Evolution: Zeroing In on the Rate of Genome Doubling

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Changes in genome copy number have occurred numerous times throughout the history of life, with profound evolutionary consequences. New experiments with budding yeast shed light on how frequently spontaneous genome doubling occurs within populations and the environmental conditions that favour cells with doubled genomes.

Given that genomes form the blueprint of life, genomic size and structure are remarkably labile over evolutionary time [1]. Particularly dramatic are shifts in ploidy — the number of genome copies carried by each somatic cell (one copy: haploid; two copies: diploid; >2 copies: polyploid). Polyploidization is frequent in plants, with angiosperm species having experienced a few to hundreds of ploidy shifts in their evolutionary history [2]. Even among animals, hundreds of polyploidization events have been documented, including early on in our evolutionary history during the radiation of vertebrates [3,4].

While the rate of polyploid formation has been measured, particularly in plants (e.g., an average of 0.56% of pollen grains are diploid [5]), such rates are influenced by both the rate of doubling and selection on doubled cells. Consequently, the precise rate at which genomes spontaneously double in size per cell generation is unknown. To estimate the rate that haploid cells spontaneously turn diploid, Harari *et al.* tracked trillions of cells of the yeast *Saccharomyces cerevisiae* to detect and quantify genomic doubling, and reported their findings recently in *Current Biology* [6].

During every mitotic cell cycle, DNA is replicated, leading haploid cells to become transiently diploid. Harari *et al.* used flow cytometry to measure the genome size of initially haploid populations of yeast, looking beyond the characteristic two-peak pattern for additional peaks that characterize cells with permanently larger genomes (Figure 1). Importantly, the authors also isolated single cells, from which colonies were grown and assayed to confirm that the additional peaks did indeed represent diploidized cells, not just cells that had failed to separate. The height of these additional peaks varied according to environment (e.g., low in hydroxyurea, intermediate in normal rich media, and high in KOH or ethanol), with additional experiments indicating that both the spontaneous rate of genomic doubling and the strength of selection on diploid cells varied across environments.

By tracking the appearance and spread of diploidized cells over time, the authors were able to tease apart the rate of spontaneous doubling from the strength of selection, estimating the rate of diploidization to be 7.1×10^{-5} per cell division. While lower than the rate at which single basepair mutations arise across the genome (4.8×10^{-3} per haploid genome per cell division from a recent estimate [7]), these rates nevertheless suggest that ~1% of mutational events involve wholesale changes to genome size.

The fact that ploidy changes occur so often is striking and helps explain why experiments in yeast have repeatedly been stymied by unintended ploidy changes, with cultures frequently shifting from haploidy to diploidy (e.g., [8–10]; see [10] for additional references) but also from diploidy to haploidy [11]. These population-wide shifts typically occur over the course of hundreds of cell generations, consistent with the findings of Harari *et al.*

For lab yeast strains, it is thought that haploid cells give rise to diploids through endoreduplication, or the failure of cell division following DNA replication. As a consequence, the diploids produced are genotypically homozygous, even at the mating-type locus (MAT). While Harari *et al.* confirmed that endoreduplication was the most common route to diploidization, mating-type switching also occurred. This is surprising, because the lab strains used lack the endonuclease responsible for MAT switching, *HO*. This endonuclease normally facilitates gene conversion between the MAT locus and silent mating cassettes in the genome, allowing haploids to give rise to cells of the opposite mating type, permitting mating and the formation of diploids in wild strains. Nevertheless, Harari *et al.* found diploid MATa/MATα cells, even without a functional copy of *HO*, presumably due to occasional DNA damage at the MAT locus and recombinational repair from the silent cassettes. Consistent with this hypothesis, Harari *et al.* found that mating-type switching was particularly common in the presence of hydroxyurea, which is known to inhibit DNA replication and increase the rate of DNA damage [12].

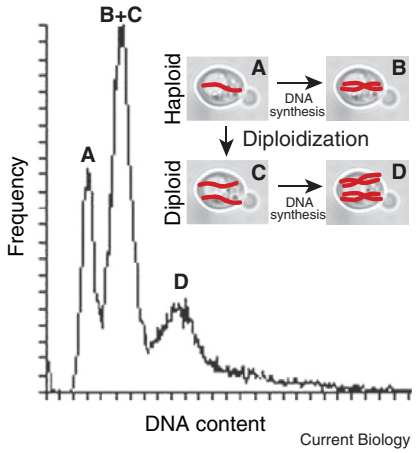

Figure 1. Detecting ploidy shifts using genome size measurements.
Flow cytometry detects shifts in genome size due both to DNA synthesis (A to B and C to D) and to diploidization (A to C). Graph used with permission from [6].

Table 1. Ploidy shifts observed under minimal selection in yeast mutation accumulation experiments, as well as the inferred rate of genome size change per cell division for comparison to the estimate of Harari *et al.* of 7.1 per 10^5 cell divisions.

Study	Species	Genetic background	Initial ploidy	Total cell divisions	Ploidy shifts	Rate (95%CI) per 10^5 cell divisions
Lynch *et al.* [9]	*S. cerevisiae*	FY10	haploid	19200	4	20.8 (5.7,53.3)
Sharp *et al.* [7]	*S. cerevisiae*	SEY	haploid	157360	0	0 (0,2.3)
Serero *et al.* [17]	*S. cerevisiae*	BY4741 (mutators)	haploid	90000	1	1.1 (0.03,6.2)
Farlow *et al.* [18]	*S. pombe*	ED668	haploid	135936	0	0 (0,2.7)
Behringer & Hall [19]	*S. pombe*	972	haploid	154208	0	0 (0,2.4)
Zhu *et al.* [20]	*S. cerevisiae*	DBY4974/4975	diploid	311000	0	0 (0,1.2)
Sharp *et al.* [7]	*S. cerevisiae*	SEY	diploid	178953	0	0 (0,2.1)

Other estimates of the rate of ploidy change come from a handful of mutation accumulation (MA) experiments, where replicate lines of yeast were subjected to repeated bottlenecks over many generations. In principle, this approach allows the rate of genomic shifts and other mutations to be estimated regardless of their effects on fitness, but rare events will be difficult to detect in these small populations. The estimate of Harari *et al.* is somewhat higher than those of several previous experiments with *S. cerevisiae* (summarized in Table 1), which range widely. Variation among studies may reflect rate differences among environments and genetic backgrounds, as well as sampling error. To our knowledge, ploidy shifts have not been observed in MA experiments with the fission yeast *Schizosaccharomyces pombe*, a species thought to be predominantly haploid in the wild and distantly related to *S. cerevisiae*. It is an open question whether species that are typically haploid have cell cycles better adapted to the haploid state and are less prone to diploidization. As far as we know, spontaneous ploidy reductions have not been observed in any mutation accumulation experiments involving diploids (Table 1), despite occurring in some evolution experiments [11]. However, any accumulation of recessive lethal alleles in diploid MA lines could make it less likely that spontaneous haploids would persist in these experiments.

What is the selective fate of the newly formed diploids? Harari *et al.* found that diploids grew faster than haploids in the presence of some stressors (ethanol, KCL) but slower with others (caffeine, hydroxyurea). This variation is in keeping with a previous study of 51 yeast strains across 33 environments that found that the relative advantage of haploids versus diploids is highly sensitive to the environment [13].

Finding a high rate of spontaneous diploidization might also shed light on evolutionary experiments where diploids spread despite having no clear fitness benefit [14]. Diploids may simply arise frequently, remaining at low frequency until beneficial mutations arise that are unique to diploids, with diploidy hitchhiking to high frequency alongside these secondary changes. A recent comparison of spontaneous mutations in haploids and diploids indeed finds that diploids have access to a different spectrum of mutations, particularly structural variants [7]. Over the course of evolutionary experiments, diploids have been observed to accumulate more structural mutations, overdominant mutations, and masked deleterious mutations [10,15,16], suggesting that the benefit of diploidy may sometimes be indirect.

With different environmental sensitivities, a different spectrum of mutations, and different selective pressures acting on these mutations, the spontaneous diploidization of haploid cells generates evolutionarily unique progeny. Diploid cells thus do not simply represent bigger versions of haploid cells. The study by Harari *et al.* indicates that yeast frequently double their genomic blueprint, accessing the distinct adaptive trajectories available to diploids.

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Social Immunity: The Disposable Individual

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Workers in an ant colony can kill fungus-infected brood, thereby protecting the rest of the colony from fungal infection. This form of social immunity is analogous to the immune system of multicellular organisms where immune cells kill infected cells.

> *"The termitary is a separate and composite animal in exactly the same way as a man is a separate composite animal. Only the power of locomotion is absent." Eugène Marais in* The Soul of the White Ant *(1937, first published as* Die Siel van die Mier *in 1925, in Afrikaans, Cape Town: Stephan Phillips (Pty) Ltd)*.

Insect societies are sometimes referred to as 'superorganisms' [1]. The most striking analogy between this superorganism and the multicellular organism, as we think of it in the traditional sense, is that a large fraction of the population — the somatic cells of the multicellular organism and the workers and soldiers of the insect colony — are sterile. They only can increase their fitness indirectly, by providing help to related, fertile colony members. To extend the analogy further, some multicellular individuals have an early irreversible sequestration of reproductive from non-reproductive cells, whereas in others, such as fungi, all cells retain the potential to reproduce. Likewise, some social insects have an irreversible caste differentiation, whereas in others, all individuals remain 'hopeful reproductives' [2].

Another aspect of the superorganism analogy is that of superorganism immunity. On one hand, cooperation facilitates division of labour and provides scale-related benefits of increased size. However, on the other hand, living in crowded societies of closely related members also increases the risk of contagious diseases. Just as multicellular organisms have an immune system to deal with those risks, recent research shows that social insects, in addition to their own organismal immune system, have a 'social immune system' to cope with infectious disease [3]. The social immune system results from cooperation between group members to combat the increased risk of disease transmission that arises from sociality and group living.

In a recent study published in *eLife*, Pull and colleagues [4] now reveal a previously unknown component of the social immune system — the killing of infested brood by workers, called destructive disinfection. In this study, the authors found that in colonies of the ant *Lasius neglectus*, pupae infected with the parasitic fungus *Metarhizium brunneum* emitted a chemical cue, which was detected by tending ants (Figure 1). Subsequently, the ants would bite an infected pupa to open its cuticle and spray it with an antiseptic poison, killing both the pupa and the fungus. These results provide a striking analogy with the innate immune system of multicellular organisms, where infection triggers the complement system to recruit inflammatory cells. In this analogy, the chemicals emitted by the brood are analogous to the signalling molecules produced by infected cells, and the workers killing and cleaning the infected pupae are analogous to the white blood cells attracted to the signalling molecules.

Like the vertebrate immune system, the social immune system of the colony is comprised of various layers of defence [5]. One line of defence is the prevention of pathogen entry. Eugène Marais compared the wall of a termite colony with the skin of a human body. Both are actively maintained, consist largely of dead matter, and serve as a first line of defence against intruders. Upon entry of parasites, a second line of defence can prevent the establishment and spread of the parasite between the body's cells or the social insect workers. One way to achieve this is via the cleaning of