Genetic variation in the clonal vertebrate *Poecilia formosa* is limited to few truly hypervariable loci

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Simple repeat oligonucleotides have proven to be useful tools for establishing multilocus DNA fingerprints in mammals and also in virtually all eukaryotic species investigated, ranging from fungi and plants to man (Epplen et al., submitted). Simple repeat oligonucleotide probes for DNA fingerprinting in teleost fish gained our special interest because of their usefulness in elucidating the mechanisms of sex chromosome evolution (Nanda et al. 1990 a,b), their potential in assessing paternal relationships, e.g. with respect to mating success as a central question of social-behavioural genetics, and their suitability to study the overall stability or instability, respectively, of the vertebrate genome on the level of the individual. For such questions, teleost fish provide a number of model systems, e.g. for studying the stability of an individual genome. Unisexual fish species like the Amazon molly (*Poecilia formosa, Teleostei: Poeciliidae*) can be analyzed in which without genetic recombination a single genome is transmitted through different generations (Monaco et al., 1984).

For this study two different clones of *Poecilia formosa* were studied. Our laboratory lines were derived both from a single female each and propagated in populations of 40 to 100 individuals. The generation time was approximately 4-6 months. For the apomictic breeding of these lines always a single male of an ornamental black molly strain was used. Males give only the physiological stimulus for gynogenesis but generally do not contribute to the gene pool of the *P. formosa* line (Kallman, 1962). Line I is derived from a field collection in 1953 by C. P. Haskins at Brownsville, Texas and is maintained in our laboratory since 1983. Line II given to us as a substrain of line I which was separated before 1985 and was bred independently in our laboratory since 1986.

Using *Hae*III, *Hin*fI, *Sau*3AI and *Alu*I digested DNA from 10 species of poeciliid fish for in gel hybridization with different simple repeat oligonucleotide probes (Nanda et al., 1990), generally the $(ggat)_4$ probe was most informative for individual differences (see table).

Informative simple repeat oligonucleotide probes for individualization in poeciliid fish.

Species	Probes
Poecilia reticulata	$(ggat)_4$, $(gata)_4$, $(gaca)_4$
Poecilia sphenops var. melanistica	$(ggat)_4$, $(ca)_8$
Poecilia velifera	$(ggat)_4$, $(ca)_8$
Poecilia peruglae	$(ggat)_4$, $(gaca)_4$
Poecilia formosa	$(ggat)_4$, $(gata)_4$, $(gaca)_4$
Poecilia latipinna	$(ggat)_4$, $(gaca)_4$
Xiphophorus maculatus	$(ggat)_4$, $(gaca)_4$
Xiphophorus helleri	$(ggat)_4$, $(gata)_4$
Xiphophorus montezumae	$(gaca)_4$
Xiphophorus cortezi	$(gaca)_4$

When specimen from both *P. formosa* strains were compared it was found that both lines had similar but divergent hybridization patterns. The band sharing was - depending on the probe used - approximately 80%. This is indicative of mutations that have occured after the separation of both strains and have been clonally transmitted (data not shown). Different individuals from one line showed overall identical fingerprints with a single exception. Using *Hae*III and *Hin*fI as restriction enzymes and $(gata)_4$ as probe subtle differences in certain regions of the entire fingerprint pattern were detected. To determine the extent of hypervariability, siblings from a single brood were analyzed (see figure). A hypervariable locus was recognized as a single band. At present it is difficult to decide whether these variations are due to germ line or/and somatic mutations. Our findings demonstrate, that individual genetic variation in the clonal vertebrate *P. formosa* is limited to rare truly hypervariable loci.

P. formosa is proposed to be a hybrid species, which is thought to be the product of interspecies matings of *P. latipinna* and *P. mexicana* (Hubbs and Hubbs, 1946). The observation that feral populations of *P. formosa* consist of multiple different clones, as analyzed by isozyme polymorphisms (Turner et al., 1980), histocompatibility studies (Kallman 1962), and by fingerprinting (Turner et al., 1990), may be explained by the reasoning that such clones originated by multiple hybridization events of *P. latipinna* with *P. mexicana*. An alternative - although not mutually exclusive - hypothesis is that mutation subsequent to the founding of clonal lineages is an important source of variation in these populations, as has been suggested by Turner et al. (1990). This problem, however, could not be solved with the specimen collected from the field. The availability of the two clonal sublines and the possibility to use DNA fingerprinting for an estimation on genome stability offers the possibility to approach these questions experimentally.

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