Corrections

EVOLUTION

The authors note that Fig. 1 appeared incorrectly. Some ABO polymorphism statuses have been corrected. The corrected figure and its legend appear below.

![Phylogenetic tree of primate species with ABO phenotypes and genotypes](https://www.pnas.org/cgi/doi/10.1073/pnas.1304029110)

**Fig. 1.** The phylogenetic distribution of ABO phenotypes and genotypes. Shown is a phylogenetic tree of primate species, with a summary of phenotypic/genotypic information given in the first column, and the genetic basis for the A versus B phenotype provided in the second column (functionally important codons at positions 266 and 268 are in uppercase letters). See Dataset S1 for the source of information about phenotypes/genotypes. Only species with available divergence times are represented here (34 of 40). The phylogenetic tree is drawn to scale, with divergence times (on the x axis) in millions of years taken from ref. 29. OWM, Old World monkeys; NWM, New World monkeys. Under a model of convergent evolution, these data suggest that A is the ancestral allele, and a turnover (e.g., a neutral substitution) occurred on the branch leading to Old World monkeys. If instead, B were ancestral, all Old World monkeys would have had to serendipitously converge from ATG to TTG to encode a leucine, whereas all New World monkeys and hominoids would have had to converge to the CTG codon.

www.pnas.org/cgi/doi/10.1073/pnas.1304029110

The authors note the following: “The genus name Lamiasaurus, which we proposed for a new lizard from the Late Cretaceous of Wyoming, is preoccupied by Lamiasaurus Watson 1914 (1), a tapinocephalid therapsid from the Permian of Africa. We therefore propose the name Lamiasaura for the Wyoming lizard; its type species is Lamiasaura ferox, also proposed in our paper. Furthermore, holotypes were figured for this and other newly proposed species but not explicitly identified in the text. We designate types and provide diagnoses as follows. Cerberophis robustus, holotype: United States National Museum 25870. Diagnosis: large stem varanoid characterized by a deep, massive jaw, teeth short, unserrated, robust, and labiolingually expanded. Cerberophis robustus, holotype: University of California Museum of Paleontology 130696. Diagnosis: medium-sized (~2 m) alethinophidian, trunk vertebrae with broad, flat ventral surface, hypertrophied synapophyses, large, massive prezygapophyses with rudimentary prezygapophyseal processes and anterior ridges; neural arch with dorsolateral ridges, moderately tall neural spine. Lamiasaura ferox, holotype: University of Wyoming 25116A, left dentary with four teeth. Diagnosis: dentary straight, tapered in lateral view; teeth widely spaced, crowns weakly recurved, crowns with bottleneck constriction between the base and apex, low mesial and distal cusps, and ridged lingual surface. Lonchisaurus trichurus, holotype: American Museum of Natural History 15446. Diagnosis: dentary long, low, and weakly bowed in lateral view; tooth crowns robust, weakly recurved, with weakly pointed crowns; teeth bases wider labially than lingually, tooth replacement reduced, coronoid overlaps dentary laterally. Obamadon gracilis, holotype: University of California Museum of Paleontology 128873. Diagnosis: small polyglyphanodontian characterized by the following combination of characters: dentary slender, symphysis weakly developed, tooth implantation subpleurodont, teeth lack basal expansion, tooth crowns with a tall central cusp separated from accessory cusps by deep lingual grooves. Pariguana lancensis, holotype: American Museum of Natural History 22208. Diagnosis: small iguanid: teeth tall, slender, with tapering crowns and weak accessory cusps; coronoid extended onto lateral surface of jaw below last tooth, Meckelian groove constricted suddenly ahead of anterior inferior alveolar foramen. Socognathus brachyodon, holotype: Yale Peabody Museum (Princeton University Collection) 16724. Diagnosis: Socognathus with posterior teeth having strongly swollen, weakly tricuspid crowns.”

“This correction formally validates the taxa proposed in our 2012 paper; thus, those taxa should be attributed to this note and accordingly dated as March 19, 2013.”

“We thank Christian Kammerer and Christopher Taylor for bringing these two issues to our attention.”

The ABO blood group is a trans-species polymorphism in primates

Laure Ségurel1(b,1,2, Emma E. Thompson1(a,1, Timothée Flutre1(a,c, Jessica Lovstad3, Aarti Venkat3, Susan W. Margulis3(d,3, Jill Mosey3, Steve Ross3, Kathryn Gamble3, Guy Sella3, Carole Ober3,4, and Molly Przeworski1(b,1,2,4

1Department of Human Genetics, Howard Hughes Medical Institute, and 2Department of Ecology and Evolution, University of Chicago, Chicago, IL 60637; 3Department of Genetics and Plant Breeding, Institut National de la Recherche Agronomique, Unité de Recherche 1164, 78026 Versailles, France; 4Lincoln Park Zoo, Chicago, IL 60614; and 5Department of Ecology, Evolution and Behavior, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Edited by Marcus W. Feldman, Stanford University, Stanford, CA, and accepted by the Editorial Board September 12, 2012 (received for review June 22, 2012)

The ABO histo-blood group, the critical determinant of transfusion incompatibility, was the first genetic polymorphism discovered in humans. Remarkably, ABO antigens are also polymorphic in many other primates, with the same two amino acid changes responsible for A and B specificity in all species sequenced to date. Whether this recurrence of A and B antigens is the result of an ancient polymorphism maintained across species or due to numerous, more recent instances of convergent evolution has been debated for decades, with a current consensus in support of convergent evolution. We show instead that genetic variation data in humans and gimbons as well as in Old World monkeys are inconsistent with a model of convergent evolution and support the hypothesis of an ancient, multiallelic polymorphism of which some alleles are shared by descent among species. These results demonstrate that the A and B blood groups result from a trans-species polymorphism among distantly related species and has remained under balancing selection for tens of millions of years—to date, the only such example in hominoids and Old World monkeys outside of the major histocompatibility complex.

Balancing selection pressures can maintain two or more alleles in the population for long periods of time—so long, that the polymorphism may be shared due to identity by descent among distinct species, leading to a trans-species polymorphism. In this scenario, the time to the most recent common ancestor (tMRCA) of the selected alleles will predate speciation times. Due to linkage, sites near the selected ones may also have old tMRCAAs, resulting in unusually high diversity within species and shared alleles between species (1). Because of recombination events between the two allelic classes in the history of the sample, however, the tMRCA at linked sites can also be much more recent than at the selected sites, so diversity levels need not be unusually high. Moreover, the old tMRCA provides many opportunities for recombination, which will erode the segment that carries the high diversity signal (2, 3); as a result, ancient balancing selection will leave only a narrow footprint in genetic variation data and will often be hard to detect (1, 3).

Perhaps for this reason, only a handful of examples of trans-species polymorphisms are known at the molecular level. The most famous examples are the self-incompatibility loci in plants and the major histocompatibility complex (MHC) in vertebrates. The self-incompatibility loci prevent self-fertilization, and variation is likely maintained by negative frequency-dependent selection (4, 5). In turn, the nature of selective pressures at the MHC are unclear, but are believed to result from its central role in the recognition of pathogens (for a recent review, see ref. 6). Within humans, the MHC is the only region known to harbor variation shared identical by descent with other species. Variants at other loci have also been found to be shared with hominoid species, potentially indicating shared balancing selection pressures, but patterns of genetic variation do not support the hypothesis that the alleles are identical by descent as opposed to due to recurrent mutations [e.g., the phenylthiocarbamide (PTC) alleles in humans and chimpanzees] (7).

Arguably the best studied case of apparent convergent evolution is the ABO blood group (A, B, AB, O) (1, 8), the first molecular polymorphism to be characterized in humans. ABO blood groups are defined by the presence or absence of specific antigens that circulate in body fluids and are attached to lipids at the surface of various epithelial and endothelial cell types (notably in the gastrointestinal tract, but also, in hominoids only, on red blood cells) (9, 10). These antigens are associated with complementary immune antibodies produced in the gut after contact with bacteria and viruses carrying A-like and B-like antigens (11). Whereas the biological significance of ABO outside of its role in transfusion is unclear (12), histo-blood antigens in general are known to act as cellular receptors by which pathogens can initiate infections (13–15). Furthermore, variation in ABO has been associated with susceptibility to a number of infectious diseases (reviewed in ref. 16), pointing to a role of ABO in immune response.

The presence or absence of the A, B, and H (O) antigens result from allelic variation at the ABO gene, which encodes a glycosyltransferase. The A transferase, able to transfer N-acetyl-D-galactosamine to the H acceptor substrate, is encoded by the A allele and the B transferase, able to transfer α-D-galactose to the same acceptor substrate, by the B allele. A and B alleles at ABO are codominant, whereas the null O alleles are recessive (17). Two amino acids at positions 266 and 268 in exon 7 are responsible for the A and B enzymatic specificity in humans (18) and are surrounded by a peak of nucleotide diversity (3, 19, 20). As an illustration, the diversity level in exon 7 is 0.0058 in a Yoruba sample, a value equalled or exceeded in only 0.08% of comparable exonic windows in the genome (SI Methods, SI Note ST). This peak of diversity provides support for long-lived balancing selection acting on this locus (20, 21).


The authors declare no conflict of interest.

This article is a PNAS Direct Submission. M.W.F. is a guest editor invited by the Editorial Board.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. JQ857042–JQ857076).

1. L.S. and E.E.T. contributed equally to this work.
2. To whom correspondence may be addressed. E-mail: lsegurel@uchicago.edu, c-ober@genetics.uchicago.edu, or mfp@uchicago.edu.
4. C.O. and M.P. contributed equally to this work.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1210603109/-/DCSupplemental.
Remarkably, the A, B, and H antigens exist not only in humans but in many other primates (reviewed in ref. 22), and the same two amino acids are responsible for A and B enzymatic specificity in all sequenced species (8, 18, 23–25). Thus, primates not only share their ABO blood group, but also the same genetic basis for the A/B polymorphism. O alleles, in contrast, result from loss-of-function alleles such as frame-shift mutations and appear to be species specific (26). That different species share the same two A/B alleles could be the result of convergent evolution in many lineages or of an ancestral polymorphism stably maintained for millions of years and inherited across (at least a subset of) species.

The two possibilities have been debated for decades, with a consensus emerging that A is ancestral and the B allele has evolved independently at least six times in primates (in human, gorilla, orangutan, gibbon/siamang, macaque, and baboon) (8, 25, 26), in particular, that the human A/B polymorphism arose more recently than the split with chimpanzee (8, 20) (SI Methods, SI Note S2). We show instead that the remarkable distribution of ABO alleles across species reflects the persistence of an old ancestral polymorphism that originated at least 20 million years (My) ago and is shared identical by descent by humans and gibbons as well as among distantly related Old World monkeys.

Results

Previous to ours, 31 studies reported phenotypic and/or genetic data on ABO in non-human primates (Dataset S1 and S2). To supplement these data, we sequenced exon 7 of the ABO gene in four hominoid species (10 bonobos, 35 western chimpanzees, 31 lowland gorillas, and 12 orangutans from both Sumatra and Borneo), in two previously uncharacterized Old World monkeys (five colobus and four vervet monkeys), as well as, for the first time, in two New World monkeys (four marmosets and three black howler monkeys) (Methods, SI Acknowledgments and Datasets S3, S4, and S5). Among 40 non-human species, the ABO polymorphism is ubiquitous, with 19 species in 10 genera polymorphic for A and B, as well as 10 species in seven genera without a B in our sample and 11 species in six genera without an A (Dataset S1, SI Methods, SI Note S3, and Fig. 1); 15 species in 11 genera also have an unambiguous O allele. Contrasting sequences obtained for marmosets (which are all A in our sample) and black howler monkeys (all B in our sample) reveal that the genetic basis for A/B specificity is the same in New World monkeys as in hominoids (Fig. 1). For the A allele, we further observed that, compared with hominoids, colobus and vervet monkeys use a different codon to encode the same amino acid at one of the two functional positions, as was previously noted for macaques (24) and baboons (27) (Fig. 1). This phylogenetic pattern is consistent with a synonymous substitution in the A lineage leading to Old World monkeys.

To distinguish between the maintenance of an old trans-species polymorphism (Fig. 2A) and more recent convergent evolution (Fig. 2B), we first sought to gain a sense of the length of the segment that should carry a signal of a trans-species polymorphism. To this end, we approximated the expected length of the (two-sided) segment contiguous to the focal sites, for plausible values of the salient parameters (see Methods, SI Methods, SI Note S4, and Fig. S1 for details). Consistent with previous modeling work (2), we found that it should be at most a few hundred base pairs (bp) in length, depending notably on the pair of species considered and the recombination rate (Fig. 2C and Fig. S2). One implication is that previous studies that considered larger windows or segments far from the selected sites may have missed the footprints of a trans-species polymorphism (SI Methods, SI Note S2).

Based on our calculations, we generated trees of ABO haplotypes for short regions around the functional sites, i.e., 300 bp in hominoids and 200 bp in Old World monkeys (SI Methods, SI Note S5). Strikingly, in the trees within hominoids (other than orangutan) and within Old World monkeys, A and B alleles cluster by type rather than by species (Fig. 3). The clustering by A and B type is consistent with a scenario in which the A and B allelic classes had a most recent common ancestor long ago and persisted across species (Fig. 2A). The lack of clustering for the O alleles could then reflect frequent turnovers (i.e., replacement) of these alleles within a balanced polymorphism, as expected from the high mutation rate to null alleles (28). Alternatively, the phylogenetic trees could reflect convergent evolution of multiple functional mutations in numerous lineages (Fig. 2B).

Because of recombination, diversity among ABO alleles should be highly heterogeneous along the sequence, with at most a few (and possibly no) short segments showing unusually high diversity levels (2). We therefore focused on small, sliding windows along exon 7 (see Figs. S3 and S4 for a slightly larger window choice), comparing the pairwise synonymous diversity among ABO allelic classes to the 95% confidence interval for synonymous divergence between the same allele (A or B) from different species (Methods). In comparisons of lineages from different species, a model of convergent evolution predicts that, near the selected sites, the divergence between allelic classes (e.g., A and B) will be the same as divergence within allelic classes (e.g., A and A), whereas a model of trans-species polymorphism predicts that the former will exceed the latter. The excess will be subtle, however, because the divergence between lineages from the same allelic class sampled in different species (e.g., A and A) is also inflated close to an ancient balanced polymorphism (due to recombination; see figure 1 in ref. 2). We note further that because O is a null allele, there is a high mutation rate to O, such
that the current O could be derived from any functional allele, including some that have not persisted to the present. As a consequence, divergence between O and A (or O and B) may be deep, possibly even deeper than A versus B, even if the O allele is itself recent.

Within Old World monkeys, there is tremendous synonymous diversity between A and B alleles in macaques, exceeding the divergence between macaque and baboon, and consistent with the divergence between macaque and colobus monkey ~18 Mya (29) (Fig. 4A). Similarly high synonymous diversity is visible between A and B alleles within colobus monkeys, notably around the functional sites (Fig. 4B). A similar but weaker pattern is seen in baboons (Fig. 4C), possibly due to greater erosion of the ancestral segment by recombination. In accordance with the inheritance of an ancestral segment identical by descent across macaques and baboons, two synonymous polymorphisms are shared between the two species (Fig. 4B). Further evidence that the origin of the polymorphism predates their split comes from the comparison between baboon A and macaque O (or baboon O and macaque B), which reveal deeper coalescent times between allelic classes than seen within an allelic class (i.e., baboon and macaque A or B). Together, these findings strongly support the hypothesis of a trans-species polymorphism shared among macaque, baboon, and colobus monkey, with an origin as old as 18 Mya (or greater).

In hominoids, in turn, human ABO diversity is over an order of magnitude higher than typical polymorphism levels of ~0.1% (30) (Fig. 4B), with synonymous diversity between ABO alleles similar to divergence levels among African apes and chimpanzees. Furthermore, divergence among African apes and chimpanzees is mirrored in gibbons (Fig. 4B), as expected if humans and gibbons share A and B alleles identical by descent. Furthermore, divergence among chimpanzee A and human B alleles significantly exceeds the divergence among African ape lineages (Fig. 4B), which is predicted only if the A/B polymorphism predates the species’ split. In orangutan, in contrast, A and B lineages are highly similar (Fig. S4), suggesting they are recently derived; because this case could represent a turnover event in this lineage, however, it does not preclude the possibility that the A/B balanced polymorphism is older (28). Thus, synonymous pairwise differences in hominoids point to an origin of the A/B polymorphism before the divergence of extant African apes ~8 Mya, and are consistent with the maintenance of a balanced polymorphism since the root of hominoids ~20 Mya, which persisted in humans and gibbons.

Because of the stochasticity of recombination and mutation events in a small window, the divergence levels are noisy and do not establish whether the polymorphism is significantly older than 8 My, i.e., whether A and B alleles arose twice in hominoids, once in the ancestor of the African apes and once in the ancestor of gibbons and siamangs, or only once, before the human–gibbon
Our results indicate that ABO is a trans-species polymorphism inherited identical by descent in humans and gibbons as well as among all Old World monkeys studied (macaques, baboons, and colobus monkeys), and which was therefore maintained over tens of millions of years. ABO is the second example of a locus at which human variation traces back to the origin of hominoids. Moreover, given that the signal of a trans-species polymorphism is expected to decrease over time, eventually becoming undetectable (2, 3) (Fig. 2C), we cannot exclude an older origin of the balanced polymorphism, which led to allele sharing among hominoids and Old World monkeys (albeit with a turnover of the A allele), or possibly even with New World monkeys. This finding points to selection pressures that have remained strong relative to genetic drift throughout the evolution of these species (28).

As remains the case for the MHC (6), the selection mechanism maintaining this polymorphism across so many primate species is largely unknown. One possibility, heterozygote advantage (seen e.g., in the HBB gene in response to malaria and sickle-cell anemia) (32), may be unlikely to underlie long-lived balancing selection, instead representing a transient solution to balancing selection pressures until a single allele that confers the selected phenotype is fixed in any of the surveyed species and are rare in humans. In support of this argument, the AB phenotype can be created by single “cis-AB” alleles (34) and yet such alleles have not reached fixation in any of the surveyed species and are rare in humans (Dataset S3). Moreover, the presence of the ABO polymorphism throughout primates implies that the selected phenotype is probably not tied to the expression of antigens on red blood cells, a trait restricted to hominoids (35). Humans are known to have many histo-blood subgroups (notably among A types), which are interchangeable for transfusion purposes, but differ in quantity and quality of antigens (36, 37); similarly, chimpanzees, gorillas, orangutans, and gibbons have been reported to have variable A and B subgroups, respectively, that differ in antigenic properties (26, 38). Thus, although A, B, and O are clearly of functional importance and may denote the strongest fitness differences
among variants of the ABO gene, these histo-blood labels are unlikely to provide a complete description of the allelic classes acted on by natural selection. These considerations suggest that variation at ABO reflects a multiallelic balanced polymorphism, with cryptic differences in function among A and B alleles. As expected from an ancient multiallelic balanced polymorphism (21, 28), there was occasional turnover within allelic classes, including of a codon in the lineage leading to Old World monkeys and of A and B alleles within orangutan, as well as frequent turnovers of O alleles in all species (28). Numerous losses of A and B have also occurred (Fig. 1), possibly as a result of bottlenecks in the history of the species (39) or due to differences in selection pressures among lineages.

In any case, the maintenance of ABO across so many primates reveals previously unknown and important functions of a heavily studied gene. More generally, this study illustrates a general approach that can be used to scan for ancient balancing selection in the genome and raises the possibility that, with the availability of genome-wide polymorphism data from closely related species, this mode of selection will turn out to be more common than currently believed.

Methods

Resequencing Data for ABO. We used previously published data: in humans, 60 individuals of European ancestry (CEU), 60 individuals of South Asian ancestry (CHB + JPT) and 59 individuals of Sub-Saharan ancestry (YRI) (30), 31 olive baboons (Papio anubis) (27), 13 macaques (seven cynomolgus or crab-eating macaques, Macaca fascicularis and six rhesus macaques, Macaca mulatta) (24), 17 gibbons (five agile gibbons, Hylabates agilis and 12 white-handed gibbons, Hylabates lar), and six siamangs (Symphalangus syndactylus) (25). In addition, we sequenced hominoid samples from Lincoln Park Zoo (40). (S1 Acknowledgments) 10 bonobos (Pan paniscus), 35 western chimpanzees (Pan troglodytes), 31 lowland western gorillas (Gorilla gorilla), and nine orangutans (Pongo pygmaeus: three orangutans from Sumatra, two from Borneo, and four hybrids). Three black howler monkeys (Alouatta caraya) samples were also obtained from Lincoln Park Zoo. Additionally, samples from three Sumatran orangutans were purchased from the San Diego Zoo, five colobus monkeys (three Colobus angolensis, one Colobus polykomos, and one Colobus guereza) from the Integrated Primate Biomaterials and Information Resource (IPBIR) through the Coriell Institute, four vervet monkeys (Chlorocebus aethiops) from Alpha Genesis, and four marmosets (Callithrix jacchus) from the Southwest Foundation for Biomedical Research.

When needed, genomic DNA was extracted from blood using the Puregene DNA isolation kit (Gentra Systems). Amplification of exon 7 was performed using primers and conditions described in Dataset S5. Sequencing reactions were carried out using the Big Dye Terminator v3.1 Cycle sequencing kit (Applied Biosystems). Chromatograms were aligned and analyzed using the Phred-Phrap-Consed package (41).

Haplotypes were estimated in each species separately using PHASE2.1 (42). The program was run twice, with different seeds (option --5); the two outputs were identical other than for six SNPs in colobus monkeys (of which five SNPs were in perfect linkage disequilibrium) and one SNP in humans (in the CHB + JPT population). Trees and diversity plots rely on the first output, but identical conclusions were obtained with the second one. The substitutions and polymorphisms requiring more than one mutation given the accepted species tree (29) are listed in Dataset S3 and the numbers of polymorphic sites found per species are listed in Dataset S4.

Estimating the Length of the Segment Carrying a Signal of a Trans-species Polymorphism. The presence of a trans-species polymorphism at one site does not necessarily mean that genetic variation data at linked sites, but only over a short distance (2). To estimate this distance, we assumed that balancing selection has maintained two alleles, A and B, at a selected site (without turnover) in two species since before the time of their split, T generations ago. Specifically, we assumed that, at the selected site, the time to the most recent common ancestor for two A alleles or two B alleles sampled from different species is less than the coalescent time for an A and B allele sampled from different species. To estimate the expected length of the segment contiguous to the selected site on which we expect to see various signals of a trans-species polymorphism (SI Methods, SI Note 4).

To estimate the expected segment length for specific species pairs, we used a generation time of 15 and an ancestral effective population size N0 of ~30,000, a typical value for ancestral hominoids (43, 44). This value is also roughly equivalent to the current effective population size of rhesus macaques (4500, based on diversity estimates of 0.29% per base pair), and a mutation rate of 2 x 10^-8 per base pair per generation, which is higher than in hominoids and consistent with the higher synonymous divergence in Old World monkeys. This value of N0 leads to an expected pairwise coalescence time of 2gN0/μ = 0.9 My, and, using divergence times from refs. 25 and 29, to the following split time estimates: ~7 My for macaque-baboon, ~17 My for macaque–colobus, ~31 My for macaque–human, and ~19 My for human–gibbon. In turn, the split between human and chimpanzee was taken to be ~5 My, between human and gorilla ~7 My, and between human and orangutan ~12 My (44, 46–49).

Calculating Synonymous Pairwise Differences, dS. The average synonymous pairwise differences between alleles (dS) per 201 bp (Fig. 4 A and B) and 300 (Figs. 5 and S4) sliding window in ABO exon 7 were calculated with the maximum likelihood method (50) implemented in the program codeml from PAML (51). The codon substitution model was one in which the equilibrium codon frequencies are estimated from the average nucleotide frequencies in the sequence (option CodonFreq = 1). The pairwise comparison was used (runmode = -2), to avoid relying on an underlying species tree for all of the sequences. To obtain a more precise estimate, the transition-to-transversion ratio was estimated for all species all together from 585 bp (the sequence for which we have data for all species). The value we have for all species (λ = 5.8) was then fixed when estimating the dS per window. Using the approximation from ref. 52, implemented in the program yn00 from PAML (51) instead, i.e., allowing the transition-transversion bias to vary along the sequence and estimating each equilibrium codon frequency from the data (option CodonFreq = 3), had a considerable effect on the denominator of dS, but did not change any qualitative difference (i.e., the ordering of the comparisons). In humans, we used the low coverage pilot data from the 1000 Genomes YRI (30); however, instead considering data generated by the Seattle SNP project (http://pga.gs.washington.edu) using Sanger sequencing yielded highly similar results.

The 95% confidence intervals for dS were calculated as follows: we estimated the mean dS, in 585 bp for the A alleles using codeml with the trees species specific, then divided it by the length of the tree to obtain the expected number of mutations per base pair per million years. We did the same for the B alleles, and took the mean of the estimates from the two trees, m, which was 0.0013/My/bp in hominoids and 0.0025/My/bp in Old World monkeys. We then assumed that synonymous mutations are neutral, so that the number of synonymous mutations in a lineage is Poisson distributed with mean λ, where λ = mL*T, T is the divergence time between lineages from different species and L is the number of synonymous sites (estimated by PAML). T* values were taken from previously published estimates, notably from ref 29: 20 My for human–gibbon, 18 My for colobus–macaque, 17 My for human–orangutan, 12 My for vervet–macaque, and 8 My for baboon–macaque. For African apes, a synonymous allele (G at nucleotide 813) is fixed in human and gorilla but absent from chimpanzee, suggesting that gorilla is closer to human than chimpanzee/bonobo in this regard. Indicating a possible lineage sorting event. The comparison of lineages from humans, chimpanzees, and gorillas coalesced before the split of the three species. To account for this possibility, we used 8 My as the divergence time between human, chimpanzee, and gorilla lineages. This is conservative for our purposes, as a more recent divergence would imply a lower 95% CI and an even greater signal of trans-species polymorphism.

Test of Convergent Evolution Using the Internal Branches in the Hominoid Tree. Using the 585-bp sequence for which we have data for all species, we inferred where synonymous substitutions occurred along the tree of hominoids by parsimony, verifying that PAML output yielded similar ancestral sequences (using the maximum likelihood method based on a priori species tree, with gorilla closest to human). We then calculated the rate of synonymous substitutions on extant lineages that are monomorphic (chimpanzee, gorilla, and siamang) or polymorphic (human and gibbon). For this purpose, we ignored the bonobo lineage, because the addition of the bonobo lineage (which split from chimpanzee <1 Mya (53) does not provide much time for neutral mutations to arise and fix, so is relatively uninformative. In addition, the orangutan lineage was excluded from this analysis because it appears to have experienced a recent turnover.

We assumed that B arose ~8 Mya (the minimum age indicated by Fig. 2B) and tested a null model in which there was only one ABO class during the first 12 My of hominoid evolution. Specifically, we compared rates of substitutions in exon 7 on internal branches (one substitution in 24 My) to that seen in monomorphic lineages (six substitutions in 24 My). Assuming that...
We thank P. Andolfatto, G. Coop, R. Hudson, G. Perry, J. Pritchard, and M. Stephens for helpful discussions, and G. Coop and J. Pritchard for comments on an earlier version of the manuscript. This study used biological materials obtained from the Southwest National Primate Research Center, which is supported by National Institutes of Health National Center for Research Resources Grant P51 RR013986. This work was supported by a Rosalind Franklin award and R01 GM72861 (to M.P.). C.O. was partially funded by Grant R01 HD21244. E.E.T. was supported by Grant K12 HL090003. G.S. was funded by a Flegg fellowship and Israel Science Foundation Grant 1492/10. M.P. is a Howard Hughes early career scientist.