred specifically on the human lineage, on the chimpanzee lineage, or on both of these lineages. However, under the assumption that changes occurred on the human lineage, a biological explanation for the selective advantage was proposed by Rogers et al. (2004). Increased pigmentation would provide protection from sunlight in an environment where hominids would be increasingly exposed to sunlight (as a consequence of their loss of hair, and living in a savanna environment).

If evolution of *MC1R* was adaptive in the lineage leading to humans, then this changes the interpretation of the MacDonald-Kreitman test. The similarity between Eurasian polymorphism and human-chimpanzee divergence found in the MK test could reflect adaptive evolution at *both* between species and within species levels (i.e. between humans and chimpanzees and within Eurasians), rather than neutrality (Rogers et al., 2004). This possibility serves as a reminder that the results of the MacDonald-Kreitman test may allow alternative interpretations. That is, similar ratios of nonsynonymous to synonymous variation for both polymorphism data and divergence data could indicate neutral evolution at both these levels or, alternatively, it could indicate selection occurring at both these levels. This indicates that it may be premature to conclude that variation in Eurasians occurred neutrally, and we should not reject the possibility of diversifying selection at *MC1R* among Europeans and Asians.

Conclusions on selection at *MC1R*

In conclusion, the population analyses of *MC1R* variation allow us to minimally state the following. African and non-African populations have strikingly different patterns of genetic variation. There is little doubt that African populations experience purifying selection, but the pattern of variation among non-Africans can be accounted for by either of two scenarios: relaxed selective constraint or diversifying selection. In order to evaluate the importance of these alternatives, future studies will need to collect data from more populations and will need to carry out joint analyses of *MC1R* variation with that of additional loci, which can serve as controls for the demographic history of populations and help separate the relative effects of population history and natural selection.

Other genes involved in skin color variation

This discussion has focused largely on the role of *MC1R* in explaining the genetic basis of variation in human skin color. This emphasis owes itself to the fact that, until recently, this was the only gene implicated in normal variation in human and hair color (Rees, 2004). However, several observations indicate that skin color is a multigenetic trait. These include the following: the finding that twins that share identical *MC1R* genotypes differ considerably in skin color, the observation that among Eurasians many individuals of light skin color carry the same alleles as those of African individuals (Rana et al., 1999; Harding et al., 2000), and the finding that human skin color variation is most certainly a result not only of the relative proportion of pheomelanin and eumelanin but also of the total amount of pigment produced, and its distribution in the skin (Barsh, 2003; Rees, 2004). Interestingly, skin color shows more within-population diversity in sub-Saharan Africa than in other regions of the world (Relethford, 2000), a result apparently at odds with the reduced variation of *MC1R* among Africans. This may be interpreted as a consequence of our poor knowledge of the complete repertoire of genes influencing pigmentation.

Knowledge of the biochemical pathways that play a role in human pigmentation has allowed novel candidate genes to be investigated, and promising results have been obtained. Graf et al. (2005) investigated the role of variation in the Membrane Associated Transporter Protein (*MATP*) in human pigmentation. They found a significant association between specific nucleotide variants detected in Caucasians and phenotypic variation in hair and skin color within this population. Lamason et al. (2005) investigated the *SLC24A5* gene, which was selected as a candidate because it was found to play an important role in determining the number, size, and density of melanosomes of the zebrafish (i.e. the melanin containing vesicles within melanocytes). Within human populations, an extremely high population differentiation was found at a specific polymorphic site within this locus (that corresponds to amino acid 111 in the third exon of the gene). A derived variant coding for threonine is virtually fixed in Europeans, whereas among Africans, East-Asians, and Indigenous American populations the ancestral variant coding for alanine is at high frequencies, yielding an extremely high difference in allele frequency between populations (Lamason et al., 2005). In addition, variation among Europeans was extremely low, suggesting that this locus may have experienced a selective sweep. Finally, Lamason et al. (2005) were able to show that in admixed European African populations the variant at this site explains between 25 and 38% of the variation in pigmentation.

The result obtained for the *SLC24A5* gene is illustrative of the fact that different genes underlying a single phenotypic trait can be under different modes of selection. Diversifying selection (or alternatively, neutral evolution after relaxation from a functional constraint) characterizes *MC1R* evolution, while the *SLC24A5* gene appears to be evolving under positive selection in European populations. Recent surveys of SNP polymorphism employing the extended haplotype homozygosity approach have uncovered additional loci involved in pigmentation as candidates for selection, emphasizing the multigenic basis of variation in pigmentation. For example, a genome scan by Voight et al. (2006; see subsection *Haplotypic diversity-based approaches* in the section *Scanning the genome for*

genes shaped by natural selection) found clear evidence for positive selection in Europeans not only at *SLC24A5* but also at four additional loci involved in pigmentation: *OCA2, MYO5A, DTNBP1*, and *TYRP1*. Another recent study (Izagirre et al., 2006), also employing the EHH approach, found evidence for selection at the *LYST, TP53BP1*, and *RAD50* loci, which are involved in various aspects of photoprotection against UV damage. Interestingly, these three loci were found to be positively selected specifically in the African population samples (Izagirre et al., 2006), indicating that recent adaptive evolution in the repertoire of genes involved in pigmentation may indeed have taken place in African populations. These recent findings indicate that in the near future we can expect the study of natural selection on human skin color to grow more complex, and to incorporate analyses - both genetic and functional - of a wider array of genetic loci.

THE SENSATION OF BITTER-TASTE

Discrimination of taste has long been of interest to geneticists and anthropologists as it mediates our proximate decisions about which foods we eat and, evolutionarily, it undoubtedly has been important in shaping the dietary and ecological specializations of different human groups (Hladik and Simmen, 1996; Molnar, 2002). Humans are generally regarded as having five categories of taste perception including sweet, sour, bitter, salty and unami (the ability to taste glutamate) (Drayna, 2005). Of these, the sense of bitter-taste has probably received the most attention (at least by anthropologists), particularly with respect to the trait for tasting phenylthiocarbamide (PTC). This trait was discovered serendipitously in the early 1930s, when Fox found that some people find the substance intensely bitter while others do not (Fox, 1932). Studies by Harris and Kalmus (1949) and many others since then (see Guo and Reed, 2001) have extensively documented the frequencies of taster and nontaster individuals within populations.

PTC is itself not a component of any known human foods, but it shares a chemical moiety (—N=C=S) with many plant foods that elicit the same taste response as does PTC (Molnar, 2002; Meyerhof et al., 2005). These compounds are found in the Cruciferae family (including turnips, cabbage, broccoli, mustard greens and others) as well as in cassava. Based on observations of similar proportions of taster versus nontaster phenotypes in chimpanzees and other great apes, as in humans, the British geneticist R.A. Fisher and colleagues (Fisher et al., 1939) were the first to hypothesize balancing selection in the evolution of PTC tasting:

> Without the conditions of stable equilibrium it is scarcely conceivable that the gene ratio should have remained the same over the million or more generations that have elapsed since the separation of the anthropoid and hominid stocks. The remarkable inference follows that over this period the heterozygotes for this apparently valueless character have enjoyed a selective advantage over both the homozygotes, and this, both in the lineage of the evolving chimpanzees and that in evolving man. Wherein the selective advantages lie, it would at present be useless to conjecture, but of the existence of a stably balanced and enduring dimorphism determined by this gene there can be no room for doubt. (p. 750)

Although Fisher and coresearchers did not speculate on the selective mechanism that maintained the two phenotypes in balance, a form of selection favoring heterozygote individuals could offer an explanation. With respect to such an explanation, it is interesting to note that several recent studies have hypothesized that the allele for the so-called "non-taster" phenotype is in fact functional, and codes for a receptor that binds a bitter compound not yet discovered (Wooding et al., 2004; Drayna, 2005; Kim and Drayna, 2005). One could thus argue that heterozygous individuals might indeed have a phenotype capable of tasting a broader range of compounds, thus explaining the maintenance of the polymorphism.

In general, an explanation of a selective advantage for bitter-taste sensation is that it helps individuals avoid ingesting toxic substances. Many bitter substances in plants are known to be toxic (Meyerhof et al., 2005), a presumed adaptation by the plant to prevent its consumption. The interaction is therefore an example of plant–herbivore co-evolution. A more specific selective hypothesis has been proposed for PTC discrimination and dates from the 1940s (Mackenzie and Mackenzie, 1943; Harris and Kalmus, 1949). It was discovered that there is a correlation between thyroid disease and the PTC nontaster phenotype. The causal explanation is that PTC interferes with the normal uptake of iodine by the thyroid gland and in turn reduces thyroid function, resulting in lowered metabolism in children and lowered fertility in adults (Jackson, 1993). This particular explanation is common in genetic and anthropological texts (Cavalli-Sforza and Bodmer, 1971; Cavalli-Sforza et al., 1994; Molnar, 2002) and proposes that the taster for PTC gains a selective advantage by being able to avoid the ingestion of plants that contain compounds that interfere with thyroid function.

Although the ability to *avoid* bitter compounds is usually believed to be an advantageous trait, in certain environmental circumstances it is conceivable that ingesting bitter substances is advantageous. Jackson (1990, 1996), for example, proposed that individuals living in regions where malaria is prevalent might gain a selective advantage by ingesting plants containing cyanide compounds because these compounds, at subacute levels, would reduce the severity of *Plasmodium falciparum* infection (Nagel et al., 1980). To our knowledge this intriguing hypothesis has never been rigorously studied.

Today, the genetics of bitter-taste sensation is much better known. We now know that besides the specific gene associated with PTC-tasting, there exists an entire repertoire of bitter-taste genes that encode receptors on the tongue for detecting bitter substances (Drayna, 2005). Our improved understanding of the molecular genetics of bitter-taste has permitted recent analyses that compare DNA sequences of these genes between and within species. In what follows, we discuss analyses surveying the repertoire of bitter-taste genes (Wang et al., 2004; Kim et al., 2005), as well as analyses that target two specific bitter taste genes, the *PTC* gene (also known as *TAS2R38*) and the *TAS2R16* gene (Wooding et al., 2004; Soranzo et al., 2005).

Genetic localization and inheritance pattern

Humans possess 33 bitter taste genes (called *TAS2R*s) of which 25 are presumed to be functional genes, and 8 are presumably pseudogenes. The majority of bitter taste genes are found on either of two chromosomes (12 or 7) with a single gene on chromosome 5 (Shi et al., 2003; Kim et al., 2004). The genes consist of short intronless sequences (approximately 1,000 base pairs in length) coding for G protein-coupled receptors believed to be expressed on

the taste-buds of the tongue (Kim et al., 2004; Drayna, 2005). The receptors are typically comprised of seven transmembrane domains, with extracellular regions thought to bind tastants (Shi et al., 2003; Drayna, 2005).

The coding structure of human *TAS2R*s, as well as their similarity to the bitter taste genes in mice (which are better studied), has led researchers to believe they are involved in bitter-taste sensation in humans. For only a subset of human *TAS2R* genes do we have evidence of a specific compound, or group of compounds, that stimulates the gene's receptor. Generally, therefore, uncertainty exists concerning the extent to which bitter taste genes are expressed on the tongue, and which compounds bind and activate specific receptors. The exceptions include *TAS2R38*, *TAS2R16*, *TAS2R10*, and *TAS2R14*, for which specific agonist chemicals have been identified through studies performed in vitro (Kim et al., 2004; Meyerhof et al., 2005). The first two genes (*TAS2R38* and *TAS2R16*) code for taste-receptors that bind the ligands PTC and β-glucopyranocides, respectively (Bufe et al., 2002, 2005; Drayna, 2005;). β-glucopyranocides are a class of chemicals widespread in nature that are found in plants and some insects, and that can have cyanogenic effects (Drewnowski and Gomez-Carneros, 2000). *TAS2R10* has been shown to bind the bitter compound strychnine, and *TAS2R14* binds a more diverse array of bitter chemicals including naphthalaldehydic acid, picrotin, picrotoxinin, sodium benzoate and others (Meyerhof et al., 2005).

With respect to inheritance, we have the best information for PTC phenotypes. PTC tasting has long been thought to conform to a simple Mendelian mode of inheritance with the nontaster phenotype due to an autosomal recessive genotype. Indeed, the discovery of the *PTC* gene (Kim et al., 2003) showed that it accounts for about 75% of the total phenotypic variance for the trait. However, relatively frequent reports of non-Mendelian inheritance (i.e. two nontaster parents having offspring with taster status) as well as inconsistent linkage results have led researchers to believe the trait might also be influenced by other genes (Drayna, 2005).

Evidence for selection on the repertoire of bitter-taste genes

Recently, two studies have analyzed DNA polymorphism in the entire repertoire of bitter-taste genes in geographically diverse samples (Wang et al., 2004; Kim et al., 2005). The studies are similar in their sampling strategy and analytical methods, though they have drawn contrasting conclusions regarding the role of natural selection in the evolution of human bitter-taste genes.

The argument by Kim et al. (2005) is based on three main ways in which the pattern of diversity at human bitter-taste genes contrasts with the average patterns reported over many human genes. First, the mean F_{ST} value for bitter-taste genes (0.22) is significantly higher than the value estimated in a recent genome-wide study (0.123; see Akey et al., 2002). In addition, of 25 genes, 18 had F_{ST} values >0.12, and 7 had very high values (ranging between 0.26 and 0.80). This indicates that human populations differ from each other more markedly at bitter-taste genes than at the majority of genes in the genome (Kim et al., 2005). Second, the mean dN/dS ratio between human alleles for the bitter-taste genes in humans (0.94) is greater than an average over 152 human genes ($dN/dS = 0.11$; Nekrutenko et al., 2002). Third, the mean level of nucleotide diversity for bitter-taste genes ($\pi = 0.11\%$) was significantly higher than values from genome-wide studies ($\pi = 0.075\%$, see Sachidanandam et al., 2001; and $\pi = 0.056$, see Schneider et al., 2003). A high proportion of nonsynonymous polymorphisms (as indicated by the high dN/dS ratio), as well as overall high nucleotide diversity, was proposed by Kim et al., (2005) to indicate that *TAS2R* alleles differ in their functional properties.

Wang et al. (2004) also found a high mean dN/dS ratio between human alleles. However, differently from Kim et al. (2005), they attribute the excess of amino acid polymorphism to reduced functional constraints. They believe, in contrast to both Wooding et al. (2004) and Kim et al. (2005), that many bitter-taste genes have lost their function, and are therefore free to accumulate amino acid changes. In support of their interpretation, Wang et al. (2004) point to evidence indicating an apparent increase in the number of bitter-taste pseudogenes in the human lineage compared to the chimpanzee lineage, a process referred to as "pseudogenization."

An important challenge is how to interpret high dN/dS ratios, since these can result from either a relaxation of selective constraints, or the effects of directional selection. To distinguish between these alternatives, Wang et al. (2004) reasoned that whereas relaxation of functional constraints would increase the dN/dS throughout entire genes, positive selection would target specific functional domains of genes in which amino acid changes can lead to functional changes. The authors therefore carried out an analysis of the dN/dS ratios for three different functional domains of each gene—the intracellular, transmembrane, and extracellular domains. Between mouse and rat, the dN/dS ratios for all three regions were significantly less than one, indicating purifying selection. In humans, the ratios for all three regions were considerably elevated compared to the ratios for the mouse–human comparison, pointing to relaxed constraint on human bitter-taste genes overall. In a functional gene, the three domains are expected to vary in their dN/dS ratios because certain domains play more important functional roles than others, and are therefore expected to be under more intense purifying selection. In the mouse-rat comparison, different domains displayed significantly different dN/dS ratios. In contrast, in humans different domains were not significantly different. For this reason, as well as the fact that the extracellular domains of human bitter-taste genes (the presumed tastant-binding domains) do not show significantly higher dN/dS ratios compared to other regions, Wang et al. (2004) argued that relaxation of selective constraints drives the increase in dN/dS ratios in humans.

Using a different approach, with an emphasis on differences among primate species, Fischer et al. (2005) also addressed the question of whether a high average dN/dS ratio in human bitter-taste genes indicates positive selection or relaxed constraint. Their results stood in contrast to Wang's et al. (2004) findings in two respects and yielded evidence supporting a role for positive selection. First, while Wang et al. (2004) found no heterogeneity of dN/dS ratios among functional domains in an analysis of human polymorphism, Fischer et al. (2005) found higher dN/dS ratios in the extracellular domain in their contrasts among primate species. Second, Fischer et al. (2005) found no significant differences between the number of pseudogenes between apes and humans, and no evidence indicating a higher rate of loss of bitter-taste genes in humans.

The different interpretations described above lead to two rather different scenarios regarding the importance of bitter-taste receptors in human evolution and population

differentiation. On the one hand, Kim et al. (2005) propose that positive natural selection has been a dynamic force in adapting taste receptors in local populations to specific bitter compounds encountered in particular geographic regions. The source of bitter compounds is suggested to be plant toxins, and the ability to recognize these compounds is hypothesized to be adaptive. In contrast, Wang et al. (2004) posit that loss of function in bitter-taste receptors along the human lineage has resulted in a decrease in the number of bitter compounds we can taste. They correlate the reduction in taste sensitivity to a dietary shift in human evolution in which plant foods became less relied upon, and eating meat and cooking became more important. Selection for bitter-taste detection, they argue, became less important because of the reduced toxins contained in meat and cooked foods.

Balancing such contrasting conclusions seems difficult especially when the data are not considerably skewed in favor of one or the other scenarios. Indeed, Wang et al. (2004) make the qualification that "loss of selective constraint does not result in loss of function instantly," and point out that "many human *TAS2R* genes may still be functional" (p. 2676). Nevertheless, the conclusion of relaxed constraints on the gene repertoire analyzed as a whole would not exclude the possibility that strong selection has acted on a subset of bitter-taste genes, especially if these genes were of critical importance in discriminating specific bitter substances that have toxic effects.

Evidence for selection in candidate bitter-taste genes

As noted above, we know the particular substances that stimulate several bitter-taste receptors, and we know that these substances can have potentially toxic effects. Two examples of such receptors are coded by the genes *PTC* (or *TAS2R38*) and *TAS2R16*. For the *TAS2R38* gene, we also know that the so-called "taster" and "non-taster" phenotypes are maintained at near intermediate levels in many populations worldwide. Therefore, these genes are ideal choices for a "candidate-gene" approach for studying the effects of natural selection and, as such, both genes have recently been sequenced in diverse population samples.

In population studies of *TAS2R38*, a total of seven haplotypes were detected but two widely divergent haplotypes named PAV and AVI (the letters indicating the amino acids that distinguish them; i.e. PAV: proline, alanine, valine; AVI: alanine, valine, isoleucine) are found at intermediate frequencies in most populations worldwide (Wooding et al., 2004). Recently, in a series of receptor-sensitivity experiments carried out both in vitro and in vivo, these two haplotypes have been confirmed to underlie the taster and nontaster phenotypes, respectively (Bufe et al., 2005). In population studies of *TAS2R16* (Soranzo et al., 2005), four haplotypes (A–D) have been identified, though two similar haplotypes (A and B) together dominate in most of the world (>96.0%). An exception to this occurs in African populations, where haplotype B is at relatively lower frequencies, and haplotypes C and D are together at relatively much higher frequencies (13.8%) than anywhere else in the world (to be further discussed below).

One of the principal ways positive directional selection and balancing selection can be distinguished in DNA data is by the opposite skews they can leave in the frequency spectrum of variants: positive directional selection leaving an excess of low-frequency and high-frequency *derived* variants, and balancing selection leaving an excess of intermediate-frequency variants (see *Theory and analytical methods* section). However, revealing the signal of selection can be problematic since demographic factors, such as population growth, population subdivision, and background selection can also result in skews in the frequency spectrum.

In the study of *TAS2R38*, Wooding et al. (2004) were only able to demonstrate that Tajima's D (1.55), and other measures of the frequency spectra were significantly positive and indicate an excess of intermediate-frequency variants, when *population growth* was taken into account. They argued that population subdivision, which can also result in an excess of intermediate frequency variants (Wakeley, 2000), was unlikely to explain the results since F_{ST} between and among human populations at *TAS2R38* is low.

Conversely, at *TAS2R16,* Soranzo et al. (2005) found a significant excess of high-frequency *derived* variants in 19 out of 60 populations when linked sites were examined using Fay and Wu's H statistic. In addition, they observed that in 38 populations the derived alleles were completely fixed. High frequencies of derived variants are not expected in neutrally evolving populations, and these results are in agreement with the hypothesis that the *TAS2R16* gene has experienced a global selective event.

Two other aspects of variation were suggestive of positive directional selection at *TAS2R16*. First, there was no indication of decay in LD throughout a 222 kb region surrounding the gene. This is a much larger region than is normally found as a single block of LD (e.g. average block size in Africans is 22 kb and about 44 kb in European populations; see Gabriel et al., 2002; also see International HapMap Consortium, 2005). Although a low local recombination rate could also account for the high LD, the pattern is consistent with positive directional selection at *TAS2R16*. Further evidence of selection can be seen in the high F_{ST} values found for certain variants within *TAS2R16*. In particular, one site (516, which has a nonsynonymous change) displayed an F_{ST} of 0.45, a value comparatively high in relation to empirical determinations of F_{ST} at other sites genome-wide.

In searching for the favored variant(s) at *TAS2R16*, several pieces of evidence came together as suggestive of a particular variant. First, one of the three high-frequency derived variants (the G to T substitution at site 516) found on both common haplotypes (A and B), is responsible for an amino acid replacement (from lysine (K) to asparagine (N) at codon 172). Second, this variant falls within the extracellular domain of the *TAS2R16* receptor, likely affecting interaction between the receptor and its tastant (ligand). The third line of evidence comes from in vitro sensitivity studies performed on cells transfected with genes bearing different DNA variants found on haplotypes A and B. Soranzo et al. (2005) were able to determine that the specific amino acid change from lysine to asparagine, resulting from the DNA variant at site 516, causes cells to have a 2-fold increase in sensitivity to β-glucopyranoside compounds.

Time frame of selection at *TAS2R38* and *TAS2R16*

Using an empirically determined mutation rate of 10^{-9}/site/year, and assuming a chimpanzee/human divergence date of 5 miilion years, Wooding et al. (2004) estimated that the divergence between the taster and nontaster haplotypes at *TAS2R38* occurred ~1.5 million years ago (Ma). Although the variance around the age-estimate was high, it considerably postdates the inferred divergence between chimpanzees and humans (~7.0 Ma), making it unlikely that the taster/non-taster polymorphism repre-

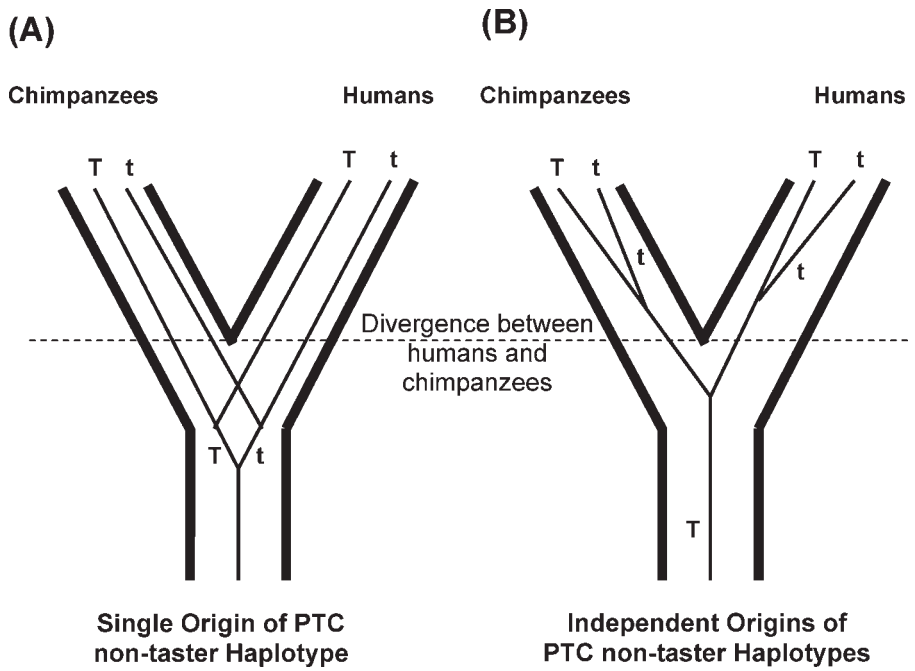

Fig. 5. Hypotheses proposed to explain the origins of the nontaster PTC haplotypes in humans and chimpanzees. Species trees are represented by thick lines, whereas gene trees of the PTC taster (T) and nontaster (t) haplotypes are indicated by thin lines within the species tree. **A:** Shows the single origin hypothesis proposed by Fisher et al. (1939) that proposed that the nontaster haplotype arose once in the common ancestor of humans and chimpanzees from the taster haplotype. Each haplotype was subsequently inherited by both species and was maintained by balancing selection to the present day. **B:** Shows the hypothesis supported by recent population genetic data (Wooding et al., 2004, 2006) in which there was independent evolution of the nontaster haplotype from the taster haplotype in both humans and chimpanzees after they diverged from each other.

sents an ancient polymorphism retained from the ancestral chimpanzee-human population. Thus, while R.A. Fisher's (1939) suggestion that balancing selection has maintained taster/non-taster status—at least in human populations—appears supported by the DNA evidence, his suggestion that the polymorphism was maintained "over the million or more generations which have elapsed since the separation of the anthropoid and hominid stocks" is apparently not true.

Recently this second scenario was further addressed in a population-level study of 86 chimpanzees from each of three different subspecies (*Pan troglodytes troglodytes, P. t. verus*, and *P. t. schweinfurthii*). A total of seven polymorphic sites were identified in *TAS2R38*. One particular nucleotide change found at intermediate frequency (0.52)—a T → G substitution in the second position of the initiation codon (a substitution not found in humans)—was predicted to truncate the *TAS2R38* receptor protein, and was therefore hypothesized to be the nontaster haplotype. Through a series of expression studies and functional assays, Wooding et al (2006) were able to confirm that this DNA variant was indeed responsible for the nontaster haplotype. Since the T → G variant is not found in humans, it was concluded that the molecular basis of the nontaster haplotypes in humans and chimpanzees is different (Wooding et al., 2006). This implies that the PTC polymorphism in humans and chimpanzees is not shared from their common ancestor (*contra* Fisher et al., 1939; see Fig. 5A), but instead that the polymorphism evolved independently along the separate lineages leading to these species (Fig. 5B).

The fact that the nontaster haplotype in chimpanzees is found at intermediate frequencies suggested that it might be maintained by balancing selection, as it is in humans. However, Tajima's D was negative (-0.59) and was not significantly different from the distribution of values under neutrality. This fact was taken to indicate that balancing selection has not operated in chimpanzees. Thus, the proposal of Fisher et al. (1939) that balancing selection has maintained the PTC taster/non-taster polymorphism at intermediate frequencies in each of these species, while supported for humans due to an excess of intermediate-frequency variants (Tajima's $D = 1.55$; Wooding et al., 2004), evidently lacks convincing support in chimpanzees, where no excess of intermediate-frequency variants is detected.

The age of the putatively selected variant *TAS2R16* was estimated using the BATWING computer program (Wilson et al., 2003) that uses a Bayesian Markov chain Monte Carlo method to estimate the ages of nodes on a gene tree. One of the major advantages of the program is that it allows the user to assume different demographic scenarios including models of population growth and substructure. The analysis was based on a subset of the entire sequence data set (the African Yoruban samples only) and estimated the age of the node immediately descendent from the mutation causing the presumed advantageous amino acid replacement from lysine (K) to arparagine (N). The estimates of the age of the mutation ranged from 83 thousand years (Ka) ago to 791 Ka (under models of constant population size) and from 78 Ka to 685 Ka (under models of population growth). As Soranzo et al. (2005) point out, although these estimates are associated with wide confidence intervals, even the most recent dates occur prior to large-scale human evolutionary innovations such as plant/animal domestication, agricultural, and the Neolithic revolutions. These dates also predate the large scale expansion of humans out of Africa and subsequent

differentiation among non-African populations (Lahr and Foley, 1998; Tishkoff and Verelli, 2003) and would appear to place the origin of the mutation at a period when humans were hunter-gatherers and plant foods made up a large portion of the diet. Due to the presumed functional significance of the mutation, as well as its high frequency worldwide (as the variant is present on both haplotypes A and B), it was suggested that selection probably acted on the variant soon after it arose (Soranzo et al., 2005).

Scenarios of selection on the bitter-taste genes, *TAS2R38* and *TAS2R16*

As R.A. Fisher pointed out, a hypothesis of balancing selection at *TAS2R38* requires that the nontaster haplotype (AVI) offer an advantage in the heterozygous state that is greater than either homozygote. Although the AVI haplotype might be inferred to be nonfunctional, several lines of evidence indicate that it is functional and expressed. The haplotype shows no stop codons or deletions (Drayna, 2005) and a recent study indicated that the haplotype is expressed at the mRNA level in human tongue cells at nearly equal levels to the taster haplotype (Bufe et al., 2005). Based on this evidence, several researchers have suggested that the nontaster haplotype codes for a distinct receptor that binds some unknown ligand (Kim et al., 2004; Wooding et al., 2004; Drayna, 2005). A selective advantage has been proposed for the taster haplotype: the sensitivity to PTC-like compounds (i.e. compounds having the −N=C=S chemical moiety) helps one avoid plant chemicals potentially causing thyroid malfunction, an advantage that may be greater in individuals living in iodine-deficient regions (Boyce et al., 1976). However, the heterozygote would presumably gain a selective advantage by being sensitive not only to these compounds but also to other aversive compounds that the AVI haplotype would be capable of detecting (Wooding et al., 2004; Drayna, 2005). It should be noted that several additional haplotypes at *TAS2R38* were also found to respond to PTC, but at intermediate response levels, suggesting that multiple haplotypes might determine a range of PTC sensitivities within populations (Bufe et al., 2005). Polymorphisms for a range of sensitivities might have been driven by evolutionary forces promoting variability at the receptor (Bufe et al., 2005). The enhanced detection of bitter compounds by some individuals may have been advantageous for them to avoid harmful compounds, yet in other individuals a diminished bitter-sensitivity might also have been advantageous. Certain known benefits are known (e.g. anticancer effects) from ingesting natural PTC-like compounds in plants (Bufe et al., 2005). It has also been suggested that other haplotypes at *TAS2R38* might also be functional, and might enable the detection of a wider array of bitter compounds (Wooding et al., 2004; Drayna, 2005).

The bitter compounds that are detected by *TAS2R16* (known as β-glucopyranosides) include compounds with known cyanogenic toxic effects (Drewnowski and Gomez-Carneros, 2000). Physiological response in humans (Soranzo et al., 2005) is triggered by a variety of compounds found in plants: salicin (willow trees), arbutin (bear berry) and amygdalin (bitter almonds) linimarin (manioc), prunasin (almonds), osmoronin epoxide (Rosacea) and 4-glucosyoxymandelonitrile (Berberidaceae). Since the functional effects of the putative advantageous variant (N172) were equivalent for all bitter compounds tested, it was not possible for researchers to determine whether one specific compound caused the selective sweep. Therefore, Soranzo et al. (2005) concluded that it was a generalized response to β-glucopyranosides in plants that brought the N172 variant to high frequencies worldwide.

An interesting observation was that the frequency of the ancestral amino acid K172 at *TAS2R16* (which results in a 2-fold less sensitive protein) was found at moderate frequencies (13.8%) in central Africa compared to its very low frequency or total absence in most other regions of the world. Soranzo et al. (2005) observed that its distribution is very similar to the distribution of malaria-resistance alleles in other genetic systems. This is a tantalizing finding since there is some evidence indicating dietary-derived cyanide compounds may help curtail *Plasmodium falciparum* infection in humans (Nagel et al., 1980; Jackson, 1996). An initial test of the association appeared to be fairly strong—those African populations in which indices of malaria risk were greatest tended to be the same populations that had higher frequencies of the ancestral amino acid K172 (Soranzo et al., 2005). It was hypothesized that variation in the *TAS2R16* bitter-taste gene "results from a balance between protection against malaria and protection against toxins in malaria-free zones." Testing of this hypothesis might entail further analysis of how plant-derived cyanide compounds interact with *Plasmodium falciparum*, as well as analyses of the levels of blood-stage infection in individuals bearing the ancestral K172 type compared to those bearing the N172 type.

If the hypothesis is true, we may infer that selection for increased frequencies of the K172 allele in central African populations occurred around 6,000 years ago when *Plasmodium falciparum* became widespread in Africa due to the spread of agriculture. Thus, selection on the K172 type in Africa appears to have occurred much more recently than positive selection on the N172 type.

DUFFY BLOOD GROUP POLYMORPHISM

The Duffy blood group was first discovered in 1950 by Cutbush and Mollison (Cutbush and Mollison, 1950). The Duffy gene encodes a receptor on the surface of erythrocytes and is characterized by three alleles, *FY*A*, *FY*B*, and *FY*O*, with *FY*O* corresponding to the absence of the *Fy* receptor. It is known that the *FY*O* allele confers almost complete resistance to the malarial parasite, *Plasmodium vivax* (Miller et al., 1976). It is also known that the *FY*O* allele is nearly fixed in sub-Saharan Africans (East Africans, ∼.95; Pygmies, ∼1.0; West Africans, 1.0) where the *P. vivax* parasite is rare, which is a rather counterintuitive pattern (Livingstone, 1984). The *FY*A* and *FY*B* alleles are at approximately intermediate frequencies in Europe. However, in Eastern Asia and the Pacific, the *FY*A* allele is nearly fixed. When the degree of genetic differentiation among world regions is estimated for each of the Duffy alleles, extremely high values are found: the F_{ST} value for the *FY*O* allele is 0.7831 (the highest known F_{ST} for any human gene) and is 0.3325 for the *FY*A* allele, much higher than the average F_{ST} of ∼0.15 estimated based on the analyses of classical markers (Cavalli-Sforza et al., 1994) as well as a more recent genome-wide estimate ($F_{ST} = 0.123$) based on surveys of DNA sequence diversity (Akey et al., 2002).

Marked geographical differentiation at the Duffy locus, as well as the known *P. vivax* resistance of the *FY*O* allele, pointed early to the hypothesis that natural selection has been a dominant force in shaping the distribution of the Duffy alleles among human populations. However, the

distribution of the *FY*O* allele presents marked differences when compared to other red cell polymorphisms presumed to confer resistance to malaria (e.g. β-globin, *G6PD*) (Livingstone, 1984). In these cases, the traits are found at high frequencies in populations in the same regions where malaria is endemic. However, as noted above, the Duffy *FY*O* allele is found at highest frequencies in tropical Africa exactly where *P. vivax* malaria is either entirely absent or at extremely low frequencies. Livingstone (1984) devised two possible explanations for this pattern: 1) *vivax* malaria was formerly present in tropical Africa, but has been almost completely eliminated through the process of human genetic adaptation to the parasite; 2) frequencies of the Duffy *FY*O* allele were already high in tropical Africa, perhaps due to some previous and different selective agent, which prevented the *vivax* parasite from becoming endemic in these regions. Though Livingstone (1984) favored the second explanation, he considered the matter unresolved.

Genetic basis of phenotype and gene localization

The Duffy locus encodes the Duffy antigen receptor for chemokines (*DARC*) found on the membrane of erythrocytes and that binds chemokines of both the C-C and C-X-C families (Hadley and Peiper, 1997). Although it has been well established that *P. vivax* requires the DARC to be present on erythrocytes to gain entry into them, the normal physiological function of the DARC remains unclear. Somatic expression of *DARC* is not confined to erythrocytes but is widespread, being found in endothelial cells of post-capillary venules, and epithelial cells of the collecting ducts of the kidneys, lung alveoli, thyroid, and in Purkinje cells of the cerebellum (Hadley and Peiper, 1997).

The gene for the Duffy receptor has been localized to chromosomal region 1p21-q22 (OMIM database, Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db = OMIM). The Duffy human transcriptional unit encompasses 1,572 base pairs including exon 1 (55 base pairs in length), a single intron (479 base pairs in length), and exon 2 (1,038 base pairs in length). Interspecies comparisons with the orthologous sequence in nonhuman primates indicates that *FY*O* and *FY*A* result from single mutations on an ancestral *FY*B* background (Seixas et al., 2002). The mutation producing the *FY*O* allele has been localized to a single nucleotide base substitution a (T → C transition) that impairs the promoter activity in erythroid cells by disrupting a binding site for the GATA1 erythroid transcription factor (Tournamille et al., 1995). Interestingly, the elimination of the receptor is specific to erythrocytes, while other cells known to bear the receptor are apparently unaffected (Hadley and Peiper, 1997). A nucleotide base substitution (A → G) at nucleotide 131 causes an amino acid change (Glycine → Aspartic acid) at codon 44 that accounts for the difference between the *FY*A* and *FY*B* alleles (Tournamille et al., 1995).

Natural selection at the Duffy locus

The Duffy locus has long been suggested to be a gene targeted by natural selection as evidenced by the extreme geographic differentiation among its three major alleles (Bodmer and Cavalli-Sforza, 1976). Only recently, however, have we been able to examine the nature of the footprint of selection at the Duffy locus. Two recent surveys (Hamblin and Di Rienzo, 2000; Hamblin et al., 2002) have analyzed genetic variation in a large set of DNA sequences from diverse populations, in the genomic region immediately surrounding the *FY* locus. Here, we discuss the results of these studies, first in relation to the nature of selection on *FY*O* in Africans, and then with respect to the possibility that selection has also acted on the *FY*A* allele in non-Africans.

The signature of selection in Africa where *FY*O* dominates

Several patterns of DNA polymorphism point to positive directional selection in sub-Saharan Africans. These are detailed below.

High F_{ST} values of DNA variants. If directional selection on the *FY*O* variant is responsible for marked population differentiation of the *FY*O* variant, then we should expect measures of population differentiation (F_{ST}) for nearby variants to diminish with increasing distance from the presumed selected site. Hamblin et al. (2002) measured F_{ST} between Hausa and Italian population samples at polymorphic sites at various distances on either side of the *FY*O* variant over a ~34 kb distance. The F_{ST} values at sites within this region were compared to F_{ST} values found at sites within nine unlinked and presumed neutral loci that had been sequenced in the same populations as for Duffy (Frisse et al., 2001). F_{ST} values for polymorphic sites in the region surrounding the *FY*O* variant, markedly exceed the highest F_{ST} value obtained for any of the variants within the nine neutral loci (see Fig. 6, lower panel). As expected, F_{ST} values were greatest for the polymorphic sites nearest the presumed selected variant (*FY*O*) but diminish with increasing distance from the variant.

African samples show significantly reduced levels of nucleotide variation. Hamblin and Di Rienzo (2000) sequenced a 19 kb region centered on the *FY*O* mutation, and a 1 kb region approximately 6 kb upstream of the *FY*O* mutation. They compared standard measures of nucleotide diversity (π and θ_s) among five sub-Saharan populations (the Beti and Hausa from Cameroon, the Mbuti Pygmy from the Central African Republic, the Luo from Kenya, and the Mandika from Gambia) and an Italian sample (from central Italy). The DNA variant that defines the *FY*O* allele was detected in all African samples and was absent in all Italian samples. Compared to the Italian samples, the African samples show two to threefold less nucleotide variation (π). Such a pattern is unusual compared to the pattern observed at most human genes in which African populations usually contain approximately twofold more variation (Yu et al., 2002; Tishkoff and Kidd, 2004). At Duffy, the reduced level of polymorphism in Africans is maintained in the upstream region.

To test whether reduced nucleotide variation in Africans represents a significant departure from neutral expectations, the HKA test was performed (Hambin and Di Rienzo, 2000). This test is based on the neutral expectation that polymorphism within humans (in the Duffy region) is proportional to the interspecies divergence between humans and an outgroup species (in this study the Orangutan was used; for background on the HKA test see *Theory and Analytical Methods* section). Positive directional selection within humans is expected to reduce the amount of polymorphism relative to the divergence. The HKA test was performed in two ways: 1) Hamblin and Di Rienzo (2000) compared the Duffy locus with the presumed neutral intron 44 of the *DMD* gene (Nachman and Crowell, 2000); 2) they compared the Duffy locus with the nine unlinked and presumed neutral loci as described above

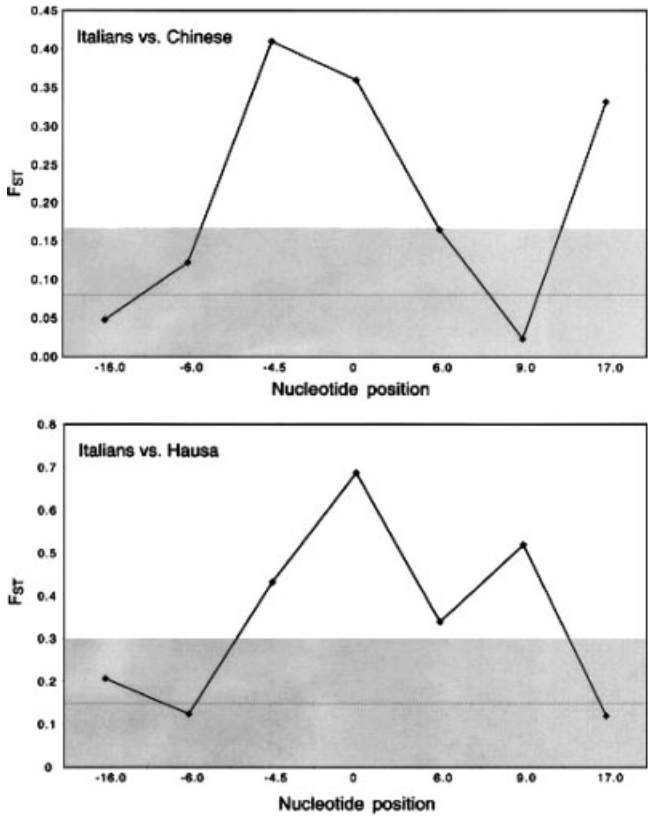

Fig. 6. Comparison of F_{ST} in the *FY* region and in the 10 locus-pairs regions. Blackened diamonds represent F_{ST} at various distances from the *FY* gene. Position 0 represents the location of the *FY*A* mutation in the top panel and the *FY*O* mutation in the bottom panel. Regions II and III were pooled and then divided into three regions of ~2.5 kb, the average size of the locus-pairs regions. The shaded area represents the range of values of F_{ST} observed, between these populations, in the locus-pairs regions. The dashed line represents the average F_{ST} in the locus-pairs regions. (Figure from Hamblin et al., Am J Hum Genet 2002;70 and used by permission from publisher.)

(Frisse et al., 2001). In comparisons with *DMD* intron 44, all African populations (except the Beti) showed significant reductions in polymorphism compared to neutral expectations. The higher relative polymorphism in the Beti is explained to be due to a haplotype in this population that is apparently a recombinant with a *FY*B* haplotype. This recombination event (also detected in the Hausa) most likely occurred prior to the fixation of the *FY*O* variant in Africa when non-*FY*O* haplotypes were still present (Hamblin and Di Rienzo, 2000). In the HKA test, in which Duffy was compared with the nine presumed neutral loci, Africans (specifically the Hausa sample) were also found to have significantly reduced levels of polymorphism. Furthermore, since Hamblin et al. (2002) sequenced a much larger area surrounding Duffy than in their earlier study, they were able to show that the pattern of reduced variation in the Hausa occurs over a span of 22 kb. They noted, however, that polymorphism returned to neutrally expected levels in a region 10 kb downstream from the *FY*O* mutation, indicating that the presumed signature of selection had mitigated at this distance.

Africans show an excess of high-frequency derived variants relative to neutral expectations. Immediately after a selective sweep event, the frequency spectrum of mutations is skewed towards an abundance of low-frequency DNA variants, resulting in negative values for Tajima's *D* statistic (see *Theory and Analytical Methods* section). Hamblin and Di Rienzo (2000) found that the Duffy region showed a skew towards rare variants for a combined analysis of the five African populations (Tajima's D = -.52), although this result was found not to be statistically significant. When the African populations were examined individually, Tajima's *D* values were positive in some of these populations (though not statistically significant), or were zero. This finding was confirmed by Hamblin et al. (2002) in a larger sample of Hausa, in which none of the eight distinct sub-regions around the Duffy locus showed significantly negative values. In fact, Tajima's *D* was positive over much of the entire region sequenced, including the sub-region nearest the variant defining *FY*O*. It was concluded that the frequency spectrum as measured by the Tajima's *D* statistic was not consistent with any simple model of a recent selective sweep. The fact that Tajima's *D* was often positive indicated that many variants were not young mutations that arose after a selective sweep, but were in fact old variants that had survived the sweep. Although there are several different ways for old variants to survive a sweep, recombination between haplotypes (as is presumed to have occurred in Africa prior to the onset of selection) seems to be the most likely possibility. As noted earlier, the *FY*O* variant occurs on two different haplotypic backgrounds in Africa; one of these is the most common *FY*O* haplotype found in Africa, and the other is a haplotype presumably formed due to recombination between *FY*O* and *FY*B* haplotypes.

DNA polymorphism at linked sites can be maintained after a selective sweep as a consequence of recombination (Hamblin et al., 2002). If recombination has taken place after the sweep, then the frequency spectrum at Duffy is expected to be very different from the frequency spectrum expected under the assumption of a sweep without recombination (as is assumed by Tajima's *D* test; see Fay and Wu, 2000). If it is possible to determine the derived states at variable sites within a sequence (e.g. by comparison to an outgroup sequence) then a model that incorporates limited recombination predicts a frequency spectrum in which there is an excess of both low-frequency and high-frequency *derived* variants (Fay and Wu, 2000). The *H* statistic (Fay and Wu, 2000) was developed to measure the excess of high-frequency derived variants within a DNA region. As described earlier, this test is relatively powerful compared to other tests of the frequency spectrum because not many high-frequency derived variants are expected under neutrality. Therefore, even only a few high-frequency variants will lead to rejection of the null hypothesis of neutrality (Fay and Wu, 2000). For the Duffy region in the Hausa, the *H* statistic was significantly negative in the region containing the *FY*O* variant, and for regions extending on both sides of the *FY*O* variant (Hamblin et al., 2002). However, it is no longer significant at distances greater than ~9 kb downstream of the *FY*O* variant. Such a pattern is consistent with positive directional selection having acted to increase the frequency of the *FY*O* variant, and having also acted to increase the frequencies of linked derived variants through hitch-hiking effects.

The time frame of selection on the *FY*O* allele in Africa

Subsequent to the fixation of a beneficial mutation due to positive selection, the genealogy for a locus will consist

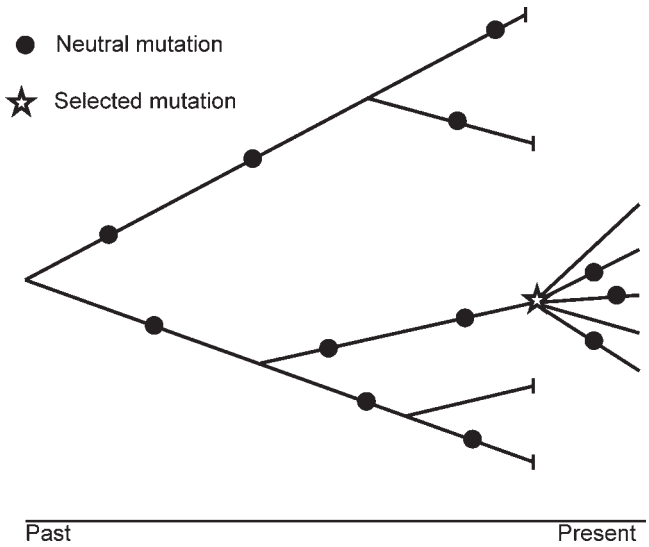

Fig. 7. A geneaological tree of a genomic region that has been affected by a selective sweep. Observe the difference between the shape of the tree before and after the sweep. Before the selective event, the genealogy shows a regular branching pattern of lineages. For some time subsequent to the selective event the genealogy resembles a "star-shaped" tree in which there are many long and unbranching lineages. The favorable mutation is inherited by all of these lineages, and new neutral mutations begin to accumulate on some of these lineages. Lineages of the genealogy that have not been selected terminate in a short vertical line, and the mutations that occurred along these lineages in the past are lost (adapted from Hamblin and Di Rienzo (2000)).

of many long and nonbifurcating lineages that trace back to the fixation event (this is commonly described as a "star-shaped" tree; see Fig. 7). Any mutations that arise after fixation will occur on these lineages, and will therefore appear in the data as low-frequency variants (see *Theory and Analytical Methods* section). Therefore, Hamblin and Di Rienzo (2000) assumed that the time since fixation of the *FY*O* variant in Africa is most accurately reflected by the number of low-frequency polymorphisms in the data set. In the set of DNA sequences in the African sample, there were very few ($n = 3$) low-frequency variants (an expected finding if selection was recent). Based on an estimated orangutan–human divergence date of 14.0 MY, and the 54 nucleotide differences found between humans and orangutans, the mutation rate at the Duffy locus was estimated to be 1.93×10^{-6}. Since 47 human chromosomes were sampled, the average mutation per branch was 0.0638. Dividing 0.0638 by 1.93×10^{-6} yields an average branch length of 33,075. The 95% confidence interval around this date is 6,500–97,000 years.

In the context of recent human evolution, these dates occur at a time period subsequent to when humans are believed to have first migrated out of Africa (around 100,000 years ago; see Jorde et al., 2001). Hamblin and Di Rienzo (2000) reasoned that this makes sense in light of the observation that *FY*O* is present at very low frequencies in non-Africans. (In the Chinese, Italian, and Pakistani samples sequenced by Hamblin et al. (2002), the *FY*O* variant only occurred in 4 of the 28 Pakistani samples). If, alternatively, the *FY*O* variant had been fixed in Africa prior to the major migration out of Africa then we would expect to observe appreciable frequencies of *FY*O* in non-Africans when in fact we do not. (It was considered unlikely that strong negative selection in non-Africans could have reduced the frequency of *FY*O* in these populations since no known disadvantages are known to be associated with the variant.)

The signature of selection outside Africa

As with the *FY*O* allele, the high F_{ST} value for the *FY*A* allele (0.3325; Cavalli-Sforza et al., 1994), as well as the fact that it is at near fixation levels in Eastern Asia and the Pacific, strongly suggests that positive selection has acted on it. However, in contrast to *FY*O*, the *FY*A* mutation has no known functional consequence. Therefore, it remains unclear where in the gene to search for a signature of selection. For example, while the target of selection could be the *FY*A* mutation itself, it is also possible that some other variant linked to the *FY*A* mutation was the target of selection. Nevertheless, Hamblin et al. (2002) found that F_{ST} values are highest for variants nearest the *FY*A* mutation, but diminish with increasing distance from it (see Fig. 6 upper panel), possible evidence of hitchhiking effects. The F_{ST} values at these variants are only about half as great as the F_{ST} values for variants linked to *FY*O* (see Fig. 6 lower panel), but even so they exceeded even the highest F_{ST} values found for variants in any of the nine presumed neutral loci.

A sample of 32 chromosomes from a Han Chinese population was analyzed by Hamblin et al. (2002). The *FY*A* variant is nearly fixed in this population. Throughout an approximately 34 kb region surrounding the Duffy locus, nucleotide variation was found to be significantly reduced (as determined by the HKA test) in the region nearest to the Duffy locus. Over the entire region, however, the pattern of variation is very complex and does not fit any simple model of selection. For example, although reduced variation is observed in several regions of the gene, these regions are interrupted by regions where variability is much higher. Thus, we do not observe a long stretch of consistently reduced variation flanking the Duffy locus as observed for the African samples. Such a heterogeneous pattern is not expected under a simple model of positive selection. Furthermore, certain regions flanking the *FY*A* mutation within the Chinese sample show significantly positive Tajima's *D* values indicating an excess of intermediate-frequency mutations, which can arise due to balancing selection. However, other regions of the gene show distinctly contrasting frequency spectra (though not significant), indicating an increase in low-frequency mutations. This leads to an unusually large variance in the frequency spectra from region to region within the gene region analyzed. Overall the pattern that emerges from the Chinese sample is a complex one, in which heterogeneity is observed in both patterns of polymorphism, and in the frequency spectrum of mutations.

While levels of polymorphism in and near to the *FY*A* mutation clearly violate the neutral model, the pattern of DNA polymorphism within the 34 kb region analyzed does not fit any simple model of positive selection. Hamblin et al. (2002) have suggested two possible historical factors that might have given rise to such a heterogeneous pattern in the Chinese sample. First, it is possible that Asian populations have experienced nonequilibrium demographic histories in which selection occurred in populations that were geographically structured. Second, it is possible that during the history of the Duffy gene in Asian populations, multiple advantageous mutations arose within the gene in Chinese populations (perhaps adapta-

tions against different pathogens), and that the heterogeneity we see today represents footprints left by multiple different selective episodes. Once more, it is possible that different selective episodes left contrasting signatures of selection (e.g. positive directional selection versus balancing selection).

Our understanding of the evolution of the *FY*A* allele in Asia will probably necessitate several advances and/or developments. First, if we learn that the *FY*A* variant, or some other variant linked to *FY*A*, has a functional consequence then this will motivate our search for a signature of selection to a specific region within the gene. Second, it will be helpful to undertake sequencing in additional populations within Asia. Third, since the pattern within the Chinese sample is complex and does not fit any current model of demography or selection, better understanding of the evolution of *FY*A* allele will likely require the development of more sophisticated theoretical models and statistical tests that can tease out signals of selection from complex gene histories.

Discussion of Duffy polymorphism

How do these new findings of selection, particularly the finding of directional selection on *FY*O* in Africa, influence our interpretations of the hypotheses put forward by Livingstone (1984)? In discussions of the origins of human malaria, the advent of slash-and-burn agricultural practices in Africa (<7.0 Ka; see Livingstone, 1958) is thought to be the crucial causal factor, not only in creating favorable ecological conditions for the spread of the *Anopheles* mosquito vector, but also in leading to the increased human densities necessary to maintain malarial transmission (Livingstone, 1958). The average time and confidence range (33,075 years; 6,500–97,200 years) estimated by Hamblin and Di Rienzo (2000) since the fixation of the *FY*O* variant predates the advent of agriculture in Africa. This estimate implies that the rise in frequency of the *FY*O* variant is unlikely to be associated with the effects of the transition to agriculture in Africa. However, it should be noted that Seixas et al. (2002) analyzed microsatellite diversity on the *FY*O* haplotype and using two different analytical methods, estimated somewhat more recent dates; 14,700 years (range 5,100–31,800) under one strategy and 9,300 years (range 4,350–15,750) under another, placing the fixation date closer to the introduction of agriculture in Africa. Such recent dates would be surprising, however, since there is a theoretical reason to suspect that selection on *FY*O* took place over a much longer period of time. Livingstone (1984) first pointed out—and others have since (Carter, 2003—that selection on the *FY*O* allele would have been initially relatively weak, making the short time since the introduction of agriculture insufficient to bring the variant to its current high frequencies in Africa. This is explained as follows: after a beneficial *recessive* mutation arises (such as *FY*O*), it is present at such low frequencies that only rarely will it occur in the homozygous state, and therefore it will only rarely come under positive selection (Carter, 2003). Comparatively speaking, the rate of selection for higher frequencies of the *FY*O* variant would have been much slower compared with the rate of selection for the sickle cell trait (against *P. falciparum*) since in this case the advantage favors the heterozygotes (Carter, 2003). Whereas selection for the sickle cell trait is thought to have occurred on the order of 2,000 to 3,000 years ago, it probably took many thousands, or tens of thousands of years, for positive selection to drive *FY*O* to near fixation levels in Africa (Carter, 2003). This is consistent with the considerably lower coefficient of selection ($\sim$0.2%) estimated for *FY*O* (Hamblin et al., 2002) compared with values estimated for the sickle-cell trait (5.0–18.0%; see Li, 1975) and for *G6PD* (2.0–5.0%; see Tishkoff et al., 2001).

Finally, the assumption that the spread of *P. vivax* is associated with agriculture and increased population densities needs reexamination (Hamblin and Di Rienzo, 2000). The life-history of *P. vivax* is such that sporozites can enjoy long incubation or dormant periods within the liver (up to 6 to 12 month), when they are known as hypnozoites (Sattabongkot et al., 2004). This contrasts with the life cycle of *P. falciparum* in which incubation stages are much shorter (9–14 days) (Sattabongkot et al., 2004). As a consequence, *P. vivax*, does not require the increased host-densities that other malarial parasites need (e.g. *P. falciparum*). This could indicate that malaria due to *P. vivax* infection represented a much older and persistent selective pressure in humans compared with *P. falciparum*. In fact, Carter (2003) has proposed a scenario in which *P. vivax* (or its immediate ancestor) was present within Africa during roughly the last 1.0 million years, and therefore was likely to be the selective agent that drove *FY*O* to near fixation levels. More specifically, Carter (2003) hypothesized that during Pleistocene Ice ages the pathogen (which was widespread in the Old World during warm periods) became periodically isolated in sub-Saharan African refuges during glacial periods. It was during a recent glaciation period, he proposes, that *P. vivax* became the selection pressure that drove the *FY*O* variant in African populations to increased frequencies.

Origins of Plasmodium vivax. The evolutionary interaction between the Duffy receptor and the parasite *P vivax* is an example of co-evolution between a pathogen and a host. Therefore, we can ask: What do phylogenetic and population genetic studies of the *P. vivax* parasite tell us about its time and geographical place of origin? Leclerc et al. (2004) found markedly reduced levels of genetic variation within 13 fast-evolving microsatellites, and eight short tandem repeats in *P. vivax*. At 12 of the 13 microsatellites they tested, *P. vivax* was nearly completely monomorphic. This was interpreted to favor a scenario in which *P. vivax* arose from a small progenitor population (with geographical location unknown) and only recently experienced worldwide expansion less than 10,000 years ago (Ayala et al., 1999; Leclerc et al., 2004).

In a more recent analysis, however, Imwong et al. (2006) found abundant variation at 11 di-nucleotide microsatellites in *P. vivax* from Colombia, India, and Thailand. The discrepancy between the results of these studies (Leclerc et al., 2004 and Imwong et al., 2006) has been explained as due to a difference in repeat length in the different sets of microsatellites analyzed (Imwong et al., 2006). For instance, one of the most important factors affecting mutation rate at microsatellites appears to be microsatellite length (Ellegren, 2004). Mutation rate increases with increasing number of repeated units, probably because more repeated units allows more opportunities for replication slippage, the presumed mutational process at microsatellites. In the analysis by Leclerc et al. (2004), the microsatellites had very few repeats, with a median of 5.5 repeats, and a range of 4–13, whereas in Imwong et al. (2006) the microsatellites had a median repeat length of 16 and a range of 12–18. Therefore, based on the considerably lower repeat length of the microsatel-

lites analyzed by Leclerc et al. (2004), high levels of variation might not be expected (Imwong et al., 2006). Consequently, the abundant variation detected in *P. vivax* calls into question Leclerc's et al. (2004) hypothesis of a recent expansion for *P. vivax* < 10,000 years ago, and may point to the pathogen's considerably older history.

The presence of abundant variation in *P. vivax* has been confirmed in more recent studies of diverse genetic systems including entire mitochondrial genomes (Jongwutiwes et al., 2005; Mu et al., 2005), as well as nuclear and plastid genes (Escalante et al., 2005). Based on these data sets, the most recent common ancestor of *P. vivax* was estimated to fall between 45,680 and 81,605 years ago by Escalante et al. (2005), 53,000 years ago by Mu et al. (2005), and between 200,000–300,000 years ago by Jongwutiwes et al. (2005). Furthermore, when Jongwutiwes et al. (2005) reanalyzed Escalante's et al. (2005) data, they obtained dates ranging from 206,000–314,000 years, which more closely agreed with their own dates. Thus, *P. vivax* shows abundant variation, and this variation points to its old history.

Phylogenetic analyses, comparing *P. vivax* with other related pathogens, have been performed based on these same data sets. All analyses (Escalante et al., 2005; Mu et al., 2005; Jongwutiwes et al., 2005) have indicated that the human *P. vivax* parasite falls within a monophyletic clade of *Plasmodium* parasites of Asian primates, specifically various species of *Macaca*. This finding supports an "Out of Asia" model for the origin of human *P. vivax*, a hypothesis originally proposed by Livingstone (1984).

Although the date of *P. vivax* entry into Africa is not definitively established, the plausibility of early dates has important implications. For instance, the older history of the pathogen increases the possibility that it arrived in Africa early enough to have been the selective agent that drove *FY*O* to near fixation in many sub-Saharan Africans populations. If *P. vivax* made an early arrival into Africa, how did it get there? One problem is that anatomically modern humans are believed to have been restricted to Africa during the earliest part of their evolutionary history therefore making it unlikely that they brought *P. vivax* from Asia into Africa. Furthermore, the fixation of the *FY*O* variant in Africa would have required an extended period during which *P. vivax* and humans coevolved (Carter, 2003). Therefore, Carter (2003) has hypothesized that the host-transfer of *P. vivax* (or its immediate ancestor) from Asian primates to Asian hominids occurred during a time (>1.0 Ma) when *H. erectus* is known to have inhabited Asia (Antón, 2003). This would have allowed the parasite to disperse back into populations of *Homo* in Africa, presumably through contact between Asian and African hominid populations. The feasibility of this scenario needs further investigation.

Based on mitochondrial DNA evidence, Jongwutiwes et al. (2005) also conjecture a long association between *P. vivax* and hominids. The mismatch distribution between pairs of *P. vivax* mitochondrial sequences revealed a pattern characteristic of populations that experienced population expansion after a bottleneck. Interestingly, they found a very similar level of variation and mismatch distribution for *P. falciparum* (the pathogen responsible for human *falciparum* malaria) leading them to propose that these pathogens had closely similar evolutionary histories. They noted that the timing of these demographic events coincides with the genetic bottleneck and subsequent population expansion that has been proposed for humans (Harpending et al., 1998). Based on the coincidence of evidence, they propose that both *Plasmodia* were parasites of *Homo* long before the evolutionary transition from early hominids to anatomically modern humans, and that both parasites underwent a bottleneck and population expansion concomitantly with their human hosts (Jongwutiwes et al., 2005). This hypothesis seems quite conceivable for *P. falciparum* since its sister species is *P. reichenowi*, a chimpanzee parasite (Escalante et al., 2005). The scenario does not as easily fit the evidence for *P. vivax*, since its sister species is believed to be a *Plasmodia* of Asian macaques, although Carter's (2003) hypothesis of an early transfer of the pathogen from Asian monkeys to Asian hominids could offer an explanation.

While it remains a strong possibility that *P. vivax* was the original selective agent that drove *FY*O* to fixation in Africans, there are other possibilities. One question is, if *P. vivax* was the selective agent that drove the *FY*O* allele to fixation in Africa, then why hasn't selection driven the *FY*O* allele to high frequencies outside Africa where *P. vivax* is known historically? A common explanation is that there has not been enough time for this to occur, because selection is slow to change allele frequencies when the advantageous phenotype is due to a homozygous recessive genotype (Carter, 2003). However, it is interesting to note that Zimmerman et al. (1999) have recently detected possible selection on the *FY*O* mutation in Papua New Guinea. Here, a *FY*A* haplotype (known as $FY*A^{\text{null}}$) appears to have independently evolved the *FY*O* causal mutation, and there is evidence that this $FY*A^{null}$ haplotype reduces infection due to *P. vivax*. The new allele was only detected in heterozygotes ($FY*A/FY*A^{null}$), and is at low frequency (0.022). However, it appears to show a dosage effect in which heterozygotes show a twofold reduced expression of the Duffy receptor on RBCs and also show less blood-stage infection with *P. vivax* than do *FY*A/FY*A* homozygotes. There may, therefore, have been a greater degree of selection on Duffy alleles outside Africa than is usually appreciated. The case appears to represent independent adaptation against *P. vivax* via the same mutational mechanism (*FY*O*), and it indicates that *P. vivax* can in fact be directly involved in the evolution of this RBC polymorphism.

Independent evolution of the *FY*O* mutation in Papua New Guinea raises the possibility that the mutation could have arisen more than once in Africans. As noted earlier, the *FY*O* variant in Africans occurs on two haplotypic backgrounds, which Hamblin and Di Rienzo (2000) and Hamblin et al. (2002) attributed as most likely due to recombination. Indeed, by subdividing African *FY*O* haplotypes using microsatellite markers, Seixas et al. (2002) found evidence supporting the hypothesis that the *FY*O* variant in Africans had arisen twice by independent mutational events.

What can we say about Livingston's (1984) second hypothesis, that the spread of *P. vivax* in Africa was curtailed by the prior fixation of *FY*O* in that region? Since cell-surface proteins are frequently used by pathogens to infect cells, it is possible that some receptors were repeatedly targeted by different pathogens. Thus, one possibility is that some pathogen other than *P. vivax* may have driven the *FY*O* allele to near fixation in Africans (Hamblin and Di Rienzo, 2000; Hamblin et al., 2002; Escalante et al., 2005; Mu et al., 2005). Escalante et al. (2005) noted that this could be a possible example whereby current utility does not necessarily indicate reason for origin (Gould and Vrba, 1982). A possible example of this is seen with the 32 base pair deletion (*CCR5-Δ32*) in the *CCR5*

gene (a chemokine receptor on T cells). It has been proposed that a past selective agent drove this mutation to high frequencies in some European populations (275–1,875 years ago), and that this mutation fortuitously provides natural resistance to HIV infection today (Stephens et al., 1998; but for an alternative view see Sabeti et al., 2005). If such a situation also occurred in the case of the Duffy gene in Africa, then it may be difficult to ascertain the original selective pathogen. It should be noted, however, that even if *P. vivax* was not the original selective agent, this would not preclude *P. vivax* from being an important selective agent in the recent past or even today, particularly in peripheral regions where the *FY*O* allele is not fixed and where *P. vivax* transmission is high.

There are a number of areas for future research that could help to resolve some of the questions raised above. First, increased genetic sampling of African populations could lead to improved estimates of the time frame of selection on *FY*O*, and would allow investigation of the possibility that the mutation arose more than once in these populations. Second, a broader and increased sampling of *P. vivax* is necessary (Escalante et al., 2005) to refine estimates of the time frame of the pathogen's existence, as well as to decipher the demographic and selective forces that may have influenced its evolution. Third, increased understanding of the evolutionary history of the pathogen's other host, the *Anopheles* mosquito, is necessary. Several future findings could shed more light on the question of the original selective agent of the *FY*O* allele in Africa. First, a newly discovered pathogen would be suspected if its distribution (or paleo-distribution) closely matched the distribution of *FY*O* in sub-Saharan Africa. Second, there is the chance that ancient DNA analyses could reveal a pathogen that contains genes encoding a Duffy-specific binding protein and whose history matches the time frame of selection of *FY*O* in humans.

SCANNING THE GENOME FOR GENES AFFECTED BY NATURAL SELECTION

The examples of human genetic adaptation that we have discussed are cases whereby genetic signatures of selection have been identified on the basis of a "candidate-gene" approach. In such an approach there are a priori functional reasons, as well as marked population differences in allele frequencies, that lead researchers to study particular genes. To date, the best examples of recent selection in humans have been discovered using the "candidate-gene" approach.

An alternative approach is to perform genome-wide surveys that aim to identify genes or genomic regions showing evidence of positive selection. Such studies are facilitated by the completion of several large sequencing initiatives that have collected DNA data on two levels – population and between-species levels. At the population level, several recent projects have collected DNA polymorphism data (SNPs) within and among human populations (e.g. the HapMap project, Hinds et al., 2005). At a between-species level, there are projects aimed at completing the sequencing of entire primate genomes. For example, draft-sequences of the chimpanzee (*Pan troglodytes*; see Chimpanzee Sequence and Analysis Consortium, 2005) and Rhesus macaque (*Macaca mulatta*) genomes have recently been completed and the genomes of the Orangutan (*Pongo pygmaeus*) and White-tufted marmoset (*Callithrix jacchus*) are underway. As more primate genomes become available it becomes possible to search for those genes underlying adaptations in specific evolutionary lineages, including the lineage leading to our species (Goodman et al., 2005).

The methods employed in scans for selected genes are similar to those used in "candidate-gene" studies. In the scanning approach, however, the methods are applied over a much greater set of DNA sequences or polymorphic sites, spanning various regions of the genome. Two different yet complementary genome-scanning approaches have been used to date: between-species comparisons (usually contrasting humans and chimpanzees) and within-species comparisons of polymorphism data (analyzing human populations of different geographic origins). The between-species contrasts yield information concerning the nature of adaptive change along lineages since two species diverged. A within-species approach, on the other hand, focuses on adaptive changes at more recent time-scales as human populations differentiated.

Genome-wide scans for positive selection by contrasting genes between humans and chimpanzees

Recently, the genome sequences of chimpanzees and humans have been used to perform scans for selection that took place since these two species diverged from a common ancestor. A recent study by Nielsen et al. (2005a) scanned large numbers of genes for evidence of positive selection by comparing orthologous genes between humans and chimpanzees. A previous analysis by Clark et al. (2003) had scanned a somewhat smaller set of genes in humans, chimpanzees and the mouse. By including the mouse as an outgroup species, Clark et al. (2003) were able to specify which changes had taken place along the lineage leading to humans and which had taken place along the lineage leading to chimpanzees. The analytical methods employed to detect positive selection involve comparing nonsynonymous and synonymous substitution rates for the divergence between humans and chimpanzees. Positive selection is inferred if the ratio of nonsynonymous to synonymous substitutions is statistically greater than 1 ($dN/dS > 1$) (see *Theory and Analytical Methods* section). The study by Nielsen et al. (2005a) restricted analysis to comparisons between chimpanzees and humans only, and therefore was unable to specify along which lineage positive selection had occurred

Initially, Nielsen et al. (2005a) analyzed 20,361 genes, but after eliminating many of these because they contain too few mutations, or because the gene-sequence was too short (i.e. < 50 base pairs), 8,079 genes remained for analysis. Of these genes, 733 evolved with $dN/dS > 1$ (although not all values were significantly greater than one). For each gene, a likelihood ratio test of the null hypothesis of neutrality, against the alternative of positive selection, was performed, yielding a *P*-value. Low *P*-values are indicative of positive selection. To test whether specific classes of genes showed overall greater evidence for positive selection, genes were assigned to groups according to the PANTHER classification database (http://www.pantherdb.org). A test was performed to determine if any category was significantly enriched (i.e. showed an over-representation of putatively selected genes as indicated by their low *P*-values). The same strategy was also applied to a classification of genes based on the Novartis Gene Expression Atlas database (http://expression.gnf.org). Genes with maximal expression in a particular tissue (e.g. the brain) were evaluated for evidence of selection. The

TABLE 2. Biological processes categories with a significant excess of putatively positively selected genes determined by the ratio of dN/dS between chimpanzees and humans[1] *(Table adapted from Nielsen et al. 2005a)*

Biological process	Number of genes	*P*-value
Immunity and defense	417	0.0000
T-cell-mediated immunity	82	0.0000
Chemosensory perception	45	0.0000
Biological process unclassified	3,069	0.0000
Olfaction	28	0.0004
Gametogenesis	51	0.0005
Natural killer-cell-mediated immunity	30	0.0018
Spermatogenesis and motility	20	0.0037
Inhibition of apoptosis	40	0.0047
Interferon-mediated immunity	23	0.0080
Sensory perception	133	0.0160
B-cell- and antibody-mediated immunity	57	0.0298

[1] The putative positive selection could not be assigned specifically to either the chimpanzee or the human lineages.

approach assumes that genes with the highest expression in a specific tissue are genes with the greatest functional importance in that tissue.

Biological processes inferred to be under selection in the divergence between humans and chimpanzees

Table 2 is a list of the 12 categories of biological processes that Nielsen et al. (2005a) identified as showing evidence of positive selection in chimpanzee–human contrasts. Each biological process category is ranked according to strength of positive selection. The numerous genes within each category are potential candidates for further genotyping or sequencing in population-level samples of humans and chimpanzees, as well as in additional primate species.

Among the functional groups surveyed, the categories "immunity and defense" and "T-cell-mediated immunity" showed the greatest proportion of genes with low *P*-values, indicating they have an over-representation of putatively selected genes. Positive selection on genes in these categories probably results from the coevolutionary arms race that occurs between viruses (and other pathogens), and our mechanisms of defense against them.

Other categories that were enriched with positive selected genes were "spermatogenesis" and "gametogenesis" (Nielsen et al., 2005a). This result is consistent with the previous finding of Wyckoff et al. (2000) that male reproductive genes in primates generally show evidence of positive selection. In this study, male reproductive genes were found to show higher rates of nonsynonymous substitutions in the chimpanzee and human lineages, but not in the gorilla lineage. Since chimpanzees show greater reproductive promiscuity compared to gorillas, and are therefore thought to experience greater sperm competition, this finding may point to a correlation between types of genes under positive selection and types of mating systems. Confirmation of an excess of positively selected reproductive genes along the human lineage may offer us a fascinating view of the mating system and reproductive behavior of early hominids.

Three additional biological process categories were enriched for positively selected genes in the lineage leading to humans—"olfaction," "chemosensory perception," and "sensory perception." This assignment was possible because Clark et al. (2003) compared the chimpanzee and human genes to their mouse orthologue. The olfaction category is made up largely of olfactory receptor genes (OR) that are the basis for the sense of smell. In mammals, there are generally >1,000 OR genes, representing the largest gene superfamily (Gilad et al., 2005), although there has been a general reduction in functional OR genes in primates and in humans particularly (Gilad et al., 2005). Humans have ~862 OR genes, but the majority (56%) have coding-region disruptions and are thought to be pseudogenes (Gilad et al., 2005). Great apes, on the other hand, have a larger fraction of functionally intact OR genes. Most pseudogenes in humans were found to be evolving with no selective constraints. When comparisons of d*N*/d*S* ratios among mouse, chimpanzee and human orthologous OR genes were made, a small group of genes (*OR52H1*, *OR6K2*, *OR51G1*, *OR4D11*, *OR1P1P*, *OR4F13P*) show evidence of positive selection along the human lineage (d*N*/d*S* ratios ranging from 1.35 to 2.80, see Gilad et al., 2005). In the chimpanzee, at least three different OR genes (*OR1L8*, *OR4F29*, *OR56B2P*) were identified to have experienced positive selection (Gilad et al., 2005) and so may indicate lineage-specific positive selection for particular OR genes in the two lineages. For several of these genes (*OR4D11* and *OR1P1P* in humans, and *OR1L3* in chimpanzees) amino acid replacements are situated at putative receptor-binding sites and therefore could be functionally important. There is, however, one potential difficulty in analyzing positive selection in OR genes. The problem is in distinguishing a gene that has been under positive selection, from a gene that has merely evolved under relaxed selective constraint. Eyre-Walker (2006) suggested that evidence for extensive positive selection on OR genes in humans may be an artifact of the statistical-method Clark et al. (2003) employed to test the significance of d*N*/d*S* ratios (i.e. the model 2 test), a method that could not distinguish between positive selection and relaxed selective constraint (see further discussion below in *Future prospects for scans of selected genes)*.

Whereas the previous approach involved testing for overrepresentation of selected genes in *a priori* defined categories, another approach was to examine those genes with the lowest *P*-values (i.e. the strongest evidence for positive selection, Nielsen et al., 2005a). Many of these genes fell into functional categories already identified as showing evidence of positive selection, such as olfaction, host-pathogen interactions and immunology, spermatogenesis, as well as testis- or sperm-specific functions. However, a moderate number of genes with low *P*-values appear to be involved in tumor-progression, tumor-suppression, apoptosis or cell-cycle control and are therefore cancer-related genes. It is worth noting that Greaves (2002) recently argued that genes associated with the persistent and proliferative activity of mammary or prostate stem-cells may have offered a fertility advantage to individuals in the evolutionary past, and might therefore have been positively selected. He suggested that the association of these genes with the development of cancer in individuals today could be related to a mismatch between our genes and the current environment in which we live, as well as to our increased life spans. Interestingly, several of the cancer-associated genes (*TSARG1*, *DFFA*, *HYAL3*, and *PEPP-2*) identified by Nielsen et al. (2005a) are also involved in apoptosis during spermatogenesis, so it is possible (though speculative) that some forms of cancers could be related to sperm competition.

Genome-scanning efforts, like the study by Nielsen et al. (2005a), provide lists of genes that can be further analyzed to better characterize the pattern and timing of positive selection. One example is the follow-up analysis by Wildman et al. (in prep) of the progesterone receptor gene (*PGR*), which Nielsen et al. (2005a) identified as showing evidence of positive selection. Wildman et al. (2006) sequenced all coding regions for *PGR* orthologues in a representative species from all major primate lineages. Evidence of positive selection (as ascertained from increased dN/dS ratios) was found on several different primate lineages, and in the human-chimpanzee clade.

Gene expression patterns and positive selection

Nielsen et al. (2005a) investigated whether genes showing high expression in specific tissues (e.g. testis, whole brain, heart, cortex, cerebellum, kidney) also showed evidence of positive selection. The only category for which there was significant enrichment of genes with high d*N*/d*S* ratios was that for genes with maximal expression in the testis, consistent with findings of positive selection dominating in spermatogenesis and gametogenesis. An unexpected finding was that genes with maximal expression in the brain (e.g. in the whole brain, fetal brain, cortex, cerebellum, corpus callosum, caudate nucleus, pituitary gland, amygdala, thalamus, and cerebellum) did not show evidence of positive selection, as might be expected given the brain expansion and increase in cognitive abilities that occurred in human evolution. In fact, genes with highest expression in the brain were among those having the least evidence of positive selection as indicated by a *lack* of nonsynonymous substitutions. Such a pattern indicates functional constraint among genes expressed in the brain. It was hypothesized that adaptive changes in the brain along the human lineage may have involved changes not so much in protein-coding portions of genes, but changes instead in gene organization, gene expression, or in brain-development and regulation genes. This finding is consistent with results of gene expression studies, which indicate that up-regulation of gene-expression in the brain (and more specifically within the cortex) is one of the most important ways in which human brains differ from chimpanzee brains (Fisher and Marcus, 2006). Indeed, a scanning study of 214 nervous system genes by Dorus et al. (2004) found that genes playing roles in the development of the nervous system in humans showed the greatest degree of positive selection.

Despite the finding of overall conservation of genes maximally expressed in the brain (Nielsen et al., 2005a), several "candidate-gene" studies have revealed intriguing examples of genes associated with brain development that display footprints of selection in their coding sequences. One gene is *ASPM*, in which mutations are known to result in severe reduction in cerebral cortex size. The *ASPM* gene (abnormal spindle-like microcephaly associated; also known as *MCPF5*) may function to regulate neural stem cell proliferation and differentiation during development (Mekel-Bobrov et al., 2005). In chimpanzee-human contrasts, the d*N*/d*S* ratio (1.44) indicates an excess of nonsynonymous over synonymous substitutions along the human lineage (Evans et al., 2004a). The McDonald-Kreitman test also showed that the number of nonsynonymous differences along the human lineage was significantly greater than expected under neutrality (Evans et al., 2004a). Neither the chimpanzee or orangutan branches (gorillas were not included in analysis) show an excess of nonsynonymous change (Zhang, 2003). Therefore, *ASPM* was proposed to be an example of a gene underlying cerebral cortex expansion in human evolution. Another brain-development gene that shows evidence of positive selection is *MCPF1* (microcephalin). Mutations in *MCPF1* cause severe reduction in brain size but with overall brain structure left intact. The gene is thought to control the proliferation and differentiation of neuroblasts during neurogenesis (Evans et al., 2005). In comparisons of *MCPF1* orthologues among primate species, the gene showed signatures of strong positive selection in the lineage leading to humans (Evans et al., 2004b).

Motivated by these findings based on between-species comparisons, each of these genes (*ASPM* and *MCPF1*) has recently been analyzed in diverse population samples of more than 1,100 individuals (Evans et al., 2005; Mekel-Bobrov et al., 2005). One set of haplotypes found at *MCPF1* (termed D haplotypes, with D indicating derived), was found to be the most common set of haplotypes worldwide, except in sub-Saharan Africa where ancestral (non-D haplotypes) dominate. The D haplotypes are distinguished by a G → C mutation in exon 8 causing an aspartate to change to histidine. Three aspects of *MCPF1* polymorphism point to positive selection on the D haplotypes: 1) measures of diversity (π) are twelve times lower for D haplotypes as compared to non-D haplotypes; 2) Tajima's *D* (-2.3) is significantly negative for D haplotypes, whereas it is not significantly different from zero for non-D haplotypes; 3) LD extends over a much larger distance at the *MCPF1* gene in D haplotypes (29 kb) compared with non-D haplotypes. At the *ASPM* gene, the derived haplotypes (again known as D haplotypes) were found at moderately high frequencies (44%) in Europeans and Middle Easterners, but are typically at low frequencies in most populations in sub-Saharan Africa and the Americas (0.0 – ~10%). At this locus, D haplotypes are distinguished from non-D haplotypes by an A → G nonsynonymous polymorphism at position 44,871. There are several indications that D haplotypes experienced recent positive selection in Eurasia relative to non-D haplotypes: 1) LD extends over the entire length of *ASPM*'s coding region (62.1 kb) for the D haplotypes, but there is no evidence for increased LD at non-D haplotypes; 2) F_{ST} is high (indicating substantial population differentiation) between European (and Middle Easterners) compared with all other world populations (0.29), and is high between Europeans (and Middle Easterners) compared with sub-Saharan Africans (0.31). Positive selection on the derived haplotypes at both *MCPF1* and *ASPM* is estimated to have occurred recently. At *MCPF1*, the estimated time of coalescence of the presumed positively selected D haplotypes is 37,000 years (compared to ~1.7 million years for the coalescence of all chromosomes at this locus). At *ASPM*, the estimated coalescence of the positively selected D haplotypes is 5,800 years (compared to ~800 thousand years for all chromosomes at this locus). These times considerably postdate the time of transition from archaic hominids to anatomically modern humans (~130,000 years; see Lahr and Foley, 1998), and so the signal of positive selection we see today at these genes was probably not left by selection associated with this transition. Instead, the signatures of selection may be associated with more recent events in human prehistory. With respect to *MCPF1*, positive selection may have occurred during the initial migration of modern humans into Europe around 40,000 years, and positive selection at *ASPM* may have occurred concomitantly with the cultural innovations of agriculture, animal domestication, or with the advent of cities, states

and written language (Evans et al., 2005; Mekel-Bobrov et al., 2005).

Genome-wide scans for positive selection within human populations

Genome scans for selected genes based on comparisons between humans and chimpanzees are efficient at recovering selective episodes that took place in the deeper past of our species' history (i.e. over millions of years). However, it is undoubtedly true that selection has acted at more recent time-scales, for which tests based upon dN/dS ratios have limited power to detect. To detect such episodes of selection, three main approaches have been employed. First, searches have been made for regions of the genome that show extremely high or low values of differentiation among populations (as measured by statistics such as F_{ST}). Regions with unusually high or low amounts of differentiation are candidates for positive selection and balancing selection, respectively. Second, searches have been made using methods that summarize the site frequency spectrum (SFS), such as Tajima's D and Fay and Wu's H. Regions of the genome exhibiting extreme values are candidates for selection. Third, searches have been made of regions of the genome exhibiting unusually high values of LD and low levels of variation. Such regions may contain genes that are candidates for recent positive selection.

F_{ST}-based scans for selected genes. Early scans for selection in the human genome utilized the expected effect of selection upon inter-population differentiation to detect putatively selected genes or regions of the genome. In such studies, the difficulty lies in establishing an expected distribution of F_{ST} values based upon a neutral model. Such a distribution would obviously be sensitive to the assumptions made concerning the demographic history of these populations. For this reason, studies typically employ a strictly empirical approach, in which those loci or regions of the genome demonstrating the most extreme values of F_{ST} with respect to the genome average are considered to be associated with regions under selection (e.g. Kayser et al., 2003).

A higher resolution scan for regions of extreme F_{ST} was carried out by Akey et al. (2002), based on a set of 26,530 SNPs from three samples: 42 African Americans, 42 East Asians, and 42 European American individuals. Overall, 174 genes were detected as showing signs of selection. The largest fraction of positively selected genes were enzymes (hydrolases, kinases, transferases), whereas signal transducers (both ligands and receptors) represented the functional class with the greatest number of genes with low F_{ST} values (Akey et al., 2002).

Perhaps the greatest limitation of this, and other early F_{ST}-based studies (e.g. Kayser et al., 2003) concerns the low density of the SNPs, which would result in failure to detect selection at genes that did not have SNPs nearby. One example of this limitation (acknowledged by Akey et al., 2004) was the study's failure to identify the Duffy (*Fy*) gene as a candidate for selection, even though Duffy is known to display an extremely high F_{ST} between Africans and non-Africans (see Duffy section this paper). The failure is explained by the fact that the closest SNP to the locus was located ~80 kb away.

Approaches based on the site frequency spectrum. With the increased availability of sequence data for population samples, it has become possible to compute summary statistics such as Tajima's D for large numbers of genes. Two recent studies surveyed large numbers of genes from a data set consisting of 24 African Americans (AA) and 23 European Americans (EA). Stajich and Hahn (2005) surveyed data for 151 genes made publicly available by Seattle SNPs, and found marked differences in the mean and variance of Tajima's D between African Americans and European Americans, with AA showing higher values, and EA showing lower values and a greater variance. Differences between populations in the overall distribution of Tajima's D for a large set of genes are expected to result from differences in their demographic histories. Thus, before making any attempts to interpret whether specific genes exhibited values of Tajima's D that deviated from neutrality, it was necessary to examine which demographic processes could account for these features of the data. Two simple demographic scenarios were explored using simulations and were shown to fit the data extremely well: a bottleneck scenario for the European Americans, and an admixture scenario (involving an ~25% genetic contribution of European Americans) for the African Americans. Importantly, although these demographic scenarios accounted for the main features of the data, there were two loci that displayed extreme Tajima's D values that remained significant even after demography was accounted for, and therefore represented robust inferences of selection. These two loci were the genes for the ABO blood group (which showed a significantly positive Tajima's D, indicative of balancing selection), and *TRPV6* (a gene expressed in the kidney, placenta and intestines and thought to serve in the rate-limiting step of calcium absorption), which showed highly negative values in EA, indicative of positive selection. A more recent analysis of *TRPV6* polymorphism (Akey et al., 2006) confirmed a signal of positive selection (though found it to be more widespread in non-Africans), and also found possible evidence for balancing selection in some African populations (Akey et al., 2006). Although Akey et al. (2004) hypothesized that positive selection on *TRPV6* may be associated with dietary changes during the agricultural revolution and specifically with the use of milk, the precise functional effects of particular *TRPV6* variants (presumed to have been selected) need to be analyzed in functional studies.

Although Stajich and Hahn's (2005) study only revealed two genes to be candidates for selection, these researchers found evidence for selection in another feature of the data. There was an overall positive correlation between Tajima's D for the 151 genes in the EA and the known recombination rates for genomic regions containing these genes. This finding was interpreted to result from multiple selective sweeps that occurred as human populations migrated out of Africa. Africans showed comparatively less evidence of selection, which the researchers explained to be due to the fact that Africans remained in regions where ancestral human populations had lived (an explanation that subsequent studies have re-examined).

Akey et al. (2004) carried out a similar study in which they surveyed 132 genes in the same set of AA and EA individuals. These loci were chosen due to their involvement with aspects of the inflammatory process in humans. In addition to Tajima's D, the authors employed other methods (Fu and Li's D and D^*, and Fay and Wu's H). Initially, the sequence data was tested against the null hypothesis of neutrality-equilibrium, and the authors found 22 genes that deviated significantly from the null hypothesis for at least one of the tests. Once again, the effects of selection and demography are confounded in such analy-

ses, making it difficult to attribute the deviations to one factor or another. To address this issue, the authors employed computer simulations under a broad range of demographic scenarios to find the parameters that best accounted for the observed data (these scenarios included exponential population expansion, population bottlenecks, population structure with gene flow, and population divergence without subsequent gene flow). Conservatively, the authors considered as robust inferences of selection those cases in which a test statistic was significant when compared to the null distribution derived from all distinct demographic models (Akey et al., 2004). Of the original set of genes with significant deviations, eight remained significant in EA and none in AA, a finding highlighting the fact that demographic history alone can account for a large proportion of the deviations observed when neutral tests are applied under the assumption of an equilibrium model of population history. The finding that there was greater evidence of selection amongst EA compared to AA was again interpreted to indicate that the European-derived population was exposed to novel and recent evolutionary pressures, and that the African-derived population experienced comparatively fewer episodes of recent positive selection.

In summary, the studies of both Stajich and Hahn (2005) and Akey et al. (2004) convey similar ideas: the analysis of sequence data from large numbers of genes reveals strong signatures of the populations' demographic histories, yet signatures of selection at specific genes can be detected. In addition, both studies claimed that recent natural selection has left a stronger mark upon European-derived than African-derived populations, possibly due to the novel environmental challenges that human populations faced as they migrated from Africa and inhabited new geographic regions. One of the main limitations of these studies, however, concerns the relatively low number of genes surveyed, which consisted of less than 1% of the human genome. Thus, although these studies have been useful in demonstrating the potential of genome-scanning approaches, and in providing valuable information about the relative effects of demography and selection in shaping polymorphism, their potential to uncover novel putatively selected genes on a genome-wide scale has been limited.

A more comprehensive scan for selection would require sequencing of large numbers of genes from many individuals. Although such data is currently not available, new genome-wide data sets are becoming available that consist of genotype surveys of known SNPs (e.g. Hinds et al., 2005; International HapMap Consortium, 2005). The crucial difference between SNP data and sequence data is the way that SNPs are selected for subsequent analyses. Typically, preliminary sequencing surveys on smaller subsets of individuals detect sites that are polymorphic, and in which the minor nucleotide variant (the one which occurs at lowest frequency) is observed above some pre-established threshold. These "ascertained SNPs" are then used for subsequent analyses. When ascertained SNPs are genotyped in a larger population sample, they will be more polymorphic (i.e. have higher levels of variability) than a randomly chosen polymorphic site. Because of this feature, known as ascertainment bias, a survey of natural selection based on a statistic such as Tajima's D, which is sensitive to the frequency at which variants occur within a sequence, does not seem to be theoretically sound.

A recent study by Carlson et al. (2005) attempted to address this issue by directly investigating the effect of ascertainment bias upon Tajima's D. They assembled two data sets for a set of 170 genes that contained different types of information. For each individual, data was available both in the form of dense genotype data (i.e. ascertained SNPs) *and* resequenced data (i.e. complete sequences of the genes for each individual). Tajima's D values for the two data sets were compared. As expected, Tajima's D for the ascertained data was found to be greater than Tajima's D for the sequence-based data, because SNP ascertainment generates data sets with an excess of intermediate-frequency variants. However, despite this bias, values of Tajima's D for the two data sets were found to be strongly correlated. This implied that low values of Tajima's D, revealed by a survey of SNPs, corresponded to regions of low Tajima's D, as inferred by complete sequencing. Thus, Tajima's D could be used to scan the genome for regions of unusually low D values, even if the data analyzed consisted of ascertained SNPs.

Similarly to the previously described scans, Carlson et al. (2005) opted for an empirical approach that searches for outlier loci or genomic regions, rather than testing a null hypothesis. Their analyses relied on the Perlegen data set (Perlegen Sciences, http://genome.perlegen.com; see Hinds et al., 2005), which consists of more than a million SNPs typed in African Americans (AA), European Americans (EA) and Chinese Americans (CA). Values of Tajima's D were obtained for polymorphisms contained within windows of 100 kb, with windows being shifted over the genome in 10 kb steps. A region likely to harbor positively selected genes was defined as one in which 20 consecutive windows presented Tajima's D values that were among the bottom 1% of the overall empirical distribution for the genome. Using this approach, seven regions of unusually low Tajima's D were found among Africans, 23 regions in the Europeans, and 29 regions in the Chinese. All but four of these regions were detected in a single population group, indicating that most selection was specific to populations rather than shared between them. To verify the reliability of these inferences, eight genes that fell within these putatively selected regions were chosen for full sequence analysis. The authors found a strong trend towards negative Tajima's D in all the genes, as well as unusually low levels of nucleotide diversity (π), as expected if these genes had experienced recent selective sweeps. Taken together, these results demonstrated the potential of surveys of SNPs within large windows of the genome to detect genes under recent positive selection.

Carlson et al. (2005) did not consider it likely that African populations had been subject to less selective pressures than non-Africans, as was proposed in earlier studies (e.g. Akey et al., 2004; Stajich and Hahn, 2005). Instead, they suggested that the different demographic features of these populations might lead to differential capacities to detect recent selection in each population. For example, because non-Africans probably experienced a recent bottleneck, Tajima's D is shifted towards more positive values. This will enhance the power of detection of positively selected genes, which show a negative Tajima's D that contrasts with the background variation. On the other hand, the larger effective population size of African populations may lead to lower amounts of selection being detected in this population. One explanation for this rests on the fact that in a population of large effective size (e.g. Africans) there is more opportunity for recombination compared to a population of smaller effective size (e.g. Europeans). Higher population recombination rates result

in shorter blocks of LD surrounding selected genes, making it harder to detect selected regions in the African data. Thus, it appears that denser sets of SNPs will be required in order to detect the shorter haplotypes in Africans that bear signatures of selection (Carlson et al., 2005).

Haplotypic diversity-based approaches. Using population genetics theory it is possible to make predictions about how positive selection will affect polymorphism along a chromosome. As selection drives an advantageous variant to fixation, it is expected to reduce variation at neighboring sites. This creates a *long haplotype of low diversity,* surrounding the selectively favored variant. Thus, a variant that has reached a high frequency due to natural selection will be surrounded by a region of low variation and will be embedded in a region of extended haplotype homozygosity (Sabeti et al., 2002). On the other hand, a variant that has attained a high frequency through the vagaries of genetic drift, would have risen in frequency over longer spans of time allowing several recombination events to have taken place. As a consequence, we do not expect to find reduced haplotype diversity or extended regions of haplotype homozygosity surrounding haplotypes that have reached high frequencies due to genetic drift. Sabeti et al. (2002) illustrated the feasibility of using these predictions to detect selected genes by analyzing the extent to which diversity had been reduced on haplotypes carrying known selectively favorable mutations. They applied the Extended Haplotype Homozygosity method (EHH; described in the *Theory and Analytical Methods* section) to two different loci with prior evidence of positive selection (i.e. *G6PD* and the *CD40* ligand). The method successfully revealed a signature of positive selection at these genes.

The use of the EHH method in "genome-wide" scans requires a data set of densely typed SNPs, so that searches can then be conducted in an exploratory manner, identifying regions that appear to have recently experienced (or are currently undergoing) a selective sweep. The completion of phase I of the HapMap project provided such a data set (International HapMap Consortium, 2005). Voight et al. (2006) analyzed approximately 800,000 SNPs in each of three population groups: Asian (ASN), Yoruban (YRI), and Central European (CEU). Based on these data, haplotypes for each individual were inferred, and these were used to scan the genome for regions exhibiting significant evidence of positive selection. In brief, the statistical method employed in this genome-scan (termed *iHS*, for integrated EHH) focused on SNPs and examined the length of haplotypes of low-diversity on which they are found. A ratio is computed between the sizes of the haplotypes carrying the ancestral variant, and the sizes of the haplotypes carrying the derived variant at the focal SNP. When ancestral and derived variants sit on haplotypes of approximately equal length, this ratio is one. However, the ratio is skewed when one of the haplotypes of low diversity is longer than the other. Positive selection can generate such a skew because the haplotypes close to a selected variant will display longer stretches of extended homozygosity (i.e. regions of low variation) due to their rapid rise in frequency. Extreme values for *iHS* are indicative of recent selective episodes. As in previous genome-wide scanning analyses, the goal of the study by Voight et al. (2006) was not to formally test the data against a null hypothesis, but rather to detect regions of the genome that behaved as outliers, exhibiting unusually high levels of *iHS*.

When this approach was applied to the SNP data, several regions of the genome displayed clusters of SNPs with high *iHS* values, indicating long haplotypes of reduced diversity. Would such clustering be expected in the absence of positive selection? To answer this question, the authors examined several additional aspects of the data. Using simulations, they were able to show that the empirical *iHS* scores are more extreme and more clustered than those obtained using a variety of nonequilibrium models of population history (including bottleneck and population growth models). Additional evidence that the clustering of SNPs with high *iHS* was a consequence of selection came from application of Fay and Wu's *H* statistic, which showed that the regions of high *iHS* usually also had strongly negative values of *H*, indicative of directional selection. Furthermore, regions where SNPs with high *iHS* were clustered often contained genes known to be selected based on previous studies (e.g., the lactase gene in Europeans). Together, these results demonstrated that observed *iHS* values cannot be explained by demographic factors alone, and that genes containing many SNPs with extreme *iHS* values could be considered candidate-genes of positive selection.

The genomic regions identified by Voight et al. (2006) were further analyzed in order to identify particular genes showing signatures of selection, and to ascertain the geographic location and time frame of selection. The inferred age for the putative sweeps showed that selection events were mostly recent, for all populations. Importantly, positive selection tended to postdate the time at which these populations diverged, indicating that they are population-specific selection events. The size of swept regions was found to be larger in non-Africans than in Africans. In concordance with this, the ages of selective sweeps were estimated to be more recent in non-Africans compared with Africans. Further insights into the nature of selection were obtained by analyzing the types and functions of genes that were putatively under selection. Among the categories of genes showing significant enrichment of selected genes were several categories previously identified in scanning studies of genes selected along the lineage leading to humans (Clark et al., 2003; Nielsen et al., 2005a,b). These include genes involved in chemosensory perception, olfaction, gametogenesis, spermatogenesis and fertilization. Additionally, five distinct genes (*OCA2, MYO5A, TYRP1*, *DTNBP1,* and *SLC24A5*) involved in skin pigmentation were identified to have experienced sweeps in Europeans, consistent with the hypothesis that selection favored a phenotypic change towards lighter skin as populations migrated to higher latitudes. A number of genes involved in carbohydrate metabolism were also inferred to have been selected, as would be expected if the exploitation of food resources changed as populations moved into new geographic regions and as cultural innovations (e.g. agriculture and animal domestication) generated novel selective pressures. Several categories of genes not singled out in previous studies also showed up in this analysis such as electron transport and phosphate metabolism genes.

Future prospects for scanning-studies of positively selected genes

The survey of variation in the human genome with the goal of detecting selected genes is in its infancy, but early results are promising and the field is attracting much attention. Thus far, it has been demonstrated that the var-

iation within and between populations is shaped largely by the demographic history of populations, yet genes that have experienced recent selection show patterns of variation that cannot be accounted for by demography alone. Tests based on the presence of long haplotypes of low diversity (Sabeti et al., 2002; Voight et al., 2006), on patterns of interpopulation differentiation (Akey et al., 2002), and on site frequency spectra (SFS) have all been shown to have the potential to detect putatively selected genes (e.g. Stajich and Hahn, 2005, Carlson et al., 2005), and recent studies have shown the potential of applying tests based on SFS to data collected from large-scale genotyping efforts (Kelley et al., 2006). As the number of studies increases, it becomes possible to compare their results. The concordance in the lists of putatively selected genes between the studies of Kelley et al. (2006), a very recent scanning analysis, and Carlson et al. (2005) was extremely high, which is expected since both used extreme values of Tajima's *D* to delimit selected genes or genomic regions (the former searched for outlier genes, and the latter searched for large genomic regions with low Tajima's *D*). In addition, approximately one-third of the genes detected as candidates for selection in Kelley et al. (2006) were within 100 kb of regions with high *iHS* values (the statistic used to detect selected regions based on extended regions of haplotype homozygosity; see Voight et al., 2006). Similarly, a scanning study by Wang et al. (2006) using a novel LD-based test showed even greater overlap with the results of Kelley et al. (2006). The extent of overlap is a promising indication that different methods can detect the same genes and genomic regions. The lack of overlap, on the other hand, can have several causes. First, some methods have relative high false-discovery rates (e.g. Kelley et al., 2006). Second, methods based on testing the null hypothesis of neutrality have low statistical power (i.e. they often fail to detect selected loci). Third, tests vary as to the type and time frame of selection they have greater ability to detect.

The wide diversity of analytical methods to detect selection is an asset to investigators, since methods for detecting selection are known to differ both in their capacity to identify selection that occurred at different time-depths, as well as different selective regimes. For example, tests based on dN/dS ratios are sensitive to adaptive evolution over longer spans of time, typically corresponding to the divergence between species, but lack power to detect recent selective episodes, which have resulted in few nucleotide substitutions. Tests based on deviations from the site frequency spectrum, on the other hand, are particularly powerful to detect recent episodes of selection within human populations (Sabeti et al., 2006). Among these, Fay and Wu's *H* test (Fay and Wu, 2000) is capable of distinguishing between purifying and positive directional selection, thus complementing the results obtained by Tajima's *D*. Tests based on patterns of linkage disequilibrium are sensitive to even more recent selective episodes (e.g. on the order of 20,000 years, see Sabeti et al. 2006), but are inadequate to detect selective events that have resulted in *complete* selective sweeps (such as took place at the Duffy locus in Africa). This occurs because complete sweeps erase haplotypic diversity at the selected site, fixing the favorable variant. This makes it impossible to compare the extent of haplotypic diversity between putatively selected and nonselected haplotypes.

Limitations of the methods must also be kept in mind, so as to avoid simplistic interpretations of the results. An important point concerns the tests applied to detect selection since the divergence between humans and chimpanzees, which have relied on dN/dS ratios. These tests are only applicable to coding sequences and thus cannot provide information on selection upon noncoding regulatory elements. Given the likely importance of regulatory changes in human evolution (King and Wilson, 1975; Carroll, 2005; Khaitovich et al., 2006), the development of methods to test for selection in such regulatory elements (e.g. Keightley et al., 2005) will be of key importance in the future.

Features of the data sets assembled to test for selection are also likely to have an important influence on the results. With the exception of the study of selective sweeps based on haplotypic variation (Voight et al., 2006), all other analyses have detected significantly less evidence for selection among Africans than non-Africans. Although this has been interpreted by some (e.g. Stajich and Hahn, 2005) to be a consequence of differences in the amount of selection and nature of selective regimes between Africans and non-Africans, it seems more likely that this is an outcome of the size of the genomic regions that carry the signature of selection (which may be smaller in Africans than in non-Africans; see Carlson et al., 2005). Indeed, Voight et al. (2006) found similar numbers of genomic regions with signals of selection in all populations. Thus, it remains to be seen whether analyses based on population differentiation, and those based on the site frequency spectrum, will be able to detect selection among Africans when denser SNP data are available. In fact, the most recent study based on identifying outliers in the site frequency spectrum detected approximately equal numbers of putatively selected genes in the three major population groups studied (African Americans, Chinese, and European Americans; Kelley et al., 2006), suggesting that denser SNP data is indeed necessary to detect selected regions in populations with lower LD (as is the case for Africans).

Important developments have also taken place in the analyses of scans for selection on the lineage leading to humans. A caveat of earlier studies was the inability of tests based on dN/dS ratios to distinguish between relaxation of selective constraint and positive selection (Zhang, 2004; Zhang et al., 2005; Arbiza et al., 2006; Eyre-Walker, 2006). This led Zhang et al. (2005) to propose a method that directly tests for positive selection on specific lineages. In essence, this is accomplished by performing a likelihood ratio test that compares a model of evolution in which specific lineages are under positive selection ($dN/dS > 1$) to a null model in which these same lineages are constrained to having $dN/dS = 1$ (as expected for lineages evolving under relaxed selective constraints). The application of this new test in one recent genome-scanning study (Arbiza et al., 2006) showed that some lists of putatively selected genes resulting from previous genome-wide studies might include a considerable number of false positives (i.e. genes that actually have not been positively selected). For instance, Arbiza et al. (2006) found that many genes within the olfaction category, one of the categories identified by Clark et al. (2003) as being enriched for genes under positive selection, have instead undergone relaxation of selective constraint, not only along the human lineage but also along the chimpanzee lineage (Arbiza et al., 2006). An advantage of these new statistical methods is that they will allow not only the detection of actual positive *adaptive* changes that differentiate humans from chimpanzees, but also will allow the detection of genes that have become nonfunctional or otherwise minimized

in functional importance in particular lineages (as evidenced by the relaxation of selective constraints). The challenge of distinguishing between positive selection and relaxation of selective constraint will not be without complications, however, since distinguishing these phenomena will be difficult for genes having weak signals of positive selection (Arbiza et al., 2006).

These genetic studies will doubtless fuel investigation at the anthropological, physiological and molecular levels by providing lists of candidate genes for selection, that can be further investigated from a functional perspective. At another level, the study of selection on the human genome is also revisiting important questions that occupied evolutionary biologists for the last decades. One such question concerns the rate at which genomes experience adaptive change (Kimura, 1969). The data generated by genome sequencing allows estimates of the amount of adaptive change that our species has experienced by analyzing the amount of nonsynonymous change over the entire genome. Initial studies suggest that the human lineage is experiencing adaptive change at a substantially lower rate than *Drosophila* and microorganisms (Eyre-Walker, 2006). However, although the rate of adaptive evolution in our species appears to be lower than that of *Drosophila,* genomic scans such as that of Voight et al. (2006) indicate that a substantial number of regions in the genome have experienced partial selective sweeps. This finding has important implications in light of a model proposed by Gillespie (2000) that concerns the effect of selection upon closely linked sites. According to Gillespie, selected loci can cause fluctuations in the frequency of closely linked sites. A corollary of Gillespie's model is that changes in frequency over many regions of the genome would not be the result of genetic drift, but rather an outcome of the effects of selection at closely linked genes. If there were in fact many genes in our genomes that experienced positive selection, this would imply that human genetic variation might be shaped to a large extent by selective processes, and that standing levels of human variation will need to be interpreted in the light of both selection and genetic drift.

DISCUSSION AND CONCLUSIONS

We have reviewed evidence for natural selection contained in patterns of DNA polymorphism at genes underlying four classically studied human traits (see Table 3 for summary of selection). For several of these genes, specifically those underlying the perception of bitter-taste, lactase persistence, and the Duffy blood group, we have strong evidence indicating that natural selection has been a dominant force in their evolution. The evolution of *MC1R*, which is one of the loci underlying variation in human pigmentation, represents a more complex case, since polymorphism has been interpreted both as a consequence of selection or neutral evolution.

Much of our understanding of the nature, geographic distribution, level of polymorphism, and evolution of these traits rests on a body of data collected in the middle of the 20th century. These data include phenotype distributions, results of simple physiological assays, and surveys of allele frequencies (e.g. through studies of protein polymorphism). Such information has served as the basis on which anthropologists and human biologists have proposed various hypotheses to explain the evolution of these traits. We have reviewed many of these hypotheses, and evaluated the nature of the signature of selection with regard to our current knowledge of DNA variation. It is therefore appropriate to ask: In what ways have the DNA data allowed us new insights into human adaptation? Below, we highlight specific contributions made by genetic studies that reveal their potential to test traditional hypotheses, and also to raise new questions.

The study of lactase persistence provides a good example of how a trait originally studied in the absence of genetic information benefited from the new DNA data. A DNA variant that is likely to have a causal effect on the phenotype was detected (−13910*T) (Enattah et al., 2002), and patterns of polymorphism at the *LCT* gene were shown to statistically support a scenario of positive directional selection favoring this variant in northern European populations (Bersaglieri et al., 2004). Furthermore, using population genetics theory an estimate was provided for the age of the selective event that drove the allele for lactase persistence to high frequency, placing its rise in frequency at a recent time, consistent with the estimated origin of dairy farming in northern Europe, ~9,000 years ago. This inferred date led to the realization that the strength of selection on lactase persistence (as measured by the coefficient of selection) was as great or perhaps even greater than selection on other loci such as *G6PD* and β-globin. The genetic data also revealed that a similar phenotype of lactase persistence, present in some African populations, has a different genetic basis, and thus is likely to represent a trait that arose independently (Mulcare et al., 2004). While selection on lactase persistence appears to have been very recent, the origin of the putative causal mutation appears to be much older and probably predates the differentiation of non-African populations (Bersaglieri et al., 2004). An early origin for the mutation is inferred since it is present in many non-African populations from Asia and Europe, even if only at relatively low frequencies. Thus, several tens of thousands of years are likely to have elapsed between the origin of the mutation and the onset of selection. This underscores the pivotal role that cultural innovations (in this case the development of dairying cultures) have played in human evolutionary history by creating new selective environments.

Our understanding of the evolution of diversity in skin color has improved considerably due to recent genetic findings. First, it was only recently that a gene (*MC1R*) was shown to have an effect on pigmentation in normal human populations (Rees, 2004). The genetic analysis of this gene provided support for a model of selection favoring dark skin in Africa (Harding et al., 2000). The finding that this gene is highly polymorphic in Eurasia served to raise two alternative hypotheses regarding selection in this region: the original selective constraint may have been relaxed, so that the gene is evolving neutrally, or it may be under diversifying selection. More recently, new genes have been discovered that appear to be associated with variation in pigmentation, and for many of these there is evidence of positive selection (Izagirre et al., 2006; Voight et al., 2006). These results underscore the multigenic and adaptive nature of human skin color, and suggest that selection upon pigmentation must be studied in the context of the effects of multiple genes.

The study of the Duffy blood group polymorphism and its relation to resistance to the malarial parasite *Plasmodium vivax,* provides an additional example of how a perspective based on DNA variation offers new insights into human adaptive evolution. Key to the understanding of how this polymorphism evolved is the estimation of the

TABLE 3. Summary of the evidence for selection at each of the genes associated with trait focused on in this paper[1]

Trait/Gene	Mode of selection	Geographic focus of selection	Indicators of selection from DNA polymorphism	Time frame of selection
Lactase *LCT*	Positive directional	North Europe	High LD: High F_{ST}/P_{excess}	2,188–20,650 years in European derived population (Bersaglieri et al., 2004) 1,625–3,188 years in Scandanavian population (Bersaglieri et al., 2004) 7–12 Ka (Coelho et al., 2005)
Duffy *Fy*	*FY*O* Positive directional	Sub-Saharan Africa	High F_{ST} Reduced polymorphism Africans (HKA test) Excess of high frequency derived mutations (*H* test)	33,075 years (6,500-97,000; 95% confidence interval) (Hamblin and Di Rienzo, 2000)
	*FY*A* Possible positive directional	Asia	High F_{ST} Pattern is heterogeneous over the gene region and is not consistent with any simple model of selection or demography	
Bitter-taste *TAS2R38 (PTC)*	Balancing selection	Worldwide	Excess intermediate frequency polymorphisms (Tajima's *D*, Fu and Li's *D* and *F* statistics under pop growth scenarios)	~1.5 Ma (Wooding et al., 2004)
TAS2R16	Positive directional	Worldwide	Excess low and high frequency derived mutations (Fay and Wu's *H* test) High LD High F_{ST}	83–791 Ka 78–685 Ka (Soranzo et al., 2005)
Skin Color *MC1R*	Purifying selection	Africa	Low nonsynonymous polymorphism (MK test)	Along the humans lineage, after split from chimpanzees
	Balancing selection (favoring loss of function mutations) or neutrality	Eurasia	High nonsynonymous polymorphism	<40 Ka years ago

[1] For each gene, we summarize the regime of selection supported by the DNA data, the inferred geographic focus of selection, and the range of different time-estimates that have been hypothesized for when selection occurred.

age of the selective event that explains the high frequency of the Duffy null allele (*FY*O*) in Sub-Saharan Africa. The molecular genetic data allowed such estimates to be made, and a relatively old date ($\sim$33,000 years) for the selective event was inferred (Hamblin and Di Rienzo, 2000). This date could imply that selective pressures that shaped the distribution of the Duffy null allele in Africa may be unrelated to the presence of *Plasmodium vivax*, the pathogen usually associated with selection on Duffy. Accordingly, an improved understanding of the evolution of the Duffy blood group, and evaluation of *P. vivax* as the potential selective agent, will require information on the evolution and biogeography of this pathogen. In this context, recent analyses of *Plasmodium* genetic variation have provided critical information on the ancestry and age of the pathogen among Africans.

The study of DNA polymorphism within bitter-taste receptor genes has enhanced our understanding of the evolution of human taste sensation. Study of the repertoire of bitter-taste genes has shown that humans have not undergone diminishment in the number of these genes compared to other primates, as has been shown for other sensory gene families (e.g. olfaction, see Gilad et al., 2003). Many bitter-taste genes in human populations show an abundance of functional variants (Kim et al., 2005). This has been interpreted in two ways. It may point to a greater degree of variation in bitter-taste sensation among individuals and between populations than once appreciated, and could indicate that different functional variants were maintained by balancing selection (Kim et al., 2005). Alternatively, it may indicate that many bitter-taste genes are under relaxed selective constraints and are therefore free to evolve in a neutral fashion (Wang et al., 2004). This matter remains unsettled, but clearly both interpretations may hold true for specific genes.

At least two bitter-taste genes (*TAS2R38* and *TAS2R16*) were intensely studied for signatures of selection in their patterns of DNA polymorphism. For one of these genes (*TAS2R38*; or *PTC* locus), RA Fisher's (1939) hypothesis, that balancing selection has acted to maintain the taster/nontaster polymorphism at intermediate frequencies in human populations, has been supported (Wooding et al., 2004). It should be emphasized, however, that the effect of selection at this gene was only detectable after the effects of demography were accounted for. This underscores the importance of incorporating realistic demographic models into the neutral hypothesis against which we look for selection. On the other hand, Fisher's hypothesis that the taster/nontaster polymorphism is ancient and predates the separation of human and chimpanzees was not

supported by the DNA data, which instead indicates that the human taster and nontaster haplotypes diverged approximately 1.5 Ma (Wooding et al., 2004; 2006). Studies of *TAS2R38* in chimpanzees have indicated that their nontaster haplotype has a different molecular basis from that in humans, and have indicated that this haplotype arose independently along the chimpanzee lineage (Wooding et al., 2006). Another bitter-taste gene (*TAS2R16*) was also found to carry a clear signature of selection. For this gene, positive selection appears to have acted to bring two similar haplotypes (A and B) to near fixation frequencies in many populations worldwide (Soranzo et al., 2005).

The studies of the *TAS2R16* and *TAS2R38* bitter-taste genes illustrate another way in which DNA polymorphism data have aided the study of selection. The discovery of functional DNA variants in these genes through population analyses made it possible to experimentally identify (through *in vitro* and *in vivo* studies) precisely which DNA variants are responsible for differences in receptor-sensitivity to toxins (Bufe et al., 2002; 2005; Soranzo et al., 2005; Wooding et al., 2006).

These success stories clearly demonstrate the utility of investigating human adaptations from a genetic perspective. However, they also serve as a reminder that genetic analyses would be of limited utility in constructing adaptive arguments in the absence of additional sources of information. For all four case studies presented in this review, an understanding of the patterns of genetic variation required careful analysis of data from various biological fields. For example, the analyses of selection on skin color used information regarding nutrient degradation due to UV radiation, skin cancer susceptibility as a function of pigmentation, vitamin D synthesis dependence on UV radiation, archaeological information on recent human migrations, as well as the biochemical consequences of specific mutations on the *MC1R* gene (Barsh, 2003). Similarly multidisciplinary approaches were employed in the discussion of selection upon the Duffy null alleles, the lactase persistence allele, and the evolution of bitter-taste receptors.

The most common approach in the study of adaptation involves studying a phenotypic trait and then trying to understand its underlying genetic basis. Indeed, this approach forms the basis of what we have called the "candidate-gene" approach, in which *a priori* information about the function of the gene and its association with a variable trait led to its being analyzed for DNA polymorphism. We believe such approaches will continue to be useful in searching for the signature of selection for classically identified adaptations for the human species as a whole, as well for population specific adaptations. Although the candidate gene approach has been traditionally employed in genetics, the results obtained by genome scans for selected genes are likely to have a large impact on the field and may result in a reverse flow of information. That is, candidate genes are detected, and their phenotypic expression must subsequently be understood. Advances in gene-expression methods in which the link between genotype and phenotype can further be understood will complement this approach. Although genome scans for selected regions are providing us with lists of candidate genes for natural selection, they clearly cannot exhaust our understanding of human adaptation. Candidate genes that emerge from scanning studies are on their own simply points of departure for research of human adaptive evolution, and must be placed within a multidisciplinary context so that adaptive hypotheses can be formulated and tested.

Within the field of genetics, several developments will further enable the study of adaptive evolution. With respect to genome scans, data sets consisting of more densely typed SNPs will provide greater power to detect selected regions of the genome. Future studies will also benefit from increased geographic sampling of populations. Current studies rely on broadly-defined and, in some cases, admixed populations (e.g. Akey et al., 2004; Carlson et al., 2005; Nielsen et al., 2005b; Stajich and Hahn, 2005; Voight et al., 2006; Zhang et al., 2006). Samples from populations with less admixture may provide increased power to detect selection, since population specific signatures will not be diluted by the presence of genes from other populations. On the other hand, studying admixed populations may in some cases be a valuable approach for establishing correlations between specific genetic variants and phenotypes (e.g. Lamason et al., 2005). Nonetheless, assembling population samples at a finer resolution will allow for the detection of episodes of positive selection that are restricted geographically. For instance, uncovering selection at genes underlying adaptations to life at high-altitude in Tibetans (Beall, 2000 and Gelfi et al., 2004) will benefit by intensive screening in particular populations.

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