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### Permalink

<https://escholarship.org/uc/item/7zs9c6zt>

### Journal

Journal of Fish Biology, 56(3)

### ISSN

0022-1112

### Authors

DeWoody, JA  
Avisé, JC

### Publication Date

2000-03-01

### DOI

10.1111/j.1095-8649.2000.tb00748.x

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## REVIEW PAPER

# Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals

J. A. DEWOODY\* AND J. C. AVISE

*Department of Genetics, University of Georgia, Athens, GA 30602, U.S.A.*

*(Received 28 September 1999, Accepted 2 December 1999)*

From a total of 524 microsatellite loci considered in nearly 40 000 individuals of 78 species, freshwater fish displayed levels of population genetic variation (mean heterozygosity,  $h=0.46$ , and mean numbers of alleles per locus,  $a=7.5$ ) roughly similar to those of non-piscine animals ( $h=0.58$  and  $a=7.1$ ). In contrast, local population samples of marine fish displayed on average significantly higher heterozygosities ( $h=0.79$ ) and nearly three times the number of alleles per locus ( $a=20.6$ ). Anadromous fish were intermediate to marine and freshwater fish ( $h=0.68$  and  $a=11.3$ ). Results parallel earlier comparative summaries of allozyme variation in marine, anadromous, and freshwater fishes and probably are attributable in part to differences in evolutionarily effective population sizes typifying species inhabiting these realms.

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Key words: microsatellite loci; simple sequence repeats (SSRs); effective population size; heterozygosity; allelic diversity.

## INTRODUCTION

The term microsatellites refers to a class of co-dominant DNA markers which are inherited in a Mendelian fashion. Microsatellite loci are widely dispersed along and among chromosomes, and each locus is characterized by a known DNA sequence. These sequences consist of both unique DNA (which defines the locus) and of repetitive DNA motifs (which may be shared among loci). The repetitive elements consist of tandem reiterations of simple sequence repeats (SSRs) and are typically composed of two to four nucleotides such as  $(AC)_n$  or  $(GATA)_n$  where  $n$  lies between 5 and 50.

In localized population samples, variation in the number of repeats at particular SSR loci often produces an abundance of alleles distinguishable by molecular size. Following the discovery of microsatellite DNA markers about a decade ago (Litt & Luty, 1989; Tautz, 1989; Weber & May, 1989), thousands of these loci have been described in diverse eukaryotic organisms, including humans (Dib *et al.*, 1996).

Due to a tendency for hypervariability and because only small amounts of tissue are required for typing, microsatellites have supplanted allozymes in recent years as the genetic markers of choice for many biological problems including forensics, parentage assessment and genomic mapping (Schlötterer & Pemberton, 1994; Rubinsztein *et al.*, 1995; Jarne & Lagoda, 1996; Goldstein

\*Author to whom correspondence should be addressed. Tel.: 706-542-1456; fax: 706-542-3910; email: dewoody@arches.uga.edu

& Schlötterer, 1999). Although alleles at both microsatellite and allozyme loci are identified through altered electrophoretic mobility, there are substantial biological differences between the two classes of markers. For instance, whereas DNA length alterations (in SSR regions) are the basis of variation detected by microsatellite assays, allozyme methods detect only DNA-level changes that result in an alteration in the charge (or in some cases size) of a gene's protein product. Other substantial differences include the fact that allozymes are often functional metabolic enzymes, whereas the functional significance (if any) of variation in SSR numbers usually is unknown. The recent advent of microsatellite assays permits a re-examination of various population-level issues, such as magnitudes of genetic variation, from the perspective of a more highly mutable set of markers.

Despite the gross disparity in mean polymorphism levels between allozyme and microsatellite loci, patterns of genetic variation in these two marker systems none the less might parallel one another across taxa or among organisms in different environments. Suppose, for example, that evolutionary effective population sizes differ consistently between phylogenetic groups or among species inhabiting different environmental regimes. Then, parallel trends in neutral genetic variation might be present even if the sets of monitored loci differ consistently in mutation rate and, hence, in mean levels of contemporary variation.

In our own genetic analyses of mating behaviours in several fish species (Jones & Avise, 1997a,b; DeWoody *et al.*, 1998, 2000; Jones *et al.*, 1998a,b,c, 1999; Zane *et al.*, 1999), we have been impressed by an apparent difference in mean levels of microsatellite variation within localized samples of marine *v.* freshwater taxa, despite the fact that similar laboratory methods and levels of effort were used to isolate and characterize microsatellite loci from populations in these two realms. A tendency toward greater genetic variation within local populations of marine *v.* freshwater fishes likewise was reported in comparative reviews of multi-locus allozyme data (Gyllensten, 1985; Ward *et al.*, 1994). Here, a developing literature on microsatellite variation has been summarized to address the possible generality of this suspected trend. Also, the estimates for fishes have been placed in a broader context of values reported for non-piscine taxa.

## MATERIALS AND METHODS

The literature search was conducted in May 1999. Although not exhaustive, it was intended to capture any major patterns of microsatellite variation in natural populations. A total of 78 relevant publications was identified (see References), from which observed allelic numbers and estimates of heterozygosity were extracted from each of 524 microsatellite loci. The mean number of individuals scored per population was 53, and no locus was included in this summary at which genotypes had been scored from <10 specimens. This review was restricted to microsatellite loci cloned from the target species.

Single-locus heterozygosities ( $h_e$ ) were those reported by the original authors, or where necessary were recalculated as expected values from published allele frequencies:

$$h_e = 1 - \sum f_i^2,$$

where  $f_i$  is the frequency of the  $i$ th allele. In some cases, allele frequencies were not published and the reported heterozygosities were values observed ( $h_o$ ) rather than those

TABLE I. Genetic variation at microsatellite loci in fish and other animals

Organismal group	No. of loci	No. of species	No. of individuals	<i>a</i>	<i>h</i> †	<i>A</i>	<i>H</i>
Freshwater fish	75	13	7755	7.5 (8.1)	0.46 (0.34)	9.1 (6.1)	0.54 (0.25)
Anadromous fish	43	7	5393	11.3 (10.1)	0.68 (0.22)	10.8 (7.2)	0.68 (0.12)
Marine fish	66	12	6005	20.6 (11.8)	0.79 (0.26)	19.9 (6.6)	0.77 (0.19)
			Combined:	13.1 (11.5)	0.63 (0.32)	13.5 (8.1)	0.66 (0.22)
Other animals	340	46	20 567	7.1 (5.6)	0.58 (0.25)	7.7 (4.6)	0.60 (0.16)

†For comparison, the following heterozygosity values (within subpopulations) based on allozyme data were reported by Ward *et al.* (1994):  $0.046 \pm 0.005$  (for 49 freshwater species);  $0.052 \pm 0.008$  (for seven anadromous species); and  $0.059 \pm 0.004$  (for 57 marine species).

*a* refers to allelic diversity when each locus is treated independently; likewise, *h* refers to heterozygosity averaged across all loci within a category. *A* and *H* are the average number of alleles per locus per species and average heterozygosity per species across loci, respectively. Numbers in parentheses indicate 1 s.d.

expected under Hardy-Weinberg equilibrium (HWE). These estimates nonetheless were included in the summary because, in most studies where comparisons could be made,  $h_o$  and  $h_e$  were in reasonably close agreement. Mean heterozygosities per species across *n* loci were calculated as  $H = \sum h n^{-1}$ . Mean numbers of alleles per locus per species were tallied as  $A = \sum a n^{-1}$  where *a* is the number of different-size alleles observed at a locus.

Levels of genetic variation in different groups of organisms were compared using a Kruskal–Wallis analysis of variance. This conservative rank-sum test is non-parametric and makes no assumptions about the underlying distributions. Tests were conducted on data compiled in two ways: as the per-locus *h* and *a* values; and as mean *H* and *A* values per species. Fewer data points (and degrees of freedom) are entailed in the latter compilation so it is a more conservative statistical approach. Furthermore, the latter procedure probably provides a more meaningful outcome biologically if estimates of genetic variation across multiple microsatellite loci are correlated (i.e., not entirely independent) within a species. This might be the case if genetic variation is governed by evolutionarily effective population sizes or other genomically-pervasive factors more so than by locus-specific influences such as, for example, the evolutionary age or the size of a microsatellite locus. In all cases, the null hypothesis underlying the Kruskal–Wallis tests was that mean genetic variability did not differ among the organismal groups under comparison.

This report deals exclusively with the within-population component of genetic variation. In large part, this was necessitated by the nature of the available literature, the vast majority of which reports the isolation and characterization of microsatellite loci from only one or a few localized population samples of a species. In a few published studies, widely-separated conspecific populations were surveyed, in which case the estimates of genetic variation presented here represent the mean of within-population values (rather than estimates from pooled population samples).

## RESULTS

The observed number of alleles per locus ranged from one (in many species) to 50 (in salmon), with a grand mean of 9.2 different size-detected alleles per gene (Table I; Fig. 1). Single-locus heterozygosities ranged from 0 to 1.00, with a mean value of 0.60 across all loci (Figs 1 and 2).

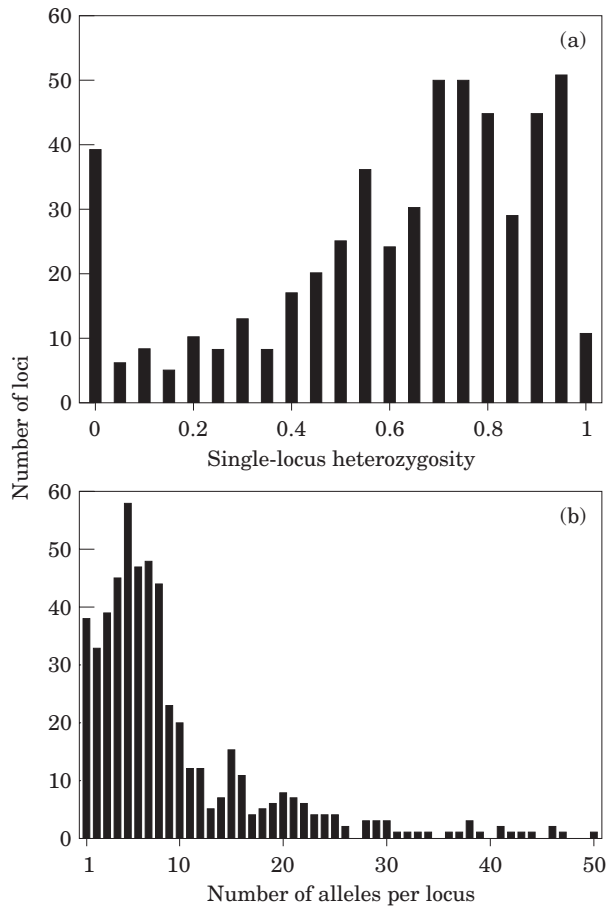


FIG. 1. Frequency distributions of (a) single-locus heterozygosities and (b) number of alleles per locus at each of 524 microsatellite loci within local populations of 78 fish and other animal species.

In most comparisons (the only exception involving  $h$  values), mean levels of genetic variation in the freshwater fishes ( $a=7.5$ ;  $A=9.1$ ;  $h=0.46$ ;  $H=0.54$ ) were similar to and did not differ significantly from the corresponding values ( $a=7.1$ ;  $A=7.7$ ;  $h=0.58$ ;  $H=0.60$ ) reported in the non-piscine taxa surveyed (Tables I and II). By contrast, significantly higher mean numbers of alleles per locus ( $a=20.6$ ;  $A=19.9$ ) and mean heterozygosities ( $h=0.79$ ;  $H=0.77$ ) characterized the assayed marine fishes. This trend is illustrated by a noticeable clustering of the single-locus variation values for marine fishes near the upper ends of the distributions of  $h$  and  $a$  in other animals (Fig. 2).

The difference between marine and freshwater fishes can be seen [Fig. 3(a)] as due primarily to the considerable proportion of loci with heterozygosities near zero in freshwater species and the rather high proportion of loci with heterozygosities  $>0.90$  in the surveyed marine taxa. Surveyed populations of anadromous fishes (primarily salmon) were generally intermediate to marine and freshwater species with respect to mean levels of microsatellite variation (Tables I and II; Fig. 3).

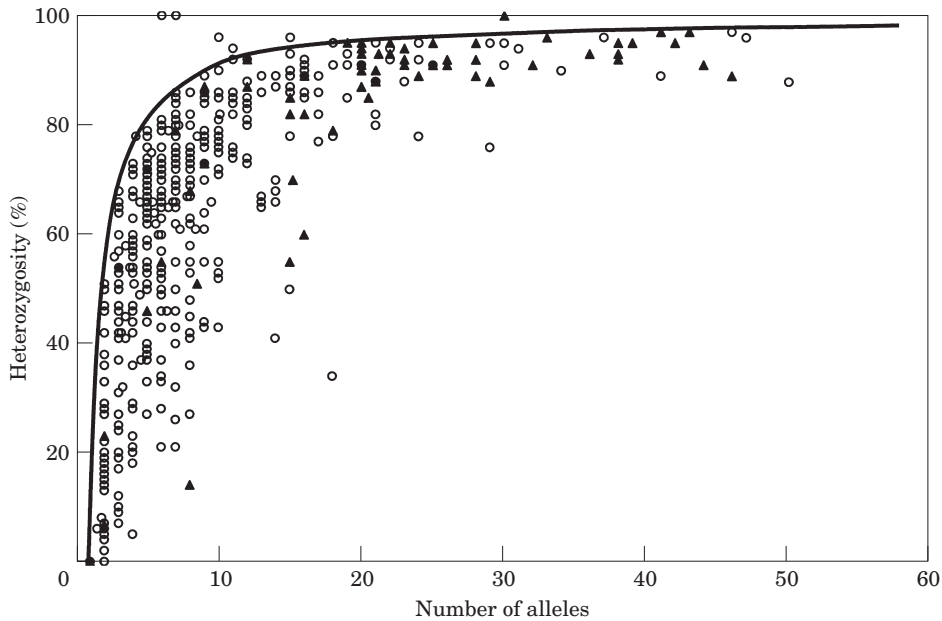


FIG. 2. Scatter plot of heterozygosity *v.* observed numbers of alleles across 524 loci in local populations of 78 animal species. The solid curve shows the expected relationship of heterozygosity to the effective numbers of alleles (i.e., when all alleles are equally frequent; Crow & Kimura, 1970). Reported heterozygosities normally fall beneath this curve, indicating inequitable allelic frequencies. The few points above the line involve the observed rather than the heterozygosities expected under HWE, and presumably reflect sampling errors associated with small numbers of individuals assayed. ▲, Marine fish; ○, other animals.

TABLE II. Statistical comparisons of genetic variation in fishes from three environmental regimes, and in other animals

Comparison	<i>P</i> values (Kruskal–Wallis test) for			
	<i>a</i>	<i>h</i>	<i>A</i>	<i>H</i>
Freshwater <i>v.</i> marine fishes	<0.01**	<0.01**	<0.01**	0.02*
Freshwater <i>v.</i> anadromous fishes	<0.01**	<0.01**	0.38	0.17
Anadromous <i>v.</i> marine fishes	<0.01**	<0.01**	0.03*	0.09
Fishes <i>v.</i> other animals (overall)	<0.01**	<0.01**	<0.01**	0.07
Freshwater fishes <i>v.</i> non-piscines	0.08	<0.01**	0.77	0.48
Anadromous fishes <i>v.</i> non-piscines	0.02*	0.01**	0.29	0.27
Marine fishes <i>v.</i> non-piscines	<0.01**	<0.01**	<0.01**	<0.01**

Probability (*P*) values are shown in comparisons treating the data on genetic variability as single-locus estimates (columns *a* and *h*) and as mean values per species (*A* and *H*).

### DISCUSSION

The salient finding of this literature review is that local population samples of marine fishes tend to display, on average, significantly higher heterozygosities and numbers of alleles per locus than do freshwater fish. This is probably not an

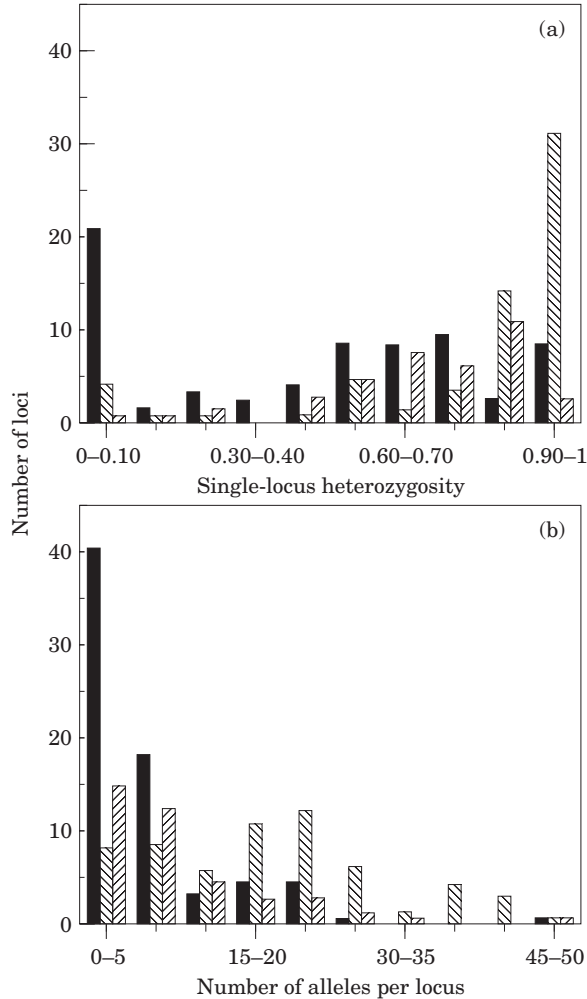


FIG. 3. Frequency distributions of (a) single-locus heterozygosities and (b) number of alleles per locus in localized populations of fish species inhabiting different environmental realms. ■, Freshwater fishes (13 species); ▨, marine fishes (12 species); ▩, anadromous fishes (seven species).

artifact of different protocols for genetic screening because standardized laboratory procedures were used to assay nearly equal numbers of species, individuals, and loci from these two realms. Yet, in the available studies, a new microsatellite allele was detected on average for each 4.4 additional conspecific marine specimens assayed, compared to about 13.7 such specimens in the freshwater fishes.

All mean estimates of within-population microsatellite variation summarized here are far higher than those recorded in earlier allozyme surveys. For example, whereas many microsatellite loci harboured in excess of 20 detected alleles, seldom were  $>c. 5$  alleles per locus reported in any protein-electrophoretic study. Furthermore, within-population heterozygosities averaged more than an order-of-magnitude higher at microsatellite loci ( $h$  and  $H \approx 0.60$ ) than at allozyme genes (0.05; Ward *et al.*, 1994). Some of this disparity probably can be attributed

to a reporting bias in the literature. In multi-locus allozyme surveys, typically data from monomorphic as well as polymorphic genes are provided, whereas frequently microsatellite studies describe variation only at the polymorphic loci that proved useful in the genetic assessments that were the subject of the study. Nonetheless, reporting bias alone clearly cannot account for the huge disparity between microsatellite and allozyme loci in terms of heterozygosities and allelic numbers at the middle and upper tiers of the spectrum.

With respect to comparisons at microsatellite loci, there is a trend toward increased genetic variation in the progression from freshwater to anadromous to marine fishes (Tables I and II). This is evidenced in the mean heterozygosity values for fishes in these three realms ( $h=0.46, 0.68$  and  $0.79$ , respectively;  $H=0.54, 0.68$  and  $0.77$ ) as well as in mean numbers of alleles per locus ( $a=7.5, 11.3$  and  $20.6$ , respectively;  $A=9.1, 10.8$  and  $19.9$ ). These tendencies generally mirror the trend reported previously in allozyme surveys ( $H=0.046, 0.052, 0.059$ ) for fish species inhabiting these respective environmental settings (Ward *et al.*, 1994).

It is suspected that this inclination toward greater genetic variation in local populations of marine as compared to freshwater fishes may reflect larger evolutionarily effective population sizes of marine fish species, on average, which in turn may be related to the larger and more continuous nature of the marine environment. Populations of freshwater fishes are confined to particular drainages over short to moderate evolutionary time, and, therefore, should be smaller in size (all else being equal) than many marine populations which are open to potential genetic exchange with conspecifics over much larger areas.

With respect to the expected level of genetic variation, a local population in the marine realm probably stretches over a much larger area and encompasses many more individuals in an extended temporal sense than does a typical freshwater fish population confined to a particular lake or river drainage for long periods of time. A related possibility is that across centuries or millennia, the sea often may be a less hostile environment than lakes and rivers, meaning that its populations in general might be less susceptible to dramatic population reductions (such as those mediated by Pleistocene glacial effects) or other forms of variation-reducing selection (Mitton & Lewis, 1989).

Microsatellite data can be used to calculate provisional estimates of evolutionarily effective population size ( $N_e$ ) given particular assumptions about mutation rates and patterns (Forbes *et al.*, 1995). For example, under a classic stepwise mutation model (Ohta & Kimura, 1973), heterozygosity at equilibrium is equal to  $1 - (1 + 8 N_e \mu)^{-0.5}$ , where  $\mu$  is the mutation rate to neutral alleles. Using this formula, and assuming microsatellite mutation rates in the range  $10^{-2}$ – $10^{-4}$  (Weber & Wong, 1993; Primmer *et al.*, 1996; Schug *et al.*, 1997), the mean  $h$  values in Table I yield estimates of  $N_e=250$ – $25\,000$  for the surveyed marine fish *v.*  $N_e=30$ – $3000$  for freshwater fish, about a 10-fold difference for a given mutation rate. Several uncertainties apply to assumptions underlying such calculations, so probably the relative rather than absolute estimates of  $N_e$  should be afforded greater credence.

Even if differences in evolutionarily  $N_e$  generally account for the higher levels of genetic variation reported in marine *v.* freshwater fishes, any parallel trends between allozymes and microsatellite loci should be far from perfect, in part



because the two sets of loci presumably are affected by population demographic factors operative over different historical time frames. For example, a prolonged population bottleneck probably would leave a much longer-lasting genetic footprint for allozymes than for microsatellite loci because the latter can recover variation far more quickly due to higher mutation rates. Instances certainly are known in which extensive polymorphism at microsatellite loci contrasts sharply with low variation at allozyme genes (e.g., Hughes & Queller, 1993).

For reasons mentioned earlier, the current review does not consider explicitly the between-population component of microsatellite variation (which should be an object of increased empirical attention in the future; Hauser & Ward, 1998). However, both protein-electrophoretic and mitochondrial DNA data clearly document significantly greater population genetic structure on average in freshwater as compared with marine fish species over equivalent geographic scales (Ward *et al.*, 1994; Avise, 2000). Given the large number of alleles often observed at microsatellite loci, sample sizes in comparable geographic surveys based on microsatellites will have to be much larger to distinguish *bona fide* population structure from sampling artifacts.

It remains to be seen whether the provisional trends revealed in this early review of the microsatellite literature will be supported as more data sets become available. With additional surveys, surely some marine fishes with severely constrained microsatellite variation will be found, because natural populations in any environment must be susceptible to bottlenecks and other diversity-reducing evolutionary forces. Conversely, at least some freshwater fishes are likely to be identified with levels of genetic variation rivaling those of the most polymorphic marine species. This being the case, and given the great variety of molecular, ecological, and evolutionary factors that are known to impact levels of variation at microsatellite loci (Goldstein & Schlötterer, 1999), it seems surprising (and thus intriguing) that recognizable patterns nonetheless seem to emerge across taxonomically diverse organisms inhabiting alternative environmental regimes.

Work in the Avise lab is supported by the Pew Foundation and by funds from the University of Georgia. We thank two anonymous reviewers, A. Fiumera, A. Keyser, M. Mackiewicz, B. McCoy, B. Nelson, D. Pearce, B. Porter, D. Promislow, C. Spencer, and D. Walker for helpful comments on the manuscript.

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