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Short Review

Estimation of spontaneous genome-wide mutation rate parameters: whither beneficial mutations?

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Empirical estimates of genome-wide mutation rates and of the distribution of mutational effects are needed to illuminate various topics ranging from evolutionary biology to conservation. Methods for inferring genome-wide mutation parameters are presented, and results stemming from these studies are reviewed. It is argued that, although most if not all mutations detected in mutation accumulation experiments are deleterious, the question of the rate of favourable mutations (and their effects) is still a matter for debate.

Keywords: adaptation, compensatory mutations, deleterious mutations, fitness, genetic load, mutational meltdown.

Mutations, why do we care?

Mutation is the ultimate source of heritable variation. As such it conditions the response to selection and adaptation through natural selection. Most researchers agree that mutations with phenotypic effects are usually deleterious. Indeed, when considering a population that has evolved for a long time in a constant environment, one can postulate that the population is composed of genotypes finely tuned with respect to a myriad of biotic and abiotic conditions and that a random mutation will probably disrupt such fine tuning (Fisher, 1999, pp. 38–42). This has been empirically shown for bacteria evolving in a constant glucose-limited environment for about 10 000 generations (Elena *et al.*, 1996). Although the result depend largely on the ecology and history of the population considered, this assertion is used as a working hypothesis in numerous evolutionary genetics models. These models address different issues that include: (1) the evolution of genetic systems such as sex, recombination, selfing rates and ploidy levels (Otto & Marks, 1996; Barton & Charlesworth, 1998; Charlesworth & Charlesworth, 1998); (2) the maintenance of genetic variability at both the phenotypic (Barton & Turelli, 1989) and DNA levels (Charlesworth *et al.*, 1993); (3) the fate of small natural or managed populations (Kondrashov, 1995; Lynch *et al.*, 1995; Lande, 1998; Schoen *et al.*, 1998); (4) sexual selection (Burt, 1995); and (5) evolutionary explanations for ageing (Partridge & Barton, 1993).

The number of these models has grown exponentially in the last decade but there have been relatively few studies providing empirical estimates for the rates and effects of spontaneous mutations affecting traits related to fitness. Recent reviews have focused either broadly on spontaneous mutations at both the molecular and genome-wide level (Drake *et al.*, 1998) or exclusively on deleterious mutations (Kondrashov, 1998; Keightley & Eyre-Walker, 1999; Lynch *et al.*, 1999). The aim of this review is to (1) present the methods currently available for inferring genome-wide mutation parameters; (2) assess our current ability to detect beneficial mutations; and (3) to propose some alternative experimental designs that will allow us to quantify the flux and distribution of beneficial mutational effects.

I define U as the sum of the haploid mutation rates across the (unknown) set of loci affecting either fitness or a fitness component. Mutational effects have then to be defined by assuming a relationship between the number of mutations carried by an individual and its genotypic value. Most studies assume that mutations act in a multiplicative or additive fashion (which are equivalent when mutations are assumed to have sufficiently small effects). The mutation effect attributed to a mutation in the homozygous (heterozygous) state is denoted by s (hs) and represents the shift in the expected genotypic value of the individual carrying the mutation relative to a value of 1 for wild type. Estimating U, h and especially s is the primary goal of empirical studies. I will concentrate on two approaches for estimating mutational parameters. One, the so-called mutation accumulation (MA) approach, uses controlled designs where mutations are allowed to accumulate *de novo* in a quasi neutral fashion while the other is based on the comparative analysis of molecular data (hereafter called the DNA-based method). Other methods based on characterizing levels of inbreeding depression in natural populations have been carried out in several plant species and *Daphnia* (Charlesworth *et al.*, 1990, 1994; Johnston & Schoen, 1995; Deng & Lynch, 1996, 1997). However, these methods provide only indirect estimates of mutational parameters and make two limiting assumptions. First, the size of the population sampled will impose a threshold selection coefficient below which mutation will not be detected (Bataillon & Kirkpatrick, 2000). Second, inbreeding depression must be solely due to recessive deleterious alleles produced by mutation, and not by maladapted migrant alleles or by overdominant alleles.

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Mutation accumulation experiments

The MA approach lets mutations randomly accumulate under benign conditions in a series of sublines derived from an inbred base population (ideally a single completely homozygous individual). The sublines are maintained by close inbreeding, ideally by selfing which ensures an effective population size (N_e) of 1, or by brother–sister mating. In such a design, drift will dominate selection within each subline and all but very detrimental mutations will be fixed at random (Keightley & Caballero, 1997). After several generations of mutation accumulation, the genotypic value of ancestral lines is compared with that of derived lines (Fig. 1). Based on the observed distribution of line values, inferences can be made on the amount of heritable variation produced by mutation and on the type of mutations causing it.

The traditional method for analysing MA experiments is the Bateman-Mukai technique (hereafter BM), which is based on regressing mean genetic value and observed between-line variance on generation number (Bateman, 1959; Mukai *et al.*, 1972). By assuming that mutations arise at a rate U per haploid genome, are fixed neutrally within each subline, and that each new mutation acts additively and shifts the genotypic value of a line by a fixed increment s, estimators of U and s are obtained as simple functions of the change in mean genetic value per generation ΔM and the mutational variance V_m (Fig. 1). If mutation effects are variable, then the BM method is biased by a factor equal to 1 plus the coefficient of variation of the distribution of effects (e.g. 2 for an exponential distribution). BM estimators are undefined if no shift in mean genetic value of MA lines is observed, despite between-line divergence.

Alternatively methods based either on minimum distance (Garcia-Dorado, 1997) or maximum likelihood (ML)

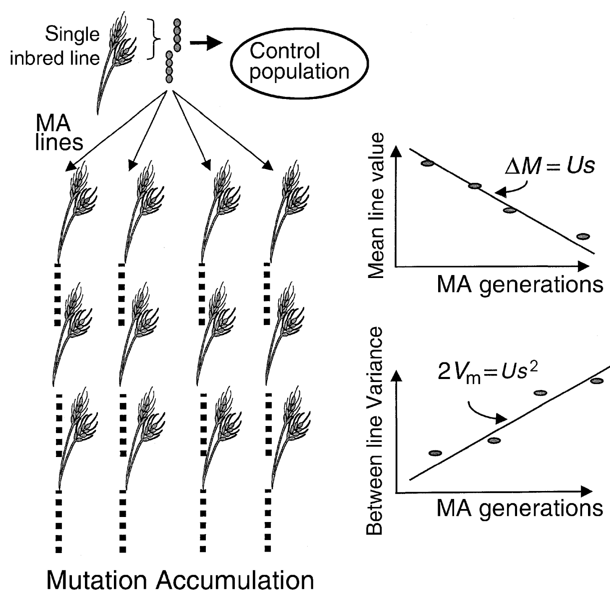

Fig. 1 Estimation of genome-wide mutation parameters using MA experiments.

(Keightley, 1994, 1998) have been developed which seek to extract more information from the distribution of MA line values. These methods can be either based on the same assumptions as BM (in which case they are biased if mutation effects are variable) or assume a parametric distribution for mutational effects, $\phi(s)$ (e.g. an exponential or Gamma distribution). In the latter case, estimates are provided for both U and the parameters describing ϕ. Simulation work and reanalysis of two recent MA experiment using *Caenorhabditis elegans* shows that, even when analysing data under the assumption of a constant effect of mutations, ML estimators of U and s yield estimates with lower sampling variances than traditional BM estimators (Keightley & Bataillon, 2000).

At any rate, all methods of analysis require a substantial level of divergence between lines and/or a high level of replication in order to estimate accurately line values for the trait of interest. The statistical power of a MA experiment can be summarized by the heritability achieved at the line level,

$$h_L^2 = V_L/(V_L + V_r),$$

where V_r represents the error variance (across the replicates used to estimate MA line value) and V_L the between-line variance. A value of 1/2 is the minimum for reliable and independent estimation of U and s.

Historically, MA experiments have been carried out on *Drosophila melanogaster*, mostly by Mukai and collaborators (reviewed in Simmons & Crow, 1977; see Keightley & Eyre-Walker, 1999 for a recent historical account). MA experiments used a marked chromosomal inversion and exploited the lack of male recombination in *D. melanogaster* to keep the entire chromosome II free of recombination. Control populations consisted of large outbred populations. The MA lines were assayed by monitoring viability. MA experiments have recently been performed on several different species (Table 1).

In all the studies, the mean fitness or mean of fitness related traits declined over time, suggesting that the net effect of spontaneous mutation is indeed deleterious (an exception is Shaw *et al.*, 1999). Mean decline of the fitness components of MA lines ranged from 0.1% to 1–2% per generation. Although the reliability of control populations used to assess erosion of components of fitness has been questioned (especially for Mukai's experiments: Keightley, 1996); fitness erosion seems to be the rule over a broad range of organisms.

Among the factors contributing to observed variation in U and s estimates are: (1) the quality of the control population used; (2) the activity of transposable elements; and (3) naturally varying levels of mutation rates. Control populations may consist of: (1) large populations that are supposed not to evolve significantly during the course of the experiment (but where a small amount of adaptive evolution can nevertheless occur), or (2) frozen controls (seeds, worms or bacteria) where any evolution is halted. We may expect that a greater number of genes in the organism studied or a longer generation time will cause greater U. But despite similar gene number, *D. melanogaster* and *C. elegans* U estimates differ at least by a factor of 10 (Keightley & Bataillon, 2000). A convincing correlation was found between V_m and generation time (Lynch *et al.*, 1999),

Table 1 Genome-wide haploid mutation rates (U_{min}) and the mean homozygous effect of mutation (s_{max}) estimates from MA experiments

Organism	Trait studied	U_{min}	s_{max}	Favourable mutations?	Reference
E. coli	*r*	0.0002	0.01	Not tested	Kibota & Lynch (1996)
C. elegans	*r*	0.0035	0.10	No	Keightley & Caballero, 1997
C. elegans	*r*	0.008	0.20	Not tested	Vassilieva & Lynch (1999)
Arabidopsis thaliana	LRS	0.05	0.23	Not tested	Schultz *et al.* (1999)
A. thaliana	LRS	0.1	?	Probably	Shaw *et al.*, 1999
Triticum durum	LRS	0.04	0.2	Not tested	Bataillon *et al.* unpublished
D. melanogaster	Egg-to-adult viability*	0.35	0.03	Not tested	Mukai (1963)
	Egg-to-adult viability*	0.45	0.03	No	Mukai *et al.*, 1972
	Egg-to-adult viability*	0.14	0.03	Yes	Ohnishi (1977)
	Egg-to-adult viability†	0.02	0.16	?	Garcia Dorado *et al.* (1999)
	Egg-to-adult viability*	0.052	0.11	Not tested	Fry *et al.* (1999)

LRS, life-time reproductive success; *r*, Intrinsic rate of growth.
*Competitive viability (based on the accumulation of mutations on chromsome II and adjusted by a factor 2.5 for extension to the whole genome).
†Non-competitive viability (MA performed using full sib mating).

but it is hard to know whether it is caused by differences in U or different distributions of mutational effects.

A fundamental problem with such phenotypic methods is that, even in instances where the major changes in the distribution of line values were caused by a few mutations with large effect, the presence of a large class of mildly deleterious mutations can never be ruled out. A mutagenesis experiment on the N2 strain of *C. elegans* (Davies *et al.*, 1999) is particularly revealing. The number of mutations induced by ethyl methyl sulphonate (EMS) at the genomic level could be estimated directly from rates of mutations scored at a known set of genes. The authors estimated that about 50 new amino acid altering mutations had been induced (80% of which are predicted to be deleterious). In parallel, the EMS lines were assayed for productivity and a ML estimator of the induced U was used. However, the ML estimator based on productivity data gave $U = 1$! When reanalysing the phenotypic data by assuming a U equal to 45 mutations/line, the best fitting distribution for $\phi(s)$ was bimodal with the vast majority of induced mutations (43.4 out of 45) having a very small effect ($s = 0.0007$) on productivity. Even competition experiments in models such as *Escherichia coli* will fail to detect fitness differences between MA lines, that are below 0.001. Potentially, one would like to detect mutations with effects as small as $1/N_e$, where N_e for the species under consideration can be as large as 10^6.

The DNA-based method

This method (Kondrashov & Crow, 1993) is based on the neutral theory of molecular evolution (Kimura, 1983, pp. 43–46 and chapter 5) and the assumption that mutations are either neutral or deleterious. In neutral regions of the genome, mutations are substituted at the rate of mutation μ_t, while in selectively constrained regions substitutions occur at a rate $f\mu_t$, where f represents the proportion of mutations that are selectively neutral (the fraction $1 - f$ that are deleterious will not be substituted). The method uses sequence data from a pair of species with known levels of divergence and generation times (Fig. 2). A random sample of orthologous genes is used to estimate K_c the average sequence divergence in selectively constrained regions of the genome. The divergence for this set of functional sequences is then compared with the level of divergence K_n for non-functional (presumably neutral) sequences such as pseudogenes. This allows the fraction $1 - f$ of mutations that are deleterious to be calculated. By extrapolating to the whole genome, one can then derive an estimate of U (Fig. 2). Eyre-Walker & Keightley (1999) recently applied a modified version of this technique to a sample of 46 human–chimp orthologous proteins and used synonymous substitutions in the sequences for estimating K_n and inferring the total mutation rate. They found that $U = 0.8$. Their estimate did not include mutations arising in non-coding sequences.

There are several caveats with respect to this method. First, the method requires an independent estimate of the total mutation rate or must rely upon indirect estimation of the total mutation rate through levels of divergence at 'neutral' sequences K_n. Second, it ignores the existence of favourable mutations which will bias downward the estimation of U by inflating K_c. More importantly, this method yields no information about the effects of deleterious mutations other than the fact that their effects are greater than the reciprocal effective population size, which may be very large.

On detecting favourable mutations…

Recent experiments involving retroviruses show that despite their elevated genomic mutation rates (Drake & Holland, 1999), adaptive evolution can occur even in small populations by means of beneficial or compensatory mutations (Burch & Chao, 1999). Such mutations may have crucial consequences

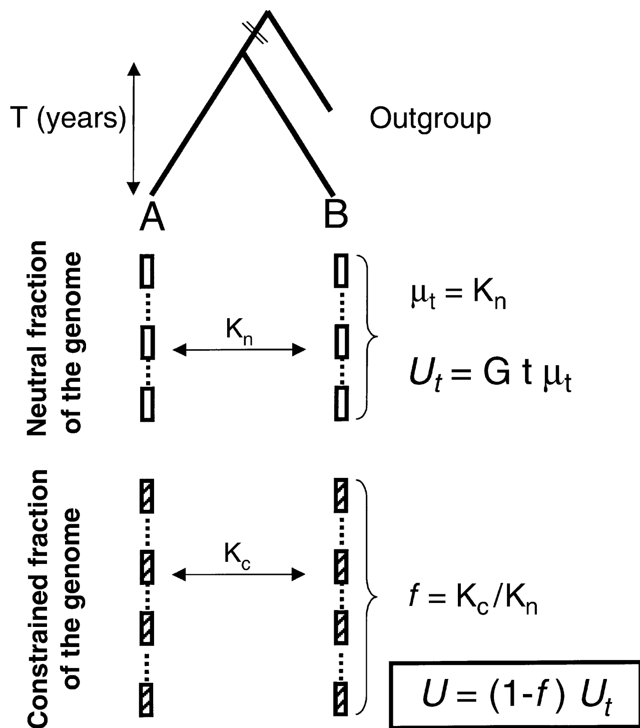

Fig. 2 The method uses a pair of recently derived species (A,B) with known divergence times (T). For the species of interest (say B), a direct estimate of the total mutation rate U_t is either known or is inferred from levels of substitution (K_n) at 'neutral' sequences (represented as empty boxes), the generation time (t) and the total genome size (G). A set of orthologous gene sequences, representing the constrained fraction of the genome (hatched boxes), is used to estimate the rate of substitution for selectively non-neutral regions (K_c) and the fraction f of neutral mutations throughout the genome. An estimate of U is then obtained as $U = (1 - f)U_t$.

for models seeking to predict the persistence of small populations (Whitlock & Otto, 1999). Yet most experiments looking at multicellular organisms have so far failed to produce any information on such mutations.

This has been largely overlooked in MA experiments. The BM technique ignores beneficial mutations but minimum distance or ML techniques are versatile enough to incorporate a non-null probability of favourable mutations. Studies that have looked for favourable mutations include the reanalysis of three *Drosophila* experiments (Garcia-Dorado, 1997) and one *C. elegans* experiment (Keightley & Caballero, 1997). Of these, one MA experiment fitted a model where 10% of mutations were beneficial (Garcia-Dorado, 1997). The question remains: if 10% of mutations are favourable has the MA method any power to detect them? Although the properties of ML estimators have been explored in detail (Keightley, 1998; Keightley & Bataillon, 2000), the situation where beneficial mutations may be fixed in MA lines has never been studied.

Simulations of MA experiments were performed where a small proportion of beneficial mutations (0%, 1% or 10% of mutations) are fixed in the MA lines, which were then analysed using the constant effect of mutation model or its ML version (see Keightley & Bataillon, 2000, for details of the simulation protocol). First results indicated that line value distributions, and BM or ML estimators traditionally used, are barely affected by the presence of beneficial mutations. Although the analysis is very crude, it indicates that the power of MA, designs to detect such mutations is probably quite low. A full analysis of the behaviour of ML estimators incorporating mutations of variable effects (both positive and negative), although computationally cumbersome, would be interesting in that regard.

An alternative design, potentially useful for the detection of beneficial mutations, would be to practise directional selection in an initially homogenous population. Such designs have traditionally been used as a way to estimate mutational variance for quantitative traits (Hill & Caballero, 1992). Here selection should be practised on fitness-related traits as those typically eroded in MA experiments. Replicating such selection experiments with widely varying population sizes, would provide some information on the distribution of favourable mutations. In such a design, population size should sieve beneficial/deleterious mutations as a function of their selective effect. Monitoring the evolution of the distribution of fitness should provide some information on the relative rates and sizes of effects of beneficial vs. deleterious mutations.

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