Genetic Evidence for the Convergent Evolution of Light Skin in Europeans and East Asians

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Human skin pigmentation shows a strong positive correlation with ultraviolet radiation intensity, suggesting that variation in skin color is, at least partially, due to adaptation via natural selection. We investigated the evolution of pigmentation variation by testing for the presence of positive directional selection in 6 pigmentation genes using an empirical F_{ST} approach, through an examination of global diversity patterns of these genes in the Centre d'Etude du Polymorphisme Humain (CEPH)-Diversity Panel, and by exploring signatures of selection in data from the International HapMap project. Additionally, we demonstrated a role for *MATP* in determining normal skin pigmentation variation using admixture mapping methods. Taken together (with the results of previous admixture mapping studies), these results point to the importance of several genes in shaping the pigmentation phenotype and a complex evolutionary history involving strong selection. Polymorphisms in 2 genes, *ASIP* and *OCA2*, may play a shared role in shaping light and dark pigmentation across the globe, whereas *SLC24A5*, *MATP*, and *TYR* have a predominant role in the evolution of light skin in Europeans but not in East Asians. These findings support a case for the recent convergent evolution of a lighter pigmentation phenotype in Europeans and East Asians.

Introduction

Skin pigmentation shows remarkable variation both within and among human populations. This variation is often explained in terms of natural (Blum 1961; Loomis 1967; Walter 1971; Branda and Eaton 1978; Kollias et al. 1991; Jablonski and Chaplin 2000) or sexual (Darwin 1871; Diamond 1992; Aoki 2002) selection. Recent work (Jablonski and Chaplin 2000; Chaplin 2004) has confirmed a strong positive correlation between skin pigmentation and ultraviolet radiation (UVR) intensity, suggesting that global variation in skin pigmentation may be the result of localized adaptation to different UVR conditions via natural selection. Although a large number of genes have been identified that explain human Mendelian pigmentation disorders and mouse coat color variability (Bennett and Lamoreux 2003), very few of these have been shown to have effects on normal variation in human skin pigmentation (Box et al. 1997; Smith et al. 1998; Flanagan et al. 2000; Kanetsky et al. 2002; Shriver et al. 2003; Bonilla et al. 2005; Graf et al. 2005; Lamason et al. 2005). Although there is strong evidence that pigmentation variation has been influenced by natural selection, it is currently unknown how selection has affected the genetic architecture of pigmentation loci in different populations, even when such populations have experienced similar levels of UVR over their evolutionary histories. For example, the dark skin that characterizes many sub-Saharan African and Island Melanesian populations may be due to shared ancestral variants or to novel genetic adaptations in the ancestral Island Melanesian population. Similarly, the light skin of Europeans and East Asians may have a common genetic origin or instead may be the result of independent adaptations to low-UVR environments.

The pigmentation candidate genes that have been identified to date have effects at various stages of the pigmentation pathway, ranging from melanogenesis, the stabilization and transport of enzymes in the melanin production pathway, the production and maintenance of melanosomes and the melanosomal environment, and the switch between the production of eumelanin and pheomelanin. In this study, we have focused on 6 of these genes. The first of these, *TYR*, produces the critical enzyme tyrosinase, which catalyzes the first 2 steps in the melanin synthesis pathway (Spritz 1994). A second gene in our study, *MATP*, has been implicated in the trafficking and intracellular processing of this critical enzyme (Costin et al. 2003). The production of melanin takes place in specialized cellular organelles known as melanosomes, and there is evidence that melanin synthesis may be dependent upon pH within these organelles (Ancans et al. 2001; Fuller et al. 2001). Puri et al. (2000) suggested that the product of the *OCA2* gene, a melanosomal membrane protein, may serve as an anion transporter, thus helping to regulate melanosomal pH. More recently, it has been suggested that melanogenesis and the development of the melanosomes themselves may also be dependent on calcium levels in the melanosome, regulated by the *SLC24A5* gene (Lamason et al. 2005). The final 2 genes that we examined, *MC1R* and *ASIP*, are involved in the production of the 2 types of melanin, darker eumelanin and the lighter pheomelanin. *MC1R* encodes for the melanocortin-1 receptor, a 7-pass transmembrane G-protein–coupled receptor that binds the hormone α-MSH. When the MC1R is activated by α-MSH, cyclic adenosine 3′,5′-monophosphate (cAMP) levels are increased and eumelanin production is stimulated through the cAMP/protein kinase A signaling pathway (Busca and Ballotti 2000). Alternatively, agouti-signaling protein, the product of *ASIP*, can also bind to the MC1R, blocking α-MSH and causing the production of pheomelanin rather than eumelanin. Of these 6 candidate genes, 5 (*TYR*, *OCA2*, *ASIP*, *MC1R*,

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and *SLC24A5*) have been previously associated with normal variation in skin pigmentation (Box et al. 1997; Smith et al. 1998; Schiöth et al. 1999; Flanagan et al. 2000; Bastiaens et al. 2001; Shriver et al. 2003; Bonilla et al. 2005), and here we demonstrate an association for the sixth gene, *MATP*.

In this study, we examine the role of 6 pigmentation candidate genes in explaining global pigmentation clines primarily by looking for evidence of directional selection in patterns of variation in a geographically diverse set of populations that exhibit a range of pigmentation phenotypes. We have applied an empirical F_{ST}-based approach (Akey et al. 2002) using allele frequency data from 11,078 autosomal single nucleotide polymorphisms (SNPs) for comparison to test for population divergence in 7 different SNPs in these genes and also examined their patterns of global variation in the CEPH-Diversity Panel. We also examine several statistics that are sensitive to directional selection (locus-specific branch length [LSBL], Tajima's *D*, lnRH) in these and other candidate genes using data from the HapMap project and directly investigate the functional relevance of the *MATP* gene using an admixture mapping approach. Our results provide new insights into the genetic mechanisms underlying the human pigmentation phenotype and their evolution.

Materials and Methods

Samples

Twenty individuals each from the following populations were typed on the Affymetrix 10K whole-genome sampling assay (WGSA) Mapping Array (Santa Clara, CA): West African (Mende from Sierra Leone), Island Melanesian (Nasioi from Bougainville), South Asian (Indians from Andhra Pradesh), Native American (Nahua from Mexico), East Asian (Chinese and Japanese from Coriel Human Cell Repository), and European (Spanish from Valencia) using the methods described in Shriver et al. (2005).

Pigmentation candidate SNPs were typed in the same 20 individuals from each population typed on the Affymetrix Arrays as well as on an average of 40 additional individuals from the same or similar populations (for a total of ~60 individuals per population). It was possible to sample individuals from the same regions as the populations typed on the Affymetrix Chip for the Island Melanesians ($n = 44$) and Europeans ($n = 42$). The additional 40 East Asian and South Asian individuals were unadmixed Chinese ($n = 46$) and South Asian Indian ($n = 45$) residents of Trinidad and Tobago (collected by Tamiko Brown, University of the West Indies). The additional 45 West African individuals were African-American individuals of the Gullah population of South Carolina measured to have 100% West African ancestry using 10 ancestry informative markers (AIMs) (Parra et al. 2001). The additional Native American samples comprised 14 Nahua speakers (from the same population typed on the Affymetrix Array) and 33 Mayans (collected and kindly provided by Ken Weiss and Anne Buchanan, Penn State University).

To determine the linkage of *MATP* with normal variation in skin pigmentation, the C374G SNP was genotyped in 202 African-American and 122 African Caribbean individuals for whom quantitative measures of skin pigmentation as well as individual ancestry estimates were available (Shriver et al. 2003). These populations were selected because admixed populations are well suited for gene mapping due to the long-range linkage disequilibrium that is generated by admixture.

Five SNPs in these pigmentation genes that showed signals of selection in the pairwise locus-specific F_{ST} study were also typed in the full CEPH Diversity Panel (Cann et al. 2002) consisting of 53 global populations and 1059 individuals in total.

Pigmentation Gene Marker Selection and Genotyping

Pigmentation candidate SNPs were selected for inclusion in the F_{ST} portion of this study because of either previously reported allele frequency differences between populations, their location within genes believed to have an effect on pigmentation variation, or previously reported associations with normal variation in pigmentation variation. Four are nonsynonymous coding SNPs, 2 (*OCA2* A355G and *MC1R* G314A) are synonymous, and *ASIP* A8818G is located in the 5′ promoter region. Each SNP was assayed using the McSNP genotyping assay (Akey et al. 2001; Ye et al. 2002). Reference SNP numbers, polymerase chain reaction, and genotyping conditions can be found in supplementary table 1 (Supplementary Material online).

F_{ST} Estimation and Percentile Rank Calculation

Unbiased estimates of Weir and Cockerham's F_{ST} were calculated as in Akey et al. (Akey et al. 2002). We determined the rank percentile of the locus-specific F_{ST} values for each candidate SNP using the following equation:

$$\text{Rank percentile}\,(x) = \text{number of loci} > \text{observed pairwise locus-specific}\,F_{ST}(x) / \text{total number of loci.}$$

The higher the pairwise locus-specific F_{ST} value for a pigmentation SNP relative to the appropriate empirical distribution, the lower its percentile rank. SNPs for which the pairwise locus-specific F_{ST} value had a rank percentile value of 0.05 or less were classified as showing significant divergence. Thus, all *P* values reported for F_{ST} comparisons are empirical *P* values indicative of the percentile rank of the candidate SNP relative to the appropriate pairwise F_{ST} distribution. For example, the East Asian–West African pairwise F_{ST} value for the *ASIP* A8818G polymorphism is 0.071. This value is then compared against the empirical distribution of pairwise F_{ST} values between East Asians and West Africans calculated for the 11,078 SNPs typed on the Affymetrix Chip. Similarly, the pairwise F_{ST} value of this SNP between the European and West African samples is 0.818. This value is compared with the empirical distribution of pairwise F_{ST} estimated for Europeans and West Africans. A 1-tailed test is appropriate in this instance as we are investigating loci showing evidence of directional selection; these would fall in the upper tail of the distribution. We calculated *P* values using the full data set of 11,078 SNPs, as well a subset composed of genic ($n = 3159$) and nongenic ($n = 7127$) SNPs. The results, regardless of which

Table 1
Allele Frequencies by Population

	n	TYR 192*A, rs1042602	ASIP 8818*A, rs6058017	OCA2 355*A, rs1800404	MATP 374*G, rs16891982	MC1R 92*G, rs2228479	MC1R 314*G, rs2228478	SLC24A5 111*A, rs1426654
Island Melanesian	120	0.00	0.52	0.18	0.00	0.83	0.26	0.07
East Asian	122	0.00	0.72	0.37	0.01	0.73	0.31	0.00
South Asian	126	0.04	0.77	0.29	0.07	0.97	0.24	0.52
Native American	128	0.01	0.98	0.38	0.06	0.96	0.07	0.08
European	84	0.52	0.86	0.58	0.86	0.94	0.11	0.96
West African	130	0.01	0.15	0.04	0.05	0.99	0.52	0.09

data set is used, are qualitatively the same, with the exception of a single interpopulation comparison at *TYR* 192. In the full data set, the F_{ST} value of 0.500 between the Europeans and West Africans has a P value of 0.043. In the genic data set, the P value rises to 0.057, but it remains significant in the nongenic data set ($P = 0.042$). This discrepancy might be due to the higher average F_{ST} values for genic SNPs relative to nongenic SNPs (Hinds et al. 2005) or also to the smaller sample size of our genic data set.

Phylogenetic Tree Construction

Population trees were constructed using the Neighbor-Joining method (Nei and Saitou 1987) as implemented in MEGA 2.1 (Kumar et al. 2001) using average pairwise F_{ST} values from the panel of 11,078 autosomal SNPs as a distance measure. Locus-specific trees were constructed in the same manner, using the pairwise locus-specific F_{ST} values for each pigmentation SNP separately.

Admixture Mapping

We tested for linkage between the *MATP* C374G SNP and skin pigmentation in the African-American and African Caribbean samples using the program ADMIXMAP (Hoggart et al. 2003). ADMIXMAP uses a combination of Bayesian and classical approaches to model the associations of ancestry between linked marker loci and the association of a particular phenotypic trait of interest with individual admixture or ancestry at a linked marker locus. Individual ancestry estimates used in this program were based on allele frequencies at 34 AIMs for West African, Native American, and European ancestry (Shriver et al. 2003). Skin pigmentation was measured using the DermaSpectrometer (Cortex Technology, Hadsund, Denmark), a narrow band reflectance spectrophotometer, following methods previously described (Shriver et al. 2003). The DermaSpectrometer estimates the concentrations of hemoglobin and melanin in the skin after the work of Diffey et al. (1984). By utilizing the differences in the absorption properties of these 2 chromophores, the DermaSpectrometer estimates the amount of skin reflectance due to melanin content of the skin and quantifies this as the M (melanin) index. Higher M index values indicate darker pigmentation, whereas lower M index values indicate lighter pigmentation. The M index values in both the African American and African Caribbean samples were normally distributed.

Analysis of Pigmentation Genes in HapMap Data

Data from a total of 3,458,541 SNPs in Release 20/Phase II of HapMap project were examined in 3 world populations: West African Yoruba (YRI), Northern Europe CEPH (CEU), and a pooled East Asian (EAS) group composed of Chinese and Japanese individuals. The genome was divided into 587,233 overlapping 25-kb sliding windows advanced by 5-kb (in other words a 20-kb overlap between adjacent windows) containing on average 29 SNPs. The position of each window was described according to the central base pair on the NCBI35 build of the human genome.

Three measures of sequence diversity indicative of the action of positive selection were calculated for each of the windows in each of the 3 populations. LSBLs and lnRH were calculated for each SNP within a window and averaged. LSBL was calculated using the methods described in Shriver et al. (2004). When calculating lnRH, we have substituted 0.0001 for the zero value that occurs when an allele is fixed in one population (Storz et al. 2004). The Tajima's D statistic was also calculated for each window. Separate distributions were constructed for autosomal and X-chromosome windows. Windows were mapped to candidate pigmentation genes using Refseq coordinates (NCBI35 build of the human genome) of the largest transcript including an additional 10 kb of upstream and downstream sequence.

Results

Pairwise F_{ST} in Pigmentation Genes

In all, 11,078 autosomal SNPs were typed, using the Affymetrix 10K Mapping Array Chip (Shriver et al. 2005), in 6 populations (representing Europeans, East Asians, Native Americans, South Asians, Island Melanesians, and West Africans) that encompass a range of geographic origins and pigment phenotypes. These formed an empirical distribution of values against which allele frequencies of 7 SNPs in 6 pigmentation candidate genes (*TYR*, *MATP*, *SLC24A5, MC1R, ASIP, OCA2)* in the same or similar populations (table 1) could be compared. The concept of using F_{ST} distributions to detect signals of natural selection was first proposed by Lewontin and Krakauer (1973) and is based on the fact that although demographic processes will affect all regions of the genome equally, selection will act on specific loci and linked neutral variants (Cavalli-Sforza 1966). F_{ST}, like many statistics of genetic variation, can be sensitive to the underlying demographic history of the populations in question (Lewontin and Krakauer 1975; Nei and Maruyama 1975; Robertson 1975). As it can be very difficult to model this history accurately, comparisons of F_{ST} values with empirical F_{ST} distributions drawn from the same populations (which will reflect the average

Table 2
Average Pairwise F_{ST} Values Based on the 11,078 Autosomal SNPs on the Affymetrix 10K whole-genome sampling assay (WGSA) chip and Locus-Specific Pairwise F_{ST} Values for the 5 SNPs That Showed At Least One Value Falling into the Top Fifth Percentile in At Least One Population Comparison

	Island Melanesian	East Asian	South Asian	Native American	European
Affymetrix chip					
East Asian	0.112				
South Asian	0.111	0.067			
Native American	0.157	0.092	0.104		
European	0.141	0.103	0.048	0.123	
West African	0.182	0.157	0.125	0.189	0.137
TYR					
East Asian	0.000 (1.000)				
South Asian	0.038 (0.569)	0.038 (0.437)			
Native American	0.000 (1.000)	0.000 (1.000)	0.019 (0.608)		
European	**0.515 (0.045)**	**0.515 (0.017)**	**0.430 (0.004)**	**0.499 (0.035)**	
West African	0.000 (1.000)	0.000 (1.000)	0.019 (0.652)	0.000 (1.000)	**0.500 (0.043)**
ASIP					
East Asian	0.071 (0.446)				
South Asian	0.115 (0.361)	0.000 (1.000)			
Native American	0.424 (0.115)	0.221 (0.147)	0.173 (0.231)		
European	0.226 (0.244)	0.048 (0.503)	0.018 (0.438)	0.075 (0.467)	
West African	0.260 (0.282)	0.489 (0.065)	**0.547 (0.023)**	**0.815 (0.011)**	**0.663 (0.011)**
OCA2					
East Asian	0.072 (0.445)				
South Asian	0.021 (0.635)	0.006 (0.589)			
Native American	0.081 (0.498)	0.000 (1.000)	0.010 (0.647)		
European	0.281 (0.188)	0.082 (0.399)	0.155 (0.097)	0.073 (0.472)	
West African	0.101 (0.525)	0.286 (0.212)	0.205 (0.237)	0.299 (0.253)	**0.516 (0.039)**
MATP					
East Asian	0.008 (0.635)				
South Asian	0.060 (0.491)	0.026 (0.493)			
Native American	0.053 (0.563)	0.020 (0.569)	0.000 (1.000)		
European	**0.855 (0.001)**	**0.836 (<0.001)**	**0.769 (<0.001)**	**0.777 (0.003)**	
West African	0.039 (0.660)	0.008 (0.727)	0.000 (1.000)	0.000 (1.000)	**0.791 (0.003)**
SLC24A5					
East Asian	0.065 (0.457)				
South Asian	0.383 (0.060)	**0.519 (0.003)**			
Native American	0.000 (1.000)	0.072 (0.397)	0.374 (0.059)		
European	**0.875 (0.001)**	**0.957 (<0.001)**	**0.389 (0.007)**	**0.870 (<0.001)**	
West African	0.000 (1.000)	0.081 (0.521)	0.358 (0.096)	0.000 (1.000)	**0.859 (0.001)**

NOTE.—Empirical P values for the latter are shown in parentheses and those <0.05 are emphasized by bold type.

demographic history) are preferable to comparisons with simulated F_{ST} distributions (Black et al. 2001; Akey et al. 2002, 2004).

Five SNPs in 5 different pigmentation genes had locus-specific F_{ST} values that fell within the top 5% of comparisons for at least one population pair gauged across our empirical distribution (table 2). The remaining 2 SNPs, both within the *MC1R* gene, did not show such interpopulation partitioning of variation. SNPs with pairwise F_{ST} values in the top 5% of the relevant empirical distributions were used to construct Neighbor-Joining trees as a way to graphically represent divergence among populations at these pigmentation loci (fig. 1).

Pairwise F_{ST} estimates for the *ASIP* A8818G and *OCA2* A355G SNPs tentatively suggest a pattern of divergence between 4 populations (Europeans, East Asians, Native Americans, and South Asians) and the relatively more darkly pigmented populations of West Africa and Island Melanesia, or possibly only between West Africans and all other populations. At both loci, West Africans and Island Melanesians have higher frequencies of the ancestral alleles than the other 4 populations. Pairwise locus-specific F_{ST} values falling in the top 5% of the empirical distributions are observed between West Africans and 3 other populations (South Asians, Native Americans, and Europeans) at *ASIP* A8818G. F_{ST} values between West Africans and East Asians at this locus are elevated but do not reach our cutoff value of 5% ($F_{ST} = 0.489$, $P = 0.065$). At *OCA2* A355G, only West Africans and Europeans show F_{ST} values falling into the top fifth percentile of relevant comparisons ($F_{ST} = 0.516$, $P < 0.05$). The low pairwise F_{ST} values and higher frequency of ancestral alleles at both SNPs studied in these loci between West Africans and Island Melanesians hint that dark pigmentation associated with both loci in these populations may have a common evolutionary origin (Mean $F_{ST(WA-IM)} = 0.182$; *ASIP* A8818G $F_{ST(WA-IM)} = 0.260$, $P = 0.282$; *OCA2* A355G $F_{ST(WA-IM)} = 0.101$, $P = 0.525$).

Three loci, *TYR* A192C, *MATP* C374G, *SLC24A5* A111G, show very strong signals of European-specific

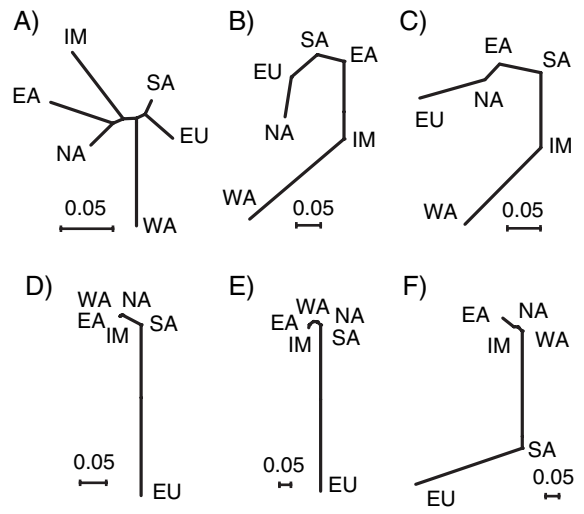

FIG. 1.—Neighbor-Joining trees based on (*a*) average F_{ST} values among the 6 populations typed on the Affymetrix 10K WGSA chip and locus-specific F_{ST} values at (*b*) *ASIP* A8818G, (*c*) *OCA2* A355G, (*d*) *TYR* A192C, (*e*) *MATP* C374G, and (*f*) *SLC24A5* A111G. Populations are abbreviated as follows: WA, West African; SA, South Asian; NA, Native American; EU, European; EA, East Asian; IM, Island Melanesian.

divergence. High F_{ST} values between Europeans and darkly pigmented populations such as West Africans and Island Melanesians are not unexpected if these genes have functional effects. However, the notably elevated pairwise F_{ST} values relative to East Asians (the population in our panel that is the most similar to Europeans in pigmentation phenotype) is striking. Populations intermediate in pigmentation (Native Americans and South Asians) also exhibit F_{ST} values falling in the top fifth percentile of their relevant F_{ST} distributions with Europeans for these 3 loci. In the case of *SLC24A5* A111G, South Asian pairwise F_{ST} values also fall in this top fifth percentile when compared with both Europeans ($F_{ST} = 0.389$, $P < 0.01$) and East Asians ($F_{ST} = 0.519$, $P < 0.01$), but not when compared with any other population. At all 3 loci, Europeans have the highest frequency of the derived alleles relative to the other 5 populations.

Admixture Mapping of *MATP*

Four of the 5 SNPs displaying potential signals of selection (*ASIP* A8818G, *OCA2* A355G, *TYR* A192C, and *SLC24A5* A111G) have been previously shown to be associated with normal pigmentation variation in admixed African-American and African Caribbean populations (Shriver et al. 2003; Bonilla et al. 2005; Lamason et al. 2005). *MATP* C374G was reported to be associated with normal variation in pigmentation in a population of European ancestry (Graf et al. 2005), but an association with variation in pigmentation between populations that differ in pigmentation levels has not been demonstrated (although observed differences in allele frequencies between such populations has led some to speculate that this may be the case [Nakayama et al. 2002; Graf et al. 2005]).

We used previously described admixture mapping techniques (McKeigue et al. 2000) to test for linkage

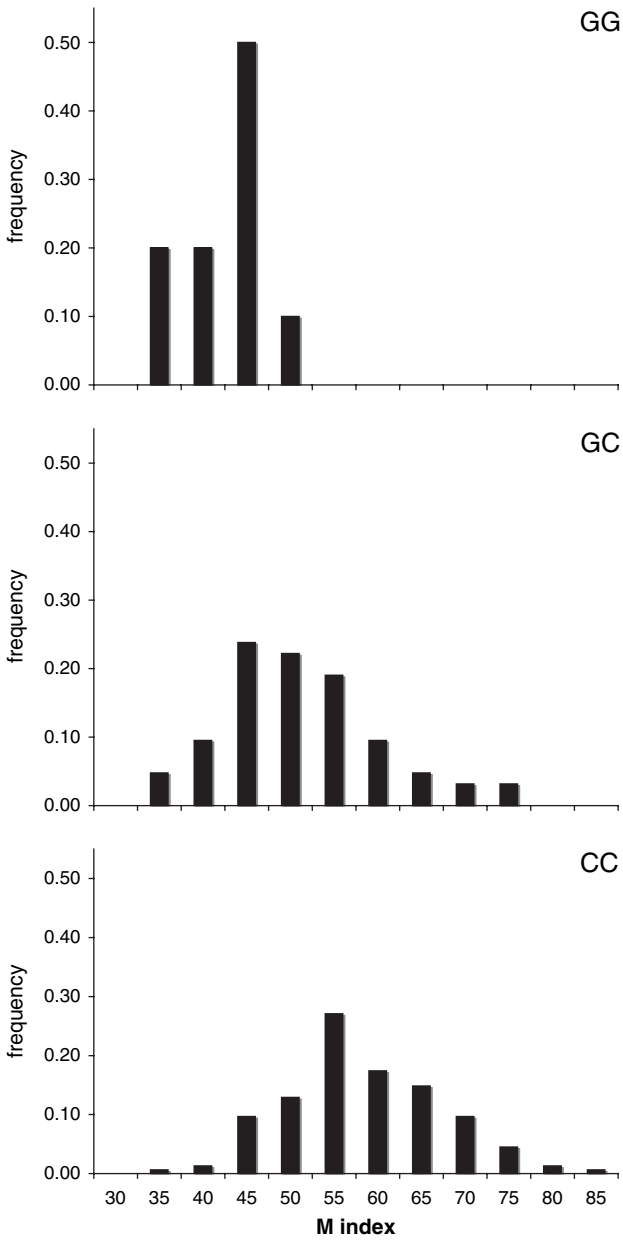

FIG. 2.—Distribution of *M* index values for each of the 3 genotype classes at *MATP* C374G in a sample of 202 African-American individuals. The ancestral allele, C, has an effect size per allele of +5 melanin units (95% CI: +2.5 to +8), and its effects are consistent with an additive mode of inheritance.

between *MATP* C374G genotype and quantitatively measured skin pigmentation in a sample of 202 African-Americans and a sample of 122 African Caribbean individuals. Admixture mapping is a test for linkage in the presence of population stratification and makes it possible to test specifically for functionally important variations between 2 particular ancestral populations that differ for the phenotype of interest. In the African-American sample, homozygotes for the *MATP* 374*G–derived allele have the lowest mean skin *M* index and hence the lightest skin pigmentation (mean $M = 39.8$). Ancestral allele homozygotes have the highest mean *M* index ($M = 55.6$), whereas heterozygotes are intermediate in skin color (mean *M* index of 49.3, fig. 2). Using the

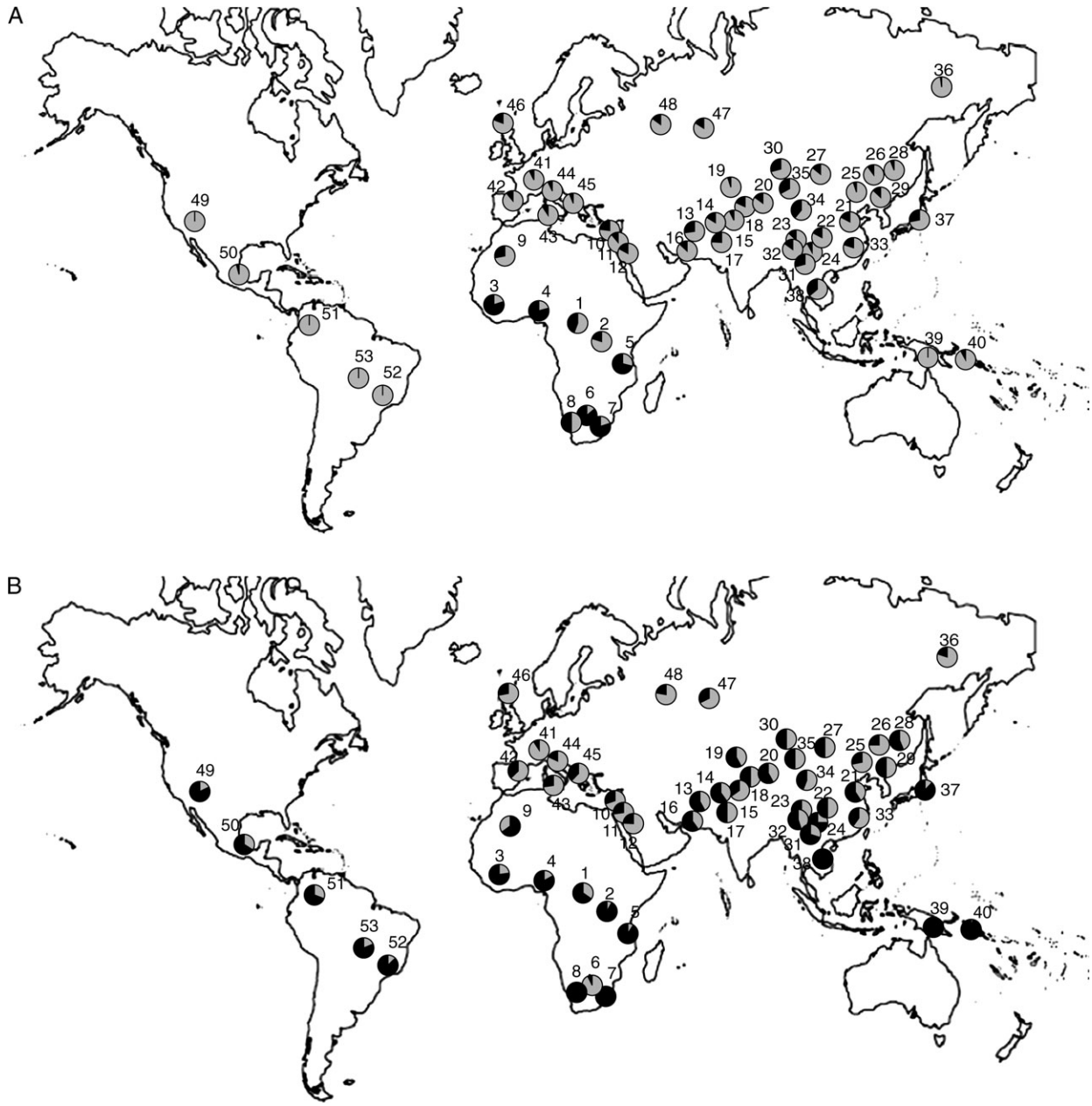

FIG. 3.—Distribution of allele frequencies in the CEPH-Diversity Panel for the 5 SNP showing elevated pairwise F_{ST} values for at least one population pair in our original population screen: (*A*) *ASIP* A8818G, (*B*) *OCA2* A355G, (*C*) *TYR* A192C, (*D*) *MATP* C374G, and (*E*) *SLC24A5* A111G. On all maps, gray shading corresponds to the frequency of the allele associated with lighter pigmentation. The numbered populations correspond to the following: 1) Biaka pygmies, 2) Mbuti pygmies, 3) Mandenka, 4) Yoruba, 5) Bantu N.E., 6) San, 7) Bantu S.E., 8) Bantu S.W., 9) Mozabite, 10) Bedouin, 11) Druze, 12) Palestinian, 13) Brahui, 14) Balochi, 15) Hazara, 16) Makrani, 17) Sindhi, 18) Pathan, 19) Kalesh, 20) Burusho, 21) Han, 22) Tujia, 23) Yizu, 24) Miaozu, 25) Orogen, 26) Daur, 27) Mongola, 28) Hezhen, 29) Xibo, 30) Uygur, 31) Dai, 32) Lahu, 33) She, 34) Naxi, 35) Tu, 36) Yakut, 37) Japanese, 38) Cambodian, 39) Papuan, 40) NAN Melanesian, 41) French, 42) French Basque, 43) Sardinian, 44) Northern Italian, 45) Tuscan, 46) Orcadian, 47) Adygei, 48) Russian, 49) Pima, 50) Maya, 51) Columbian, 52) Karitiana, 53) Surui.

program ADMIXMAP, we detected a significant linkage between genotype at *MATP* C374G and pigmentation ($P < 0.0001$). The ancestral allele has an effect size per allele of +5 melanin units (95% confidence interval [CI]: +2.5 to +8) and is consistent with an additive rather than a dominant mode of action. We did not observe a significant association between *MATP* C374G and pigmentation in the African Caribbean sample, but this may be due to both the smaller sample size and lower admixture proportions observed for this population relative to the African-American sample. Nonetheless, the 95% CIs for effect size in this population (−3 to +8) are consistent with those observed in the African-American population.

Global Patterns of Variation in Pigmentation Genes

To confirm and investigate further the patterns we observed in our original samples, we typed the 5 SNPs

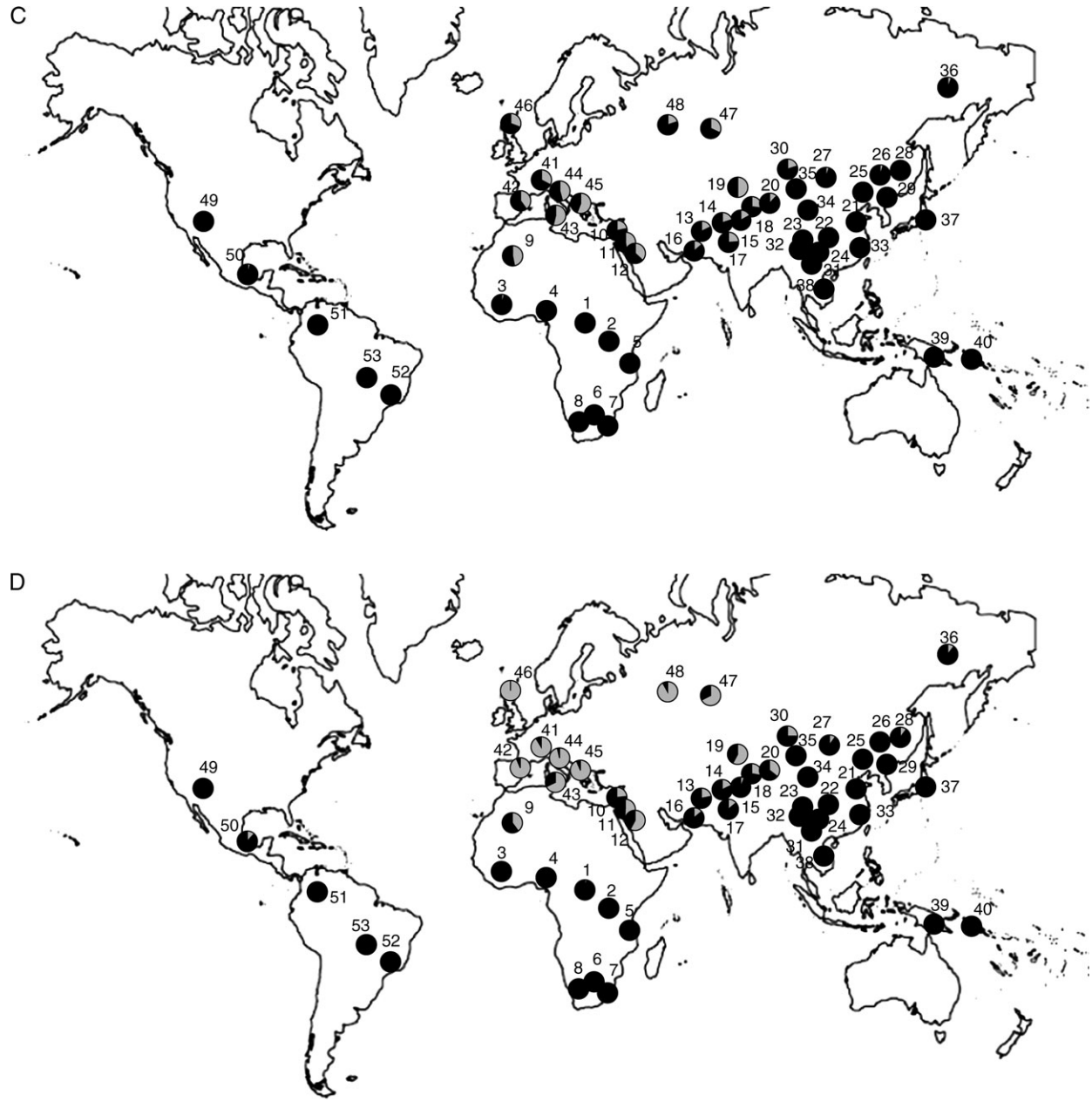

FIG. 3. Continued

showing elevated F_{ST} values relative to empirical distributions in 53 additional populations from the CEPH-Diversity Panel (Cann et al. 2002). Allele frequencies for each of these can be found in supplementary table 2 (Supplementary Material online), whereas figure 3*A–E* illustrates their global allele frequency distributions.

The pattern of diversity at *ASIP* 8818*G allele (the ancestral allele associated with darker pigmentation) indicates a role primarily in African/non-African divergence (sub-Saharan African frequency: 66%, all other populations: 14%) rather than between darkly and lightly pigmented populations. At *OCA2* 355, the derived allele (linked with lighter pigmentation) occurs at its highest frequencies across Europe and Asia but is also relatively common among Native American populations (18–34%) and is present at much lower frequencies (0–10%) among Bantu-speaking African groups. In contrast, the ancestral allele associated with dark pigmentation has a shared high frequency in sub-Saharan African and Island Melanesians. A notable exception is the relatively lightly pigmented San population of Southern Africa where the derived allele predominates (93%), although this may be simply due to small sample size ($n = 14$).

The distributions of the derived and ancestral alleles at *TYR* A192C, *MATP* C374G, and *SLC24A5* A111G are consistent with the F_{ST} results suggesting strong European-specific divergence at these loci. The derived allele at *TYR*, 192*A (previously linked with lighter pigmentation [Shriver et al. 2003]), has a frequency of 38% among European populations but a frequency of only 14% among non-Europeans. The differences between Europeans and

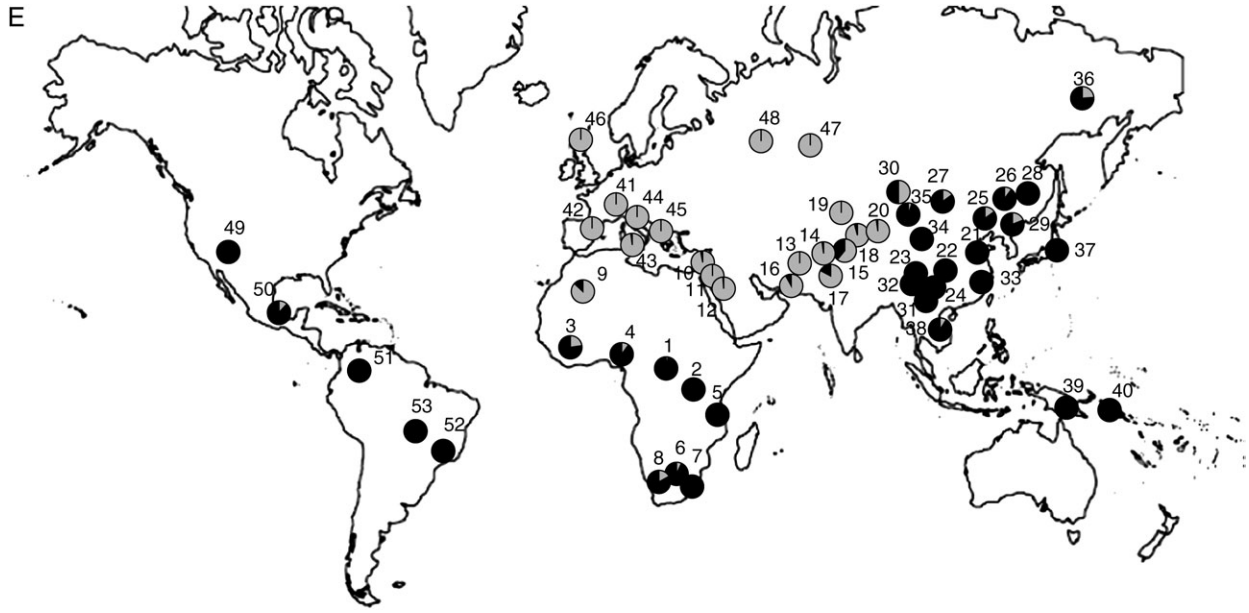

FIG. 3. Continued

non-Europeans for the *MATP* 374*G and *SLC24A5* 111*A alleles (both derived alleles associated with lighter pigmentation) were even more striking ($MATP_{\text{European}} = 87\%$; $MATP_{\text{non-European}} = 17\%$; $SLC24A5_{\text{European}} = 100\%$; $SLC24A5_{\text{non-European}} = 46\%$). The frequency of the *SLC24A5* 111*A allele outside of Europe is largely accounted for by high frequencies in geographically proximate populations in northern Africa, the Middle East, and Pakistan (ranging from 62% to 100%).

Signatures of Selection in Pigmentation Genes Using HapMap Data

To supplement our analyses, we also examined the global diversity of our 6 pigmentation genes using data from the International HapMap project. Three potential indicators of directional positive selection (LSBL, Tajima's *D*, and lnRH) were calculated in 25-kb overlapping windows in European (CEU), East Asian (EAS), and West African (YRI) populations separately and their significance gauged by an empirical genome-wide distribution.

The first statistic, LSBL (Shriver et al. 2004), decomposes F_{ST} among 3 populations into population-specific components and provides a means to quantify the degree to which a SNP (or group of SNPs in this case) has changed in allele frequency in one population relative to the other 2. Second, Tajima's *D* was used to summarize the allele frequency spectrum in each genomic window. Under neutrality, Tajima's *D* will take on values close to zero. Significantly negative values indicate an excess of rare alleles that is consistent with recent positive directional selection or a population expansion (Tajima 1989). Although Tajima's *D* is normally used in cases of full ascertainment (i.e., resequencing), previous studies have established a correlation between resequencing and dense genotyping data (Carlson et al. 2005; Voight et al. 2006). Although the HapMap data set does suffer from an ascertainment bias toward common SNPs, this should result in a skew of Tajima *D* values against negative values that are indicative of directional selection.

Finally, we calculated the related natural log of the ratio of heterozygosities (lnRH) between all pairwise population comparisons (Schlotterer 2002). Strongly negative values (i.e., a low ratio) indicate a reduced heterozygosity in one population relative to another and points to population-specific effect (not necessarily the case with significant Tajima *D* values). A simple case of strong and population-specific positive selection is expected to result in strongly negative Tajima's *D*, high LSBL, and negative lnRH values. Although these are relatively simple metrics, when used together, they may provide nuanced insight in the timing and place of more complex selective events. The full set of results can be found in supplementary table 3 (Supplementary Material online).

These data confirm the unusual European-specific patterns at *MATP* and *SLC24A5*. Both genes display long range (consecutive windows) and significant indications of positive selection for all 3 statistics. In contrast, there is little evidence of a European-specific pattern in the *TYR* locus although the nonsynonymous *TYR* A192C SNP does individually show a strongly significant CEU-LSBL ($P < 0.003$) in the HapMap data as in our original findings. The contrast may be explained by the limitations of our HapMap sliding window analyses, whereby adjacent SNPs are averaged using a method that does not consider haplotype structure.

A more complex pattern of evolution is indicated in the HapMap data for the *OCA2* gene. In line with the F_{ST}-based survey, it shows consistently strong and significant European LSBL and somewhat more erratic significance in other measures. However, it also reveals a similar if slightly weaker pattern of significance in the East Asian population consistent with our original observation of a role in lightly versus darkly pigmented

populations. Another such candidate gene, *ASIP*, also shows some tentative indications of an African-specific pattern, although this signal is weak compared with that observed for the *MATP* and *SLC24A5* genes in Europeans. The HapMap data does, however, concur with our previous finding regarding *MC1R* in failing to detect any signal of selection at this locus in any of the 3 populations.

We also examined these statistics at 28 additional pigmentation candidate loci and identified several genes showing evidence of natural selection. In particular, 2 genes (*ADAM17* and *ATRN*) showed East Asian–specific signatures comparable in strength with those observed for *MATP* and *SLC24A5* in Europeans. The *ADTB3A* gene also shows a strong and focused signature of positive selection in Africans. Several other genes showed more tentative and/or complex evidence of a selected past. These include the transcription factor *MITF*, which may have been subject to a selective sweep prior to the divergence of these 3 populations (significantly negative Tajima's *D*), and *TYRP1*, which shows strong LSBL in the European and, to a lesser extent, East Asian populations.

Discussion

Patterns of variation in the 6 candidate loci that we examined in this study suggest that darkly pigmented populations in West Africa and Island Melanesia may share some ancestral pigmentation alleles but that the lighter pigmentation observed in European and East Asian populations is due to independent genetic mutations in at least 3 loci: *SLC24A5*, *MATP*, and *TYR*. The initial set of populations that we examined occupied a range of different UVR environments and fell across a broad spectrum of pigmentation phenotypes. Although there is certainly variation within these populations (as well as overlap in pigmentation phenotype between some of them), 2 populations from high-UVR regions, West Africans and Island Melanesians, fall at the darker end of the pigmentation continuum, whereas 2 other populations from low-UVR regions, East Asians and Europeans, fall at the opposite end. Comparisons of genetic variation in these 2 pairs of populations should be helpful in determining if similarities in pigmentation phenotype are due to the same or independent genetic mechanisms.

Many hypotheses predict that natural selection will eliminate genetic variants associated with lighter skin in regions of high UVR as a protection against photodamage (e.g., sunburn, melanoma, and basal and squamous cell carcinomas) (Blum 1961; Kollias et al. 1991) and folic acid photodegradation (Branda and Eaton 1978; Jablonski and Chaplin 2000). The photoprotective properties of a highly melanized skin and the recent African origin of modern humans suggest that the ancestral phenotype is one of the relatively dark skin (Jablonski and Chaplin 2000; Rogers et al. 2004). If dark skin is the ancestral phenotype, then we may assume that the first migrants out of Africa were also relatively darkly pigmented.

There are 2 primary explanations for the evolution of lighter skin in regions of low UVR. The first suggests that light skin is merely due to the relaxation of functional constraint and that derived alleles associated with lighter pigmentation may have simply drifted to high frequency in the absence of strong purifying selection (Brace 1963). The second explanation suggests that in lower UVR regions, positive selection would have favored mutations leading to lighter skin as a way to maximize cutaneous vitamin D synthesis (Rana et al. 1999; Jablonski and Chaplin 2000). Given the relatively recent arrival and divergence of humans in and across Europe and Asia, the most parsimonious evolution of light skin would involve such mutations arising in a proto-Eurasian population soon after humans left Africa. Consequently, these mutations should be shared between modern Asian and European populations. Alternatively, if separate existing functional variants were driven to high frequency in East Asian and Europeans or independent de novo mutations arose and were selected in each population after the divergence of Europeans and Asians, then these would be obvious as high allele frequency differences between modern European and East Asian populations. Reduced levels of heterozygosity surrounding the *SLC24A5* A111G polymorphism in the European, but not East Asian, HapMap populations support the latter hypothesis (Lamason et al. 2005), as do reduced polymorphism levels based on full resequencing data from *MATP* in populations of European descent (Soejima et al. 2005).

We will first address the role of selection in influencing darker pigmentation across different populations living in high-UVR environments. Current archaeological evidence suggests human presence in Island Melanesia by at least 40,000 years ago and in other parts of Sahul by at least 45,000 years ago (O'Connell and Allen 2004). If the original migrants to Oceania arrived there via a corridor of relatively high UVR, then we might expect their descendents to share ancestral pigmentation variants with African populations. However, if the ancestors of modern day Island Melanesians spent a significant amount of time in low-UVR regions prior to arriving in Oceania, then it is possible that mutations associated with lighter pigmentation could have accumulated and a readaptation to high-UVR conditions would have been necessary, leading to potential divergence between Island Melanesians and Africans at functional pigmentation loci. In actuality, both of these scenarios may apply, as we know that modern Island Melanesian populations are descended from both early migrants (arriving ~40,000 years ago) as well as later proto-Austronesian–speaking peoples from a southeast Asian homeland ~3,200 years ago (Spriggs 1997).

The discordance between our F_{ST}-based divergence values and allele frequencies in the Melanesian CEPH populations at *ASIP* largely stem from the relatively low frequency of the ancestral allele in the 2 CEPH Island Melanesian populations relative to our original Island Melanesian sample. These discrepancies make it difficult to determine if *ASIP* truly underlies broad pigmentation differences between darkly and lightly pigmented populations or if instead interpopulation variation at this locus can largely be explained by differences between Africans and non-Africans. This discordance between the frequencies of the *ASIP* ancestral allele in our original Island Melanesian sample and the Melanesian samples from the CEPH panel may be indicative of both the complex demographic

history of Island Melanesia (involving several migratory events (Spriggs 1997) and probable extensive genetic drift (Friedlaender 1975, 1987) as well as the importance of multiple loci in determining pigmentation phenotype. Some indications of selective forces acting in the West Africans at *ASIP* in the HapMap data are consistent with a role in shaping interpopulation pigmentation, but due to the small numbers of populations surveyed in the HapMap, these data cannot resolve the role of *ASIP* across multiple darkly pigmented populations.

The results for *OCA2* using the F_{ST}-based approach, allele frequency distribution in the CEPH panel, and analysis of the HapMap data are largely consistent in pointing to a role for this gene in control of light versus dark pigmentation. In general, the derived allele (associated with lighter pigmentation) is most common in Europeans and East Asians, whereas the ancestral allele predominates in sub-Saharan Africa and Island Melanesia. The lightly pigmented hunter–gatherer San population of Southern Africa is exceptional in having a high frequency of the derived allele relative to geographically proximate and more darkly pigmented African populations (Jablonski and Chaplin 2000), further supporting the importance of *OCA2* in regulating normal variation in pigmentation. The widespread distribution of the derived allele in the CEPH-Diversity Panel suggests that it is not necessarily a new mutation, nor has it been restricted to a specific geographic area. Interestingly, derived allele frequencies at this locus are quite different between Native American (15%) and East Asian populations (45%), suggesting that perhaps the derived allele at this locus did not reach very high frequencies in East Asians until after the colonization of the Americas. Evidence from a suite of selection statistics in HapMap populations suggests that derived allele may have been selected in both East Asians and Europeans, and consequently a proportion of the light skin phenotype in both groups may be explained by the same genetic mechanism.

Although our data do not allow us to draw very strong conclusions regarding the evolution of dark pigmentation in human populations, they do provide us with compelling evidence that light skin has evolved independently in European and East Asian populations. The strong signal of selection in the *SLC24A5* and *MATP* genes, and to a lesser extent in the *TYR* gene, supports the active role of selection in shaping the patterns of diversity at these loci. Linkage between *TYR* and *SLC24A5* and pigmentation phenotype have been previously demonstrated (Shriver et al. 2003; Lamason et al. 2005). Using similar methods, we observe a strong linkage between *MATP* C374G and pigmentation in African-Americans, indicating the functional relevance of this SNP and the potentially important role that it plays in determining interpopulation phenotypic variation. We conclude that light pigmentation in Europeans is at least partially due to the effects of positive directional and/or sexual selection and not simply the relaxation of functional constraint. This is consistent with a recent resequencing study that examined variation in *MATP* (Soejima et al. 2005) in a global sample. The virtual absence of *MATP* 374*G–derived allele in the sub-Saharan African populations that we examined in the CEPH-Diversity Panel is consistent with the origin of this mutation outside of Africa after the divergence of modern Asians and Europeans. In contrast, the *SLC24A5* 111*A–derived allele is found at low frequencies in several sub-Saharan populations including the West African Mandenka and Yoruba, the Southern African San, and South West Bantu. The relatively high frequencies of the derived allele in Central Asian, Middle Eastern, and North Africa seem likely to be due to gene flow with European populations. Similarly, the presence of the derived allele (albeit at low frequencies) in some sub-Saharan African populations may be due to recent gene flow from European and Central Asian populations. Alternatively, the derived allele may have existed in the ancestral human population and was lost in the ancestors of modern East Asians but retained in the ancestral European population. The allele then rose to high frequency in Europeans following the divergence of European and East Asian ancestral groups.

These results simultaneously and strongly suggest that Europeans and East Asians have evolved lighter skin independently and via distinct genetic mechanisms, as there is an absence of any unusual pattern of diversity at *SLC24A5*, *MATP*, and *TYR* in East Asians. These observations are consistent with conclusions based on analyses of heterozygosity levels surrounding the *SLC24A5* functional polymorphism in the HapMap populations (Lamason et al. 2005), the results of a resequencing study of *MATP* (Soejima et al. 2005), and a recent work examining variation in *SLC24A5* and *MATP* in populations representing Europe, Africa, and Asia (Soejima and Koda 2006). Assuming that the lighter pigmentation phenotype of both Europeans and East Asians is due to the same selective pressure (decreased UVR), we might expect to identify other loci having phenotypic effects as strong (or stronger) than these 3 loci that exhibit patterns of reduced diversity and population-specific F_{ST} in East Asian populations. Intriguingly, analyses of HapMap data corroborate this suggestion in identifying 2 a priori pigmentation candidate genes, *ADAM17* and *ATRN*, that show evidence of strong, population-specific selection in East Asians. These loci show extended blocks of significant values at all 3 test statistics computed for the HapMap data (supplementary table 3, Supplementary Material online) in East Asian populations. Although both of these genes are associated with pigmentation variation in mice, they also have other effects outside of the pigmentation system, including the development of the central nervous system and energy homeostasis (*ATRN*) (Lu et al. 1999; He et al. 2001; Barsh et al. 2002) and development (*ADAM17*) (Peschon et al. 1998). As such, further investigation will be required to confirm the role of these genes in regulating normal pigmentation variation as well as the potential selective event that has shaped the observed patterns of genetic variation.

The *MC1R* gene was the only locus examined in detail that did not show any signal of potential positive selection. Previous sequence-based studies have reached conflicting conclusions about whether or not *MC1R* has been subject to positive selection outside of Africa (Rana et al. 1999; Harding et al. 2000; Makova et al. 2001). Although *MC1R*'s association with red hair, fair skin, freckles, and melanoma risk in European and European-derived populations primarily from the British Isles (Box et al. 1997; Smith et al. 1998a; Schiöth et al. 1999; Flanagan et al. 2000;

Bastiaens et al. 2001) clearly demonstrates the important regional role that it plays in pigmentation, *MC1R* may have (with some exceptions [John et al. 2003; Nakayama et al. 2006]) little effect on variation outside of Europe (Myles et al. 2006). Consequently, no signal will be detected using our approaches. Although the 2 SNPs that we typed in *MC1R* are not strongly associated with the red hair and fair skinned phenotype for which *MC1R* is so well known (Sturm et al. 2003), both are polymorphic in global surveys of populations (Rana et al. 1999; Harding et al. 2000). In addition, the *MC1R* G92A SNP may have a "mild" effect on pigmentation phenotype (Motokawa et al. 2006). The 92*A allele at this site is known to have a lower affinity for α-MSH than wild-type *MC1R* alleles (Xu et al. 1996), which suggests that it may contribute to normal variation in pigmentation. However, if positive directional selection has acted on *MC1R*, we would expect variation at linked sites to be affected. As such, even if we have not assayed the relevant functional SNP, we should still have observed some signal of selection, especially given the small size (~3 kb) of this gene.

Two recent papers have examined signals of selection in pigmentation candidate genes using publicly available data from the HapMap and Perlegen databases that contain genotype information from African (or African-American), European, and East Asian populations (Izagirre et al. 2006; Myles et al. 2006). Both studies examine F_{ST} and measures of the extent of long-range haplotypes (LRHs) surrounding a number of candidate loci in these 3 populations. All 3 studies have reached similar conclusions for a subset of the genes examined. Both Izagirre et al. (2006) and Myles et al. (2006) identified signals of European-specific selection at *SLC24A5*. Similarly, both studies also identified signals of selection in *MATP* between Europeans and East Asians. However, Izagirre et al. (2006) did not detect a signal at *MATP* between Europeans and Africans. We agree with Myles et al. (2006) that this discrepancy may be due to the sampling strategy of Izagirre et al. (2006), in which allele frequency information was pooled across both Africans and African-Americans. At *OCA2*, Myles et al. (2006) reported F_{ST} values that, although not statistically significant, were suggestive of European divergence at *OCA2*, similar to our F_{ST} results. All 3 studies failed to observe a signal of selection at *MC1R*. Although all 3 studies agreed on the potential role of selection in the above-mentioned genes, at other loci, there is some disagreement between the 3 works. Neither Izagirre et al. (2006) nor Myles et al. (2006) observed significant F_{ST} or LRH values at *TYR*. Although we did observe significant F_{ST} values between Europeans and all other populations at this locus, we did not observe a gene-wide signal at *TYR* in our HapMap analyses, suggesting that this difference may be due in part to the populations and types of analyses used in each study. These differences may also explain why Izagirre et al. (2006) and Myles et al. (2006) failed to detect a signal of European-African differentiation at *ASIP*. Although we only examined 6 genes for significantly high F_{ST} differences, we also examined an additional 28 pigmentation candidate loci for signals of selection using HapMap data alone. These results also show some agreement with Izagirre et al. (2006) and Myles et al. (2006), including a signal of East Asian differentiation at *ADAM17* (Myles et al. 2006) and European differentiation at *TYRP1*. Finally, Myles et al. (2006) identified *DCT* as a candidate for influencing pigmentation in East Asian populations (but importantly, not in European populations). Although we did not include *DCT* in our F_{ST} analyses, it did show significantly high LSBL in the HapMap East Asian sample.

Observed patterns of global skin pigmentation diversity and their correlation with environmental UV exposure suggest an adaptive response. Although we cannot rule out a role for sexual selection, our results support multiple genetic mechanisms for evolution of skin color. We provide evidence that at least 2 genes, *ASIP* and *OCA2*, probably play a shared role in shaping light and dark pigmentation across the globe. We have also firmly identified a further 3 genes (consistent with the results of previous studies: Lamason et al. 2005; Soejima et al. 2005), and potentially several more candidate loci, that have a significant effect in regional pigmentation phenotype. Our data strongly support independent genetic origins for the light skin phenotype in Europeans and East Asians arising after the divergence of modern European and East Asian populations.

Supplementary Material

Supplementary tables 1–3 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

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