Reports

Genetic Variation at the MC1R Locus and the Time since Loss of Human Body Hair[1]

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The melanocortin 1 receptor (MC1R) locus makes a protein that affects the color of skin and hair. At this locus, amino-acid differences are entirely absent among African humans, abundant among non-Africans (especially Europeans), and abundant in chimpanzee/human comparisons (Rana et al. 1999, Harding et al. 2000). Previous studies have found no evidence of either directional or diversifying selection, so the pattern in these data has been attributed to tight selective constraint within Africa—a defense against the strong sunlight there—and relaxed constraint in Eurasia (Harding et al. 2000). This interpretation implies that genetic differences within Eurasia have an ancient history and suggests the likelihood of admixture between Neandertals and anatomically modern humans. We reanalyze these data here and interpret them differently. We suggest that adaptive evolution for sun-resistant MC1R alleles ensued when humans first became hairless savannah-dwellers. The level of neutral MC1R variation among African humans provides a lower bound for the time since this event.

Although there are no amino-acid differences at the MC1R locus among African humans, there are ten between the human last common ancestor and the chimpanzee (Harding et al. 2000). This suggests that selective constraint may have been weaker in the distant past. Furthermore, many of these differences are nonsynonymous (cause changes in the protein). This is surprising because, in functional genes, most nonsynonymous mutations are detrimental and are quickly removed by selection. Consequently, nonsynonymous differences tend to be rare. Between chimpanzee and the human last com-

mon ancestor, the number of synonymous differences per silent site is $K_s = 6/261 = 0.0230$ and the number of nonsynonymous differences per nonsilent site is $K_a = 10/693 = 0.0144$ (Harding et al. 2000:1352). Thus, $K_a/K_s = 0.63$, an unusually large number for a functional protein (Li 1997:180–81).

One view attributes this high nonsynonymous substitution rate to weak selective constraint (Harding et al. 2000). This requires that selective constraint be weak in the distant past (to account for chimpanzee/human differences) but strong in the recent past (to account for the absence of amino-acid variation among African humans). But if the African sun causes selective constraint now, why would this constraint have been weak in the past?

There are at least two obvious answers: selective constraint may have been weak in ancient hominids because they lived in forests or because their bodies were covered with hair. Several lines of evidence suggest that hominids were making extensive use of the savannah by 1.7 million years ago (Klein 1999:248–53). Some argue that hair was lost at this time, but this is controversial (see below). Whatever the timing, at some point in the past hominid bodies began confronting the African sun without the protection of forest or fur. It seems likely that this would have triggered selection for MC1R alleles that provide protection from sun. The resulting adaptive evolution may account for the high K_a/K_s ratio seen in comparisons between modern humans and chimpanzees.

To make this hypothesis credible, we need also to account for the pattern of variation in Eurasian MC1R data. In Eurasia (and especially in Europe) the majority of nucleotide differences are nonsynonymous. Indeed, K_a/K_s is about the same in comparisons among Eurasians as in the chimpanzee/human comparisons just discussed. Because of this similarity, the McDonald-Kreitman test provides no evidence for selection (Harding et al. 2000:1352). Yet if the nonsynonymous fraction is inflated by selection in the chimpanzee/human comparison, then selection is also needed to explain its value in Eurasians. The movement into northern climates may have favored fair skin because of the increased need to synthesize vitamin D or to protect against folate deficiency (Jablonski and Chaplin 2000). If so, selection would have favored any mutant MC1R allele that did not produce dark sun-resistant skin. This hypothesis accounts not only for the high Eurasian gene diversity but also for the large fraction of nonsynonymous Eurasian mutants. In addition, it accounts for the fact that the Eurasian gene genealogy is nearly star-shaped (Harding et al. 2000: fig. 2). Such genealogies are consistent with diversifying selection but not with a long history of neutral evolution in a population of constant size.

This hypothesis does not, however, explain the result of another statistical test, which also failed to show evidence of selection. The HKA test (Hudson, Kreitman,

TABLE 1
Data Used in Pairwise HKA Tests

Locus	m	n	S	D	χ^2	p
MC1R	954	443	13	17.35	–	–
Xq13.3	10,000	47	16	5.02	5.68	0.003
ZhaoNC22	9,901	88	44	133.80	1.20	0.288

NOTE: m, the number of nucleotide sites used in estimating S and D; n, haploid size of human sample; S, number of polymorphic nucleotide sites within human sample; D, mean difference between chimpanzee and humans; χ^2, HKA goodness-of-fit statistic; p, significance of HKA test with MC1R estimated from 10,000 simulated data sets.
SOURCES: MC1R, Harding et al. (2000); Xq13.3, Kaessmann et al. (1999); ZhaoNC22, Zhao et al. (2000).

and Aguadé 1987) compares ratios of between-species to within-species differences at different loci. Absent selection, these ratios should be similar, and this is exactly what was found in a comparison between MC1R and beta-globin (Harding et al. 2000). We can suggest several reasons this analysis might have failed to detect selection even if selection were really present. First, we have suggested that selection on MC1R inflated differences both within and between these species. If so, it may have had little effect on the ratio of between-species to within-species differences. Second, the HKA test assumes that population size has been constant and may yield misleading results in a species with a complex demographic history (Hudson, Kreitman, and Aguadé 1987). Finally, beta-globin and MC1R both produce protein, so the two ratios may be similar because selection has affected the two loci in similar ways.

To investigate this last possibility, we performed two additional HKA tests (shown in table 1) using loci that do not produce protein and are outside of any known gene. One of these gave strong evidence ($p = 0.003$) for selection at MC1R, but the other result was not significant ($p = 0.288$). In aggregate, these tests provide qualified support for the view that selection has affected MC1R. They certainly provide no contrary evidence. A recent analysis by C. Huff (personal communication) provides additional evidence of selection at MC1R.

Where does this leave us? The hypothesis of relaxed selective constraint is difficult to reconcile with the star-shaped Eurasian gene genealogy, the relatively high K_a/K_s ratios, and the significant HKA result. The hypothesis of positive selection explains all this and is also consistent with the general weakness of statistical support for selection. We feel that, on balance, the evidence favors the positive-selection hypothesis. Although this case is not yet compelling, it seems worth asking how our interpretations of human history would change should the positive-selection hypothesis turn out to be correct.

The first casualty would be the inference that European alleles for red or blonde hair were derived from admixture with Neanderthals. That inference is based on the conclusion that the root of the European gene genealogy is several hundred thousand years old, a con-

clusion that was reached using a neutral model of evolution (Harding et al. 2000). If the European gene genealogy was shaped by diversifying selection, then the observed level of gene diversity could have been reached much more quickly.

But even if MC1R does not inform us about Neanderthal admixture, it may have a different story to tell. The hypothesis of positive selection implies that neutral variation at MC1R contains information about when human skin was first regularly exposed to strong sunlight. After this time, we suppose that some newly favorable allele swept to fixation under the influence of natural selection. There may have been several such sweeps, and (as discussed below) we assume that each sweep reduced gene diversity (π) to zero. After the most recent sweep, there would have been no further change in amino-acid sequence, but π would have increased gradually toward its equilibrium value, $\theta = 4Nu$, because of synonymous mutations. Here, N is diploid effective size of the African population and u is the rate of synonymous mutations per sequence per generation.

This process is illustrated in figure 1, which shows the trajectory of π under three assumptions about N. Consider first the hypothesis that African gene diversity is at mutation-drift equilibrium—that $\pi = \theta$. Since the observed gene diversity is $\hat{\pi} = 0.67$,[2] the equilibrium hypothesis

2. Harding et al. (2000) report that gene diversity is 0.0007 per nucleotide site within Africa, that the synonymous substitution rate is 2.3×10^{-9} per site per year, and that 261 of the 954 nucleotide
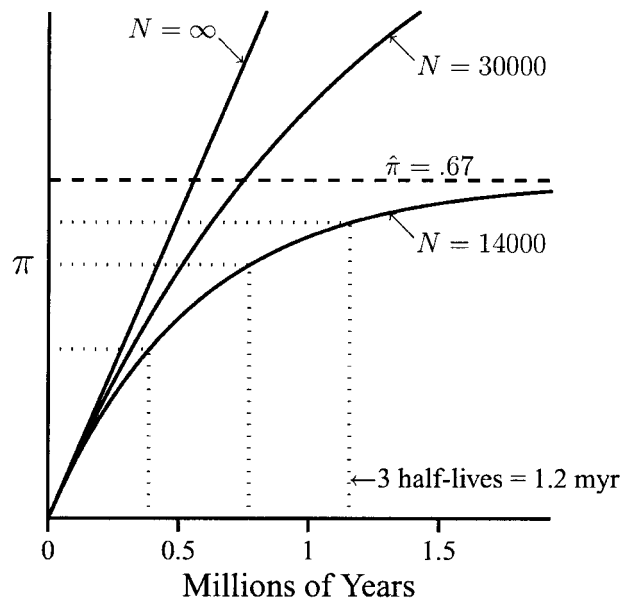

FIG. 1. *Trajectories of π following a selective sweep under different assumptions about* N, *assuming that π = 0 just after the sweep.* Dotted lines, *half-lives of the process when* N = 14,000; dashed line, $\hat{\pi}$, *the observed value of π.*

implies that $N \approx 14{,}000$. We cannot calculate the time since the selective sweep, because we cannot know how long the system has been at equilibrium. But we can calculate the time required to approach the neighborhood of equilibrium, and this will provide a minimum bound on the time since the sweep. The half-life is the time required for π to converge from any starting value halfway to θ (fig. 1). It equals $2N\ln2$ generations (Rogers and Jorde 1995), or roughly $28N$ years, and π will reach the neighborhood of equilibrium in about three half-lives, or $84N$ years. With $N = 14{,}000$, this equals roughly 1.2 million years. Thus, the hypothesis of equilibrium implies that we have been hairless savannah-dwellers for at least 1 million years.

Perhaps, however, N was some larger value and African gene diversity is still below equilibrium. For any given N and observed π, we can calculate t, the time since the selective sweep.[3] Figure 1 shows that larger values of N imply smaller values of t. For example, $t \approx 750{,}000$ years when $N = 30{,}000$. But if we allow N to increase without limit, t will converge toward a limit of about 560,000 years.[4] No matter how large we imagine the African population to have been, we will not conclude that the most recent selective sweep occurred less than 560,000 years ago.

Finally, one might propose that N was much smaller than 14,000. Then the observed π would be larger than its expected value θ. This would imply that the population had recently suffered some catastrophe, such as a decline or a bottleneck in population size, that had reduced gene diversity. To make sense of the observed gene diversity, it would be necessary to assume that the predecline (or prebottleneck) population size was at least 14,000, and the arguments of the preceding two paragraphs would then apply to that population.

In summary, the lower bound on time since the most recent selective sweep is somewhere between 560,000 and 1.2 million years. The lower figure is unreasonable because it assumes that the African population was infinitely large. The upper figure is more plausible, since it rests on an assumption (that $N \approx 14{,}000$) that is consistent with estimates from other loci (Harding et al. 2000, Zhao et al. 2000). Thus it seems likely that humans

have been hairless savannah-dwellers for at least 1.2 million years.

These results bear on a variety of published hypotheses. Some authors argue that hairlessness was either a precondition for (Newman 1970) or a response to (Wheeler 1992) the evolution of bipedal locomotion. Others argue that it was a precondition for the evolution of larger brains (Jablonski and Chaplin 2000). These views are all consistent with the present results, since bipedal locomotion evolved at least 4 million years ago and brains began to increase in size about 2 million years ago (Klein 1999). Others see hairlessness as an adaptation that dissipates the heat generated by running after prey (Montagu 1964, Carrier 1984). Humans have been hunting for at least 400,000 and perhaps 2.5 million years. Under this hypothesis, the present results would imply that hunting appeared early. Finally, some argue that hairlessness evolved in response to clothing (Glass 1966, Kushlan 1985). The most recent variant of this argument (Pagel and Bodmer 2003) holds that hairlessness evolved to reduce parasite load, an adaptation that was made feasible by clothing and control of fire. There is no evidence of tailored clothing before about 20,000 years ago (Klein 1999:536) or even of hide scraping before 300,000 years ago (Toth and Schick 1993:161). Thus, the present results indicate that humans were naked before they were clothed.

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sites are silent. With generations of 20 years, these values imply that observed gene diversity is $\hat{\pi} = 0.67$ per sequence and that the synonymous mutation rate is $u = 1.2 \times 10^{-5}$ per sequence per generation ($\hat{\pi} = 0.0007 \times 954 = 0.67$, and $u = [2.3 \times 10^{-9}] \times 261 \times 20 = 1.2 \times 10^{-5}$).

3. We assume that $\pi = 0$ just after a selective sweep. This is a simplification, since some gene diversity can survive a sweep unless the selectively favored nucleotide is initially at full linkage disequilibrium with each polymorphic silent site. Nonetheless, the assumption seems reasonable, since linkage disequilibrium is likely to be high in a locus of only 954 nucleotides. We further assume that mating is random and that mutation obeys the model of infinite sites. These assumptions imply that $\pi(t) = 4Nu(1-e^{-t/2N})$, where t is the time in generations since the selective sweep and N is the effective size of the African population (Li 1977: equation 6). For any given N and $\pi(t) < \theta$, this equation can be solved for t.

4. In the limit as $N \to \infty$, the equation in n. 3 becomes $\pi = 2ut$, a straight line as shown in figure 1. Setting π equal to its observed value and solving for t now gives $t = \hat{\pi}/2u = 27{,}811$ generations. If generations last 20 years, this is $t = 556{,}222$ years.

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Proximate Factors of Different Types of Grooming Hand-Clasp in Mahale Chimpanzees: Implications for Chimpanzee Social Customs[1]

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Whether one would call it culture (e.g., Nishida 1987) or not (e.g., Tomasello, Kruger, and Ratner 1993), it has become clear from the accumulated data of long-term field

studies of wild chimpanzees (*Pan troglodytes*) that they show quite a wide range of behavioral diversity that cannot simply be explained in terms of ecological differences (Whiten et al. 1999). Although de Waal (1999) has celebrated the maturity of "cultural primatology," studies of culture in nonhuman primates still seem to have a long way to go.

Laboratory studies have supplied us useful information on, for example, whether particular kinds of social learning occur in nonhuman animals (see review by Tomasello and Call 1997). These studies are important because culture is usually regarded as being transmitted through some kind of social means and the details of such processes are often difficult to see in the wild. However, the settings of such studies are often too restricted, unnatural, or unusual for animals to show their potentials in the wild. Wrangham and others (1994) have pointed to the importance of recording and describing behavioral diversity in wild chimpanzees and likened it to ethnography (the term used earlier by McGrew [1992]). Many researchers have compiled and reviewed data on chimpanzee behavioral diversity (e.g., Nishida 1987, Sugiyama 1997, McGrew 1998, Boesch and Tomasello 1998, Whiten et al. 2001, Humle and Matsuzawa 2001). However, commenting on a paper on cetacean culture employing similar "ethnographic" methodology, Ingold (2001) has criticized such lists of behaviors as still being a million miles from the practice of ethnography by cultural anthropologists or sociologists. His critique is too demanding in its insistence that culture requires intentions, purposes, and values. Such concepts in anthropology are usually obtained by fully language-based interviews, and even so it is hard to know another's true intentions, purposes, and values because people sometimes do not or cannot say what they really think or intend. Nevertheless, his critique is partly justified in that such lists of traits tend to leave behind the stuff of everyday life (Fox and King 2002), and to understand their "culture" we still need more detailed descriptions of what chimpanzees do in the wild.

Among the so-called cultural behaviors of chimpanzees, various types of tool use have been relatively well described (e.g., McGrew 1992), but social behaviors or social customs have received less attention (see review by Nakamura 2002). The grooming hand-clasp is the first case of social behavior documented as custom in the chimpanzees of K-group in the Mahale Mountains of western Tanzania (McGrew and Tutin 1978). It is also observed in Mahale M-group, Kibale (both Kanyawara and Ngogo), Kalinzu, Lópe, and Taï (performed only habitually) but never in Gombe, Budongo, or Bossou (see Nakamura 2002). It has also appeared in at least one captive chimpanzee colony (de Waal and Seres 1997). McGrew and others (2001) have reported that there are two different types of grooming hand-clasp: palm-to-palm and non-palm-to-palm. In the former type, the two chimpanzees truly clasp each other's hands with mutual palmar contact (fig. 1, *A*), while in the latter only one or neither hand clasps the other (fig. 1, *B–D*) and usually the hands are flexed with one limb resting on the other