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## Mutation

DAVID HOULE

ALEXEY KONDRASHOV

Mutation is the ultimate source of all genetic variation, and genetic variation is absolutely necessary for any sort of evolution to proceed (Wayne & Miyamoto, Ch. 2 of this volume). These two facts should make the study of mutation the foundation of evolutionary genetics. Unfortunately, this is very far from the case. Both the processes of mutation and the pattern of effects of those mutations are relatively little known compared with the properties of the standing genetic variation within populations and among populations and species. However, these properties can only be understood in light of mutation.

Two classes of mutation are particularly important to evolution: those with beneficial and those with deleterious effects on fitness. Evolutionary change depends on the input of beneficial mutations. Unfortunately, such mutations are usually quite rare and hard to study. The vast majority of mutations that affect fitness decrease it. Because these mutations are common, their effects in total can have very large evolutionary consequences. Such disparate phenomena as inbreeding depression, sexual selection (Moore & Moore, Ch. 22 of this volume), recombination and sexual reproduction, and senescence (Promislow & Bronikowski, Ch. 30 of this volume) are all affected by, or even explained by, the commonness of deleterious mutations.

Genetic information is encoded in nucleic acids; genetic variation is created when the sequence of these is altered. In all cellular organisms DNA is the genetic material. This makes the study of mutation

at the DNA level both natural and important. The focus at this level is on the rate at which various sorts of alterations occur. It is also necessary to understand the effects that these alterations have on the phenotype of the organism. This latter aspect of mutation is a key to resolving one of the major controversies in evolutionary biology: whether evolution is limited by the supply of genetic and phenotypic variation (Gould & Lewontin 1979), or by natural selection (Charlesworth et al. 1982).

The difficulties of the study of mutation stem largely from one simple fact: individually, mutations are very rare events. This has always made the direct study of mutation, consisting of recording new mutations as they arise, exceptionally tedious. For example, one of the best direct studies of mutation in mammals is that of Russell and Russell (1996), who report examinations of over 1,000,000 mice to find just 46 visible mutations.

The alternative to this tedium is to fit a model to genetic variation within a population or to the variation found among populations or species. These model-based approaches utilize data on contemporary variation, which are relatively easy to gather, to obtain information about mutations that happened over a considerable period of time. Consequently, much of what we know about mutation comes from model-fitting. Such efforts have a very important Achilles heel—if the model is not correct, the results can be very misleading. A model is necessary because the variation studied, while certainly due to mutation, has also been filtered by natural selection and

genetic drift. This tension between direct observation and indirect, assumption-based approaches is very common in evolutionary biology.

## CONCEPTS

### What is Mutation?

Most, but not all, changes in DNA sequence are mutations. The exception is reciprocal recombination, where both homologous DNA sequences are broken and rejoined at the same point. This does change the DNA sequence, but its reciprocal nature conserves information. Small-scale alterations of one or a few base pairs are mostly caused by errors in DNA replication and repair, and thus are unambiguous mutations. Sequences that emerge after large-scale changes often contain clearly identifiable, rearranged pieces of old sequences, so that recombination as well as errors per se may be involved. Many of these changes that can be viewed as either mutation or nonreciprocal recombination. Such processes will be considered here, because they share the rarity and irregularity of small-scale mutations.

This definitional difficulty carries over to the language of variation. A bit of DNA sequence that has been changed from the copy in its parent is clearly a “mutation,” but when two sequences differ, we cannot assume without direct knowledge of their ancestry, that one variant is the “mutation” while the other is “ancestral,” “normal,” or “wild-type,” although in practice a deleterious or low-frequency

variant is often referred to as a mutant. This is justified for deleterious variants, which are usually lost due to natural selection, and thus cannot be ancestral; in contrast, a low-frequency variant may, nevertheless, be ancestral. Genetic variants are variously called alleles when referring to alternate forms of a gene or haplotypes when referring to longer DNA sequences. We will adopt the term “variant” as the most general, and least loaded term.

Replication, repair, and recombination of DNA sequences are incredibly complex molecular processes, each involving interactions of dozens of macromolecules. However, we will ignore biophysical and biochemical aspects of these processes, and adopt a simple transmission genetics approach, concerned with inputs, outputs, and rates.

### Classification of Mutations

Mutational events may affect anything from 1 base pair to entire genomes. Small-scale mutations that affect only a few nucleotides are categorized in Table 3.1. Small-scale mutation depends on the local sequence context. The most important aspect of context is whether the sequence consists of repeated sequences (for example AGAGAG, known as “periodic”), or a more typical sequence where the progression of base pairs is more or less unpredictable (“complex”).

Mutations in short periodic sequences, called micro- and minisatellites, are mostly deletions and insertions, usually of lengths that are multiples of the period length. They often occur with rates

**TABLE 3.1.** Classification of mutational events involving small numbers of base pairs

Event	$l_1^a$	$l_2$	Example	Description
Nucleotide substitution	1	1	AGC → ATC	Any single base pair change
Transition	1	1	AGC → AAC ACC → ATC	Base pair substitution of a purine (A or G) with another purine, or of a pyrimidine (T or C) with another pyrimidine. More common than transversions
Transversion	1	1	AGC → ATC	Base pair substitution of a purine with a pyrimidine, or a pyrimidine with a purine. Less common than transitions
Deletion	≥ 1	0	AGGC → AC	One or several successive nucleotides are removed
Insertion	0	≥ 1	AC → AGGGC	Insertions of one or several successive nucleotides
Complex events —	1	> 1	AGC → ATTC	Combined indel/substitution very rare. Gene
combine substitution	> 1	1	AGGC → ATC	conversion (see Table 3.2) can cause complex
and deletion or insertion	2	2	AGGC → ATTC	changes Simultaneous substitutions do occur at appreciable rates

<sup>a</sup> $l_1$  is the length of the affected sequence before mutation;  $l_2$  is the length of the sequence after mutation.

that are orders of magnitude higher than those of mutations in complex sequences. Microsatellites are unlikely to have important functions, but their frequent mutations provide abundant data for tracing ancestry. Even within complex sequences, the context can affect mutation rate significantly (Kondrashov & Rogozin 2004). An important case is the elevated mutation rate in mammals of the dinucleotide CpG. CpG pairs consist, in both strands, of a cytosine followed by a guanine, where p refers to the phosphate that joins them. The impact of a local context is so important that, say, insertions into complex sequences versus microsatellites, or transitions within versus outside CpG are often considered as separate types of mutations.

Large-scale mutations are categorized in Table 3.2. With the exception of long deletions, these changes usually involve recombination of pre-existing segments of sequences. The reason for this is that de novo origin of a long DNA sequence is a rather unlikely occurrence. Thus, sequences added by a large-scale mutation are usually copies, often modified, of pre-existing sequences. The probability that such a sequence (which can code for a protein domain, or even an entire protein) would be functional is substantial (Thornton, Ch. 11 of this volume). Creation of homologous sequences by duplication can catalyze further large-scale events, as it creates the opportunity for nonreciprocal recombination. Transposable elements provide a particularly important example, as they can also lead to

**TABLE 3.2.** Mutational events involving large numbers of base pairs

Event	Where does new sequence come from?	Example	Details
Deletion	—	A[S <sub>1</sub> ]G → AG	Removed sequence can be very long. Rates increase if flanking sequences are similar
Tandem duplication	Neighboring sequence	A[S <sub>1</sub> ]G → A[S <sub>1</sub> ][S <sub>1</sub> ]G	S <sub>1</sub> may be a rather long sequence, sometimes a large proportion of the chromosome
Nontandem duplication LGT	Non-neighboring sequence	AG → A[S <sub>2</sub> ]G	S <sub>2</sub> is a sequence of any length from elsewhere in the genome. In practice only detected when S <sub>2</sub> is > 20 bp
Transposable element (TE) insertion	TE	AG → A[T <sub>1</sub> ]G	T <sub>1</sub> is DNA derived from a TE. T <sub>1</sub> often consists of a fragment of TE sequence. Rate may be quite high. A common mode of nontandem duplication. Often insertion is accompanied by other changes (i.e., short duplications)
Lateral gene transfer	Other genome	AG → A[S <sub>3</sub> ]G	Important in prokaryotes
Inversion		A[GT ... CC]A → A[GG ... AC]A	Rotates a sequence
Gene conversion	Homologous sequence		Sequence of one homolog converted to that of the other
Transposition	Sequence changes location		Usually through breakage and fusion of chromosomes
Chromosome break	None		Usually deleterious, but may increase chromosome number
Chromosome fusion	None		Usually deleterious. May decrease chromosome number
Aneuploidy	Chromosomal duplication or loss		Usually lethal, or very deleterious
Polyploidization	Same genome: autopolyploidy		Fixed more frequently in plants than animals
	Different genome: allopolyploidy		Often caused by hybridization. Fixed more frequently in plants than animals

small duplications through transposition (Petrov & Wendel, Ch. 10 of this volume).

### Classification of Phenotypic Effects

Variant DNA sequences may or may not have an effect on the phenotype of their carriers. Those that do may have a cascading series of effects, from the regulation or function of a gene, through the biochemical, developmental, or physiological levels, and ultimately on the readily observable phenotype of the organism. It is useful to distinguish loss-of-function variants, for example a frameshift deletion, from those mutations that leave an altered functional gene, such as an amino acid substitution. Mutations that alter function usually result in quantitative changes in amount or timing of expression. Occasionally such changes may lead to a qualitatively different effects, such as new substrate specificity of an enzyme.

The most important phenotype is fitness, the capacity to produce offspring, ~~and so~~ includes survival ~~and the ability to breed and reproduce~~. The effect on fitness of a variant sequence is an important determinant of its evolutionary fate. However, fitness has surprisingly little effect on the fate of any single variant, as all start out rare where stochastic effects are very strong (Gillespie, Ch. 5 of this volume).

Mutations can be conveniently classified according to their fitness effects into the following categories:

1. Lethal mutations kill the individuals that carry them.
2. Deleterious mutations reduce fitness relative to alternative states, but not to zero. Considerable evidence suggests that such mutations are more common than lethals.
3. Neutral mutations do not affect fitness much, either positively or negatively. These too are likely to be common.
4. Advantageous mutations increase fitness, and therefore will be favored by natural selection. These are probably the rarest type of mutation.

This categorization is context-dependent. An advantageous variant in one environment may be neutral or deleterious in other circumstances (Scheiner, Ch. 21 of this volume). Also, the fitness

of a variant might depend on the genotype it finds itself in (epistasis).

In diploid organisms, an important additional consideration is the dominance of a variant. Dominant mutations have their full phenotypic effects when present in heterozygous condition, while recessives only affect the phenotype in homozygous condition. If the mutant heterozygote is intermediate between the two homozygotes, the mutant is partially dominant. If the heterozygote is exactly intermediate, the variant is said to act additively.

The concept of dominance is itself phenotype-specific. For example, Mendel's wrinkled pea allele is recessive when the smoothness of the seed coat is examined, but when the amount of starch in the seed is measured, the heterozygote is intermediate ~~between the~~ two homozygotes. When subjected to quantitative analysis, the vast majority of even major variants seem to be neither completely recessive ~~or~~ dominant. Variants with minor phenotypic effects tend to be partially dominant, and are often nearly additive.

### How to Study Mutation

Classification of the types of mutations (Tables 3.1 and 3.2) makes the study of mutation sound altogether straightforward. It is important to realize that our ability to study these different classes of mutations varies widely depending on their rarity and the nature of their phenotypic effects. In Table 3.3, we classify the study of mutation according to two criteria suggested in Kondrashov (1998): the time scale over which mutation is studied, and the type of data that is used to detect mutations.


Three different classes of characteristics may be used profitably to detect mutations: DNA sequences, phenotypes, and fitness. Studies of mutation almost invariably cover one of three time frames. First there is the direct study of mutation through comparison of parents and offspring. At a slightly longer time scale, one can set up a mutation-accumulation (MA) experiment. To do so, one maintains the population under conditions that minimize the impact of natural selection on the fate of any mutations that may arise (see Case Studies for examples). Finally, the comparative method infers mutation rate from the rate of divergence between species.

The use of different time frames allows different aspects of mutation to be investigated. The chief reason for these differences is the degree to which we can assume a realistic model for the interaction

**TABLE 3.3.** Categorization of approaches to the study of mutation, with reviews or examples of successful studies

	Generations separating samples		
	1 Direct	10 to 10 <sup>3</sup> Mutation accumulation	>10 <sup>5</sup> Comparative
DNA	Weber and Wong 1993	Denver et al. 2004; Schug et al. 1997	Nachman and Crowell 2000
Phenotype Fitness	Kondrashov 2002 Woodruff et al. 1983	Houle et al. 1996 Mukai et al. 1972	Lynch 1990

between mutation and natural selection. With direct studies, the need for assumptions about natural selection is minimal. Almost all types of mutations may be observed in the offspring. As the time frame of the study lengthens, the necessary assumptions about natural selection become more stringent. Only mutation rates to neutral alleles can simply be inferred over long time periods. As a result, the same data can lead to very different conclusions about the overall mutation rate, depending on the assumptions chosen.

Despite the complications in applying models to divergence data, the essential neutral theory behind such models is easy to grasp. If we consider a population of genetic variants with no impact on fitness, that is neutral variants, whether they are lost from the population or will become fixed (rise to a frequency of 1) depends only on genetic drift, the luck of sampling during reproduction. Lucky variants will become fixed; the vast majority will be lost just by chance. The chance that each particular variant will be fixed in the future is proportional to its frequency right now: rare variants are likely to be lost, common ones likely to be fixed. Now, let us consider the fate of each new neutral variant. If there are  $N$  diploid individuals in the population, each new variant starts out at a frequency of  $1/2N$ , and thus has a chance of  $1/2N$  of rising to fixation. On the other hand, with a mutation rate  $m$  per gamete, the number of new mutations in each generation is  $2Nm$ . Multiplying these two together gives the surprisingly simple rate of neutral evolution:  $k = 2Nm \times 1/2N = m$ .  rate is the divergence from the ancestral sequence; species diverge at twice this rate because variants arise along both branches to the common ancestor.

In reality, variants have a range of effects on fitness from undetectable to lethality. Their ability to persist in the population and so be detected also

depends on the effectiveness of natural selection at influencing frequencies; this depends on the size of the population. In a population where  $N$  is small, genetic drift (luck) will be a relatively strong force, swamping out small differences in fitness. However, when  $N$  is large, even tiny differences in fitness reliably discriminate higher and lower fitness variants. Mutation-accumulation experiments are therefore designed to maximize the impact of drift, either by making  $N$  as small as possible, or by equalizing family sizes (Shabalina et al. 1997). Thus, the influence of natural selection is minimal in a direct study, somewhat higher in a mutation-accumulation study, and very large in a comparative study. The result is that the neutral model can be applied to an uncertain and decreasing proportion of variants as the time scale of the study increases. Even at the DNA level, it is difficult to be sure that a particular segment really evolves at the neutral rate. For example, evolutionary biologists have treated pseudogenes, altered sequences derived from functional genes, as neutral (e.g. Nachman and Crowell in Case Studies, below). However, there are at least two possible mechanisms for selection on pseudogenes. First, recombination between pseudogenes and their parent gene is deleterious, so deletions of pseudogenes may be favored by natural selection. Second, the discovery of naturally occurring nonprotein-coding genes (such as micro-RNAs) that can regulate expression of their homologous genes suggests that some apparent pseudogenes may play such a selected role.

A second major disadvantage of comparative studies is that the number of generations that separate species is usually known only very approximately. As the time scale of any comparison becomes longer, these uncertainties become very large. The number of generations in a lineage since the Mesozoic era will hardly be ever known with any

confidence at all. As a result, comparative data are usually summarized as a mutation rate per unit time. While this may be useful in some contexts, such as calibrating a molecular clock (e.g. Rodríguez - Trelles et al. Ch. 8 of this volume), it does not tell us what we want to know about mutation rates in organismal terms.

These considerations would seem to make direct studies preferable, were it not for the fact that the longer the time period, the greater the number of mutational events that can be assayed. Because mutations are individually rare, direct studies can only be informative when there is an efficient mechanism for screening enormous numbers of individuals for mutations. Such screening is available for many inherited phenotypes in human societies with advanced health care. At the other end of the biological spectrum, microbial populations can be rapidly screened for the converse sort of mutations that restore function at a defective gene (reviewed in Drake 1991). For other species, the direct data are limited. Furthermore, because the phenotypic impact of most mutations is usually small, we need to be able to infer from the minority of mutations that are observable the properties of the full spectrum of mutations.

Of the three different classes of characteristics that may be used to study mutation, DNA sequences are the most conceptually straightforward. The challenge with the use of sequence data is that care must be taken to account for the possibility of errors in scoring. This has so far limited the use of sequence data in direct or mutation accumulation studies. For example, to detect a sample of base pair mutations, which typically occur at a rate of  $10^{-8}$  per generation, over a 100 generation mutation-accumulation experiment, one needs the ability to sequence many more than  $10^6$  nucleotides with an error rate well below  $10^{-6}$ . The necessary methods are emerging and are starting to be applied (Denver et al. 2004). Exceptions are provided by sequences with especially high mutation rates, such as microsatellites or mitochondrial DNA.

The other two categories of data (phenotypes and fitness) refer to whole-organism characteristics. Fitness is, in some respects, just another phenotype, but is by definition under strong natural selection. Mutations with large effects on these phenotypes, such as genetic diseases in humans or visible and lethal mutations in *Drosophila*, can be counted. This is the basis for the direct studies in Table 3.3. By connecting such changes to the DNA changes

responsible for them, as explained below for human genetic diseases, they can be used to gain very detailed information about mutation rates.

However, mutations with the very largest phenotypic effects used in direct mutation studies are themselves rarely of evolutionary significance, as they usually reduce fitness. Quantitative trait locus and developmental studies of species differences suggest that both detectable-if-you-know-what-to-look-for and small effect variants are the major sort of variation that allows evolution. Their cumulative effects are usually studied in a mutation-accumulation experiment. In most such experiments, an initially inbred genotype is replicated and selection on each replicate minimized by lowering  $N$  for each replicate, for example by selfing or brother-sister mating. The rate at which variation in the phenotype accumulates is used to measure the increase in phenotypic variance due to a single generation of mutation,  $V_M$ . In addition, a change in the mean of the accumulation lines indicates that mutations are biased in their effects. For example, fitness and its key components of viability, fecundity and mating ability, are maximized by natural selection, suggesting that the mutations that arise will on average decrease fitness. Information on  $V_M$  and mutational bias can sometimes be combined to give a very crude estimate of the overall mutation rate of all genes affecting fitness, as in Mutation Accumulation in *Drosophila* in the Case Studies section below.

### Mutation Rates

The most detailed picture that we have of mutation rates in eukaryotes is for humans. This is due to several factors. First, human-chimpanzee is the only species pair for which the total number of generations since their divergence is reasonably well known, facilitating comparative studies (see Nachman and Crowell in Case Studies, below). Second, data on Mendelian diseases provide the only large-scale phenotypic screenings for de novo mutations in any eukaryotic species for direct estimates (Kondrashov 2002). For our species, the comparative and direct approaches suggest a very similar mutation rate of about  $2 \times 10^{-8}$  per nucleotide per generation. The fact that the two methods give essentially the same estimate is quite encouraging. Substitutions account for about 95% of this total, with short insertions and deletions accounting for almost all of the remainder. Large-scale mutations are generally rare.

This simple picture conceals substantial complexities based on the sequence that surrounds a particular site. In mammals, substitution rate at CpG sites is elevated by a factor of more than 10. The reason for this is that the C tends to be methylated, which misleads DNA polymerase. This confusion results in a transition from C to T, and the destruction of the CpG context. Oddly, this results in a lower mutation rate of noncoding DNA than in coding DNA. In coding regions, natural selection to maintain a particular sequence can preserve CpG pairs despite this high mutation rate away from them; in noncoding regions mutation pressure rapidly destroys them. Duplicated sequences are another frequent source of mutational hot spots because nonhomologous recombination between the similar sequences leads to large-scale mutations. For example, about half of the mutations causing hemophilia A are caused by recombination of the gene with its nearby pseudogene.

Another complexity in the human data is that it appears that mutation rates are substantially higher in males than females (Drake et al. 1998), contrary to the impression created by the well-known maternal age effect on Down's syndrome. Part of this effect is probably due to the fact that the spermatogonia divide continuously throughout a male's lifespan, while oocytes essentially stop dividing before birth. It has been estimated that the number of divisions in the germ line of a 30-year-old human is 31 for a female and 400 for a male; this difference increases with paternal age.

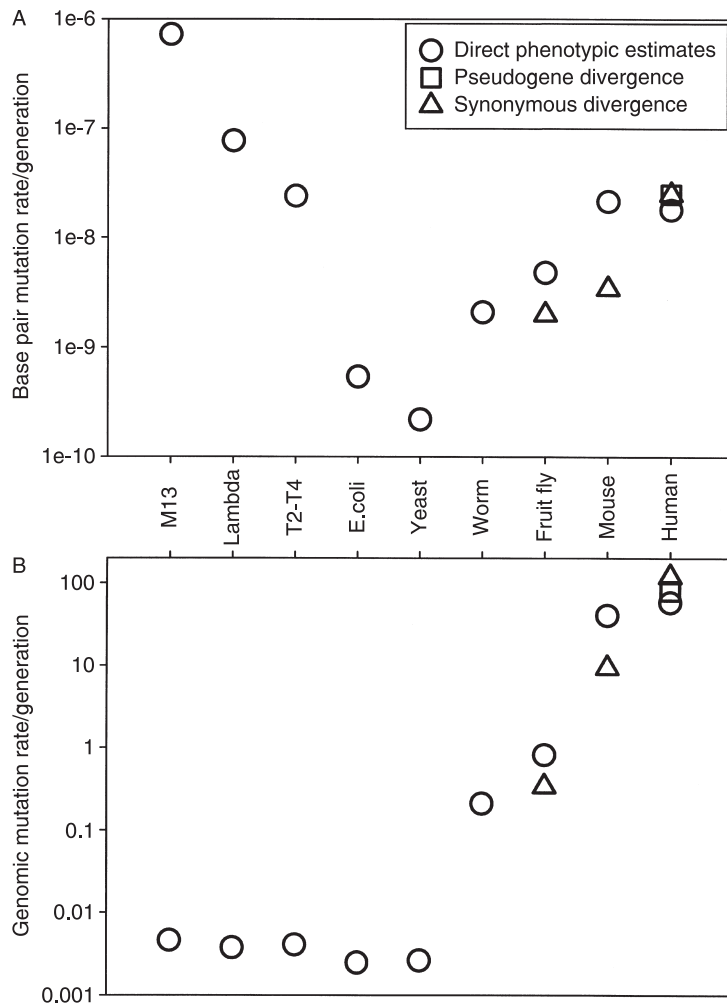
These facts point up the difficulties in generalizing about mutation rates across species. The methylation that gives rise to the CpG bias is not universal, and other biases undoubtedly arise in other groups. While human mutations are overwhelmingly single-base substitutions, mutations in *Drosophila* tend to involve more short insertions and deletions, and more large-scale mutations. The details of gametogenesis and details of life history, such as the average age at reproduction, can have a big effect on mutation rates, even when the cellular details of meiosis and replication remain the same.

With these difficulties in mind, data on mutation rates in some other well-studied DNA-based systems are shown in Figure 3.1. In the nematode worm *Caenorhabditis elegans* mutation rates were obtained by sequencing random nuclear sequences in a mutation-accumulation experiment (Denver et al. 2004). Mutation rates are relatively well known in viruses (phages) and bacteria from direct and

mutation-accumulation studies, as it is possible to rapidly screen huge numbers of individuals for novel phenotypes (Drake 1991). With far more investigator effort, substantial direct phenotypic mutation assays have also been performed in several model systems (Schalet 1960; Woodruff et al. 1983; Russell & Russell 1996; Drake et al. 1998). In all these studies, investigators first screen for phenotypic mutations at previously identified loci. These values are then combined with the size of the locus and an estimate of what proportion of all DNA changes will result in a mutant phenotype to arrive at an overall mutation rate. These estimates may be inaccurate; for example, the direct *C. elegans* mutation rate from sequencing is one order of magnitude higher than the estimate that Drake et al. (1998) obtained using data from direct phenotypic assays. Finally, there is a large amount of comparative data from which mutation estimates can be calculated. As an example of this sort of estimate we have used the calculations of Keightley and Eyre-Walker (2000), who calculated rates based on the assumption that synonymous sites evolve at the neutral rate.

The data in Figure 3.1 make it clear that the mutational properties of organisms are extremely different. To help interpret this variation, the genome sizes and number of replication events per generation are shown in Table 3.4. Figure 3.1a shows that mutation rates per base pair per generation vary over three orders of magnitude. Viruses seem to sacrifice accuracy for speed, while multicellular organisms with long generation times accumulate mutations over many cellular replication events. When the numbers of mutations over the whole genome are summed, it is clear that mutation rates on average rise with the genome size of the organism. Drake (1991) called attention to the fact that viruses and unicellular organisms have a fairly constant mutation rate per genome. It is now clear, however, that this relationship does not hold for multicellular organisms (Figure 3.1b; Drake et al. 1998).

A second point concerning Figure 3.1 is that the discrepancies between mutation rates obtained using different approaches can be very substantial. The mutation rates obtained from assuming that third-base-pair positions evolve at the neutral rate are substantially lower than those obtained using direct evidence in *Drosophila* and *Mus*. In humans, there is no such discrepancy, with synonymous rates being within a factor of 2 of the direct estimate. This difference may be caused by the larger population



**FIGURE 3.1.** Estimated mutation rates in 10 well-studied taxa. (A) Mutation rates per base pair per generation. (B) Haploid mutation rates per genome per generation. Circles, direct and mutation accumulation estimates; square, estimate assuming that divergence in pseudogenes is neutral (Nachman & Crowell 2000); triangle, estimates assuming that divergence in synonymous base pairs is neutral (Keightley & Eyre-Walker 2000).

sizes of *Drosophila* and *Mus* relative to that of *Homo*, allowing a larger proportion of human variants with very small deleterious effects to evolve at the neutral rate.

The above estimates do not reflect whole gene duplications, which may be critical for long-term changes in the genome (Petrov & Wendel, Ch. 10 of this volume; Thornton, Ch. 11 of this volume). Genomic data from several species suggest that

there is about a 1% chance that a duplicate copy of gene will be fixed per million years (Lynch & Conery 2000). For *Drosophila melanogaster*, this suggests that the fixation rate of duplications is about  $3 \times 10^{-10}$  per gene per generation. This rate may be higher or lower than the actual duplication rate, depending on whether duplications are deleterious (for example due to recombination between duplicates) or advantageous. Bearing in mind that



**TABLE 3.4.** Genome sizes, generation times and average number of replication events/life cycle for taxa with mutation data in Figure 1.

Taxon	Common name	Genome sizes	Generation time (days) <sup>a</sup>	Rep./gen. <sup>b</sup>
M13 phage		$6.4 \times 10^3$	0.01	1
Lambda phage		$4.9 \times 10^4$	0.01	1
T2 and T4 phages		$1.7 \times 10^5$	0.01	1
<i>Escherichia coli</i>		$4.6 \times 10^6$	0.01	1
<i>Saccharomyces cerevesiae</i>	Brewer's yeast	$1.2 \times 10^7$	0.1	1
<i>Caenorhabditis elegans</i>	Nematode worm	$1 \times 10^8$	4	9
<i>Drosophila melanogaster</i>	Fruit fly	$1.7 \times 10^8$	12	30
<i>Mus musculus</i>	House mouse	$3 \times 10^9$	275	43
<i>Homo sapiens</i>	Human	$3 \times 10^9$	7300	215

<sup>a</sup>These times are minimum generation times under ideal conditions. Such conditions may not be typical of those in nature.

<sup>b</sup>Estimated number of replication events per generation.

a gene duplication will affect many nucleotides, the overall rate of duplications may affect as many or more base pairs as do base pair mutations.

### Mutations and Fitness

Mutations that affect fitness are more likely to be deleterious than advantageous. As a result, the rate of evolution of DNA sequences is on average inversely related to their functional importance. Within protein-coding genes, synonymous sites evolve faster than nonsynonymous sites, and homologous exons are much more similar than homologous introns. Direct estimates of the fitness effects of new mutations generally corroborate this by showing a decline in fitness during mutation accumulation experiments (see Mutation Accumulation in *Drosophila* in Case Studies, below). This is hardly surprising: spoiling the product of 3.5 billion of years of evolution is easier than improving it.

However, there are parts of some genes where nonsynonymous substitutions occur faster than synonymous, suggesting that many replacements of amino acids were advantageous. For example, the antigen binding region of the HLA gene in the major histocompatibility complex in humans and mice has a higher rate of amino acid substitutions than synonymous substitutions. This suggests that positive selection for diversity or at least change in antigen-binding makes advantageous amino acid changes quite frequent (Hughes & Nei 1989).

Another key property of DNA sequences in eukaryotes is that large portions are irrelevant to fitness. Only approximately 2% of the human genome codes for proteins, and only a minority of

the remainder evolves slower than the neutral rate, and is therefore functionally important (estimates range from 15% to 3%; Shabalina et al. 2001; Dermitzakis et al. 2002). Mutations affecting the remainder are phenotypically silent and selectively neutral. Quantitatively, the fraction of neutral sequences is smaller in compact genomes of bacteria (where over 80% of sequences code for proteins) and much larger in eukaryotic genomes. From these figures, together with the data on per nucleotide mutation rates referred to above, the 100 mutations expected per human genome per generation can translate into from 4 to 14 deleterious mutations (assuming that 50% of mutations in coding sequences are deleterious).

Direct evidence makes it clear that most mutations that affect fitness have relatively small deleterious effects (see Mutation Accumulation in *Drosophila* in the Case Studies section below). A striking confirmation of this fact is that systematic knockouts of genes in eukaryotes reveal that less than 30% of all genes are essential to viability. For example, the fitness effects of knocking out nearly every gene in the yeast genome have been measured. Under typical laboratory conditions, only 18.7% of the genes are essential, while quantitative decreases in fitness are detectable in another 15% of the knockouts (Giaever et al. 2002). Since any gene that is not capable of affecting fitness will rapidly be destroyed by mutation, the remaining genes must either have effects on fitness that are too small to be detectable, or be advantageous under conditions not found in these experiments. Most spontaneous mutations must have smaller effects on fitness than the whole-gene knockouts used in this experiment.

Unfortunately, our ignorance regarding the mutation rate to beneficial mutations is quite profound. Recent work holds out hope that this ignorance is curable. For example, microbiologists have long been able to exploit the lack of recombination to detect fixation of beneficial mutations. When a novel beneficial variant arises, the genetic background in which it occurs increases along with it. Thus, perturbations of neutral allele frequencies are good markers of fixation. A recent study exploited this fact to estimate that the genomic beneficial mutation rate in a set of *E. coli* populations was  $4 \times 10^{-9}$ /replication (Imhof & Schlotterer 2001), or only one millionth of the total genomic mutation rate shown in Figure 3.1. It is not easy to generalize from this, as presumably the evolutionary history of a population and the constancy of the environment will have an impact on this rate. Comparative data can also be used to estimate beneficial mutation rates if one is prepared to accept a fairly simple set of assumptions about the distribution of effects. For example, fixation events are slightly more likely to involve amino acid substitutions than predicted from the pattern of within-species polymorphism in two *Drosophila* species. Under a simple model this suggests that about 45% of the amino acid substitutions are due to positive selection (Smith & Eyre-Walker 2002).

### Mutations and Phenotypes

The importance of mutation for evolution also depends on the precise pattern of effects on the phenotype. For example, the degree to which the evolution of two parts of the body may be decoupled depends on whether and how often mutations that affect the parts in different ways arise (Wagner & Altenberg 1996). While our knowledge of the molecular and fitness effects of mutation are far from comprehensive, we are more ignorant of these important phenotypic properties. We know a bit about the amount of phenotypic variation produced by mutation. The study of the correlated effects of mutations is just beginning (see Mutation Accumulation in *Drosophila* in the Case Studies section below).

The basic challenge is that discrete mutations of large effect, such as lethals, visibles, or human genetic diseases, can be readily observed, but these are irrelevant to long-term evolution as they have extremely large deleterious fitness effects. Mutations with small positive or negative effects on fitness are the ones that we need to understand, and it is precisely

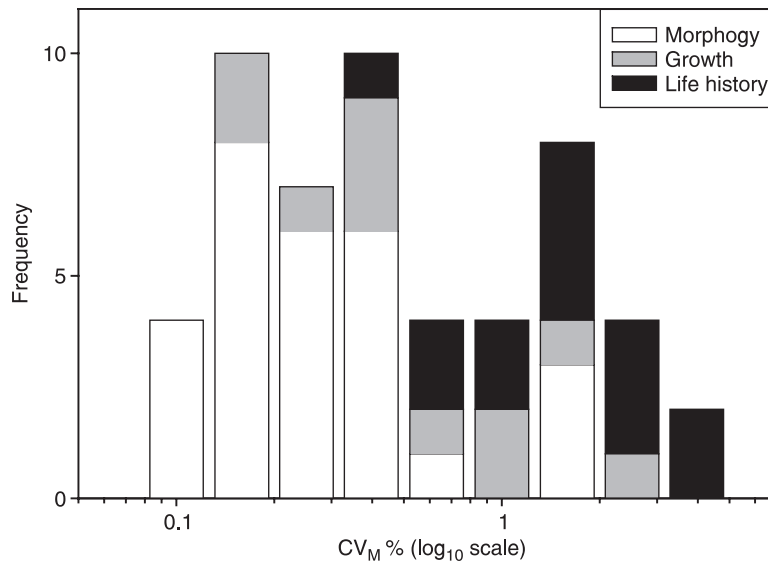
these mutations that are most difficult to detect and study. These questions have so far been addressed by relatively crude mutation-accumulation experiments in which the aggregate properties of unknown numbers of mutations are studied.

These studies make it clear that the effects of mutation differ with phenotype (Houle et al. 1996). Figure 3.2 summarizes estimates of the mutational variance,  $V_M$ , in seven species expressed as coefficients of variation,  $CV_M$ . A  $CV_M$  of 1% means that after one generation of mutation, the standard deviation among initially identical lines is 1% of the mean. The traits are classified by their presumed relationship to fitness. Life history traits are measures of viability, fecundity, or mating ability, and are expected to be closely related to fitness. Morphological traits are features such as bristle number or leaf size that are probably under stabilizing selection. Growth traits reflect the size of the organism during growth, which may or may not be closely related to fitness.

Two facts are apparent from Figure 3.2. First, the variation in  $CV_M$  is large, ranging from 0.1% to over 4%. This reflects variation both within and among species. Species-level  $CV_M$ s are correlated with generation time and genome size, suggesting that, as for molecular mutation rates, large genomes and/or large numbers of cell divisions increase mutational variance (Lynch et al. 1999). Second, it is clear that morphological traits accumulate variance less fast than life history traits (median  $CV_M$  0.24% vs. 1.47%). At least part of the explanation for this difference seems to be that larger numbers of loci affect life history traits, because they summarize variation in the overall function of the organism (Houle 1998). Thus the concept of mutational target size—the number of base pairs which, when mutated, affect a trait—can help to explain both among- and within-species variation in the impact of mutations on phenotypes.

### Why Are Mutation Rates What They Are?

Mutation rates themselves may evolve. Since the 1930s it has been clear that three major factors potentially determine the outcome of this process: the inevitability of some mutation, the costs of making replication as accurate as possible, and the possible advantages of beneficial mutations. Thus, there are three possible sorts of equilibrium mutation rates: the minimum possible, an optimal rate



**FIGURE 3.2.** Mutational coefficients of variation from a review of studies prior to 1995 (Houle et al. 1996). See text for explanation.

determined by the costs of fidelity, and an optimal rate determined by selection for evolvability (Kondrashov 1995).

It is clear that the first two processes must influence mutation rates. Mutation is unavoidable because recovery of destroyed information is generally not possible. For example, when bases on complementary DNA strands do not match, it is difficult to see how a repair mechanism could always determine which of the two bases in the mutant that should be repaired. Similarly, physical considerations show that, if one attempts to reduce the error rate in DNA replication and repair to zero, the cost of these processes, in terms of both time and energy, could be large. Thus, the first hypothesis, that mutation rates are minimal, is not tenable. This is also suggested by the inverse relationship between genome size and mutation rates among microorganisms (Figure 3.1). What is controversial is the effect of selection for evolvability on mutation rate.

The fact that mutation is essential for evolution has made the notion that its rate is tuned to allow evolution attractive to many. Furthermore, there is plenty of evidence for variation in mutation rate within species that could be exploited by natural selection. The fact that most eukaryotes employ meiotic recombination to produce genetic variation shows that variation-generating adaptations are

possible (regardless of why exactly sex is good). As indicated above, the rate of mutation depends on the DNA sequence, and the chromosomal context it is in. There is also substantial evidence for environmental effects on mutation rates. Nutritional deficiencies, the presence of mutagens, and temperature can change both rates and patterns of mutation. All this suggests that the variation necessary to alter mutation rates is abundant.

In a few cases, organisms seem to have evolved portions of their genomes to be susceptible to mutation. For example, a region of the gene specifying host recognition in the *Bordetella* bacterium mutates at a very high rate because the bacterium has harnessed a retrotransposition-like process that targets that region (Doulatov et al. 2004). The attractiveness of the notion that mutation rates are adaptive must be tempered by the evidence that the vast majority of mutations are deleterious, and therefore costly to the individual in whose genome they occur (Johnson 1999). In sexual organisms, this creates a typical conflict between group-level evolution and individual selection over the fate of a variant that increases the mutation rate. The high mutation rate variant can increase in frequency when it causes a beneficial variant; however, this advantage benefits the high mutation variant only as long as it and the beneficial variant remain together in the same genotype. This may not be long at all if the loci involved

are unlinked. On the other hand, most copies of the high mutation variant will produce a steady stream of deleterious variants that will reliably decrease the transmission of the high mutation rate variants. From the point of view of a population, more experimentation is ultimately beneficial if it can speed adaptation. However, from the viewpoint of the individual, it would be much better if another individual took the risk of experimentation. It takes special conditions for the advantages of mutation to the population to outweigh its costs to the individual (Sniegowski et al. 2000).

Closely related to the idea that the overall mutation rate is adjusted to promote evolvability is the idea that organisms can increase their mutation rate in relation to their need for variation, so-called adaptive mutation. The converse idea that mutations occur at random with respect to their usefulness has been a cornerstone of evolutionary thinking since the late nineteenth century, so evolutionary biologists reacted with outrage when adaptive mutation was revived again by Cairns et al. (1988). These researchers observed mutation rates that restored growth in nondividing "stationary phase" cells in *E. coli* and other single-celled organisms are higher than the rates of the same mutations when the cells are growing. This basic observation that has now been made in many microorganisms (Foster 2000).

There are three potential explanations for the increase in mutations observed in stationary phase. The increase can be adaptive in two senses: The strong version is that the rate of beneficial mutations can be increased at need, which we can call "directed mutation." The weak version is that the overall rate of mutation may be increased when variation is needed, or "hypermutability." Finally increased mutation may arise because of an unavoidable breakdown in normal repair and replication.

Foster (2000) and others have pinned down the mechanisms which underlie several cases of high mutation in stationary phase. In each case the effects are not confined to genes where mutations might be adaptive, ruling out the directed mutation hypothesis. However, the specific mechanisms by which the mutations arise, including DNA synthesis initiated by recombination and activation of transposable elements, do not necessarily suggest a general breakdown of fidelity. Thus hypermutability is real, but its adaptive significance is still not clear. The deleterious consequences of increases in mutation are not readily avoided, unless death of the cell is certain in the absence of mutation.

It is much more difficult to imagine adaptive increase of mutation rates in multicellular organisms. In fact, the study of mutation in cancerous cells of multicellular eukaryotes suggests that similar hypermutability may occur in tumors, where it facilitates the evolution of high tumor growth rate and resistance to chemotherapy, resulting in death of the organism. The fact that similar phenomena occur whether or not they can be adaptive favors breakdown in normal replication, recombination, and repair machinery. The isolation of the germline from the soma in most multicellular animals is a powerful argument against the generality of adaptive mutation.

### What Limits the Rate of Evolution, Mutation or Selection?

One of the central paradoxes of evolutionary biology is that most of the time, organisms do not evolve at all (reviewed in Gould & Eldredge 1993). There are two sorts of explanation for this stasis (reviewed by Hansen & Houle 2004). First, many believe that this is due to stabilizing selection which is somehow maintained over very long time periods. The weakness in this hypothesis is simply that it is difficult to see why selection should be constant over periods of tens of millions of years. The other alternative is that the kinds of variation necessary for populations to evolve in response to whatever novel selection pressures come up are often not produced (Gould & Lewontin 1979). Such limitations on variation are usually referred to as constraints, but this suggests that the necessary variation is never produced by mutation.

The common argument against the constraint hypothesis is that nearly every trait studied does display genetic variation. This is insufficient to resolve the issue because all aspects of the phenotype will be selected simultaneously. It is not enough to produce variation in each trait, the variation must also be relatively free of entangling effects on other selected traits. The capacity of the genome to produce appropriate sorts of phenotypic variation may determine which of the many pressures that natural selection places on an organism it will be capable of responding to. If mutations tend to affect a limited number of phenotypes, or phenotypes that tend to have similar selection pressures on them, the structure of variation is said to be modular (Mezey, Box 19.5 of this volume; Wagner & Altenberg 1996). Thus not only the rate but also the nature of mutation may itself be shaped by natural selection.

## CASE STUDIES

## Nachman and Crowell

Nachman and Crowell (2000) studied the rate of mutation in humans using the comparative approach. We are unusually confident of both the time since we diverged from chimpanzee lineage, and of the number of generations that this represents. Nachman and Crowell took advantage of this by studying divergence of DNA sequences that are among the most likely to be neutral and thus evolve at the mutation rate: processed pseudogenes.

A processed pseudogene is a bit of DNA that has been reverse transcribed from a messenger RNA back into DNA and incorporated into the genome. Differences of a pseudogene from the homologous gene, such as frameshift deletions or insertions, make it clear that the pseudogene cannot encode a protein, hence the name. Processed pseudogenes can be recognized because they have had their introns edited out, and often have a poly-A sequence attached. Therefore, pseudogenes are expected to have no function, and consequently to evolve at the neutral rate, although this may not always be so (see above).

A potential complication with the use of pseudogenes is that there may be many pseudogenes derived at different times from the ancestral gene. If an older pseudogene in one species were to be compared with a younger one in the other species then a very misleading picture of the rate of divergence would be obtained. Nachman and Crowell were able to guard against this possibility by a careful choice of methods. They used polymerase chain reaction (PCR) amplification to obtain material directly from genomic DNA. For each pseudogene, they chose one of their PCR primers to lie in the genomic DNA outside the pseudogene itself. Thus, only sequences that possessed the same flanking sequence in both humans and chimpanzees would amplify. This ensures that each pair of compared human and chimpanzee pseudogenes was orthologous, that is, it originated from the pseudogene already present in the last common ancestor.

In total Nachman and Crowell sequenced 18 different pseudogenes in a chimpanzee and two humans. In each individual, 16 kb was sequenced. Overall, they found 199 differences between the human and chimpanzee sequences, for a divergence of 1.2%. These differences consisted of 131 transitions, 52 transversions, and 16 insertion-deletion

variants. The insertion-deletion variants were all of 4 nucleotides or shorter. These data are biased against detection of large insertions and deletions as this would tend to preclude recognition of a pseudogene in the first place. CpG contexts accounted for about 25% of all the substitutions, with such sites having a 10-fold higher rate of substitution than non-CpG contexts. The estimated rate of substitution and mutation differed by a factor of 6 among different pseudogenes. These differences were statistically significant, suggesting that the region in which the pseudogenes inserted influenced their mutation rates.

The simple neutral divergence model outlined above assumes that a single copy of DNA is split into two lineages at the time of species divergence. In reality the ancestral species was very likely to already have DNA sequence variation at the time of speciation. This means that some of the differences fixed in each lineage after speciation are actually variants that arose before the time of speciation. To compensate for this an assumption about the effective size,  $N$ , of the ancestral species must also be made. When the number of generations since divergence is not much greater than  $N$ , which is likely to be the case for humans and chimps, the effect of this adjustment can be substantial.

Thus, there are three unknown factors that still must be taken into account to convert the 1.2% divergence into an estimate of the mutation rate per generation:  $N$  before speciation, the time since divergence, and the average generation time since divergence. Nachman and Crowell considered  $N$  values up to  $10^5$ , divergence times between 4.5 and 6 million years ago, and generation times of 20 and 25 years. This gives an estimate of the number of generations separating chimps and humans of between 360,000 and 600,000. The range of possibilities suggests mutation rates per base per generation pair between  $1.3$  and  $3.4 \times 10^{-8}$ . This agrees very well with direct estimates of human mutation rates (Kondrashov 2002), and more recent analyses of a much larger human-chimp data set.

Mutation Accumulation in *Drosophila*

Studying mutations with small effects on the phenotype and fitness is both important for understanding evolution, and difficult experimentally. Much of the data on such mutations come from

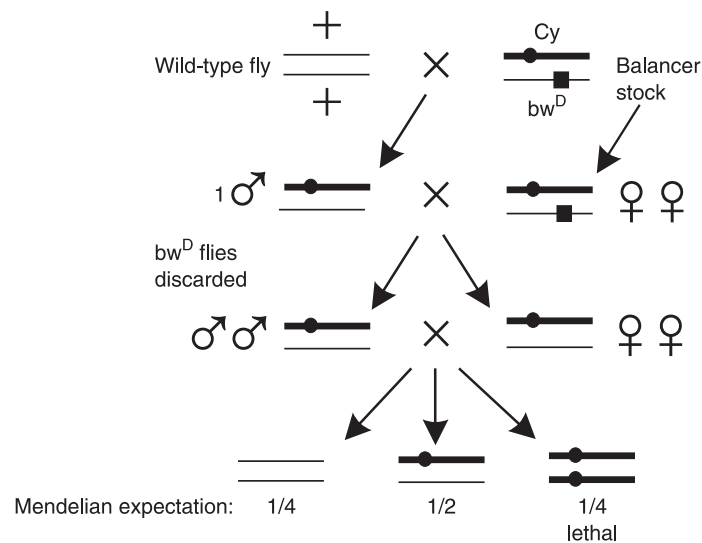
mutation-accumulation experiments on *Drosophila melanogaster*, due to its 2 week generation time, readily observed morphology and life history, and the genetic tools available.

The most important such tools are balancer chromosomes, so-called because they allow the preservation of a sampled chromosome from the disruptive effects of recombination. Balancer chromosomes have three key properties: a multiply inverted gene order, a dominant morphological marker, and a recessive lethal mutation. When an individual is heterozygous for chromosomes with very different gene orders, chromosomes can pair at meiosis, but recombination between them results in duplications and deficiencies in the products, and inviable gametes. Therefore, a fly heterozygous for a balancer chromosome will only give rise to gametes that carry the unrecombined balancer or wild-type chromosomes. As shown in Figure 3.3, this fact can be exploited to capture (or “extract”) and replicate single chromosomes from any population, allowing their properties to be studied.

In the mutation-accumulation experiments we want to discuss (Mukai et al. 1972; Houle et al.

1994) a second chromosome balancer was used to extract and replicate a single test chromosome, then to preserve independent copies in heterozygous condition (Figure 3.3). The second chromosome in *D. melanogaster* contains about 40% of the genome. These copies start out genetically identical, but diverge over time as each copy independently accumulates spontaneous mutations. If the heterozygous fitness effects of mutations are small, then they will accumulate at very close to the mutation rate. To detect the effects of mutation, inversion heterozygotes are crossed, and the ratio of test chromosome homozygotes to inversion heterozygotes observed in the offspring, as shown in the last two rows of Figure 3.3.

Mukai utilized this basic design several times to study the effects of mutation on egg-to-adult viability, the probability that an egg survives to become an adult. In his 1972 paper, three test chromosomes were each replicated 50 times to make sublines. Each subline was then subjected to the accumulation process. Every 10 generations, the relative homozygous viability of each subline was measured. From this, Mukai estimated the rate at which viability went



**FIGURE 3.3.** The use of balancer chromosomes to extract intact chromosomes and to serve as standards for the measurement of viability. The thick line denotes a multiply inverted chromosome (the balancer), while thin lines are chromosomes with the usual gene order. The circle denotes the dominant mutation Curly (*Cy*), which causes the adults to have wings that curl upwards. The square denotes the mutation brown-dominant (*bw<sup>D</sup>*), which causes its carriers to have brown rather than red eyes.

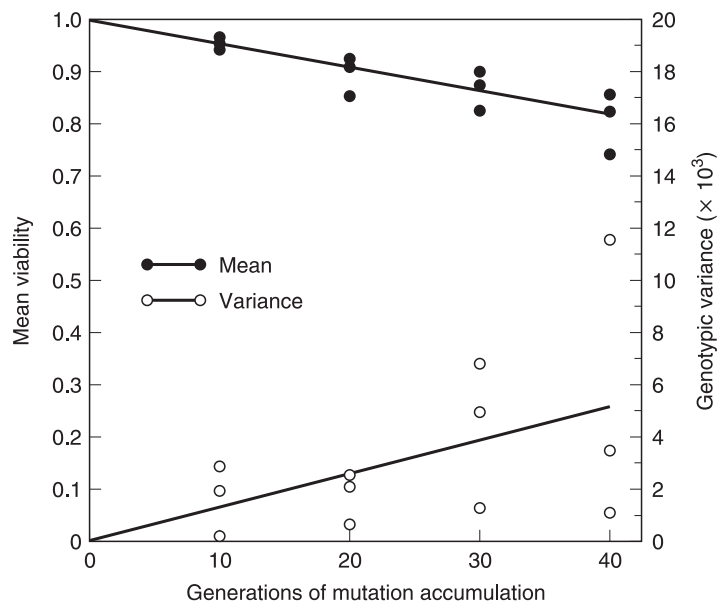
down over generations ( $M$ ), and the rate at which the variation among sublines went up ( $V_M$ ).

The distribution of homozygous viabilities showed a minority of chromosomes acquired lethal recessive mutations, while most chromosomes showed modest declines in fitness, with very few chromosomes having reductions of viability between 50% and 100%. The rate at which chromosomes acquired lethal mutations was 0.006 per chromosome per generation. Even with the lethals excluded,  $M$  was negative, as shown in Figure 3.4, as expected due to a preponderance of deleterious mutations, while  $V_M$  was positive as expected since each line accumulates its own unique set of individually rare mutations.

These two quantities,  $M$  and  $V_M$ , together give some indication of the mutation rate and the effects of the nonlethal mutations. To see this, imagine two cases with the same decline in the mean: if the variance among sublines had not increased at all, this would indicate the presence of a very large number of mutations that each had very small effects. Conversely, if the variation among sublines was high, this would indicate that a few mutations with

large effects must have occurred. If all mutations had exactly the same effect on homozygous viability this relationship would be mathematically precise:  $M = Us$ , where  $U$  is the total mutation rate on the chromosome to alleles affecting viability by amount  $s$ , and  $V_M > Us$ . In reality,  $s$  does vary from variant to variant, and this increases  $V_M$ , so Mukai et al. could set a lower limit to the mutation rate  $U < M^2/V_M$  as an upper limit on the average  $s$ ,  $s < V_M/M$ . For this experiment  $U > 0.06$  when all the nonlethal chromosomes were considered, or even  $> 0.17$  when the few chromosomes with homozygous viabilities near 0 were excluded. Thus the mutation rate to variants with small homozygous effects on viability is at least 10 times greater than the recessive lethal mutation rate. When extrapolated to the whole genome, this suggests a deleterious mutation rate greater than 0.4 per genome per generation.

The high genomic deleterious mutation rate estimates of Mukai et al. (1972) have proved to be quite controversial. Many subsequent studies have undertaken similar estimates in *Drosophila* and other organisms; some broadly support Mukai's results, while others do not (Lynch et al. 1999).



**FIGURE 3.4.** Changes in the mean viability (left scale, filled circles) and genetic variance (right scale, open circles) in viability over 40 generations of mutation accumulation in *Drosophila melanogaster*. Mukai et al. (1972), reprinted with permission of the Genetics Society of America.

More troubling is that estimates of the genomic mutation rate in *Drosophila*, such as the direct estimate of 0.8 shown in Figure 3.1, are not much larger than this estimate. If the direct estimate is correct, then half of all mutations in *Drosophila* must be deleterious, ~~an implausibly large proportion~~. The direct estimate is itself subject to uncertainty but where the discrepancy lies is not certain.

Two other potential gaps in the Mukai et al. study are less well known. Mukai et al. assumed that balancer heterozygotes provide a standard for viability to compare with that of the heterozygotes. This is almost certainly incorrect due to partially dominant or epistatic effects of the mutations themselves. Second, early development in *Drosophila* is primarily driven by maternal message, so that the homozygous effects of maternal-acting genes are not assayed with their technique.

Houle et al. (1994) used the same technique as Mukai et al. to accumulate mutations, but focused instead on the pleiotropic effects of mutation on life history traits. This work was undertaken largely to test the mutation-accumulation theory for senescence, one of the major theories concerning the evolution of lifespan (Promislow & Bronikowski, Ch. 30 of this volume). The mutation-accumulation theory requires that many mutations occur that decrease fitness of old individuals, while leaving early-life fitness unimpaired. Natural selection is less effective at removing mutations that act late in the lifespan than those that act earlier, potentially causing a decrease in lifespan. To test for such mutations, Houle et al. studied female fecundity in young flies (5 and 6 days old) and old flies (27 and 28 days old), as well as average male and female lifespan. If the mutation-accumulation hypothesis is correct, there should be variation in late fecundity that does not affect early-life fecundity.

After 44 generations of mutation accumulation, there was significant genetic variation due to mutation for all four traits. The mutational correlation of early and late fecundity was not different from a perfect correlation of 1, but was significantly higher than 0, suggesting that mutations tended to affect early- and late-life fitness components similarly. Consistent with this, the correlations of both fecundities with lifespan were also positive and not different from 1. Mutations that affect one aspect of fitness also seem to impair all aspects of fitness — a fly with poor fecundity early in life will also live less long and have lower fecundity late in life.

This substantially undermines the mutation-accumulation hypothesis for the evolution of lifespan.

## FUTURE DIRECTIONS

Knowledge of the rates and properties of mutation is critical to understanding evolution. The study of mutation is difficult, and so has been relatively neglected by evolutionary biologists. The result is that our generalizations about mutation rest on somewhat shaky ground. Consequently, we expect that the study of mutation will be among the most productive areas of evolutionary genetics in the future. Here are a few areas where we hope to see great progress over the next 10 years.

1. Improvements in sequencing technology have now made it possible to directly assay mutations in DNA sequences after a mutation-accumulation experiment. Such studies should increase rapidly in number. These data should remove much of our uncertainty about the molecular aspects of mutation, as it will no longer be necessary to find phenotypic evidence for a mutation before molecular analysis.
2. Availability of complete genome sequences for closely related species pairs will tell us what proportion of genomes are subject to natural selection. Combined with direct data on mutation rates, this will reveal what proportion of mutations affect fitness.
3. Automated phenotyping is being pursued in several systems, making it possible to rapidly screen for mutants or increases in variation in many traits simultaneously. This will be necessary to understand the correlation structure of mutational effects.
4. After many years of relative eclipse, the study of advantageous mutations should take its rightful place as the most important aspect of evolutionary biology. New theory and large amounts of molecular data should make it much easier to detect the signature of positive selection, for example in bacterial cultures, or from population survey data.
5. One of the most neglected issues in evolutionary genetics is what limits evolutionary progress (Hansen & Houle 2004), with one of the major possibilities being limits on the phenotypic effects of mutation. Progress in



the previous three areas may make it possible to test this idea.

#### SUGGESTIONS FOR FURTHER READING

We know of very few general works on the role of mutation in evolution, that is those that cover the range of issues raised in this chapter. Molecular mechanisms of mutation are introduced in Lewin's series of *Genes* books (e.g., 2004). Graur and Li (2000) provide an overview of molecular evolution with a reasonable emphasis on mutation as a driving force. For information on molecular mutation rates the review of Drake et al. (1998) and the recent paper by Denver et al. (2004) introduce the important literature. For phenotypic mutation rates Drake et al. (1998) and Lynch et al. (1999) provide reviews. For the evolution of mutation rates generally, Kondrashov (1995) gives an overview of hypotheses. Foster (2000) and Sniegowski et al. (2000) provide a quick summary of the evidence for and against the adaptive values of environmental responsiveness of mutation rate.

- Denver DR, Morris K, Lynch M & WK Thomas 2004 High mutation rate and predominance of insertions in the *Caenorhabditis elegans* nuclear genome. *Nature* 430:679–682.
- Drake JW, Charlesworth B, Charlesworth D & JF Crow 1998 Rates of spontaneous mutation. *Genetics* 148:1667–1686.
- Foster PL 2000 Adaptive mutation: implications for evolution. *BioEssays* 22:1067–1074.
- Graur D & W-H Li 2000 *Fundamentals of Molecular Evolution*. Sinauer Assoc.
- Kondrashov AS 1995 Modifiers of mutation–selection balance: general approach and the evolution of mutation rates. *Genet. Res.* 66:53–69.
- Lewin B 2004 *Genes VIII*. Oxford Univ. Press.
- Lynch M, Blanchard J, Houle D, Kibota T, Schultz S, Vassilieva L & J Willis 1999 Perspective: Spontaneous deleterious mutation. *Evolution* 53:645–663.
- Sniegowski PD, Gerrish PJ, Johnson T & A Shaver 2000 The evolution of mutation rates: separating causes from consequences. *BioEssays* 22:1057–1066.