characterize many carnivorous non-mammalian synapsids[24]. The molariform teeth at the back of the dentition of *Repenomamus* are small with blunt crowns; they probably played a minor role in food processing. Although mammals are considered definitive chewers within amniotes[25], the dental morphology and large pieces of prey in the stomach of *Repenomamus* suggest that chewing as a derived feature in mammals was probably not present in *Repenomamus*.

It is not easy to assess whether *Repenomamus* was a predator or scavenger. Scavengers are relatively rare among mammals—among extant carnivorous mammals, only two species of hyenas are habitual scavengers[12,26]. Compared to their hunting cousins, these hyenas have smaller second upper incisors and less jaw muscle leverage, which probably reflect their inability to capture and handle live prey. In contrast, the enlarged incisors and strong jaw muscles of *Repenomamus* are well shaped for catching prey, favouring it as a predator rather than a scavenger.

For fossil mammals, body size is one of the most important factors influencing life history strategy[27]. Early mammals or their close relatives, such as morganocodontids and kuehneotheriids in the Late Triassic to Early Jurassic periods, were small and considered to be nocturnal insectivores[2,3]; the same is true of most later Mesozoic mammals[28] (Fig. 4). The reason for the very small size of Mesozoic mammals is uncertain[5], but it has often been hypothesized that well-established larger (and presumably diurnal) reptilian carnivores and herbivores, particularly dinosaurs, prevented mammals from invading those niches[29]. *Repenomamus* extend significantly the upper limit of body size of Mesozoic mammals (Fig. 4) and are actually larger than several small dinosaurs, particularly dromaeosaurid dinosaurs, from the same fauna[11]. Larger animals can live longer and move faster, but they also need a larger food supply and broader home range[30]. Judging from their body size, *R. giganticus* could feed on larger prey and forage a wider area for food. These large Mesozoic mammals were probably carnivores that competed with dinosaurs for food and territory. □

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The simplicity of metazoan cell lineages

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Developmental processes are thought to be highly complex, but there have been few attempts to measure and compare such complexity across different groups of organisms[1–5]. Here we introduce a measure of biological complexity based on the similarity between developmental and computer programs[6–9]. We define the algorithmic complexity of a cell lineage as the length of the shortest description of the lineage based on its constituent sublineages[9–13]. We then use this measure to estimate the complexity of the embryonic lineages of four metazoan species from two different phyla. We find that these cell lineages are significantly simpler than would be expected by chance. Furthermore, evolutionary simulations show that the complexity of the embryonic lineages surveyed is near that of the simplest lineages evolvable, assuming strong developmental constraints on the spatial positions of cells and stabilizing selection on cell number. We propose that selection for decreased complexity has played a major role in moulding metazoan cell lineages.

Biological systems are obviously complex in both structure and

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LETTERS

Sexual reproduction selects for robustness and negative epistasis in artificial gene networks

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The mutational deterministic hypothesis for the origin and maintenance of sexual reproduction posits that sex enhances the ability of natural selection to purge deleterious mutations after recombination brings them together into single genomes[1]. This explanation requires negative epistasis, a type of genetic interaction where mutations are more harmful in combination than expected from their separate effects. The conceptual appeal of the mutational deterministic hypothesis has been offset by our inability to identify the mechanistic and evolutionary bases of negative epistasis. Here we show that negative epistasis can evolve as a consequence of sexual reproduction itself. Using an artificial gene network model[2,3], we find that recombination between gene networks imposes selection for genetic robustness, and that negative epistasis evolves as a by-product of this selection. Our results suggest that sexual reproduction selects for conditions that favour its own maintenance, a case of evolution forging its own path.

A century of genetic research has revealed two general properties of spontaneous mutations with detectable effects on fitness: most of them are deleterious, and they frequently interact with each other[4,5]. Many types of interactions are possible, including directional epistasis, in which the average effect of spontaneous mutations changes in the presence of other mutations in the genome[6]. Directional epistasis can be either negative (synergistic) or positive (antagonistic), depending on whether the average effect of mutations becomes more or less harmful, respectively, as the number of other mutations in the genome increases (Fig. 1). Directional epistasis holds particular interest for evolutionary biologists because it is expected to determine the outcome of multiple evolutionary processes, notably the evolution of sex and recombination[1]. Empirical studies on a variety of organisms have reported every conceivable form of directional epistasis: negative[7–9], positive[6,10] and no significant directional epistasis[11,12]. These mixed results have not helped to clarify either the mechanistic or evolutionary causes of directional epistasis[13].

In contrast, evolutionary simulations using computational models of RNA secondary structure[14], viral replication[15] and artificial life[14] have demonstrated that the average strength and direction of epistasis can be shaped by natural selection. One mechanism by which epistasis evolves in these models[13] is through a negative correlation among genotypes between the extent of genetic robustness (or genetic canalization, measured as the insensitivity of a phenotype to mutation) and the direction of epistasis. As a consequence, selection for higher robustness produces a correlated response in the strength of epistasis in all three models, towards either weaker positive or stronger negative epistasis[14,15]. The repeatability of this result in models of different biological systems suggests that the strength and direction of epistasis observed in living organisms depend on their history of selection for genetic robustness.

Theory predicts that traits can evolve to be robust to genetic perturbations (that is, mutation and recombination) under a variety of selective regimes[16–18], as long as the following two conditions are met: genes must interact to determine the trait[17–19], and the population must contain sufficient genetic variation[18]. Whereas the former condition is inherent to particular organisms, the latter condition will depend on population genetic parameters such as the mutation and recombination rates. Experimental tests of these predictions using computational models confirm that high mutation rates, such as those experienced by RNA viruses, favour the evolution of genetic robustness[2,3,18,20]. Sexual reproduction (that is, increased recombination) is also expected to impose stronger selection for genetic robustness than asexual reproduction[21,22], but this hypothesis has never been tested experimentally[21].

To test this hypothesis, and to determine whether the evolution of genetic robustness is accompanied by the evolution of negative epistasis, we return to the computational model of genetic networks used in two previous studies[2,3]. We chose this model primarily because it explicitly incorporates one of the key characteristics required for the evolution of robustness[17–19]—genetic interactions. Furthermore, empirical data from biological systems has consistently suggested that extant gene networks are robust to changes in biochemical rate parameters and levels of gene activity[19,23]. Previous work with this model has shown that genetic robustness (again, measured as robustness to mutation) evolves readily if networks are subjected to selection for the production of a stable gene expression pattern[2,3]. Here we explore the extent to which recombination contributed to the evolution of genetic robustness in this model, and ask whether recombination, through its effect on robustness[2,21,22], can cause the direction of epistasis to evolve.

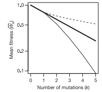

Figure 1 | Types of directional epistasis for deleterious mutations. Three hypothetical relationships between fitness (log scale) and number of deleterious mutations are plotted. All relationships depicted have the same mutational robustness ($\overline{W}_1 = 0.78$) but different directions of epistasis: negative epistasis (plain line, concave downwards; $1 - \beta < 0$), no directional epistasis (bold, straight line; $1 - \beta = 0$) and positive epistasis (dashed line, concave upwards; $1 - \beta > 0$).

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PLoS COMPUTATIONAL BIOLOGY

Redundancy and the Evolution of *Cis*-Regulatory Element Multiplicity

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Abstract

The promoter regions of many genes contain multiple binding sites for the same transcription factor (TF). One possibility is that this multiplicity evolved through transitional forms showing redundant *cis*-regulation. To evaluate this hypothesis, we must disentangle the relative contributions of different evolutionary mechanisms to the evolution of binding site multiplicity. Here, we attempt to do this using a model of binding site evolution. Our model considers binding sequences and their interactions with TFs explicitly, and allows us to cast the evolution of gene networks into a neutral network framework. We then test some of the model's predictions using data from yeast. Analysis of the model suggested three candidate nonadaptive processes favoring the evolution of *cis*-regulatory element redundancy and multiplicity: neutral evolution in long promoters, recombination and TF promiscuity. We find that recombination rate is positively associated with binding site multiplicity in yeast. Our model also indicated that weak direct selection for multiplicity (partial redundancy) can play a major role in organisms with large populations. Our data suggest that selection for changes in gene expression level may have contributed to the evolution of multiple binding sites in yeast. We conclude that the evolution of *cis*-regulatory element redundancy and multiplicity is impacted by many aspects of the biology of an organism: both adaptive and nonadaptive processes, both changes in *cis* to binding sites and in trans to the TFs that interact with them, both the functional setting of the promoter and the population genetic context of the individuals carrying them.

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Introduction

Promoters frequently contain multiple functional regulatory elements [1]. For example, the regulatory region for stripe 2 of *even-skipped* (*eve*) of the fruit fly *Drosophila melanogaster* comprises 17 binding sites for four transcription factors (TFs), including five binding sites (B1–B5) for the activator *bicoid* (*bcd*) [2]. How does *cis*-regulatory element multiplicity evolve? There are three possibilities. First, perhaps "more is better" when it comes to TF binding sites. Multiple binding sites may cause changes in the level of gene expression or in its robustness against variation in TF concentrations [1,3–5]. Second, multiplicity might be favored by selection, but independently of its functional consequences. For example, genotypes with many binding sites may be more likely to produce viable offspring after mutation or recombination with genotypes with fewer binding sites [6–9]. Third, *cis*-regulatory element multiplicity may arise by nonadaptive processes [9–11]. Stone and Wray [10] have shown that a population of 10^6 diploid individuals could evolve two identical copies of a 6 base pair (bp) binding site in a 200-bp promoter every 5.4×10^5 generations through random mutation and genetic drift alone. The intergenic regions of *Saccharomyces cerevisiae* are ~400 bp long on average, whereas those of multicellular eukaryotes can be orders of magnitude longer.

The common thread to all the evolutionary scenarios listed above is redundancy, the ability of structurally identical elements to contribute to the same function [12–16]. Redundancy is thought to be widespread in biological systems. In eukaryotes, a large proportion of genes are duplicates, and deletion of one copy often has little or no phenotypic effect because the other copy can compensate for the loss of function [17]. Functionality and redundancy are more difficult to establish for the case of multiple *cis*-regulatory elements [1]. The five *bcd* binding sites in *eve* the stripe 2 enhancer are not fully redundant because loss-of-function mutations to B1, B2 or B3 cause reduced *eve* stripe 2 expression and gain-of-function mutations to B4 and B5 lead to increased expression [2,18]. However, redundancy was likely important in the evolution of these sites. When Ludwig and colleagues [3] compared the stripe 2 enhancers of different species of *Drosophila*, they found that some of them lacked the B3 site (Figure 1). This observation implies that the B3 site evolved recently in the lineage leading to the last common ancestor of *D. melanogaster* and *D. simulans*. Furthermore, the B3 site was probably redundant when it first appeared because the stripe 2 enhancers of three species lacking the B3 binding site were able to drive expression of a reporter gene in *D. melanogaster* embryos coincident with native *eve* stripe 2 (Figure 1). Thus, redundant transitional forms can, in principle, play an important role in the evolution of *cis*-regulatory element multiplicity [1,19]. In this paper we develop a model of binding site evolution and use it to evaluate the plausibility of different scenarios for the evolution of *cis*-regulatory element redundancy and multiplicity. We then test predictions obtained from our model using data from yeast.

Low Base-Substitution Mutation Rate in the Germline Genome of the Ciliate *Tetrahymena thermophila*

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Abstract

Mutation is the ultimate source of all genetic variation and is, therefore, central to evolutionary change. Previous work on *Paramecium tetraurelia* found an unusually low germline base-substitution mutation rate in this ciliate. Here, we tested the generality of this result among ciliates using *Tetrahymena thermophila*. We sequenced the genomes of 10 lines of *T. thermophila* that had each undergone approximately 1,000 generations of mutation accumulation (MA). We applied an existing mutation-calling pipeline and developed a new probabilistic mutation detection approach that directly models the design of an MA experiment and accommodates the noise introduced by mismapped reads. Our probabilistic mutation-calling method provides a straightforward way of estimating the number of sites at which a mutation could have been called if one was present, providing the denominator for our mutation rate calculations. From these methods, we find that *T. thermophila* has a germline base-substitution mutation rate of 7.61×10^{-12} per-site, per cell division, which is consistent with the low base-substitution mutation rate in *P. tetraurelia*. Over the course of the evolution experiment, genomic exclusion lines derived from the MA lines experienced a fitness decline that cannot be accounted for by germline base-substitution mutations alone, suggesting that other genetic or epigenetic factors must be involved. Because selection can only operate to reduce mutation rates based upon the "visible" mutational load, asexual reproduction with a transcriptionally silent germline may allow ciliates to evolve extremely low germline mutation rates.

Key words: drift-barrier hypothesis, mutation accumulation, micronucleus, macronucleus, microbial eukaryote, Oligohymenophorea.

Introduction

Mutation is the ultimate source of all genetic variation, and the rate, molecular spectrum, and phenotypic consequences of new mutations are all important drivers of biological processes such as adaptation, the evolution of sex, the maintenance of genetic variation, aging, and cancer. However, because mutations are rare, detecting them is difficult, often requiring the comparison of genotypes that have diverged from a common ancestor by at least hundreds or thousands of generations. Further, interpreting the results of such comparisons is complicated by the fact that mutations are frequently eliminated by natural selection before they can be studied.

Mutation accumulation (MA) is a standard method for studying mutations experimentally. In a typical MA experiment, many inbred or clonal lines are isolated and passed repeatedly through bottlenecks. This reduces the effective population size and lessens the efficiency of selection, allowing all but the most deleterious mutations to drift to fixation (Bateman 1959; Mukai 1964). The genome-wide mutation rate and mutational spectrum can then be estimated by comparing the genomes of MA lines with those of their ancestors.