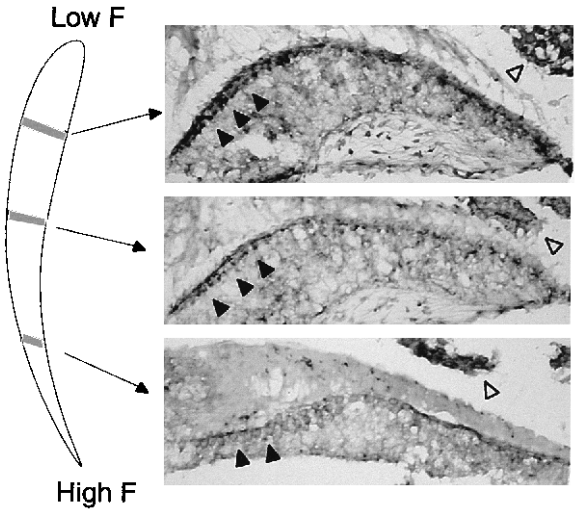

Fig. 4. *Slo*-β mRNA is expressed in quail hair cells. In situ hybridization was performed on cochlear cross sections (*23*). Solid arrowheads point to the rows of hair cells in the basilar papilla. The tonotopic positions of the sections are shown on the schematic cochlear duct [low frequency (F), apical end on top] at left. The tonotopic axis of the chick extends from 100 to 5000 Hz. Hair cell *slo*-β decreases from lowest to highest frequency regions. Label intensity in the tegmentum vasculosum (open arrowheads) did not vary systematically.

were studied here, and other alternate exons (*7–9*) may provide still further variation. Also, additional β subunits, as yet unknown, could provide still other forms of modulation to hair cell channels. The challenge remains to match particular channel proteins to the functional properties of an identified hair cell. Finally, the impressive conservation of channel function among the hair cells of amphibia, reptiles, and birds raises expectations that related molecular mechanisms will be found in the mammalian cochlea, where developmental changes in hair cell excitability (*25*) parallel the embryonic acquisition of BK channels in chick cochlear hair cells (*26*).

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13. *cSlo1* was cloned from a chick cochlear cDNA library (*5*). PCR of the cochlear cDNA library and RT-PCR of cochlear tissue identified the 61–amino acid insert. A chimeric construct incorporating the 61–amino acid insert was made by restriction digest and ligation of the PCR product with *cSlo1* and was packaged into pcDNA3.1 vector (Invitrogen, San Diego, CA) for transfection into mammalian cells.
14. HEK293 cells were transiently transfected by calcium phosphate precipitation. Inside-out patches (IOPs) were excised and analyzed with standard voltage clamp protocols (*5*). Ionic conditions were symmetrical, with the exception of buffered calcium, and contained 140 mM KCl, 0.5 mM $MgCl_2$, and 5 mM Hepes (pH = 7.2).
15. "Cytoplasmic" calcium was changed by perfusing IOPs with solutions buffered with 2 mM of EGTA, Br_2 BAPTA, or nitrilotriacetic acid [the free calcium concentration was determined with MaxChelator software (*27*)]. These concentrations were measured with a calcium electrode (Microelectrodes, Bedford, NH) calibrated with standard solutions obtained from World Precision Instruments (Sarasota, FL).
16. Half-activation voltages were determined by fitting normalized conductance (g/g_{max}) (Figs. 1C and 2, B and D) with a Boltzmann function given by ($\%g/g_{max} = 100/[1 + e^{-(V - V_{1/2})qF/RT}]$), where q is the gating charge, F is Faraday's constant, R is the universal gas constant, and T is the absolute temperature.
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19. Ratios of α cDNA to β cDNA were varied from 1:1 to 1:10 with no differences seen, implying that the effect of β was saturated at the lowest ratio. In most experiments a ratio of 1:2 was used.
20. In these experiments, activation rates were slightly faster upon β combination. This observation is consistent with the hypothesis that β subunits principally affect channel open states, with lesser influence on closed-to-open transitions. Activation steps were made from negative voltages at which the channels were fully closed. Symmetrical slowing of activation and deactivation by β would be expected for small excursions about a voltage level at which some fraction of channels are in the open state, as observed in hair cells (*4*).
21. K_D and δ were determined from the dependence of $V_{1/2}$ on $[Ca^{2+}]$ (Fig. 1D), where $V_{1/2} = -(2.303RT/2\delta F)\log[Ca^{2+}] + (2.303RT/2\delta F)\log[K_D]$ (*28*). δ is the electrical distance traveled by calcium into the membrane; it varied between 0.3 and 0.5.
22. RT-PCR was performed on quail cochlear RNA with the use of primers flanking the untranslated regions of *slo*-β. A full-length product (800 bp) was subcloned and sequenced to confirm its identity.
23. Frozen tissue sections of fixed and decalcified quail temporal bone (16 μm thick) were hybridized with digoxigenin-labeled cRNA (*29*) obtained by in vitro transcription of the full-length RT-PCR product of *slo*-β from quail cochlea. Alkaline phosphatase (AP)–conjugated sheep antibody to digoxigenin was used to detect cRNA-mRNA hybrids. The labeling was visualized with the AP substrates 4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolylphosphate. All reagents were purchased from Boehringer-Mannheim (Indianapolis, IN).
24. The gradient in β expression, as well as the fact that BK channels are fewest in apical hair cells (*2, 6*), implies that the ratio of β to α is highest in apical hair cells and falls toward the high-frequency base. The stoichiometry of αβ combination was not explicitly tested in these experiments (*19*).
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30. We thank C. Oberst and K. Bister for their gift of quail *slo*-β. This work was supported by grant DC00276 from the National Institute of Deafness and Communication Disorders.

28 July 1998; accepted 19 November 1998

Female × Male Interactions in *Drosophila* Sperm Competition

Andrew G. Clark,* David J. Begun, Timothy Prout

In several organisms, the success of a male's sperm in multiply inseminated females depends on the male's genotype. In *Drosophila*, the female also plays a role in determining which sperm are successful. Pairwise tests among six isogenic lines of *Drosophila melanogaster* were performed to determine whether there is a genotype-specific interaction in the success of sperm. The success of a particular male's sperm was found to depend on the genotype of the female with which he mates, providing evidence for an interaction with profound evolutionary consequences.

Males and females face very different problems in trying to assure that their gametes are used maximally. Males that can co-opt females into using their sperm are at an advantage over other males. Ignoring the complexities of mating behavior, there is an enormous opportunity for variation in the success of different males' sperm in multiply mated females. Genes that give such an advantage to one male's sperm are expected to increase in the population, even if this increase causes a decline in the viability or fertility of the mother or offspring (*1*). Females cannot afford to let males be the sole determinant of which gametes are used, especially if evolution in males results in deleterious consequences for the female (*2–4*).

In the case of *Drosophila*, females mate before stored sperm are exhausted, resulting in sperm from more than one male being present in the storage organs of females (*5*). Extensive variation among male genotypes in the competitive success of sperm in multiply mated females has been documented for *Drosophila melanogaster* (*6, 7*). Similarly, females differ widely among genotypes in their tendency to exhibit last-male precedence (*8*). A preclusion of the evolutionary response by females results in the increased competitiveness of sperm, which comes at a cost to the female's life-span (*3*). This result suggests that not only is there substantial genetic variation among males in sperm competitiveness, but also that females are normally evolving in response to male variation in these traits. These results beg the question of whether there are specific interactions between the genotypes of males and females that determine the outcome of sperm competition. We designed a study to quantify interactions in gametic use and found that these interactions are quite strong.

Sperm displacement by a pair of male genotypes (*9*) was tested in both mating orders, with the order (bw^D, wild) considered as a test of the "offense" ability of the wild male's sperm to displace the resident tester (bw^D) male's sperm (*10*) (Table 1). The other order (wild, bw^D) was considered as a test of "defense" and scored how well the wild male's sperm resisted being displaced by the tester male (*11*). Additional tests were done to verify that multiple mating within any trial time was rare (*12*). If the different genotypes varied in viability, this might appear as a spurious sperm displacement effect (*13*). The segregation ratios were used to score viabilities in four separate crosses, including $+_i/bw^D \times +_j/+_j$, $+_j/+_j \times bw^D/+_i$, $bw^D/+_i \times +_j/+_j$, and $+_j/+_j \times +_i/bw^D$, where the female genotype is given first, the allele of maternal origin is given before the slash, and i and j represent all six lines. Segregation ratios were scored in more than 1500 vials (>45,000 flies) and were found to be heterogeneous among the 36 crosses by an analysis of variation (ANOVA) (not shown), but the magnitude of the differences was not great (Fig. 1). If the fractions of wild and *bw* offspring were k and $(1 - k)$, then dividing the counts of wild and *bw* offspring by $2k$ and $2(1 - k)$, respectively, will compensate for the segregation effects.

Variability among the 36 crosses in the degree of sperm displacement is extensive (Fig. 2). An inspection of the line means suggested that both male and female effects on P1 and P2 are evident, which is consistent with earlier studies. (P1 is the fraction of offspring sired by the homozygous wild-type male when he is the first male to mate and is generally well under ½; P2 is the fraction of offspring for each cross that were fathered by the second male, when the second male to mate is wild type.) A three-way ANOVA was used to test the null hypothesis that the degree of sperm precedence was the same in all crosses (Table 2). The tests were performed both with and without compensation for the segregation differences. Test location (California, Pennsylvania, or Texas) was treated as a cross classification (along with the line), because environmental conditions were different in the three locations and line × location interactions are equivalent to biologically interesting gene × environment interactions. On average, 19.3 replicate tests were done for each of the 36 crosses at each location, and 124,881 offspring from 6246 vials were scored.

The location × female × male interaction was significant in both tests, meaning that the rank order of female × male interactions in P1 and P2 differed at the different sites. A conservative test of the female × male interaction was to use a composite MS, including the three-way interaction MS as the denominator, and this test yielded a significant female × male interaction for P1 only. When the ANOVA was performed for each location separately, the tail probabilities for the female × male interaction in the defense tests were 0.2787, 0.0001, and 0.0518 for California, Pennsylvania, and Texas, respectively; the offense tests had respective tail probabilities of 0.2085, 0.0001, and 0.0102. The likely cause for the lower significance of the California

A. G. Clark, Department of Biology, Pennsylvania State University, University Park, PA 16802, USA. D. J. Begun, Department of Zoology and Institute for Cellular and Molecular Biology, University of Texas, Austin, TX 78712, USA. T. Prout, Center for Population Biology, University of California, Davis, CA 95616, USA.

*To whom correspondence should be addressed. E-mail: c92@psu.edu

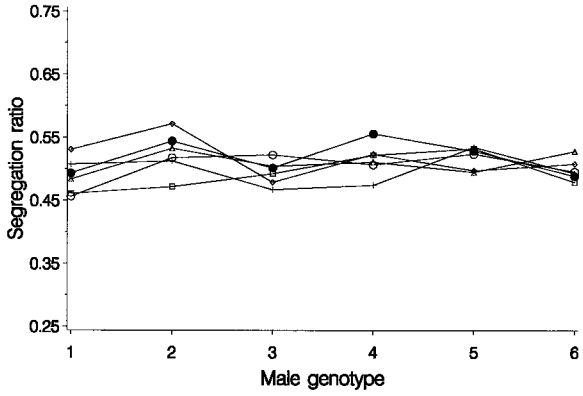
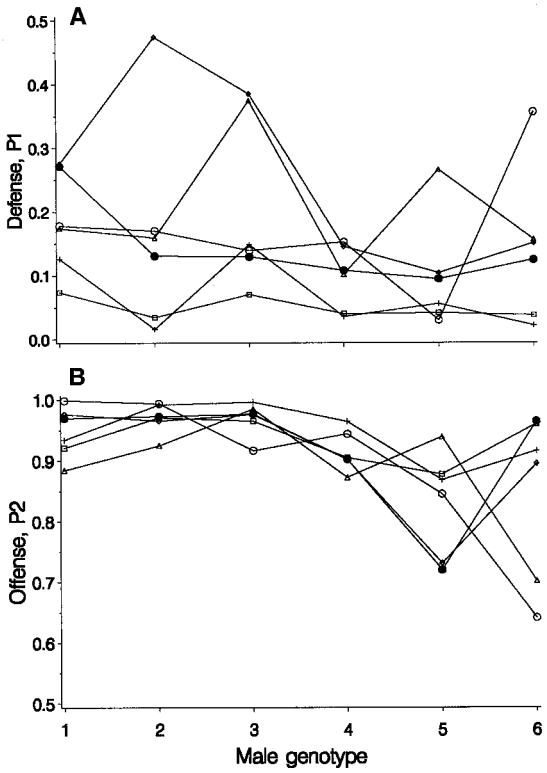


Fig. 1 (left). Segregation ratios for the 36 pairwise combinations of six lines. Each point represents the average across replications of the four segregation tests, and each has an average standard error of 0.014. The six lines represent the six female genotypes (solid circle, 1; solid diamond, 2; open circle, 3; open square, 4; cross, 5; and open triangle, 6). The means were found to be significantly heterogeneous, so departures from Mendelian segregation of offspring counts were compensated in estimates of the sperm displacement parameters P1 and P2. **Fig. 2 (right).** (**A**) The defense component of sperm displacement, P1, plotted for all 36 combinations of male and female genotypes. The interaction between male and female genotypes is evident from the crossing of the lines connecting female genotypes. Symbols are as in Fig. 1. (**B**) The offense component, P2, is plotted as in Fig. 2A.



samples was that the California sample size was 20% that of the Pennsylvania sample and 30% that of the Texas sample.

We also constructed the null distribution of the interaction MS by randomly permuting the observed data, shuffling the observed measure (P1 or P2 in this case) in relation to the classification variables that were being tested. For each permuted data set, the MS was calculated again. With sufficient replication (n = 10,000), the permuted samples provided the best picture of the null distribution that was compatible with the sample structure, although ideally the test would conserve the marginal male and female effects (*14*). The MS of female × male interaction was found to fall in the null distribution with 0 out of 10,000 replicates having a greater P1 and 12 out of 10,000 having a greater P2. The case for female × male interaction in both components of sperm competition is quite strong.

The relative success of the sperm from the same or different strains is compared in Fig. 2. The rank of competitive ability of the line 1 sperm in offense tests in females of line 1 was 3 (out of 6). The ranks for the other five lines were 3, 4, 5, 6, and 6 (out of 6). A permutation test showed that the probability of getting ranks as high or higher than these by chance was <0.002, so it appears that females are avoiding sperm from their own line, which is consistent with an inbreeding avoidance. There was no such association in the defense tests [ranks were 1, 1, 5, 4, 3, and 5 (out of 6)]. Offense and defense components appear to be physiologically distinct, because, consistent with previous findings (*11*), offense and defense components of sperm displacement were uncorrelated [Spearman rank correlation of the line means of P1 versus P2 was 0.155 ($P < 0.365$)].

Physiological mechanisms suggest that female × male interactions in the manifestation of sperm precedence ought to be expected. The uptake of seminal proteins by females varies widely across species, and often, radiolabel incorporation is seen in somatic tissue (*15*). There is abundant evidence that male accessory gland proteins, which are transmitted to the female in seminal fluid, mediate egg laying, sperm use, remating, and other behavioral changes in the female (*16*). There also is apparently strong conspecific recognition of sperm, which may be important in the evolution of isolating mechanisms (*17*). Seminal proteins are highly variable and undergo rapid molecular evolution, which is consistent with a male-female coevolutionary arms race (*18*).

In order to extend models of sperm competition (*19*) to the situation wherein sperm success depends on both the female and male genotypes, a matrix of sperm displacement parameters must be constructed for all mating combinations. Computer simulations of the simplest two-allele model with female × male interactions demonstrate that not only can polymorphisms be maintained by this model, but this model can exhibit complex cycling dynamics. The mode of selection suggests that rapid allele turnover would occur and that polymorphism among alleles with strong differences in sperm competitive ability is not unlikely.

When laboratory populations were allowed to evolve in such a way that female-specific responses could not occur, remating was faster and female fitness was reduced (*3, 4*). This implies that evolution results in male traits that are in conflict with female fitness. Females normally respond to this evolution to keep the deleterious effects of male evolution in check, and evolutionary changes in females, in turn, change the males' mating and sperm competitive ability. The male-female signaling that is evidently involved in decisions about remating and sperm use is not simple, but it may have profound fitness consequences. The resulting antagonistic coevolution may be responsible for the rapid rate of molecular evolution of genes encoding seminal proteins.

Table 1. Experimental design.

	Female	First male	Second male
Defense test	female *i*	male *j*	bw^D
Offense test	female *i*	bw^D	male *j*

Table 2. Analysis of variance of sperm displacement parameters P1 and P2. df, degrees of freedom; MS, mean square; F_{comp}, F statistic for segregation-compensated data; F_{uncomp}, F statistic for uncompensated data; dash, not applicable.

Source	df	MS	F_{comp}	F_{uncomp}
	Defense component (P1)			
Location	2	0.1901	1.28	3.11
Female	5	0.1189	4.46**	7.21***
Male	5	0.9321	16.85***	16.34***
Female × male	25	0.1580	2.01**	2.03**
Location × female × male	52	0.0914	1.78**	1.75**
Error	516	0.0514	–	–
	Offense component (P2)			
Location	2	0.0548	1.16	1.02
Female	5	0.0540	1.00	1.55
Male	5	0.2835	13.03***	12.37***
Female × male	25	0.0840	1.02	0.98
Location × female × male	44	0.1078	3.20***	3.31***
Error	474	0.0336	–	–

**$P < 0.01$. ***$P < 0.001$.

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9. Six lines were selected to cover a range of sperm displacement values, which were based on previous assays (*6, 8*). The selected lines were B3-09 (Beltsville, MD), B3-225 (Beltsville), D2-1 (Davis, CA), D2-29 (Davis), D2-55 (Davis), and NC3-107 (Raleigh, NC) and were coded as 1 to 6, respectively, in this study. The Beltsville lines were provided by B. Charlesworth, the Davis lines were extracted by D. Begun in summer 1995, and the Raleigh line was provided by T. Mackay.
10. For the "offense" tests, virgin 4- to 5-day-old females from each of the six homozygous lines were mated first to same-aged virgin bw^D males en masse for 2 hours. Females were then aspirated into individual shell vials (vial 1), where they were allowed to oviposit for 2 days. Each of the six female lines was divided into six groups, with each group getting one of the six extracted lines of males for the second mating. Then, two or three males of the same extracted line were placed in each vial for the second mating and left overnight. Second males were then removed, and females were transferred by aspiration to vial 2. After 4 days, females were transferred again without anesthesia to vial 3, and 1 week later, females were discarded. All three vials were scored for eye-color phenotype (wild versus brown) on the 17th day after oviposition began. The fraction of all progeny in vials 2 and 3 that were sired by the second male is denoted by the statistic P2 [E. Boorman and G. E. Parker, *Ecol. Entomol.* **1**, 145 (1976)].
11. The defense test is the same as the offense test, except the females were mated first to the wild extracted-line males. Following the same protocol for times of transfer and so forth, we then mated the females to virgin bw^D males. The sperm displacement was scored as the fraction of wild-type progeny, which corresponds to the fraction of offspring sired by the first male (P1).
12. Multiple mating by males in the vial 2 was tested with a *Lobe* strain. In no case (0 out of 121 trials) did a female accept more than one male in the overnight trial, given that she had already mated with the first male. The entire experiment was repeated at the Pennsylvania State University, the University of Texas at Austin, and the University of California at Davis.
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25 August 1998; accepted 8 December 1998

A Nonhyperthermophilic Common Ancestor to Extant Life Forms

Nicolas Galtier,* Nicolas Tourasse, Manolo Gouy

The G+C nucleotide content of ribosomal RNA (rRNA) sequences is strongly correlated with the optimal growth temperature of prokaryotes. This property allows inference of the environmental temperature of the common ancestor to all life forms from knowledge of the G+C content of its rRNA sequences. A model of sequence evolution, assuming varying G+C content among lineages and unequal substitution rates among sites, was devised to estimate ancestral base compositions. This method was applied to rRNA sequences of various species representing the major lineages of life. The inferred G+C content of the common ancestor to extant life forms appears incompatible with survival at high temperature. This finding challenges a widely accepted hypothesis about the origin of life.

A remarkable feature of genomic sequences is their ability to retain traces of extremely ancient evolutionary events, including the very first steps of life on Earth. By sequencing small-subunit (SSU) rRNA genes from various eukaryotic and prokaryotic species in the late 1970s, Woese and colleagues could construct for the first time a comprehensive picture of the universal tree of life (*1*). This work gave rise to conjectures about the nature of the most recent common ancestor (MRCA) of extant life forms: A hot, auxotrophic origin of life was hypothesized (*1*). The information contained in molecular data, however, has been obscured by numerous base substitution events that occurred during thousands of millions of years of diverging evolution. Realistic modeling of the molecular evolutionary processes is required to discriminate between phylogenetic signal and noise (*2*). Here, we devised a Markov model accounting for three major forces governing DNA sequence evolution: unequal transition/transversion rates, unequal evolutionary rates among sequence sites, and varying G+C contents among lineages. Maximum likelihood inference based on this model applied to large-subunit (LSU) and SSU rRNA sequences yields insights about early molecular evolution. Our results cast doubts on one commonly accepted hypothesis, namely the thermophilic nature of the MRCA.

The designed Markov model of DNA sequence evolution generalizes Galtier and Gouy's nonhomogeneous model (*3*) by accounting for variable substitution rates among sites. In this model, the assumed substitution process in a given branch of the tree (that is, the probability of change from one nucleotide to another) depends on two parameters, namely transition/transversion ratio and equilibrium G+C content, that is, the G+C content that would be reached after infinitely long evolution (*4*). The latter parameter is allowed to vary between branches, so that G+C content can diverge with time and among lineages. This assumption appears necessary given the observed range of G+C content (40 to 75%) in actual rRNA sequences. The resulting model is nonhomogeneous (variable substitution process), nonstationary (equilibrium is not reached), and irreversible (*5*), in contrast to usual models of DNA sequence evolution. Substitution rates are highly variable among sites in rRNA molecules as a consequence of unequal selective constraints (*6*). Neglecting this point may lead to biased phylogenetic estimators (*7*). Following Yang (*8*), a discretized gamma distribution of rates was assumed to account for among-site rate variability.

In usual homogeneous-stationary models, the ancestral base composition of the compared sequences is deduced from the assumed substitution rate matrix. Here, ancestral G+C content is a free parameter that can be estimated by fitting the model to data. In a previous study, surprisingly accurate estimates of ancestral G+C contents were found from simulated data

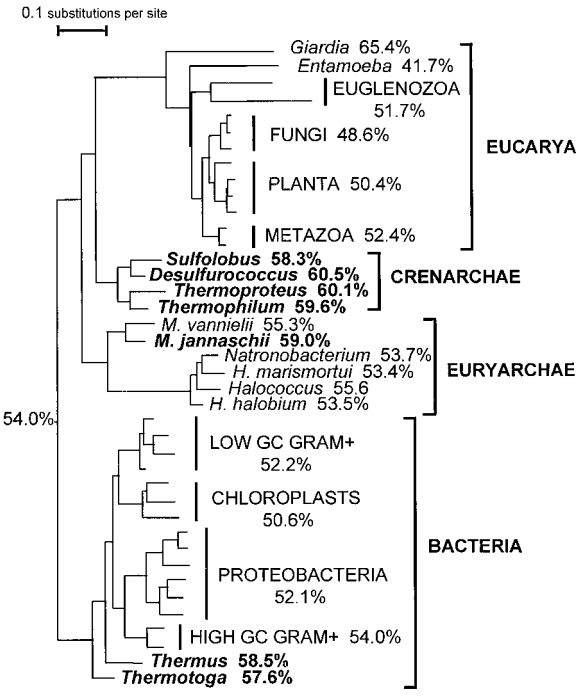

Fig. 1. Maximum likelihood tree reconstructed from 40 LSU rRNA sequences (*17*). G+C contents of (groups of) sequences are given next to taxon names. The inferred ancestral G+C content appears next to the root. Two hundred topologies obtained by rearranging an initial neighbor-joining tree were evaluated. The monophyly of Bacteria, Eucarya, Crenarchaea, Euryarchaea, Euglenozoa, animals, green plants, fungi, high-GC Gram-positive bacteria, low-GC Gram-positive bacteria, proteobacteria, and chloroplasts was kept in all trees, but the remaining branching orders were randomly shuffled; 1409 complete, unambiguously aligned sites were used. The names of thermophilic species are in boldface. *M*, *Methanococcus*; *H*, *Halobacterium*.

N. Galtier, Laboratoire de Biométrie, Génétique et Biologie des Populations, Université C. Bernard Lyon 1, France, and Laboratoire "Génome et Populations," Université Montpellier 2, 34095 Montpellier, France. N. Tourasse, Human Genetics Center, University of Texas, Houston, TX 77030, USA. M. Gouy, Laboratoire de Biométrie, Génétique et Biologie des Populations, Université C. Bernard Lyon 1, 69622 Villeurbanne, France.

*To whom correspondence should be addressed.