Evolutionary History of Microsatellites in the Obscura Group of Drosophila

Mohamed A. F. Noor, Richard M. Kliman,†[1] and Carlos A. Machado†*

*Department of Biological Sciences, Louisiana State University; and †Department of Genetics, Rutgers University

The evolutionary origins of microsatellites are not well understood. Some investigators have suggested that point mutations that expand repeat arrays beyond a threshold size trigger microsatellites to become variable. However, little empirical data has been brought forth on this and related issues. In this study, we examine the evolutionary history of microsatellites in six species within the obscura group of Drosophila, tracing changes in microsatellite alleles using both PCR product size and sequence data. We found little evidence supporting a general role of point mutations triggering initial microsatellite expansion, and no consistent threshold size for expansion was observed. Flanking region length variation was extensive when alleles were sequenced in distantly related species, and some species possessed altogether different repeat arrays between the same primer binding sites. Our results suggest extreme caution in using microsatellite allele sizes for phylogenetic analyses or to infer divergences between populations.

Introduction

Microsatellites, or simple sequence repeats, differ from most DNA sequences in that they have a distinct "birth." These repeats likely originate from an initial expansion of an existing sequence motif, perhaps from a replication slippage or unequal-crossing-over event, although few cases of microsatellite births are documented (see review in Schlötterer 2000). In one relevant study, Messier, Li, and Stewart (1996) used a phylogenetic approach to identify a putative ancestral sequence for a microsatellite in primates. Messier, Li, and Stewart (1996) results suggest that "once a critical number of repeat units has arisen in a given species, that locus can become hypervariable, with mutations occurring on [greater timescales]." A base-pair mutation appears to have created this critical number of repeats in the microsatellites examined by Messier, Li, and Stewart (1996) (see also Gordon 1997). Similarly, Rose and Falush (1998) analyzed the accumulated *Saccharomyces cerevisiae* sequence data and suggested that they observed "a minimum size threshold for slippage mutation" of approximately 8–10 nt. Based on these findings, thresholds have been used in microsatellite mutation models (e.g., Kruglyak et al. 1998, 2000). However, in a separate analysis of *S. cerevisiae* sequences, Pupko and Graur (1999) disagreed, suggesting "no critical point exists" for microsatellite expansion. Hence, shorter microsatellites have the potential to expand, but with a correspondingly lower probability than longer microsatellites.

To address whether a threshold size for expansion exists and to identify other patterns apparent in microsatellite evolution, we amplified and sequenced eight microsatellites in several species within the obscura group of Drosophila, which has a well-established phylogeny (reviewed in Gleason et al. 1997; O'Grady 1999). Multiple alleles of microsatellites showing size polymorphisms were sequenced to verify that the observed variability came from polymorphism in the identified repeat array and not solely from flanking-region variation. We then attempted to identify patterns of microsatellite evolution or ancestral allele states where possible.

Materials and Methods

The microsatellites surveyed were originally identified in *Drosophila pseudoobscura* (Noor, Schug, and Aquadro 2000): *DPSX002, DPSX004, DPSX006, DPSX010, runt* (5′ region), *bicoid* (exon), *decapentaplegic* (*dpp,* 3′ polyA region), and *DPS4002.* All of these microsatellites are variable in *D. pseudoobscura* (Hamblin and Aquadro 1999; Noor, Schug, and Aquadro 2000). We attempted to amplify all of these microsatellites in the following species: *D. pseudoobscura* (48 F_1 females from Utah), *Drosophila persimilis* (14 F_1 females from Mather and 7 from Mount St. Helena, Calif.), *Drosophila miranda* (1 F_1 female from Mather and 1 from Mount St. Helena, Calif.), *Drosophila affinis* (10 wild-caught males from Baton Rouge, La.), and *Drosophila subobscura* (10 F_2 females from Lagrasse, France). *DPSX002* and *DPS4002* were also amplified and sequenced in *Drosophila lowei* (1 wild-caught male from Mount Lemon, Ariz.) by Andy Beckenbach.

Allele sizes were scored on an acrylamide gel. One primer was ordered with an M13 tail at the 5′ end, and PCR was performed in a 10-μl reaction volume with 0.5 pmol of each primer, 0.4 pmol fluorescent-dye-labeled M13, 200 μM dNTP's, 1 μl 10 × buffer (100 mM Tris [pH 8.3], 500 mM KCl, 15 mM $MgCl_2$), 1 U *Taq* polymerase, and 1 μl from a 50 μl single fly squish preparation (Gloor et al. 1993). PCR was executed using a touchdown cycle (Palumbi 1996). Following PCR, 3 μl of LiCor stopping buffer was added to the reactions, and 1 μl was loaded onto an acrylamide gel (National Diagnostics Sequagel) on a LiCor 4200 DNA sequencer for visualization.

Two or more size alleles from each species were selected for sequencing to confirm that variation in PCR product size stemmed, at least in part, from variation in

[1] Present address: Department of Biological Sciences, Kean University.

Key words: microsatellites, simple sequence repeats, threshold, mutation, phylogenetics.

Address for correspondence and reprints: Mohamed A. F. Noor, Department of Biological Sciences, 508 Life Sciences Building, Louisiana State University, Baton Rouge, Louisiana 70803. E-mail: mnoor@lsu.edu.

Mol. Biol. Evol. 18(4):551–556. 2001
© 2001 by the Society for Molecular Biology and Evolution. ISSN: 0737-4038

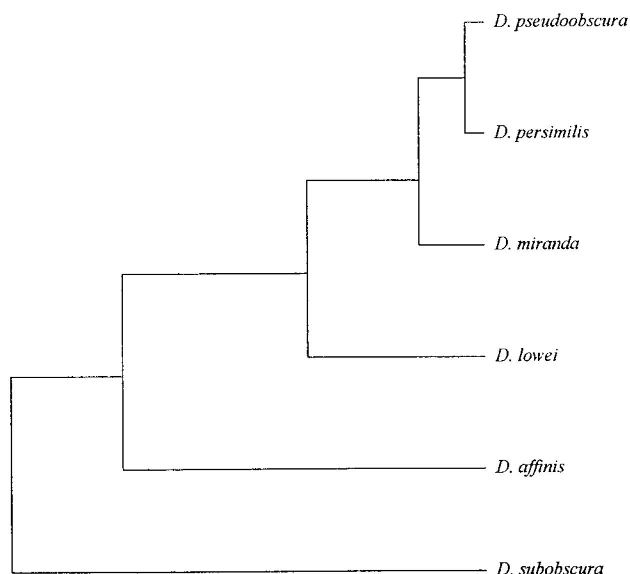

FIG. 1.—Representative phylogeny of the obscura group of Drosophila, from Aquadro et al. 1991 and Beckenbach, Wei, and Liu (1993).

number of repeats in the microsatellite. Individuals that were chosen for sequencing were homozygous or hemizygous for a particular size of allele. PCR for sequencing was performed as above but in 50-μl reaction volumes. Except for *bicoid* and *DPSX010,* PCR products were cleaned using the QiaQuick PCR purification kit (Qiagen) and sequenced in both directions using ABI's BigDye terminator kit. Sequencing reactions were then visualized on the ABI377 automated sequencer at the Louisiana State University Museum of Natural Science. Sequence alignments were performed manually.

For *bicoid,* a 1.5-kb fragment was amplified using single individuals of highly inbred strains (15–17 generations of sibling mating); secondary PCR amplified two overlapping smaller segments, which were then used as template for fluorescent bidirectional sequencing on a LiCor 4200 in the Hey laboratory at Rutgers University. No heterozygosity was evident for any of the sequences reported. For *DPSX010* and *DPS4002,* a ~1-kb fragment was similarly amplified and sequenced.

We were unable to amplify some of these microsatellites in some of the species more distantly related to *D. pseudoobscura* despite several attempts. Homology of amplified microsatellite sequences surveyed was inferred by similarity in the flanking-region sequences. We cannot completely exclude the possibility that duplications or other forms of redundancy in the genome may have caused us to occasionally amplify a nonhomologous locus, particularly in *D. subobscura* or *D. affinis,* where amplification was occasionally weak with our primers. However, our sequence electropherograms were clear, suggesting that multiple templates were not present.

Sequences were aligned and compared using the phylogenies of Beckenbach, Wei, and Liu (1993) and Barrio and Ayala (1997). The topologies of these phylogenies are consistent with one another. Estimated divergence times from *D. pseudoobscura* are as follows: *D. persimilis,* 500,000 years; *D. miranda,* 3.3 Myr; *D. lowei,* 8.4 Myr; *D. affinis,* 17 Myr; *D. subobscura,* 22 Myr (Aquadro et al. 1991; Beckenbach, Wei, and Liu 1993) (see fig. 1). Other, similar, divergence time estimates have been suggested (Beckenbach, Wei, and Liu 1993; Wang, Wakeley, and Hey 1997), but we chose these figures as representative. Following Messier, Li, and Stewart (1996), we identified ancestral states of microsatellites as those with identical short arrays in multiple outgroup species, but we expand on their qualification by only using alleles that are fixed within those outgroup species (see *Discussion*). Given that the species we surveyed represented progressively increasing genetic distances from *D. pseudoobscura* rather than a homogeneous spread of pairwise relationships, we did not follow Zhu, Queller, and Strassmann (2000) in inferring the most common motif as ancestral.

Results

We noted that the flanking regions of the microsatellites were frequently repetitive in nature and often variable in length. However, the patterns of evolution of the microsatellites were quite different from one another. Table 1 presents a summary of all sequences. *DPSX002* appears to have become variable only after the split between *D. pseudoobscura* and *D. persimilis. Drosophila lowei* had a shorter repeat without the interruption present in *D. pseudoobscura, D. persimilis,* and *D. miranda,* but we cannot distinguish whether this is an ancestral state or the result of a deletion.

The variability of *DPSX004* appears much older than that of *DPSX002,* possibly consistent with its longer size. While this microsatellite is long and variable in *D. pseudoobscura, D. persimilis,* and *D. miranda,* it is fixed for one allele in *D. affinis.*

DPSX006 has a complicated evolutionary history. In *D. pseudoobscura* and *D. persimilis,* the microsatellite is long and variable, but there is a shorter, nearly invariant repeat motif upstream of it. In *D. affinis,* the shorter upstream repeat is long and variable, while the downstream repeat is absent entirely. *Drosophila subobscura,* in contrast, basically has neither microsatellite: the upstream one is even shorter than in *D. persimilis,* and the downstream one is gone, as in *D. affinis.*

Our conclusions from *DPSX010* are necessarily limited because we were only able to amplify it in three species. Amplification was extremely difficult in *D. miranda* as well. While *D. pseudoobscura* apparently has fairly long alleles, *D. persimilis* is fixed for a shorter allele. *Drosophila miranda* has a longer allele than *D. persimilis.* This suggests either that the shorter allele in *D. persimilis* became fixed by either drift or selection, or that there were two independent origins of variability in this microsatellite in this group.

Runt had substantial differences among the three subgroups in the flanking region, yet the potential for variability of this microsatellite was not permanently lost over 40 Myr of independent evolution in multiple groups. Polymorphisms for interruptions were noted in

Table 1
Microsatellite Allele Sequences in the obscura Group of Drosophila

	Sequence	No. of Alleles	n	No. of Sequences
DPSX002				
D. pseudoobscura ...	CTGTGTGTAT(TG)$_n$C	9	(TG)$_8$, (TG)$_{10}$, (TG)$_{12}$GGTG, (TG)$_{14}$GGTG	4
D. persimilis	..........(TG)$_6$.	1	NA	1
D. miranda	..........(TG)$_6$.	1	NA	3
D. lowei	.---------(TG)$_5$.	1	NA	1
DPSX004				
D. pseudoobscura ..	TGTAC-AAA---------(CA)$_n$AA	12		
D. persimilis	.....-...---------(CA)$_n$..	8	(CA)$_{12}$, (CA)$_{13}$	2
D. miranda	.....-...---------(CA)$_n$..	2	(CA)$_{11}$, (CA)$_{14}$	2
D. affinis	.....G...GCACACACT(CA)$_{10}$..	1	NA	1
DPSX006				
D. pseudoobscura ..	C(TB)$_{5-6}$------------AGTCTCCCTCCCTGGCTA(TG)$_n$T	11	(TG)$_9$, (TG)$_{14}$, (TG)$_7$TA (TG)$_2$	4
D. persimilis	.(TB)$_5$------------..................(TG)$_n$.	7	(TG)$_{12}$, (TG)$_{13}$	2
D. miranda	.(TG)$_5$------------..................(TB)$_7$.	1	NA	2
D. affinis	.TGAG(TG)$_n$TCTA(TG)$_6$..............---------.	7	(TG)$_9$, (TG)$_{14}$	2
D. subobscura	.TGTA(TG)$_2$----------..C........A..---------.	1	NA	1
DPSX010				
D. pseudoobscura ..	CA(TG)$_n$AA	9	(TG)$_{7,8,9,10,12,13,17}$ (TG)$_8$CG(TG)$_4$	25
D. persimilis	..(TG)$_6$..	1	NA	14
D. miranda	A.(TG)$_8$..	1	NA	2
runt				
D. pseudoobscura ..	CC-TCTCTGCCAC----------------(TG)$_n$CGC	17	(TG)$_9$, (TG)$_{11}$, (TG)$_5$CG(TG)$_{9,10,11}$	6
D. persimilis	..-..........----------------(TG)$_n$...	12	(TG)$_8$, (TG)$_{17}$	2
D. miranda	..-..........----------------(TG)$_n$...	2	(TG)$_9$, (TG)$_7$TT(TG)$_6$	2
D. affinis	..G.AAG..TAT.----------------(TG)$_n$...	5	(TG)$_7$, (TG)$_3$TC(TG)$_7$	2
D. subobscura	..TCTG...TGTGG(TG)$_4$CAGC(TG)$_3$AG(TG)$_n$...	7	(TG)$_8$, (TG)$_{12}$	2
bicoid				
D. pseudoobscura ..	CTTTCAGACACAA---(CAG)$_n$CTCCAT(CAG)$_3$(CAACAG)$_1$CA	2	(CAG)$_4$, (CAG)$_5$	6
D. persimilis	.............---(CAG)$_n$......(CAG)$_3$(CAACAG)$_1$..	4	(CAG)$_4$, (CAG)$_5$	6
D. miranda	T............---(CAG)$_5$......(CAG)$_3$(CAACAG)$_1$..	1	NA	1
D. affinis	.......G.....CAA(CAG)$_5$......(CAG)$_3$(CAACAG)$_n$..	4	(CAACAG)$_1$, (CAACAG)$_2$	2
dpp				
D. pseudoobscura ..	TTTATTTAAAA---CAAA(CA)$_n$TATTTTTGATGAT	7	(CA)$_5$, (CA)$_8$, (CA)$_9$	5
D. persimilis	...........---....(CA)$_n$.............	4	(CA)$_7$, (CA)$_8$	2
D. miranda	...........---....(CA)$_5$.............	1	NA	1
D. affinis	...........---....(CA)$_3$.............	1	NA	1
D. subobscura	...........AAA....(CA)$_3$......C......	2	NA	1
DPS4002				
D. pseudoobscura ..	CCT(CA)$_n$ATT	7	(AC)$_{12}$, (AC)$_{13}$, (AC)$_{15}$	5
D. persimilis	...(AC)$_n$...	4	(AC)$_8$, (AC)$_9$, (AC)$_{10}$	4
D. lowei	...(AC)$_7$...	—	NA	1

NOTE.—The number of alleles is the number of size alleles observed in all the PCR products surveyed. ''n'' indicates a sequence variable in a repetitive element, and the alleles are presented in the right column. The number of strains sequenced for each species is listed under ''No. of Sequences.'' Typically, species which did not exhibit PCR product size variability at a particular marker were represented by just a single sequence.

D. pseudoobscura, D. miranda, and *D. affinis,* although these do not appear to have severely disrupted the continued variability of this microsatellite. Indeed, in *D. pseudoobscura,* the interrupted microsatellite allele appears to have continued to mutate after the interruption occurred, as we noted multiple array sizes bearing the same interruption.

Bicoid has a history similar to that of *DPSX006.* Two repeat motifs are apparent, and while one is variable in *D. pseudoobscura* and *D. persimilis,* the other is variable in *D. affinis.* The hexanucleotide repeat array that was variable in *D. affinis* was invariant in the six *D. pseudoobscura* or seven *D. persimilis* samples sequenced. Furthermore, no allele from our PCR product size variability survey corresponded with its expected size. Hence, this hexanucleotide repeat array appears to be variable in *D. affinis* but invariant in *D. pseudoobscura* and *D. persimilis.*

The dinucleotide repeat array in *decapentaplegic* is highly variable in *D. persimilis* and *D. pseudoobscura* but much smaller and less variable in the other species. The ancestral size may be the smallest allele fixed in *D. affinis* and *D. subobscura* (see *Discussion*). In *D. subobscura,* a second size allele was noted in a heterozygous state in one individual. This individual was sequenced, and the size difference resulted from an indel in the region flanking the microsatellite (outside that shown in table 1).

DPS4002 was segregating for alleles in *D. persimilis* shorter than those observed in *D. pseudoobscura.* However, *D. lowei* possesses an allele shorter than those observed in the two species.

Discussion

We amplified and sequenced microsatellite size alleles both within and among species of the obscura group of Drosophila. Our results suggest that microsatellite evolution is often quite complex and cannot readily be interpreted from PCR product size data alone. Although some amplified products bearing microsatellites were variable in size among individuals across several species, we found that this variability sometimes stems from different repeat arrays in the region between the same primers. Our observations of variability in *DPSX006* were particularly compelling in that regard: two species bore one variable repeat array; one species bore a different, but also variable, repeat array; and one species bore neither repeat array.

We cannot infer the ancestral states for most of the microsatellites that we examined because we did not observe multiple outgroups fixed for an identical, short array (Messier, Li, and Stewart 1996). At *dpp,* however, we observed an identical and fixed sequence for the proto-microsatellite array in species separated by more than 40 Myr. We did not employ standard phylogenetic ancestral state reconstruction methods (e.g., Martins and Hansen 1997; Schluter et al. 1997) to derive a measure of confidence for this ancestral state reconstruction, as the underlying model would be very complex. The proto-microsatellite would initially be invariant and have a very low mutation rate, the first mutation would necessarily lead to a higher probability of a second mutation, and later mutations could lead to a greater or lesser likelihood of subsequent mutations depending on whether they result in expansions or contractions of the array (see below). However, for the common ancestor of the obscura group species to have borne a variable array, two unlikely events would have had to occur. First, the same allele would have to be fixed independently in *D. subobscura* and *D. affinis.* Second, the fixed allele would also necessarily have borne a much shorter array than is present in the other obscura group species. Instead, we interpret our data as suggesting that the microsatellite in *dpp* expanded after the split between *D. affinis* and the pseudoobscura subgroup (*D. miranda, D. pseudoobscura,* and *D. persimilis*).

First, our data for *dpp* convincingly suggest that initial microsatellite length expansion does not require a base-pair substitution incidentally increasing array length beyond a threshold. Given that neither of the regions flanking the proto-microsatellite in the outgroup species (*D. subobscura* and *D. affinis*) were incorporated into what has become the variable array in *D. pseudoobscura,* the initial expansion was not triggered by a base-pair substitution. This conclusion assumes only that there were no multiple substitutions at particular sites of the flanking regions prior to the initial microsatellite expansion. Given that only one base-pair difference in the region flanking the array was noted among all species, we consider the possibility of multiple substitutions very unlikely.

In contrast, we did not observe an unambiguous case of the facilitation of a microsatellite expansion by base-pair substitution (Messier, Li, and Stewart 1996). The initial expansion of the small array in *DPSX006* and the primary array in *DPSX004* may have been aided by single-nucleotide substitutions (see table 1), but the ancestral states for these arrays are less certain.

Our data also failed to support an expansion threshold of 8–10 nt (Rose and Falush 1998). In *dpp,* the initial expansion appears to have occurred from a three-unit dinucleotide repeat array (6 nt). Similarly, in *bicoid,* the expansion observed in *D. affinis* may have occurred from a single repeat unit that was 6 nt in length.

Rather than a threshold size for microsatellite expansion, we interpret our results as being consistent with those of Pupko and Graur (1999) and others (e.g., Weber and Wong 1993; Goldstein and Clark 1995; Schlötterer et al. 1998; Schug et al. 1998; Zhu, Queller, and Strassmann 2000) that microsatellite mutation rates increase with increasing numbers of repeats. We expand on these studies by extrapolating this result for extant microsatellites to microsatellite origins, also suggested as a possibility by Zhu, Queller, and Strassmann (2000). Hence, at very low repeat numbers (e.g., three repeats, as seen in *dpp* in *D. affinis*), the microsatellite is still prone to possible expansion, but with a length mutation rate lower than that of a longer array.

We can infer that reasonable estimates of per-repeat-unit microsatellite mutation rates could result in the expansion of the short repeat unit present in *D. subobscura* to the longer array found in *D. pseudoobscura* without invoking a true threshold size for expansion. Kruglyak et al. (1998) computed stationary allele length distributions under a model of length-dependent mutations in microsatellites and applied this to data from *Drosophila melanogaster* and other species. They predicted that the per-repeat-unit mutation rate of *D. melanogaster* dinucleotide arrays would be 2.3×10^{-7} mutations per generation. If microsatellite mutation rates are comparable in the obscura group, as some data suggest (Noor, Schug, and Aquadro 2000), then the mutation rate of the proto-microsatellite in *dpp* in *D. subobscura* would be 6.9×10^{-7} mutations per generation (3 repeats $\times$ 2.3×10^{-7} mutations per generation per repeat), or an average of approximately 1.45×10^{6} generations for a mutation that will ultimately be fixed to occur. *Drosophila miranda* and *D. subobscura* diverged from each other approximately 22 MYA (Beckenbach, Wei, and Liu 1993), or approximately 110 million generations ago if we assume five generations per year. If the effective population size of the common ancestor was similar to that predicted for *D. pseudoobscura* (approximately 4.5 million; Schaeffer 1995), then the average length of time to fixation of a new neutral mutation would be 18 million generations (four times the effective population size). A smaller population size would result in a more rapid fixation time.

If the proto-microsatellite that existed 110 million generations ago could have mutated to a longer size in only 1.45 million generations, and it would take only 18 million generations for a new mutation to become fixed within the lineage leading to *D. miranda* and *D. pseudoobscura,* the new allele could have expanded and

been fixed in this lineage over 90 million generations ago, well before the split of *D. pseudoobscura* and *D. miranda.* Hence, it is unsurprising that this proto-microsatellite would have expanded and been fixed in this lineage, perhaps multiple times, without the need for a base-pair substitution creating an array longer than a threshold size. In contrast, multiple-base-pair substitutions at the same site (only one site could have done this) creating a longer array and then mutating back to the ancestral state would not be expected in only 110 million generations, as its probability would still be below the single-base-pair mutation rate.

Of course, one should not place too much emphasis on the exact figures or calculations presented above, as each figure has a large associated error or variance, and we do not know that the distant common ancestor of these species had a population size equal to or smaller than that of *D. pseudoobscura.* This calculation merely demonstrates that an expected low mutation rate could have created a pattern similar to that observed in this species group.

Microsatellites are not the simple sequences they were once perceived to be. As size homoplasies appear common, flanking-region size variation continues to be identified, and some species even have altogether different variable regions between primer sequences (this study, and see Angers and Bernatchez 1997; Grimaldi and Crouau-Roy 1997; Orti, Pearse, and Avise 1997; Colson and Goldstein 1999; Karhu, Dieterich, and Savolainen 2000; Makova, Nekrutenko, and Baker 2000), extreme caution should be exercised in the use of microsatellite allele sizes as inferred from PCR product size. The origin of microsatellites is still not completely clear, but we hope that this study and future use of phylogenetic studies of microsatellite sequence data will help to elucidate the genetic and evolutionary mechanisms involved, as well as to clarify and interpret results of microsatellite variability studies.

Supplementary Material

The nucleotide sequences presented in this manuscript are available from the EMBL/GenBank databases under the accession numbers AF319128, AF320128-AF320189, and AY012609-AY012618.

Acknowledgments

We thank Andy Beckenbach for sequencing two of our microsatellites in his sample of *D. lowei.* We also thank John C. Larkin, David Rand, Jane Reiland, and two anonymous reviewers for helpful comments on the manuscript, and N. Crochet for technical assistance. This research was supported by NIH grant GM58060-01.

Literature Cited

Angers, B., and L. Bernatchez. 1997. Complex evolution of a salmonid microsatellite locus and its consequences in inferring allelic divergence from size information. Mol. Biol. Evol. **14**:230–238.

Aquadro, C. F., A. L. Weaver, S. W. Schaeffer, and W. W. Anderson. 1991. Molecular evolution of inversions in *Drosophila pseudoobscura*: the amylase gene region. Proc. Natl. Acad. Sci. USA **88**:305–309.

Barrio, E., and F. J. Ayala. 1997. Evolution of the *Drosophila obscura* species group inferred from the *Gpdh* and *Sod* genes. Mol. Phylogenet. Evol. **7**:79–93.

Beckenbach, A. T., Y. W. Wei, and H. Liu. 1993. Relationships in the *Drosophila obscura* species group, inferred from mitochondrial cytochrome oxidase II sequences. Mol. Biol. Evol. **10**:619–634.

Colson, I., and D. B. Goldstein. 1999. Evidence for complex mutations at microsatellite loci in *Drosophila.* Genetics **152**:617–627.

Gleason, J. M., A. Caccone, E. N. Moriyama, K. P. White, and J. R. Powell. 1997. Mitochondrial DNA phylogenies for the *Drosophila obscura* group. Evolution **51**:433–440.

Gloor, G. B., C. R. Preston, D. M. Johnson-Schlitz, N. A. Nassif, R. W. Phillis, W. K. Benz, H. M. Robertson, and W. R. Engels. 1993. Type I repressors of P element mobility. Genetics **135**:81–95.

Goldstein, D. B., and A. G. Clark. 1995. Microsatellite variation in North American populations of *Drosophila melanogaster.* Nucleic Acids Res. **23**:3882–3886.

Gordon, A. J. E. 1997. Microsatellite birth register. J. Mol. Evol. **45**:337–338.

Grimaldi, M.-C., and B. Crouau-Roy. 1997. Microsatellite allele homoplasy due to variable flanking sequences. J. Mol. Evol. **44**:336–340.

Hamblin, M. T., and C. F. Aquadro. 1999. DNA sequence variation and the recombinational landscape in *Drosophila pseudoobscura*: a study of the second chromosome. Genetics **153**:859–869.

Karhu, A., J.-H. Dieterich, and O. Savolainen. 2000. Rapid expansion of microsatellite sequences in pines. Mol. Biol. Evol. **17**:259–265.

Kruglyak, S., R. T. Durrett, M. D. Schug, and C. F. Aquadro. 1998. Equilibrium distributions of microsatellite repeat length resulting from a balance between slippage events and point mutations. Proc. Natl. Acad. Sci. USA **95**: 10774–10778.

———. 2000. Distribution and abundance of microsatellites in the yeast genome can be explained by a balance between slippage events and point mutations. Mol. Biol. Evol. **17**: 1210–1219.

Makova, K. D., A. Nekrutenko, and R. J. Baker. 2000. Evolution of microsatellite alleles in four species of mice (genus Apodemus). J. Mol. Evol. **51**:166–172.

Martins, E. P., and T. F. Hansen. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. Am. Nat. **149**:646–667.

Messier, W., S.-H. Li, and C.-B. Stewart. 1996. The birth of microsatellites. Nature **381**:483.

Noor, M. A. F., M. D. Schug, and C. F. Aquadro. 2000. Microsatellite variation in populations of *Drosophila pseudoobscura* and *Drosophila persimilis.* Genet. Res. Camb. **75**:25–35.

O'Grady, P. M. 1999. Reevaluation of phylogeny in the *Drosophila obscura* species group based on combined analysis of nucleotide sequences. Mol. Phylogenet. Evol. **12**:124–139.

Orti, G., D. E. Pearse, and J. C. Avise. 1997. Phylogenetic assessment of length variation at a microsatellite locus. Proc. Natl. Acad. Sci. USA **94**:10745–10749.

Palumbi, S. R. 1996. Nucleic acids II: the polymerase chain reaction. Pp. 205–247 *in* D. M. Hillis, C. Moritz, and B.

K. MABLE, eds. Molecular systematics. Sinauer, Sunderland, Mass.

PUPKO, T., and D. GRAUR. 1999. Evolution of microsatellites in the yeast *Saccharomyces cerevisiae*: role of length and number of repeated units. J. Mol. Evol. **48**:313–316.

ROSE, O., and D. FALUSH. 1998. A threshold size for microsatellite expansion. Mol. Biol. Evol. **15**:613–615.

SCHAEFFER, S. 1995. Population genetics in Drosophila pseudoobscura: a synthesis based on nucleotide sequence data for the Adh gene. Pp. 329–352 *in* L. LEVINE, ed. Genetics of natural populations: the continuing importance of Theodosius Dobzhansky. Columbia University Press, New York.

SCHLÖTTERER, C. 2000. Evolutionary dynamics of microsatellite DNA. Chromosoma **109**:365–371.

SCHLÖTTERER, C., R. RITTER, B. HARR, and G. BREM. 1998. High mutation rates of a long microsatellite in *Drosophila melanogaster* provides evidence for allele specific mutation rates. Mol. Biol. Evol. **15**:1269–1275.

SCHLUTER, D., T. PRICE, A. O. MOOERS, and D. LUDWIG. 1997. Likelihood of ancestor states in adaptive radiation. Evolution **51**:1699–1711.

SCHUG, M. D., C. M. HUTTER, K. A. WETTERSTRAND, M. S. GAUDETTE, T. F. C. MACKAY, and C. F. AQUADRO. 1998. The mutation rates of di-, tri- and tetranucleotide repeats in *Drosophila melanogaster*. Mol. Biol. Evol. **15**:1751–1760.

WANG, R. L., J. WAKELEY, and J. HEY. 1997. Gene flow and natural selection in the origin of *Drosophila pseudoobscura* and close relatives. Genetics **147**:1091–1106.

WEBER, J. L., and C. WONG. 1993. Mutation of human short tandem repeats. Hum. Mol. Genet. **2**:1123–1128.

ZHU, Y., D. C. QUELLER, and J. E. STRASSMANN. 2000. A phylogenetic perspective on sequence evolution in microsatellite loci. J. Mol. Evol. **50**:324–338.

DAVID RAND, reviewing editor

Accepted December 1, 2000