other studies (*16*–*20*). Agriculture accounts for 70% of British land use, strongly suggesting that this relationship is causal, though the exact drivers of extinctions are clearly multifactorial and complex. For example, for some species there may have been a mismatch in the timing of extinctions in relation to specific agricultural changes (an "extinction debt") that we cannot currently identify.

Finally, we note that the United Kingdom is on the northern and western edge of the distribution range for many Hymenoptera, resulting in the recent colonization of species that had not previously been recorded, such as *Bombus hypnorum* (*21*) and *Colletes hederae* (*22*). We might therefore expect other colonizations, extirpations, and recolonizations as part of normal background ecological processes, regardless of human activity (see supplementary materials). The consequences of climate change on species distributions provides further complications, and disentangling anthropogenic versus natural effects poses a future challenge for researchers.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Tables S1 to S4
References (*23*–*25*)

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NUTRITIONAL IMMUNITY

Escape from bacterial iron piracy through rapid evolution of transferrin

Matthew F. Barber and Nels C. Elde*

Iron sequestration provides an innate defense, termed nutritional immunity, leading pathogens to scavenge iron from hosts. Although the molecular basis of this battle for iron is established, its potential as a force for evolution at host-pathogen interfaces is unknown. We show that the iron transport protein transferrin is engaged in ancient and ongoing evolutionary conflicts with TbpA, a transferrin surface receptor from bacteria. Single substitutions in transferrin at rapidly evolving sites reverse TbpA binding, providing a mechanism to counteract bacterial iron piracy among great apes. Furthermore, the C2 transferrin polymorphism in humans evades TbpA variants from *Haemophilus influenzae*, revealing a functional basis for standing genetic variation. These findings identify a central role for nutritional immunity in the persistent evolutionary conflicts between primates and bacterial pathogens.

Iron is a precious cellular metal, sequestered by hosts and scavenged by pathogens (*1*–*3*). Vertebrate iron transport is mediated by serum transferrin, a protein that binds circulating iron and delivers it to cells via receptor-mediated endocytosis. Modern transferrin arose through a tandem duplication event in ancestral metazoans that produced two homologous domains, the N and C lobes, each of which binds a single iron ion with high affinity (*4*). Transferrin also contributes to host nutritional immunity by sequestering essential iron away from microbial pathogens. One hallmark of host immunity protein evolution is recurrent positive selection driven by diverse and rapidly evolving viruses (*5*). However, the essential nature of transferrin's role in iron transport necessitates functional conservation, which may impede adaptation against iron piracy. Indeed, the effect of nutritional immunity on evolution at host-pathogen interfaces is unclear.

To determine whether transferrin might be subject to pathogen-driven evolution in the primate lineage, we cloned and sequenced transferrin orthologs from 21 hominoid, Old World, and New World monkey species for phylogenetic analysis (Fig. 1A and fig. S1). A combination of maximum likelihood–based algorithms to assess ratios of nonsynonymous to synonymous substitution rates (dN/dS) revealed strong signatures of episodic positive selection in transferrin ($P < 0.0001$) (table S1) across several branches of the primate lineage (Fig. 1A, fig. S2, and tables S2 to S8). To date, such signatures of molecular "arms races" in mammals are primarily documented among cell surface receptors and innate pattern recognition proteins antagonized by viruses (*6*–*8*), reflecting the primacy of such host proteins as "front line" immune defenses or points of entry for viruses. These results indicate that primate transferrin has undergone bouts of rapid evolution reminiscent of canonical innate immunity factors engaged in host-pathogen arms races.

Our analysis of positive selection in transferrin revealed that 16 of 18 rapidly evolving sites map to the C lobe (Fig. 1B), despite the fact that the N and C lobes are functionally homologous for iron binding and transport. This contrast was particularly clear in the hominoid lineage, where the C lobe alone shows strong evidence of positive selection ($P < 0.0001$), whereas the N lobe does not ($P > 0.99$) (table S4) and instead has evolved under purifying selection. The transferrin N and C lobes have thus been subject to very different selective pressures during their respective evolutionary histories, despite performing identical essential physiologic functions. Previous reports indicate that the transferrin receptor (Tf-R) in rodents and carnivores has been subject to positive selection driven by viral entry proteins (*6*, *9*). However, Tf-R is subject to purifying selection in primates (fig. S3A), and only one of 18 rapidly evolving sites in transferrin makes contact with Tf-R (fig. S3B), indicating that, as expected, rapid evolution of transferrin has not been driven by coevolution with its cognate receptor.

Remarkably, 14 of 16 rapidly evolving sites in the transferrin C lobe form direct contacts with transferrin binding protein A (TbpA) from *Neisseria meningitidis* when mapped to a recently solved, high-resolution co-crystal structure of human transferrin bound to TbpA (Fig. 1, B and C, and fig. S4) (*10*). Several Gram-negative human pathogens, including *Neisseria gonorrhoeae*, *N. meningitidis*, and *Haemophilus influenzae*, scavenge host iron via surface receptors that bind and extract iron exclusively from the C lobe of transferrin (*11*–*13*). As the primary component of these bacterial receptors, TbpA is a transmembrane transporter that facilitates extraction and translocation of iron into the bacterial periplasm. Notably, the specificity of TbpA proteins for their respective host transferrin is hypothesized to restrict the host range of these bacteria (*14*, *15*). The rapidly evolving sites that

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we identified cluster within six of the seven subregions of the TbpA interface, proximal to the TbpA L3 helix finger and plug loop that have been defined as critical points of contact between these two proteins (*10*). In contrast, no rapidly evolving sites in transferrin form contacts with TbpB, a bacterial accessory co-receptor that coordinately binds the C lobe of transferrin for iron acquisition (*10*, *16*) (fig. S5). Thus, positive selection of the transferrin C lobe may have been solely driven by interactions with TbpA and ancestral TbpA-like proteins.

To define the functional consequences of rapid evolution in primate transferrin, we used competitive binding assays (see the supplementary materials) to directly assess interactions between transferrin and TbpA (*17*, *18*). For these experiments, we purified recombinant transferrin proteins from a panel of primate species (fig. S6) and expressed TbpA from the human-specific pathogens *N. gonorrhoeae* and *H. influenzae* (strains MS11 and Eagan, respectively) in nonpathogenic BL21 *Escherichia coli*, which do not possess transferrin receptors (fig. S7). Consistent with previous studies, TbpA from both pathogens bound strongly to recombinant human transferrin (*17*, *19*) (Fig. 1D). TbpA variants also recognized transferrin from gorillas but not chimpanzees, orangutans, gibbons, or baboons (Fig. 1D). The lack of binding between TbpA and chimpanzee transferrin was particularly striking given that chimpanzees represent the closest primate relative to gorillas and humans. These observations were independently corroborated by competitive binding enzyme-linked immunosorbent assays (ELISA) recapitulating transferrin-TbpA interactions (Fig. 1D, bar graphs). Thus, transferrin divergence among great apes is sufficient to dictate distinct outcomes in bacterial TbpA binding interactions.

Among the four amino acid differences between human and chimpanzee transferrin, position 591

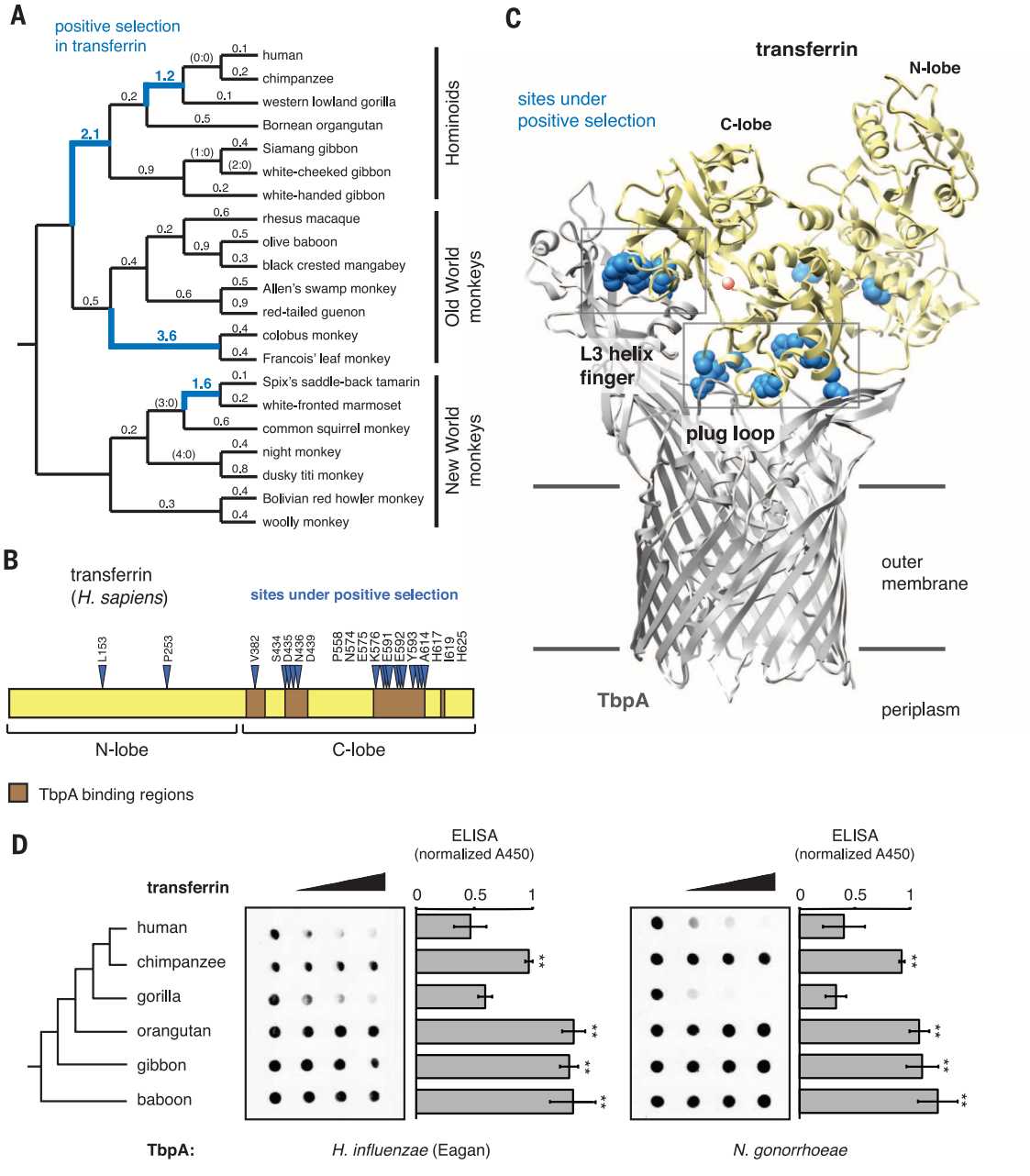

Fig. 1. Primate transferrin has undergone recurrent positive selection at the binding interface with bacterial TbpA. (**A**) A primate phylogram highlighting rapid evolution of transferrin. d*N*/d*S* ratios along each branch of the primate lineage are listed, with d*N*/d*S* values >1 highlighted in blue. Branches with no synonymous changes display N:S substitution ratios in parentheses. (**B**) Schematic of the human transferrin protein (yellow). Amino acid positions showing evidence of positive selection (phylogenetic analysis by maximum likelihood (*30*), posterior probability > 0.99) are denoted by blue arrows. Regions of transferrin spanning the *N. meningitidis* TbpA binding interface (*10*) are highlighted in brown. (**C**) Ribbons representation of the structure (Protein Data Bank no. 3V8X) of human transferrin (yellow) in complex with *N. meningitidis* TbpA (gray), with the position of the bacterial outer membrane labeled. Amino acid side chains in transferrin with strong evidence of positive selection are marked by blue spheres. The position of a ferric ion is modeled as a red dot. (**D**) Competitive TbpA binding assays performed as dot blot (left) or ELISA (right), using *E. coli* expressing indicated pathogen TbpA. Samples were incubated with horseradish peroxidase (HRP)–conjugated human transferrin alone (0.5 μg/mL), or HRP-transferrin in the presence of increasing concentrations of recombinant purified transferrin (5, 10, or 20 μg/mL) from indicated primates. Error bars represent SD of four independent experiments. ***P* < 0.01 relative to human transferrin.

(glutamic acid in humans, lysine in chimpanzees) shows strong signals of recurrent positive selection (Fig. 2A and table S6) and, intriguingly, also lies proximal to the interface with the L3 helix finger of TbpA, which plays a pivotal role in iron acquisition (*10*) (Fig. 2B and fig. S4). Competitive binding assays revealed that a glutamic acid to lysine substitution at this position in human transferrin (E591K) is sufficient to impair binding to TbpA from both *N. gonorrhoeae* and *H. influenzae*, rendering its binding affinity similar to chimpanzee transferrin (Fig. 2C). In addition, introducing a glutamic acid to lysine substitution at position 591 (K591E) in chimpanzee transferrin is sufficient to restore TbpA binding (Fig. 2C). Variation at position 591 thus directly links positive selection of transferrin to recognition by bacterial TbpA. Furthermore, closely related bonobos share glutamic acid 591 with humans and gorillas (Fig. 2A), providing clear evidence of recent transferrin adaptation in chimpanzees. These findings are surprising, given that previous studies concluded that the transferrin-TbpA interaction is largely impervious to single-point mutations in TbpA, likely owing to a substantial 2500 $Å^2$ binding interface (*10*). This result highlights the predictive power of positive selection analyses to pinpoint residues that most drastically alter binding affinity at host-pathogen interfaces. Our findings also bolster the hypothesis that transferrin is one of a handful of factors limiting the host range of human-specific bacterial pathogens (*15*, *20*), even among closely related primate species.

Given the ability of a single substitution to dictate TbpA recognition between primate transferrin orthologs, we were curious whether standing genetic variation of transferrin in human populations might provide similar protection. After the major C1 allele, C2 is the most abundant transferrin variant, found at roughly 6 to 26% allele frequency across human populations (*21*) (Fig. 2D and tables S9 and S10). C2 differs from C1 by a single C/T substitution that changes proline 589 to serine (Fig. 2D and table S9). Although this variation in transferrin has long been recognized, no appreciable differences in iron binding or other activities have been discerned between C1 and C2 (*22*). However, this polymorphism occurs only two amino acids away from position 591, which is sufficient to control TbpA binding (Fig. 2B, E). In competitive binding assays, the C2 variant was markedly resistant to recognition by TbpA from *H. influenzae* (Fig. 2F), providing a striking example of functionally adaptive consequences for genetic variation in humans. This result highlights the effect of nutritional immunity on primate evolution, from 40 million years of species divergence to a single polymorphism circulating in human populations.

To quantify differences in TbpA binding with transferrin human variants, we generated dissociation curves and calculated half-maximal inhibitory concentrations (IC_{50}). IC_{50} calculations revealed severely reduced binding by *H. influenzae* TbpA to C2 transferrin relative to C1 (fig. S8 and table S11). Unlike TbpA from *H. influenzae*, the *N. gonorrhoeae* variant bound C2 with nearly equal affinity to C1 transferrin as determined by competitive binding assays and IC_{50} calculations (Fig. 2F, fig. S8, and table S11), indicating functional variability among pathogen TbpA orthologs.

To delineate functional outcomes among TbpA proteins, we sampled variants from additional pathogenic strains isolated in clinics. We found that TbpA from *H. influenzae* isolates Eagan and strain 11 are specific for recognition of the C1 variant, potentially at the expense of C2 recognition (Fig. 2F and Fig. 3, A and B). In contrast, TbpA from *N. meningitidis* and *N. gonorrhoeae* displayed similar binding to both C1 and C2 transferrin, whereas *H. influenzae* strain 15 TbpA exhibited intermediate C2 binding affinity (Fig. 3,

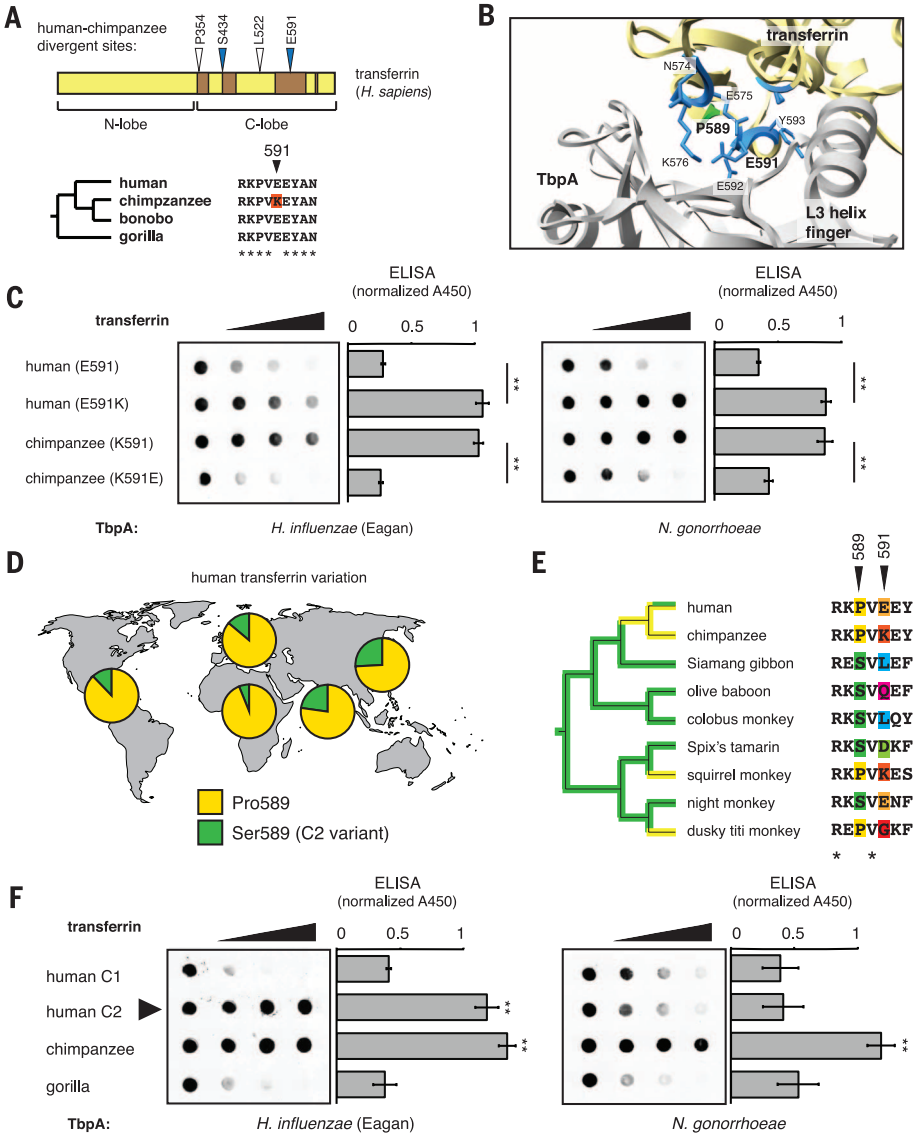

Fig. 2. Transferrin divergence in humans and chimpanzees impairs TbpA binding. (**A**) Schematic representation (top) showing divergent amino acid positions between human and chimpanzee transferrin. Blue arrows indicate amino acids that also display signatures of positive selection. Amino acid alignment (bottom) around position 591, highlighting the chimpanzee-specific E591 to K substitution. (**B**) Sites of positive selection in transferrin (blue) proximal to loop 3 of TbpA. Position 589, which is variable in human populations, is highlighted in green. (**C**) Competitive binding dot blots and ELISAs using recombinant human and chimpanzee transferrin, along with human E591K and chimpanzee K591E mutant proteins. Error bars represent SD of four independent experiments. $^{**}P < 0.01$. (**D**) Distribution of the transferrin C2 polymorphism (green) across human populations. (**E**) Primate phylogeny and amino acid alignment displaying toggling of transferrin position 589 across primates. Colors denote variable amino acids at each position. Variability at position 591 is also highlighted. (**F**) Competitive binding dot blot and ELISAs using the major transferrin variant (C1), the transferrin P589S variant (C2), chimpanzee, and gorilla transferrin. Error bars represent SD of four independent experiments. $^{**}P$ <0.01 relative to human (C1) transferrin.

A and B). Based on these findings, we speculate that there is an evolutionary trade-off between increased affinity to transferrin C2 and increased breadth of transferrin recognition. Regardless of whether such a trade-off constrains bacterial evolution, these findings reveal functionally distinct outcomes for transferrin recognition by TbpA among bacteria. Together our observations strongly suggest that both transferrin and TbpA have undergone repeated counteradaptations during the battle for iron over the course of primate evolution.

Observing functional consequences of both transferrin and TbpA evolution is consistent with the predictions of the "Red Queen" hypothesis, which posits that evolutionary arms races arise by recurrent episodes of positive selection between hosts and pathogens (*5*, *23*). To directly investigate whether TbpA has been subject to positive selection, we compared gene sequences from a large group of human-derived *Neisseria* and *H. influenzae* isolates (Fig. 3B and fig. S9). Horizontal gene transfer and recombination among bacterial strains notoriously compromise phylogenetic analyses of bacterial genes (*24*). Therefore, we relied on algorithms accounting for potential recombination break points and used combinations of structural insights and binding assays to substantiate predictions of positive selection (see the supplementary materials). We identified 10 sites among *Neisseria* and 9 among *Haemophilus* displaying strong signatures of positive selection, with one site shared between the two groups (Fig. 3, C and D, and tables S12 to S17). Nearly every site, though nonoverlapping between *Neisseria* and *Haemophilus*, lies in predicted extracellular loops of TbpA, which comprise the transferrin-binding interface (Fig. 3, C and D). These domains display marked variation even among closely related pathogen isolates, in contrast to a high degree of conservation in transmembrane domains (figs. S10 and S11).

To assess the functional implications of TbpA substitutions at rapidly evolving sites, we mutagenized *N. gonorrhoeae* TbpA at three positions under positive selection to corresponding amino acids present in *H. influenzae* (strain Eagan) TbpA (fig. S12). Of these three mutations, R365Q exhibited reduced binding to human transferrin (Fig. 3E). Thus, substitutions at rapidly evolving sites in transferrin, as well as TbpA, modulate interactions at this protein interface. By integrating phylogenetic analyses with high-quality structural data and experimental approaches, our results provide a high-resolution view of molecular genetic dynamics on both sides of host-pathogen evolutionary arms races.

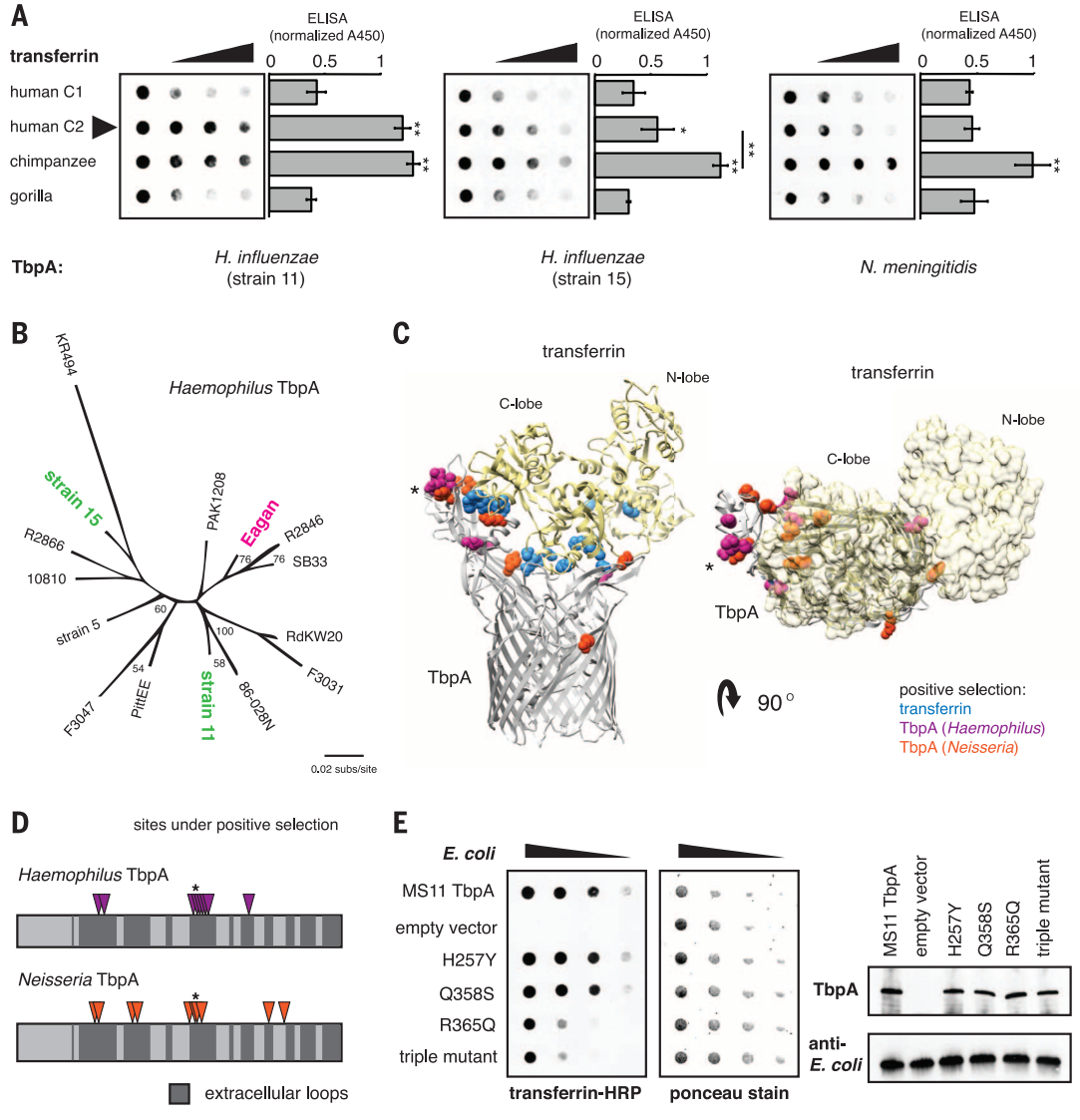

Fig. 3. Rapid evolution and functional variation of TbpA among human pathogens. (**A**) Competitive binding dot blots and ELISAs assessing TbpA binding to the human transferrin C2 variant. Error bars represent SD of four independent experiments. $^{**}P < 0.01$ relative to human (C1) transferrin, or C2 transferrin (vertical line). $^{*}P = 0.05$. (**B**) Gene tree of TbpA from *H. influenzae* isolates. TbpA from strain Eagan (magenta), as well as two divergent TbpA variants, strains 11 and 15 (green) are highlighted. (**C**) Structure of human transferrin (yellow) in complex with *N. meningitidis* TbpA (gray). Side chains of amino acids under positive selection in transferrin (blue), *H. influenzae* TbpA (purple), and *Neisseria* TbpA (orange) are denoted by colored spheres. * indicates position under positive selection in both *Haemophilus* and *Neisseria* TbpA. A "top" view of TbpA (right) shows exposure of rapidly evolving sites at the transferrin binding interface. (**D**) Schematic representation of sites among either *H. influenzae* or *Neisseria* TbpA showing evidence of positive selection, overlaid on TbpA from *N. meningitidis*. Arrows denote sites that pass multiple tests of positive selection, incorporating PhyML TbpA gene trees as well as phylogenies that account for recombination break points. Predicted extracellular loops are indicated in dark gray. * indicates a single amino acid position showing evidence of positive selection in both *Haemophilus* and *Neisseria*. (**E**) Indicated *E. coli* strains expressing mutations of TbpA were tested for interactions with human transferrin-HRP (left). A control blot was stained with ponceau (middle) as a loading control. Western blots using antibodies against *N. gonorrhoeae* TbpA or total *E. coli*. were performed with cell lysates from indicated strains (right).

In addition to the C1/C2 polymorphism in human transferrin, position 589 toggles exclusively between proline and serine across the primate lineage (Fig. 2E and fig. S13), a potential signature of antagonistic pleiotropy at a largely constrained position, as observed for other host-pathogen interfaces (*7*). Previous work has also implicated the C2 transferrin variant as a risk factor for disorders involving iron metabolism, including Alzheimer's disease; however, these associations remain controversial and appear dependent on the populations tested and interactions with other susceptibility loci (*25*, *26*). Our findings provide a functional basis for human transferrin variation and establish an important role for nutritional immunity in recent human evolution.

Although canonical innate immunity factors have been appreciated as nodes of host-virus evolution, our work demonstrates that nutritional immunity has played a fundamental role in the survival of primate populations challenged by bacterial pathogens. *H. influenzae* and *N. meningitidis* remain a major source of morbidity and mortality in regions where vaccine coverage is poor (*27*, *28*) and drug-resistant *N. gonorrhoeae* is developing into an urgent public health threat (*29*). By illuminating the battle for iron as a major driving force of host-pathogen evolution, from 40 million years of primate divergence to emerging human epidemics today, our studies reveal new reservoirs of genetic resistance to infectious diseases.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/346/6215/1362/suppl/DC1
Materials and Methods
Figs. S1 to S13
Tables S1 to S18
Movie S1
References (*30*–*36*)

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POLITICAL SCIENCE

When contact changes minds: An experiment on transmission of support for gay equality

Michael J. LaCour[1] and Donald P. Green[2]

Can a single conversation change minds on divisive social issues, such as same-sex marriage? A randomized placebo-controlled trial assessed whether gay (*n* = 22) or straight (*n* = 19) messengers were effective at encouraging voters (*n* = 972) to support same-sex marriage and whether attitude change persisted and spread to others in voters' social networks. The results, measured by an unrelated panel survey, show that both gay and straight canvassers produced large effects initially, but only gay canvassers' effects persisted in 3-week, 6-week, and 9-month follow-ups. We also find strong evidence of within-household transmission of opinion change, but only in the wake of conversations with gay canvassers. Contact with gay canvassers further caused substantial change in the ratings of gay men and lesbians more generally. These large, persistent, and contagious effects were confirmed by a follow-up experiment. Contact with minorities coupled with discussion of issues pertinent to them is capable of producing a cascade of opinion change.

Foremost among theories of prejudice reduction (*1*) is the contact hypothesis (*2*), which contends that outgroup hostility diminishes when people from different groups interact with one another. Although contact is credited with reducing prejudice toward a wide array of outgroups (*3*), in practice it is often difficult to facilitate intergroup contact of sufficient duration to dispel negative stereotypes and build empathy. For this reason, research attention has recently focused on alternative interventions that may be deployed in a more compressed time frame. Examples include brief personal contact with outgroup members during the course of a conversation (*4*) and the "extended contact" that occurs when one learns that a close friend has experienced positive contact with an outgroup (*5*). The question is whether brief or indirect contact is sufficient to produce meaningful and enduring attitude change. Recent literature reviews have been tentative on this point, noting the lack of randomized experiments that track attitudes months after the intervention (*6*).

Our theoretical contribution is to introduce the distinction between active and passive contact, which are posited to produce different effects in the context of a brief intergroup encounter. Whereas passive contact involves personal exposure to an outgroup member (e.g., through collaborative activity), active contact involves, in addition, communication about an issue that divides the two groups (e.g., discussion of recent communal violence). The effects of active contact doubtless depend on whether the conversation is respectful or accusatory, but in principle, active contact has the potential to both reduce hostility toward outgroups and to change attitudes on divisive issues. Our empirical contribution is the first field-based experimental demonstration of persistent attitude change in the wake of active

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Escape from bacterial iron piracy through rapid evolution of transferrin

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