shift from hair production to quiescence. During catagen, cell proliferation ceases, the hair-producing machinery is destroyed through apoptosis, and a club differentiates to anchor the hair in the shorter follicle. When Hr function is lost, all of these processes are disrupted: follicular cells develop an abnormal tendency to proliferate (not only during catagen but afterwards, in follicle remnants), the apoptotic program becomes disorganized (causing follicles to thin and break apart rather than progressively shorten), and differentiation defects emerge, as the club forms improperly, and follicular epithelial cells turn occasionally into sebaceous cells or (possibly) epidermal keratinocytes[9,10,12]. Given this set of abnormalities, Hr seems to promote the catagen program in general and growth arrest, apoptosis and differentiation in particular (although Hr may affect some processes more directly than others). It is conceivable that when *U2HR* function is lost, HR induces properties of catagen during anagen, the period in which hair is produced. Individuals with MUHH experience defective anagens, and inappropriate growth arrest or apoptosis would explain the loss of hair follicles. Undoubtedly, much will be learned about HR in the coming years through the comparison of MUHH with HR loss-of-function disorders.

A second question raised by the Wen *et al.* study is as follows: if the U2HR protein normally represses HR synthesis, how is this repression lifted when HR is needed (for example, during catagen)? Clues may lie in the literature. When polyamines are overproduced in murine hair follicles, abnormalities develop that closely resemble the rhino phenotype[13]. In other words, increasing polyamines is essentially identical to repressing Hr. Moreover, polyamines are known to repress the translation of *S*-adenosylmethionine decarboxylase and to do so by interacting with a uORF-encoded peptide[14,15]. The peptide and polyamines together cause ribosomes to stall at the uORF's termination codon, thereby blocking translation of the enzyme's ORF. In other words, polyamines enable the uORF's peptide to repress translation in *cis*. As polyamines seem to repress Hr function—and as the U2HR protein seems to repress Hr translation in *cis*—Hr may normally be repressed via the U2HR protein and polyamines, and a decrease in polyamines may lift this repression. As such, polyamines would provide an on-off switch for Hr translation. Whether this particular idea is right or wrong, the work of Wen *et al.* has opened new windows into HR function and regulation and is certain to inspire studies of HR in the years ahead.

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Reverse evolution and evolutionary memory

Michael M Desai

Experimental reverse evolution, in which a population is readapted to an ancestral environment, can probe the nature and extent of evolutionary memory. A new study shows that standing genetic variation is key to this memory in experimental *Drosophila* populations, where selection drives rapid but incomplete convergence to ancestral genotypes.

Evolution is intrinsically contingent on history and chance, as selective landscapes are complex and the space of possible genotypes is far too large to explore comprehensively[1]. Thus all populations maintain an evolutionary memory—their past has much to say about how they evolve in the future. This memory is stored in two related ways. A population remembers its recent past through standing genetic variation, which reflects the range of variation it could generate and maintain in the selective environment of its immediate past. As evolution progresses, this standing variation fixes or goes extinct, creating a longer-term memory reflected in the set of genotypes that are closely accessible via mutation to those currently present in the population. On page 251 of this issue, Henrique Teotónio *et al.*[2] study evolutionary memory by measuring the extent of convergence to ancestral genotypes during experimental reverse evolution of *Drosophila*, in which lines adapted to starvation resistance or altered reproductive timing were readapted to ancestral conditions. Their work highlights the importance of selection on standing genetic variation, which allows a quick but incomplete return to ancestral genotypes.

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Memory fades with time

The quality of evolutionary memory—and hence the speed and comprehensiveness of reverse evolution—declines as ancestral states fade further into the past. If a population has only recently moved to a new environment, it will retain standing genetic variation generated in the ancestral environment. When the population is returned to the ancestral state for reverse evolution, selection will act to increase the frequencies of existing genotypes that are advantageous in the ancestral environment[3,4]. As this is a nearly deterministic process, parallel populations should behave similarly. If instead the population has been away from the ancestral state long enough for the standing variation that existed in that state to disappear, then reverse evolution will depend on new mutations. Because these are rare, and their

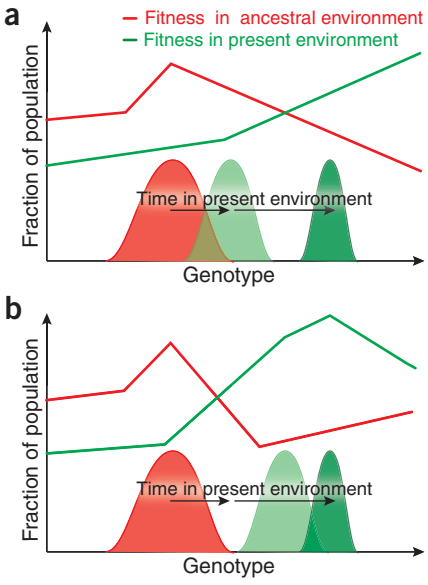

Figure 1 Schematic of the effect of selective landscape on evolutionary memory. Fitness as a function of a simplified one-dimensional 'genotype' for both ancestral (red) and present (green) environments is shown, along with the distribution of genotypes (the standing genetic variation) in the ancestral population (red). The evolution of this variation in the present environment at both early (light green) and late (dark green) times is also shown. (**a**) If the population were returned to the ancestral environment at an early time, standing variation from the ancestral state would still exist and reverse evolution would be rapid and repeatable. At longer times, reverse evolution would depend on mutations back to the ancestral genotypes. (**b**) The fitness landscape in the present environment is steeper, so the distribution of genotypes moves rightward more quickly. This rapidly eliminates the standing variation that existed in the ancestral state. If the population stays in the present environment too long, it becomes trapped away from the ancestral states, because it moves far enough rightward that mutations back toward the ancestral genotypes are deleterious even in the ancestral environment.

arrival and fate is a random process, reverse evolution will be much slower, and will not necessarily proceed similarly in parallel populations (**Fig. 1**).

Although it is clear that the ability of a population to remember and recapture adaptations to ancestral environmental conditions depends on whether it has seen these conditions recently enough, it is not obvious what 'recently enough' means. Teotonio and colleagues show that in *Drosophila* populations of approximately 1,000 individuals presented with new selective conditions for approximately 100–200 generations, reverse evolution is dominated by standing genetic variation. This leads to a relatively high degree of convergence to ancestral genotypes and significant parallelism between independent populations. By contrast, an earlier study in phage found that in populations of approximately 10^7–10^8 individuals presented with new selective conditions for approximately 1,000 generations, reverse evolution is dominated by new mutations—leading to substantially less convergence to ancestral genotypes[5].

These are two very different experimental systems, but which differences are responsible for the divergent results, and what can we learn from them? The length of evolutionary memory depends on a variety of population genetic parameters, such as population size and mutation and recombination rates[3,6], as well as on ploidy and breeding system, among other things[7]. For example, the larger phage populations should be able to create and maintain more standing genetic variation, but on the other hand, low recombination rates in phage mean that this standing variation can be more easily disrupted by selective sweeps of linked beneficial mutants.

The selective landscape

Although most of these population genetic parameters are relatively easy to measure and understand, at least in a rough sense, there is another crucial issue we know very little about: the nature of the map between genotype and fitness—the selective landscape. The local structure of this landscape in both the ancestral and new environments is essential in determining the quality of evolutionary memory and the outcome of reverse evolution[8,9]. If, for example, genotypes that are beneficial in the ancestral environment are deleterious in the present environment, memory in the form of standing genetic variation would be much shorter-lived than if those genotypes were neutral in the present. On longer timescales where standing variation is gone and memory depends on being mutationally close to favorable genotypes, the structure of the fitness landscape may make it either easy or difficult to become 'trapped' by epistatic interactions into a state where reverse evolution to an ancestral genotype is impossible. For example, one could imagine a situation where two or more mutations fix in the new environment because they are neutral or beneficial, making reverse evolution impossible, because even though reversion of all these mutations together would be advantageous in the ancestral environment, each reversion is individually deleterious. Such trapping can also occur when reverse evolution results in compensatory mutations that lead to phenotypic reversion but trap the population away from its ancestral genetic state[10,11].

The results Teotónio and colleagues report in this issue highlight the potential role of the selective landscape in maintaining and responding to standing genetic variation. They find that allele frequencies converge only about halfway toward ancestral levels, despite their earlier finding that the reverse-evolved populations fully regained ancestral fitness levels[12]. This suggests some sort of non-additive or frequency-dependent aspects of the selective landscape that inhibit reverse evolution at the genotypic level. The authors also find that although allele frequencies converge toward ancestral levels in reverse-evolved populations, these alleles do not tend to change in frequency in populations that have lived continuously in the ancestral conditions. Thus, reverse evolution is not driven by simple directional selection on standing genetic variation. Rather, their results hint at some type of balancing or frequency-dependent selection, which might be responsible for maintaining standing genetic variation for longer periods and hence improving the quality of evolutionary memory in this system.

The present study demonstrates that although reverse evolution of phenotypic characters is informative[12,13], combining this work with genotypic analysis can improve our understanding of the operation and targets of selection. Still more could be learned by connecting specific variant alleles with differences in fitness and other characters. One could then directly measure the relevant aspects of the genotype-fitness map. In simpler systems such as bacterial or viral populations, it might also be possible to systematically vary the time in and away from ancestral states, to study the time-dependence of evolutionary memory. With these approaches, we can hope to use reverse evolution as a probe into the structure of selective landscapes, and more generally the nature of evolutionary memory.

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Integrative genetical genomics in *Arabidopsis*

Wout Boerjan & Marnik Vuylsteke

An integrative genetical genomics study in *Arabidopsis* reports that six QTL hot spots have system-wide effects on a wide range of molecular and phenotypic traits, providing empirical evidence for phenotypic buffering.

The phenomenon of genetic buffering was initially described by Waddington in 1942 (ref. 1). One of the key examples of genetic buffering is that of Hsp90, a molecular chaperone that secures the proper functioning of many different developmental signaling pathways in *Drosophila* and *Arabidopsis*. In the presence of Hsp90, much of the genetic variation remains hidden, but upon impairment of Hsp90 function, novel and discrete phenotypic variants appear[2]. This evolutionarily conserved buffering system may allow organisms to accumulate mutations without negative impacts on fitness, and increase their chances for evolutionary adaptation in conditions when the genetic variation is expressed[3].

Ritsert Jansen and colleagues[4], on page 166 of this issue, provide the first system-wide evidence for phenotypic buffering in *Arabidopsis*. Using a genetical genomics approach, the authors profiled 162 L*er* × Cvi recombinant inbred lines (RIL) of *Arabidopsis thaliana* for variation in transcript, protein and metabolite abundance, and mapped quantitative trait loci (QTL) for 40,580 of these molecular traits. The data were integrated with QTL for a total of 139 publicly available phenotypic data collected for the same RIL population over many years by various research laboratories (**Table 1**). They found that only six QTL hot spots underlie variation in 16% of the transcript traits, 25% of the protein traits, 55% of the metabolite traits and 77% of the phenotypic traits for which QTL could be mapped. Although the parental lines L*er* and Cvi differ by more than 500,000 SNPs, it is notable to find that a large proportion of the variation in such a wide range of molecular and morphological traits is explained by these six QTLs, and this finding suggests that phenotypic buffering may be a mechanism of robustness to molecular variation in this system.

Table 1 Integrative analysis of *Arabidopsis* QTL

Mapping population	Traits	Method	Traits with one or more of six QTL hot spots (%)	Hierarchy of buffering
Arabidopsis RIL $n = 162$	24,065 transcripts	Microarray	16	
	2,843 proteins	2D-PAGE	25	
	13,672 metabolites	GC-TOF-MS; LC-QTOF-MS; ^{1}H-NMR	55	
	139 phenotypes	Biomass, morphology, etc.	77	

Taking an integrative genomics approach, Fu *et al.*[4] characterize a wide range of molecular (transcript, protein and metabolite abundance) and phenotypic traits using the noted methods. The percentage of traits with QTL that mapped to at least one of the six QTL hot spots is given.

Robustness and pleiotropy

As predicted previously[5], and as recently substantiated with experimental data in yeast[6], robustness to perturbations is an inherent property of biological networks[7]. Biological networks are characterized by a small number of highly connected nodes, called hubs. On a cellular level, a hub represents a transcript, protein or metabolite that either interacts or is correlated with a high number of other transcripts, proteins or metabolites. In the study of Fu *et al.*[4], correlations between transcript, metabolite and protein levels are evident, as they are mainly controlled by the same six QTL hot spots. Whether such QTL hot spots correspond to network hubs at some level needs further investigation, and a possible role of these hot spots in evolutionary adaptation should also be considered.

Fu *et al.*'s observation that QTL for 77% of all phenotypic traits with QTL map to six hot spots has important general implications. It suggests that morphological screens for mutants may be biased toward a limited number of loci with pleiotropic effects. In addition, the Fu *et al.* study[4] demonstrates that apparently unrelated phenotypes may often have some shared genetic basis, a concept that also emerges from human disease studies[8–10].

Furthermore, Fu *et al.*[4] find that these six QTL hot spots influence less of the molecular traits with QTL, which may suggest lower levels of buffering at the molecular level. QTL for 16%, 25%, 55% of all transcript, protein and metabolite traits with a QTL, respectively, map to the same six QTL hot spots, compared to 77% of phenotypic traits (**Table 1**). Consequently, screening for mutants at the molecular level will increase the probability of identifying new causal loci that could not be identified from morphological screens. One such example in *Arabidopsis* was given by a study showing that mutations in the gene encoding phenylalanine ammonia lyase (PAL), the entry point in phenylpropanoid biosynthesis, evoke far-reaching effects at the transcript and metabolite level, yet do not cause morphological abnormalities[11].

Resolving causal variation

Fu *et al.*[4] provide an ideal demonstration of how taking an integrative genetical genomics approach, in which transcriptomic,

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