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Rates and patterns of microsatellite mutations in common carp (*Cyprinus carpio* L.)

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Abstract: During genotyping 150 microsatellites in a F_1 family of common carp, six mutations were found at five microsatellite loci. The overall mutation rate of common carp microsatellites was 2.53×10^{-4} per locus per generation. At five loci, mutations increased the length of alleles by at least one repeat unit, suggesting mutations at microsatellite loci in common carp do not follow strict stepwise mutation model. The data on mutation rates and patterns can facilitate population genetics studies, and provide useful parameters for estimating a long-term effective population size of common carp.

Key words: Mutation rate; Mutation pattern; Common carp

鲤鱼微卫星突变速率和模式

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摘要：利用 150 个微卫星分子标记在 F_1 代家系的基因型分析过程中，共有 27 600 个等位基因从亲本向子代传递，其中在 5 个微卫星座位上检测到 6 个突变的等位基因。对突变的等位基因数目进行统计分析后得出：鲤鱼平均每个世代每个微卫星座位的突变速率为 2.53×10^{-4}。在发现突变的 5 个位点中，经测序发现，突变序列中插入 1 个以上的重复单元就导致了突变的发生。这些突变表明，鲤鱼的微卫星突变没有遵循严格的渐变突变模型（stepwise mutation model，SMM）。该文关于鲤鱼微卫星突变速率和模式的研究将会对统计鲤鱼有效群体的统计提供有效参数。

关键词: 突变速率；突变模式；鲤鱼
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Microsatellites are highly polymorphic repeat DNA sequences. Due to their exceptional variability and easy detection, microsatellites are now generally considered to be one of the most useful genetic markers. They have been used in genome mapping, population genetics studies, evolution genetics research, parentage and kinship analysis, genome instability detection in cancer, forensic identification, and species stock management (Goldstein & Schlotterer, 1999; Balloux et al, 2002; Laloi et al, 2004; Beacham et al, 2008; Xia et al, 2009). In general，Microsatellites gain or lose their repeat units at a high rate. The underlying mutation process has been termed “DNA replication slippage”. It is assumed that during DNA synthesis, nascent strands could dissociate and re-align with their templates out of register. When DNA synthesis continues, the repeat number at the

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microsatellite gets altered in nascent strands (Schlotterer, 2000). However, the exact mechanisms underlying microsatellite mutations are not fully understood. Microsatellite mutations can be studied using a number of approaches. The most direct and conclusive method is direct detection of mutation events in genotyping pedigree (Ellegren, 2004).

Mutation rates are useful in estimating long term effective population sizes (Nei, 1987; Lehmann et al, 1998; Beerli & Felsenstein, 2001; Ohta & Kimura, 2007). The effective population size is an important parameter in aquaculture. Although fish species represents 46% of vertebrates on the earth, data on mutation rate analysis is limited. Only in a few fish species, such as salmon (Steinberg et al, 2002), common carp (Yue et al, 2007), dollar sunfish (MacKiewicz et al, 2002) and pipefish (Jones et al, 1999), their mutation rates have been estimated. The mutation rates range from 5.56×10^{-4} to 2.0×10^{-3}. In most of these studies, mutation rates were estimated by analyzing a few microsatellite markers.

Common carp is one of the most widely cultivated and important commercial freshwater fish (Naylor et al, 2000; FAO, 2002). We have isolated a large number of microsatellite loci and constructed a linkage map (Wei et al, 2001; Sun et al, 2004; Zhang et al, 2007; Zhang et al, 2008). This article describes the mutation patterns and rates of microsatellites in common carp examination of a F_1 family using a panel of 150 microsatellites.

1 Materials and Methods

Ninty and seven microsatellites were selected from microsatellites cloned by us (see Appendix 1) and those published by others (Crooijmans et al 1997; David et al 2001) based on polymorphism, and were genotyped in 92 F_1 progeny generated from one pair of parents. PCR reactions were conducted with the following thermo-profile: An initial step at 94℃ for 2 min, followed by 30 cycles of 94℃ for 30 s, annealing at Tm temperature for 30 s, and extension at 72℃ for 30 s, with a final step of 72℃ for 10 min. Each PCR reaction consisted of 1X PCR buffer (Takara, Dalian) with 1.5 mmol/L $MgCl_2$, 200 nmol/L of each PCR primer, 50 mmol/L of each dNTP, 10 ng genomic DNA and 1 unit of Taq DNA polymerase (Takara, Dalian). PCR products were analyzed using 6% PAGE gels on a Genetic Analyzer (ABI 377, Applied Biosystems, Foster City, CA). Genotypes were determined by using a molecular size standard GS-ROX-500 (Applied Biosystems) with the software GENESCAN 2.1 (Applied Biosystems). Mutant alleles were defined as alleles detected in offspring, but absent in the mother.

The following equation was used for estimating microsatellite mutation rates: $R=N/T$, where N is the number of mutant alleles and T is the total number of transferred alleles in one generation at loci considered. Confidence intervals for the mutation rate per locus per generation were calculated assuming the mutations follow a Poisson distribution. The upper and lower limits corresponding to a number of mutations were using methods described by Rohlf & Sokal (1995). The association between mutation rates and other factors (i.e. GC contents in the flanking regions, heterozygosity, and repeat number) were analyzed using Microsoft Excel.

Each allele at a microsatellite locus with mutations were ligated into pGEM-T vector respectively (Promega, MA, USA), the ligation product was then transformed into XL-10 gold competent cells (Stratagene, CA, USA). In order to sequences true alleles of a locus, 5 − 10 clones were amplified using the original microsatellite primer pair and PCR products were separated on 6% PAGE gel. Then the length of the PCR products were compared to sizes of true alleles. Only the clones containing correct sizes of true alleles were sequenced using a M13 primer.

2 Results and Discussion

Mutation rates were estimated by counting each mutant allele detected as one mutation. A total of six mutant alleles were confirmed by polyacrylamide gel analysis. The overall average mutation rate was 2.53×10^{-4} per locus per generation in the F_1 family (6 mutations in a total of 27,600 inherited microsatellite alleles at 150 loci, 95% confidence interval=7.9×10^{-5}, 4.7×10^{-4}). The mutation rate estimated in this study is slight lower than that of Yue et al (2007) 5.56×10^{-4}.

In the F_1 generation, the average heterozygosity (1.0) of the five mutant microsatellites loci was higher than the average heterozygosity (0.68) of the other 145 microsatellites loci, but the difference was insignificant (P>0.05). The correlation between mutation rates and heterozygosity were low (r=0.32 df=149, P>0.05). GC contents in the flanking regions between the microsatellites exhibiting mutations and the ones displaying no mutations were significant different [(33.6%±1.2%) vs. (42.4%±2.3)%, P>0.05)]. However, the association between mutation rates and GC contents in the flanking regions was not statistically significant (r=0.27, df=149, P>0.05). In addition, no significant

relationship between mutation rates and repeat number was detected. There were a number of papers (e.g. Weber and Wong, 1993; Chakraborty et al, 1997) that reported the influence of microsatellite motif (i.e. dinucleotide and tetranucleotide repeats) on mutation rates. However, their conclusions were controversial (Schlotterer, 2000). In this study, we were not able to compare the difference of mutation rates at dinucleotide repeats and tetranucleotide repeats, because no tetranucleotide microsatellite were studied here. In summary, we found mutation rates at different microsatellite loci were independent of heterozygosity and GC content in flanking regions.

Sequencing analysis was carried out for the F_1-parent's alleles and at the five microsatellite loci displayed mutations. At locus *HLJ084*, the mutant allele, 232 bp, was generated by a deletion of three CA repeats; At the *HLJ101*, the mutant allele, 324 bp, was probably generated by a decrease of two CA repeats. At the *HLJ424*, the mutant allele, 151 bp, contained an insertion of four TG repeats. At the *HLJ623*, the mutant allele, 212 bp, was caused by a increase of one TG repeat. At the *HLJ919*, the mutant allele, 360 bp and 368 bp, was caused by a decrease and an insertion of four CA repeats, separately. All the mutations detected had size changes (Tab. 1). No sequence differences were found in the flanking regions. This mutation pattern of common carp microsatellites deviated from the strict stepwise mutation model (SMM) of microsatellite mutations, which was in agreement with reported in common carp before (Yue et al, 2007).

The mutation rates and patterns of microsatellites estimated, could supply a useful parameter to estimate long term effective population size of common carp. The effective population size (*N*e) is an important parameter in population genetics and the breeding practices. It is closely related to the accumulation of inbreeding in a population. Unfortunately, *Ne* has been very difficult to estimate using demographic or genetic data of natural populations. The reason is that demographic methods require information such as variance in reproductive success, and incorporate all of the factors (e.g. skewed sex ratios, changed population size and mutant rate) (Vitalis et al, 2001). Unfortunately, accurate mutation rates of genetic makers are not available in most cases. Therefore, estimating mutation rates are keys to accurately calculate *N*e. There are two methods used to calculate *N*e based on the stepwise mutation model (SMM) and infinite-allele model (IAM) when microsatellite mutantion rate and pattern of a species were known (Lehmann et al, 1998), and *N*e based on microsatellite mutation rates are shown below:

Tab. 1 Microsatellite mutations observed in a common carp

Locus	Motif	Parental genotypes		Number of genotypes in offspring					Repeat number change	Mutant rate
		Dam	Sire	a/c	a/d	b/c	b/d	Mutant		
HLJ084	$(CA)_{27}$	228/232	234/240	22	23	28	18	228/232(1)	−3	5.4×10^{-3}
HLJ101	$(CA)_{29}$	318/324	328/334	25	23	21	22	318/324(1)	−2	5.4×10^{-3}
HLJ424	$(TG)_{30}$	143/159	139/151	26	20	24	21	139/151(1)	+4	5.4×10^{-3}
HLJ623	$(TG)_7$	206/218	210/216	25	22	20	24	206/212(1)	+1	5.4×10^{-3}
HLJ919	$(CA)_{28}$	348/360	354/368	22	23	24	22	348/360(1)	−4	2.7×10^{-3}
								354/368(1)	+4	

The experiment family was F_1 line; the progeny allele was transmitted from dam and sire.

__ : Represent the mutant allele could be traced back to the allele.

*: a, b, c, d are microsatellite alleles showed in different fragment size at one locus.

SMM: $N_e=\left(\left(\frac{1}{1-H}\right)^2-1\right)\Big/(8\mu)$; IAM: $N_e=\frac{H}{4\mu(1-H)}$

These methods were described in Nei (1987), where *H* represents an unbiased and expected heterozygosity and μ represents a mutation rate. The mutation rates and patterns of common carp microsatellites are estimated here, can facilitate population genetics studies of common carp. They supply a useful parameter to estimate long term effective population size of the common carp.

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Appendix 1:

The 150 microsatellites of dinucleotide repeats genotyped in a pedigree including two parents and 92 offspring.

Koi29/30, Koi49/50, Koi63/64, Koi85/86, Koi87/88, Koi95/96, Koi107/108, Koi113/114, Koi115/116, Koi117/118 and Koi119/120 (Crooijmans et al, 1997).

MFW1, MFW2, MFW5, MFW7, MFW8, MFW12, MFW13, MFW14, MFW15, MFW 24 and MFW29 (David et al, 2001).

FJ403111, FJ403112, FJ403113, FJ403114, FJ403115, FJ403116, FJ403117, FJ403118, FJ403119, FJ403120, FJ403121, FJ403122, FJ403123, FJ403124, FJ403125, FJ403126, FJ403127, FJ403128, FJ403129, FJ403130, FJ403131, FJ403132, FJ403133, FJ403134, FJ403135, FJ403136, FJ403137, FJ403138, FJ403139, FJ403140, FJ403141, FJ403142, FJ403143, FJ403144, FJ403145, FJ403146, FJ403147, FJ403148, FJ403149, FJ403150, FJ403151, FJ403152, FJ403153, FJ403154, FJ403155, FJ403156, FJ403157, FJ403158, FJ403159, FJ403160, FJ403161, FJ403162, FJ403163, FJ403164, FJ403165, FJ403166, FJ403167, FJ403168, FJ403169, FJ403170, FJ403171, FJ403172, FJ403173, FJ403174, FJ403175, FJ403176, FJ403177, FJ403178, FJ403179, FJ403180, FJ403181, FJ403182, FJ403183, FJ403184, FJ403185, FJ403186, FJ403187, FJ403188, FJ403189, FJ403190, FJ403191, FJ403192, FJ403193, FJ403194, FJ403195, FJ403196, FJ403197, FJ403198, FJ403199, FJ403200, DQ378895, DQ378894, DQ378899 (These microsatellites were cloned by us).