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SHORT PAPER

Estimation of microsatellite mutation rates in *Drosophila melanogaster*

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Summary

Microsatellite mutations were studied in a set of 175 mutation accumulation lines, all of them independently derived from a completely homozygous population of *Drosophila melanogaster* and maintained under strong inbreeding during 80 generations. We assayed 28 microsatellites and detected two mutations. One mutation consisted of a single addition of a dinucleotide repeat and the other was a deletion of five trinucleotide repeats. The average mutation rate was $5{\cdot}1\times10^{-6}$, in full agreement with previous estimates from two different sets of mutation accumulation lines.

1. Introduction

Microsatellite loci are widely used in evolutionary and ecological studies of natural populations. The population statistics of microsatellites depend on their rates and modes of mutation. Estimates of microsatellite mutation rates from mammals (Dallas, 1992; Weber & Wong, 1993; Ellegren, 1995) range between 10^{-2} and 10^{-5}. In *Drosophila melanogaster*, the reported rates are around 10^{-6}, at least an order of magnitude lower (Schug *et al.*, 1997, 1998*b*; Schlötterer *et al.*, 1998). Models of microsatellite mutation usually assume slippage during DNA replication that produces mostly gains or losses of single repeat units. Evidence based on population distribution of alleles and pedigree analysis shows that most mutations are compatible with this simplest model, but deviations from it also occur due to multirepeat mutations, directional bias and heterogeneity of mutations between loci (Amos & Rubinstzein, 1996; Primmer *et al.*, 1996; Schlötterer *et al.*, 1998; Di Rienzo *et al.*, 1998; Xu *et al.*, 2000). In this paper, we report an analysis of the rate and type of mutation for 28 microsatellites in a set of 175 mutation accumulation lines of *Drosophila melanogaster*.

2. Materials and methods

(i) *Fly lines*

The lines were derived from a population made isogenic for the four chromosomes following a scheme of crosses to balancer chromosomes, and carried the marker *sepia* (*se*) as an indicator of contamination (Caballero *et al.*, 1991). Starting from this isogenic population, lines were established and maintained under strong inbreeding as described previously (Santiago *et al.*, 1992). A total of 175 lines inbred for 80 generations were screened for microsatellite mutations at 28 loci.

(ii) *Microsatellite analysis*

Genomic DNA that was extracted from 50 to 100 flies per line at generation 80 (Domínguez & Albornoz, 1996) was used for amplification. Each 20 μl PCR reaction contained 20–50 ng of genomic DNA, 100 μM of each dNTP, 0·5 μM of each primer, 2·5 mM $MgCl_2$, 1 × PCR Gold Buffer and 0·5 U of *Taq* polymerase (AmpliTaq Gold, PE Biosystems). Amplifications included an initial denaturing step of 12 min at 95 °C followed by 30 cycles of 1 min at 95 °C, 1 min at the annealing temperature and 1 min at 72 °C. Final extension was at 72 °C for 5 min. PCR products were electrophoresed in 6% denaturing polyacrylamide gels and visualized by silver staining (Promega).

We screened 28 microsatellite loci chosen to include perfect and imperfect, di- and trinucleotide repeats,

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Table 1. *Microsatellite loci assayed*

Locus	Repeat motif in the reference	No. of repeats in the lines	Reference
DRONANOS	$(TA)_{18}$	17	a
DMU1951	$(TA)_{16}$	18	a
DROYANETSB	$(TG)_{19}$	21	a
DROABDB	$(CA)_{19}$	19	b
DM73	$(AC)_{21}$	39	c
DM97	$(AC)_{30}$	7	c
DS06335a	$(AC)_{7}$ to $(AC)_{22}$	21	d
DS00361	$(AC)_{5}$ to $(AC)_{19}$	10	d
DS08687b	$(AC)_{8}$ to $(AC)_{15}$	15	d
DMAC1	$(AC)_{12}$	12	e
DMAC2	$(AC)_{12}$	13	e
DMAC3	$(AC)_{9}$	11	e
DMAC7	$(AC)_{10}$	12	e
DMAC9	$(AC)_{13}$	13	e
DMAC4	$(AC)_{9+10}$	22	e
G410	$(CT)_{11}(GT)_{4}$	7	f
DMANTPE1	$(CCG)_{5}$	5	b
DRODSOR1	$(ATA)_{6}$	7	b
DMZ60MEX	$(AAG)_{8}$	9	a
DROTKABL3	$(ACA)_{5}$	6	b
DMCATHPO	$(ACC)_{6}$	4	b
DROFASI	$(AGG)_{5}$	6	b
DMSGG3	$(CAG)_{11}$	9	a
GREG-5	$(CAG/CAA)_{30}(CAG)_{5}$	30	g
GREG-8	$(CAG/CAA)_{37}(CAG)_{9}$	30	g
GREG-10	$(CAG/CAA)_{18}(CAA)_{10}$	17	g
DMMASTER	$(CAG)_{8}(CAA)_{2}(CAG)_{5}$	16	a
DROMYALK	$(CAA)_{5}CAC(CAA)_{2}$	11	a

a, Goldstein & Clark (1995); b, Schug *et al.* (1997); c, Schug *et al.* (1998*a*); d, Schlötterer *et al.* (1997); e, England *et al.* (1996); f, Harr *et al.* (1998); g, Michalakis & Veuille (1996).

distributed along the three major chromosomes. Sequencing reactions of pUC18 were used as standard markers to determine approximate allele sizes. Repeat numbers were inferred from allele sizes given in the original publication and assuming that variation is due to changes in the microsatellite stretch only (Table 1). To analyse the new mutations fixed in the lines, the sequences of the alleles ancestral and mutant were compared. Sequences of both alleles of each variant locus were determined by cloning PCR products in pUC18 (SureClone Ligation Kit, Amersham Pharmacia Biotech) and cycle sequencing (Silver Sequence, Promega).

3. Results

Only two spontaneous mutations were detected among the 175 lines screened for 28 microsatellite loci. The lines have been maintained independently for 80 generations, hence the total number of allele generations was 392000 and the mean microsatellite mutation rate is $5\cdot1\times10^{-6}$. The upper and lower 95% confidence limits were calculated assuming a Poisson distribution and solving for μ such that the probabilities of Y larger than 2 and Y lower than 2 equal 0·975, respectively. The confidence limits, obtained as the corresponding value of μ divided by the number of allele generations, are $6\cdot1\times10^{-7}$–$1\cdot8\times10^{-5}$.

The two mutations occurred in lines B36 and B64 at microsatellite loci *DROYANETSB* (pure dinucleotide repeat) and *DMSGG3* (pure trinucleotide repeat), respectively. The mean mutation rate for pure dinucleotide repeats is $1/(175\times80\times14) = 5\cdot1\times10^{-6}$; 95% confidence interval $1\cdot3\times10^{-7}$–$2\cdot8\times10^{-5}$. The mean mutation rate for pure trinucleotide repeats is $1/(175\times80\times7) = 1\cdot02\times10^{-5}$; 95% confidence interval $2\cdot5\times10^{-7}$–$5\cdot7\times10^{-5}$). Sequence analysis showed that the changes affected the number of repeats only. The mutation in *DROYANETSB* increased the number of repeats by one, from $(TG)_{21}$ to $(TG)_{22}$. The mutation in *DMSGG3* reduced the number of repeats by five, from $(CAG)_{9}$ in the starting allele to $(CAG)_{4}$ in the mutant one.

4. Discussion

The mean mutation rate in this study is $5\cdot1\times10^{-6}$, a value remarkably close to $6\cdot3\times10^{-6}$, the rates published by Schug *et al.* (1997) and Schlötterer *et al.*

(1998), and slightly lower than the value of $9{\cdot}3\times10^{-6}$ obtained for dinucleotide repeats only (Schug *et al.*, 1998*b*). It is interesting to note the agreement between the different estimates, despite the fact that the average in the study of Schlötterer *et al.* (1998) is very affected by only one highly variable allele. Taking together the four published studies on direct estimates of microsatellite mutation rates, a total of 97 microsatellite loci have been assayed in three different sets of lines. Consequently the reported values, substantially lower than the estimates in mammals (Dallas, 1992; Weber & Wong, 1993; Ellegren, 1995), must be representative of *D. melanogaster*. It has been proposed that the comparatively low microsatellite mutation rate of *Drosophila melanogaster* is due to the short length of their microsatellites (Schug *et al.*, 1997) because microsatellite instability is greatly dependent on size (Wierdl *et al.*, 1997). The observation that the mutations in our study affected two 'pure' microsatellites (*DROYANETSB*, a dinucleotide with 21 repeats, and *DMSGG3*, a trinucleotide with 9 repeats) that are among the longest in the studied sample is consistent with this interpretation. Also, a very long allele of *DROYANETSB* with 28 repeats was the only variant in the study of Schlötterer *et al.* (1998). The fact that the same locus (*DROYANETSB*) was unstable in two mutation studies may be related to the large size of alleles at this locus. Schug *et al.* (1998*b*) have found that trinucleotides mutate at a rate 6·4 times slower than dinucleotides in a study based on population variation. Our results do not point to a relatively lower mutation rate of trinucleotides. This discrepancy may be related to the larger size of trinucleotides in our study. In any case, the huge errors associated with these mutation rate estimates, that render most differences non-significant, must be borne in mind when making these comparisons.

The two mutations in our study affected only the number of repeats. The trinucleotide mutation consisted of the loss of five repeats. This observation can be related to the reported instability of CAG tracts in yeast, which are prone to long deletions (Maurer *et al.*, 1996). The mutation in the dinucleotide repeat consisted of one addition. Pooling all the studies on direct spontaneous microsatellite mutation in *Drosophila*, a total of 14 mutants have been detected: eight were changes within a single repeat, and six involved more than three repeats (mean 5·8 repeats). Thus, mutations of more than one repeat unit are common, in agreement with the study by Di Rienzo *et al.* (1998) on the distribution of microsatellite mutations in human cancer cell lines. Several studies have reported a bias in the distribution of microsatellite mutations. Two studies, in humans (Amos & Rubinstzein, 1996) and swallows (Primmer *et al.*, 1996), reported a bias towards longer alleles. More recently, it was demonstrated in yeast (Wierdl *et al.*, 1997) and humans (Xu *et al.*, 2000) that the mutational bias depends on allele size. Long alleles tend to suffer large downward mutations. As already noted by Schlötterer *et al.* (1998), the mutation spectra observed in *D. melanogaster* fit well with these observations. Among the changes within a single repeat, six were additions and two were losses. The changes of several repeats involved long alleles (*DROYANETSB*, 28 dinucleotide repeats and *DMSGG3*, 11 trinucleotide repeats), and were two additions of 3 and 4 units and four losses, all of more than five repeats.

Although the number of spontaneous mutants is still limited, it can be noted that the distribution of microsatellite mutations shows a clear discontinuity that must be related to a distinct mechanism giving rise to each type of mutation. Single repeat changes are assumed to arise by DNA polymerase slippage during replication, while the origin of large deletions and additions is less clear; they could result from DNA polymerase slippage events involving the formation of large loops, or from recombination events (Wierdl *et al.*, 1997). This distribution of microsatellite mutations contrasts with that obtained by Flores & Engels (1999) in lines of *Drosophila* with deletions of *spellchecker 1* (a gene of the mismatch repair system), where 90% of all new alleles were within a single repeat unit of a parental allele. A similar difference was found between the distributions of microsatellite mutants in strains of yeast wild-type and *msh2* (a mismatch repair mutant), where the fraction of alterations representing large deletions is reduced by *msh2*. This was attributed to the effect of the mismatch repair system in repairing small loops but not other kind of events leading to larger changes (Wierdl *et al.*, 1997).

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