Journal of Fish Biology (2000) **56,** 461–473
doi: 10.1006/jfbi.1999.1210, available online at http://www.idealibrary.com on IDEAL®

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.

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

0022–1112/00/030461+13 $35.00/0

& 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, 1997*a*,*b*; DeWoody *et al.*, 1998, 2000; Jones *et al.*, 1998*a*,*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:

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

where f_i is the frequency of the ith 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	*a*	*h*†	*A*	*H*
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): $0{\cdot}046 \pm 0{\cdot}005$ (for 49 freshwater species); $0{\cdot}052 \pm 0{\cdot}008$ (for seven anadromous species); and $0{\cdot}059 \pm 0{\cdot}004$ (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 $H=\Sigma h\ n^{-1}$. Mean numbers of alleles per locus per species were tallied as $A=\Sigma a\ n^{-1}$ 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).

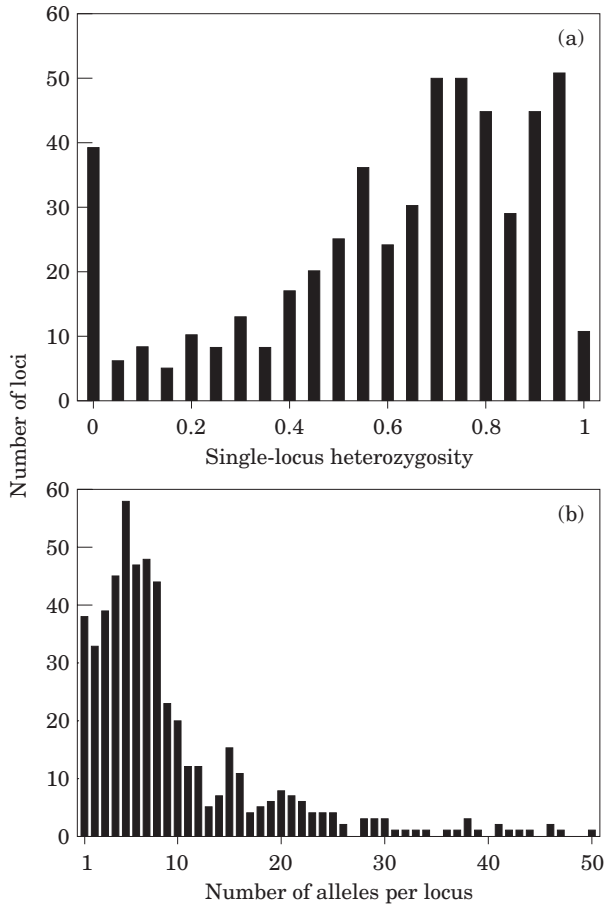

FIG. 1. Frequency distributions of (a) single-locus heterozygosities and (b) number of alleles per locus at each of 524 microsatellite loci within local populations of 78 fish and other animal species.

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).

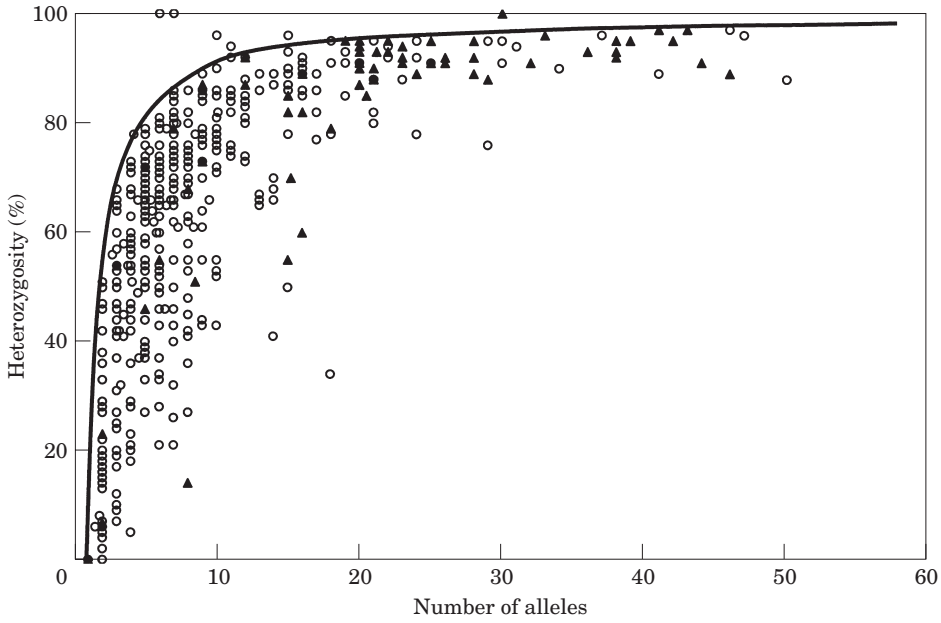

FIG. 2. Scatter plot of heterozygosity *v.* observed numbers of alleles across 524 loci in local populations of 78 animal species. The solid curve shows the expected relationship of heterozygosity to the effective numbers of alleles (i.e., when all alleles are equally frequent; Crow & Kimura, 1970). 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	*P* values (Kruskal–Wallis test) for			
	a	*h*	*A*	*H*
Freshwater *v.* marine fishes	<0·01**	<0·01**	<0·01**	0·02*
Freshwater *v.* anadromous fishes	<0·01**	<0·01**	0·38	0·17
Anadromous *v.* marine fishes	<0·01**	<0·01**	0·03*	0·09
Fishes *v.* other animals (overall)	<0·01**	<0·01**	<0·01**	0·07
Freshwater fishes *v.* non-piscines	0·08	<0·01**	0·77	0·48
Anadromous fishes *v.* non-piscines	0·02*	0·01**	0·29	0·27
Marine fishes *v.* 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

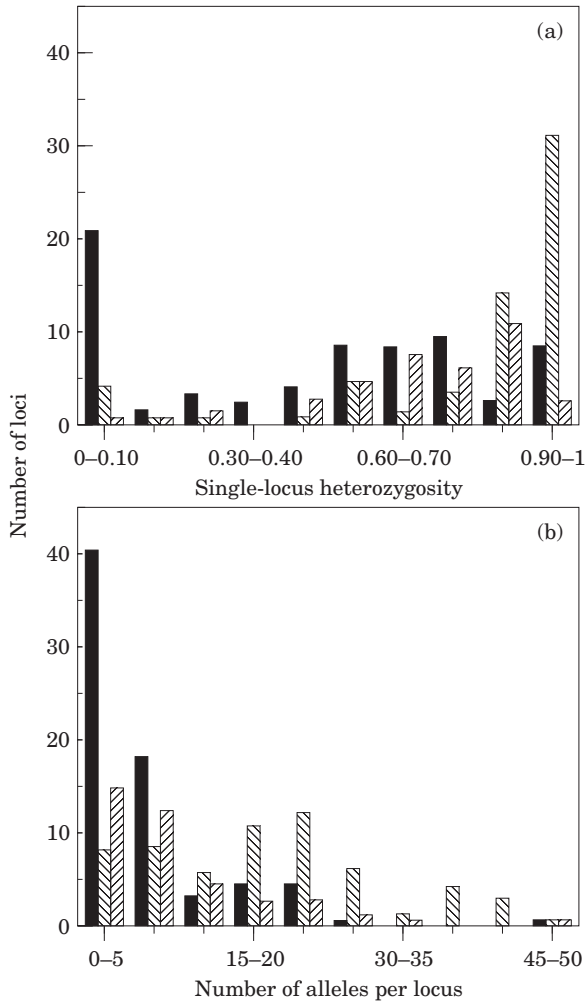

FIG. 3. Frequency distributions of (a) single-locus heterozygosities and (b) number of alleles per locus in localized populations of fish species inhabiting different environmental realms. ■, Freshwater fishes (13 species); ▨, marine fishes (12 species); ▨, anadromous fishes (seven species).

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 $H \simeq 0{\cdot}60$) 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 μ 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. Pearse, B. Porter, D. Promislow, C. Spencer, and D. Walker for helpful comments on the manuscript.

References

(*papers contributing to the database of this review)

*Allen, P. J., Amos, W., Pomeroy, P. P. & Twiss, S. D. (1995). Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. *Molecular Ecology* **4,** 653–662.

Avise, J. C. (2000). *Phylogeography: The History and Formation of Species*. Cambridge, MA: Harvard University Press.

*Bagley, M. J. & Geller, J. B. (1998). Characterization of microsatellite loci in the vermillion snapper *Rhomboplites aurorubens* (Percoidei: Lutjanidae). *Molecular Ecology* **7,** 1089–1090.

*Becher, S. A. & Griffiths, R. (1997). Isolation and characterization of six polymorphic microsatellite loci in the European hedgehog *Erinaceus europaeus*. *Molecular Ecology* **6,** 89–90.

*Blouin, M. S., Parsons, M., LaCaille, V. & Lotz, S. (1996). Use of microsatellites to classify individuals by relatedness. *Molecular Ecology* **5,** 393–401.
*Borer, S. O., Miller, L. M. & Kapuscinski, A. R. (1999). Microsatellites in the walleye *Stizostedion vitreum. Molecular Ecology* **8,** 336–338.
*Brooker, A. L., Cook, D., Bentzen, P., Wright, J. M. & Doyle, R. W. (1994). Organization of microsatellites differs between mammals and cold-water teleost fishes. *Canadian Journal of Fisheries and Aquatic Sciences* **51,** 1959–1966.
*Buchanan, F. C., Friesen, M. K., Littlejohn, R. P. & Clayton, J. W. (1996). Microsatellites from the beluga whale *Delphinapterus leucas. Molecular Ecology* **5,** 571–575.
*Cathey, J. C., DeWoody, J. A. & Smith, L. M. (1998). Microsatellite markers in Canada geese (*Branta canadensis*). *Journal of Heredity* **89,** 173–175.
*Chapuisat, M. (1996). Characterization of microsatellite loci in *Formica lugubris B* and their variability in other ant species. *Molecular Ecology* **5,** 599–601.
*Choundary, M., Strassman, J. E., Solís, C. R. & Queller, D. C. (1993). Microsatellite variation in a social insect. *Biochemical Genetics* **31,** 87–96.
*Colbourne, J. K., Neff, B. D., Wright, J. M. & Gross, M. R. (1996). DNA fingerprinting of bluegill sunfish (*Lepomis macrochirus*) using $(GT)_n$ microsatellites and its potential for assessment of mating success. *Canadian Journal of Fisheries and Aquatic Sciences* **53,** 342–349.
*Coltman, D. W., Bowen, W. D. & Wright, J. M. (1996). PCR primers for harbour seal (*Phoca vitulina concolour*) microsatellites amplify polymorphic loci in other pinniped species. *Molecular Ecology* **5,** 161–163.
*Condrey, M. J. & Bentzen, P. (1998). Characterization of coastal cutthroat trout (*Oncorhynchus clarki clarki*) microsatellites and their conservation in other salmonids. *Molecular Ecology* **7,** 789–791.
*Cooper, G., Miller, P. L. & Holland, P. W. H. (1996). Molecular genetic analysis of sperm competition in the damselfly *Ischnura elegans* (Vander Linden). *Proceedings of the Royal Society of London Series B* **263,** 1343–1349.
Crow, J. F. & Kimura, M. (1970). *An Introduction to Population Genetics Theory*. New York: Harper & Row.
*DeWoody, J. A., Honeycutt, R. L. & Skow, L. C. (1995). Microsatellite markers in white-tailed deer. *Journal of Heredity* **86,** 317–319.
*DeWoody, J. A., Fletcher, D., Wilkins, S. D., Nelson, W. & Avise, J. C. (1998). Molecular genetic dissection of spawning, parentage, and reproductive tactics in a population of redbreast sunfish, *Lepomis auritus. Evolution* **52,** 1802–1810.
*DeWoody, J. A., Fletcher, D., Wilkins, S. D. & Avise, J. C. (2000). Microsatellite analyses of parentage and nest guarding in the tessellated darter (Pisces: Percidae). *Copeia* in press.
Dib, C. & 13 others (1996). A comprehensive genetic map of the human genome based on 5264 microsatellites. *Nature* **380,** 152–154.
*Ellegren, H., Johansson, M., Sandberg, K. & Andersson, L. (1992). Cloning of highly polymorphic microsatellites in the horse. *Animal Genetics* **23,** 133–142.
*Ellegren, H., Lifjeld, J. T., Slagsvold, T. & Primme, C. R. (1995). Handicapped males and extrapair paternity in pied flycatchers: a study using microsatellite markers. *Molecular Ecology* **4,** 739–744.
*Estoup, A., Presa, P., Krieg, F., Vaiman, D. & Guyomard, R. (1993). $(CT)_n$ and $(GT)_n$ microsatellites: a new class of genetic markers for *Salmo trutta* L. (brown trout). *Heredity* **71,** 488–496.
*Estoup, A., Garnery, L., Solignac, M. & Cornuet, J.-M. (1995). Microsatellite variation in honey bee (*Apis melifera* L.) populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models. *Genetics* **140,** 679–695.
*Evans, J. D. (1993). Parentage analysis in ant colonies using simple sequence repeat loci. *Molecular Ecology* **2,** 393–397.
Forbes, S. H., Hogg, J. T., Buchanan, F. C., Crawford, A. M. & Allendorf, F. W. (1995). Microsatellite evolution in congeneric mammals: domestic and bighorn sheep. *Molecular Biology and Evolution* **12,** 1106–1113.

*Galbusera, P., Volckaert, F. A., Hellemans, B. & Ollevier, F. (1996). Isolation and characterization of microsatellite markers in the African catfish *Clarius gariepinus* (Burchell, 1822). *Molecular Ecology* **5,** 703–705.

*Garcia de Leon, F. J., Chikhi, L. & Bonhomme, F. (1997). Microsatellite polymorphism and population subdivision in natural populations of European sea bass *Dicentrarchus labrax*. *Molecular Ecology* **6,** 51–62.

*Garza, J. C., Slatkin, M. & Freimer, N. B. (1995). Microsatellite allele frequencies in humans and chimpanzees, with implications for constraints on allele size. *Molecular Biology and Evolution* **12,** 594–603.

*Gibbs, H. L., Brooke, M. L. & Davies, N. B. (1996). Analysis of genetic differentiation of host races of the common cuckoo *Cuculus canorus* using mitochondrial and microsatellite DNA variation. *Proceedings of the Royal Society of London Series B* **263,** 89–96.

*Gibbs, H. L., Miller, P., Alderson, G. & Sealy, G. (1997). Genetic analysis of brown-headed cowbirds *Molothrus ater* raised by different hosts: data from mtDNA and microsatellite DNA markers. *Molecular Ecology* **6,** 189–193.

*Glenn, T. C., Stephan, W., Dessauer, H. C. & Braun, M. J. (1996). Allelic diversity in alligator microsatellite loci is negatively correlated with GC content of flanking sequences and evolutionary conservation of PCR amplifiability. *Molecular Biology and Evolution* **13,** 1151–1154.

Goldstein, D. B. & Schlötterer, C. (Eds) (1999). *Microsatellites: Evolution and Applications*. Oxford: Oxford University Press.

*Gottelli, D., Sillero-Zubiri, C., Applebaum, G. D., Roy, M. S., Girman, D. J., Garcia-Moreno, J., Ostrander, E. A. & Wayne, R. K. (1994). Molecular genetics of the most endangered canid: the Ethiopian wolf *Canis simensis*. *Molecular Ecology* **3,** 301–312.

Gyllensten, U. (1985). The genetic structure of fish: differences in the intraspecific distribution of biochemical genetic variation between marine, anadromous, and freshwater species. *Journal of Fish Biology* **26,** 691–699.

*Hanotte, O., Zanon, C., Pugh, A., Creig, C., Dixon, A. & Burke, T. (1994). Isolation and characterization of microsatellite loci in a passerine bird: the reed bunting *Emberiza schoeniclus*. *Molecular Ecology* **3,** 529–530.

Hauser, L. & Ward, R. D. (1998). Population identification in pelagic fish: the limits of molecular markers. In *Advances in Molecular Ecology* (Carvalho, G. R., ed.), pp. 191–224. Amsterdam: IOS Press.

*Heist, E. J. & Gold, J. R. (1999). Microsatellite DNA variation in sandbar sharks (*Carcharhinus plumbeus*) from the Gulf of Mexico and mid-Atlantic Bight. *Copeia* **1999,** 182–186.

*Holmes, N. G., Mellersh, C. S., Humphreys, S. J., Binns, M. M., Holliman, A., Curtis, R. & Sampson, J. (1993). Isolation and characterization of microsatellites from the canine genome. *Animal Genetics* **24,** 289–292.

*Hughes, C. R. & Queller, D. C. (1993). Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. *Molecular Ecology* **2,** 131–137.

*Ishibashi, Y., Saitoh, T., Abe, S. & Yoshida, M. C. (1997). Sex-related spatial kin structure in a spring population of grey-sided voles *Clethrionomys rufocanus* as revealed by microsatellite DNA analyses. *Molecular Ecology* **6,** 63–71.

*Jarne, P., Viard, F., Delay, B. & Cuny, G. (1994). Variable microsatellites in the highly selfing snail *Bulinus truncatus* (Basommatophora: Planorbidae). *Molecular Ecology* **3,** 527–528.

Jarne, P. & Lagoda, P. J. L. (1996). Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution* **11,** 424–429.

*Jones, A. G. & Avise, J. C. (1997*a*). Microsatellite analysis of maternity and the mating system in the Gulf pipefish *Syngnathus scovelli*, a species with male pregnancy and sex role reversal. *Molecular Ecology* **6,** 202–213.

*Jones, A. G. & Avise, J. C. (1997*b*). Polygynandry in the dusky pipefish *Syngnathus floridae* revealed by microsatellite DNA markers. *Evolution* **51,** 1611–1622.

Jones, A. G., Kvarnemo, C., Moore, G. I., Simmons, L. W. & Avise, J. C. (1998*a*). Microsatellite evidence for monogamy and sex-biased recombination in the Western Australian seahorse *Hippocampus angustus*. *Molecular Ecology* **7,** 1497–1505.

*Jones, A. G., Östlund-Nilsson, S. & Avise, J. C. (1998*b*). A microsatellite assessment of sneaked fertilizations and egg thievery in the fifteenspine stickleback. *Evolution* **52,** 848–858.

*Jones, A. G., Stockwell, G. A., Walker, D. & Avise, J. C. (1998*c*). The molecular basis of a microsatellite null allele from the White Sands pupfish. *Journal of Heredity* **89,** 339–342.

*Jones, A. G., Rosenqvist, G., Berglund, A. & Avise, J. C. (1999). The genetic mating system of a sex-role-reversed pipefish (*Syngnathus typhle*): a molecular inquiry. *Behavioral Ecology and Sociobiology* **46,** 357–365.

*Kayser, M., Ritter, H., Bercovitch, F., Mrug, M., Roewer, L. & Nürnberg, P. (1996). Identification of highly polymorphic microsatellites in the rhesus macaque *Macaca mulatta* by cross-species amplification. *Molecular Ecology* **5,** 157–159.

*Kellogg, K. A., Markert, J. A., Stauffer, J. R. Jr & Kocher, T. D. (1995). Microsatellite variation demonstrates multiple paternity in lekking cichlid fishes from Lake Malawi, Africa. *Proceedings of the Royal Society of London Series B* **260,** 79–84.

*Lade, J. A., Murray, N. D., Marks, C. A. & Robinson, N. A. (1996). Microsatellite differentiation between Phillip Island and mainland Australian populations of the red fox *Vulpes vulpes*. *Molecular Ecology* **5,** 81–87.

*Largiader, C. R., Fries, V., Kobler, B. & Bakker, C. M. (1999). Isolation and characterization of microsatellite loci from the three-spined stickleback *Gasterosteus aculeatus*. *Molecular Ecology* **8,** 342–344.

Litt, M. & Luty, J. A. (1989). A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics* **44,** 387–401.

*MacHugh, D. E., Loftus, R. T., Bradley, D. G., Sharp, P. M. & Cunningham, P. (1994). Microsatellite DNA variation within and among European cattle breeds. *Proceedings of the Royal Society of London Series B* **256,** 25–31.

*McConnell, S., Ruzzante, D. E., O'Reilly, P. T., Hamilton, L. & Wright, J. M. (1997). Microsatellite loci reveal highly significant genetic differentiation among Atlantic salmon (*Salmo salmar* L.) stocks from the east coast of Canada. *Molecular Ecology* **6,** 1075–1089.

*Michalakis, Y. & Veuille, M. (1996). Length variation of CAG/CAA trinucleotide repeats in natural populations of *Drosophila melanogaster* and its relation to the recombination rate. *Genetics* **143,** 1713–1725.

Mitton, J. B. & Lewis, W. M. (1989). Relationships between genetic variability and life-history features of bony fishes. *Evolution* **43,** 1712–1723.

*Morin, P. A., Wallis, J., Moore, J. J. & Woodruff, D. S. (1994). Paternity exclusion in a community of wild chimpanzees using hypervariable simple sequence repeats. *Molecular Ecology* **3,** 469–478.

*Mundy, N. I. & Woodruff, D. S. (1996). Polymorphic microsatellite markers in the loggerhead shrike *Lanius ludovicianus* isolated from a library enriched for CA repeats. *Molecular Ecology* **5,** 811–813.

*Naish, K. A. & Skibinski, D. O. F. (1998). Tetranucleotide microsatellite loci for Indian major carp. *Journal of Fish Biology* **53,** 886–889.

*Neumann, K. & Wetton, J. H. (1996). Highly polymorphic microsatellites in the house sparrow *Passer domesticus*. *Molecular Ecology* **5,** 307–309.

*O'Connell, M., Wright, J. M. & Farid, A. (1996). Development of PCR primers for nine polymorphic American mink *Mustela vison* microsatellite loci. *Molecular Ecology* **5,** 311–312.

*O'Connell, M., Dillon, M. C. & Wright, J. M. (1998). Development of primers for polymorphic microsatellite loci in the Pacific herring (*Clupea harengus pallasi*). *Molecular Ecology* **7,** 358–360.

Ohta, T. & Kimura, M. (1973). A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genetical Research* **22,** 201–204.

*Olsen, J. B., Bentzen, P. & Seeb, J. E. (1998). Characterization of seven microsatellite loci derived from pink salmon. *Molecular Ecology* **7,** 1087–1089.

*O'Reilly, P. T., Hamilton, L. C., McConnell, S. K. & Wright, J. M. (1996). Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Canadian Journal of Fisheries and Aquatic Sciences* **53,** 2292–2298.

*Paetkau, D. & Strobeck, C. (1994). Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology* **3,** 489–495.

*Paetkau, D., Calvert, W., Stirling, I. & Strobeck, C. (1995). Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology* **4,** 347–354.

*Parker, A. & Kornfield, I. (1996). Polygynandry in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. *Environmental Biology of Fishes* **47,** 345–352.

*Parker, K. M., Hughes, K., Kim, T. J. & Hedrick, P. W. (1998). Isolation and characterization of microsatellite loci from the Gila topminnow (*Poeciliopsis o. occidentalis*) and their utility in guppies (*Poecilia reticulata*). *Molecular Ecology* **7,** 361–363.

*Paxton, R. J., Thorén, P. A., Tengö, J., Estoup, A. & Pamilo, P. (1996). Mating structure and nestmate relatedness in a communal bee, *Andrena jacobi* (Hymenoptera, Andrenidae), using microsatellites. *Molecular Ecology* **5,** 511–519.

*Piertney, S. B. & Dallas, J. F. (1997). Isolation and characterization of hypervariable microsatellites in the red grouse *Lagopus lagopus scoticus. Molecular Ecology* **6,** 93–95.

*Pope, L. A., Sharp, A. & Moritz, C. (1996). Population structure of the yellow-footed rock-wallaby *Petrogale xanthopus* (Gray, 1854) inferred from mtDNA sequences and microsatellite loci. *Molecular Ecology* **5,** 629–640.

*Primmer, C. R., Møller, A. P. & Ellegren, H. (1995). Resolving genetic relationships with microsatellite markers: a parentage testing system for the swallow *Hirundo rustica. Molecular Ecology* **4,** 493–498.

Primmer, C. R., Ellegren, H., Saino, N. & Møller, A. P. (1996). Directional evolution in germline microsatellite mutations. *Nature Genetics* **13,** 391–393.

*Prodohl, P. A., Loughry, W. J., McDonough, C. M., Nelson, W. S. & Avise, J. C. (1996). Molecular documentation of polyembryony and the micro-satial dispersion of clonal sibships in the nine-banded armadillo, *Dasypus novemcinctus. Proceedings of the Royal Society of London Series B* **263,** 1643–1649.

*Richard, K. R., Whitehead, H. & Wright, J. M. (1996). Polymorphic microsatellites from sperm whales and their use in the genetic identification of individuals from naturally sloughed pieces of skin. *Molecular Ecology* **5,** 313–315.

Rubinsztein, D. C., Amos, W., Leggo, J., Goodburn, S., Jain, S., Li, S.-H., Margolis, R. L., Ross, C. A. & Ferguson-Smith, M. A. (1995). Microsatellite evolution—evidence for directionality and variation in rate between species. *Nature Genetics* **10,** 337–343.

Schlötterer, C. & Pemberton, J. (1994). The use of microsatellites for genetic analysis of natural populations. In *Molecular Approaches to Ecology and Evolution* (Schierwater, B., Streit, B., Wanger, G. & DeSalle, R., eds), pp. 203–214. Basel: Birkhauser Verlag.

Schug, M. D., Mackay, T. F. C. & Aquadro, C. F. (1997). Low mutation rates of microsatellite loci in *Drosophila melanogaster. Nature Genetics* **15,** 99–102.

*Scribner, K. T., Arntzen, J. W. & Burke, T. (1994). Comparative analysis of intra- and interpopulation genetic diversity in *Bufo bufo*, using allozyme, single-locus microsatellite, minisatellite, and multilocus minisatellite data. *Molecular Biology and Evolution* **11,** 737–748.

*Spencer, C. C., Chlan, C. A., Neigel, J. E., Scribner, K. T., Wooten, M. C. & Leberg, P. L. (1999). Polymorphic microsatellite markers in the western mosquitofish, *Gambusia affinis. Molecular Ecology* **8,** 157–159.

*Spencer, P. B. S., Odorico, D. M., Jones, S. J., Marsh, H. D. & Miller, D. J. (1995). Highly variable microsatellites in isolated colonies of the rock-wallaby (*Petrogale assimilis*). *Molecular Ecology* **4,** 523–525.

*Stockwell, C. A., Mulvey, M. & Jones, A. G. (1998). Genetic evidence for two evolutionary significant units of White Sands pupfish. *Animal Conservation* **1,** 213–225.

*Strassman, J. E., Solís, C. R., Barefield, K. & Queller, D. C. (1996). Trinucleotide microsatellite loci in a swarm-founding neotropical wasp, *Parachartergus colobopterus* and their usefulness in other social wasps. *Molecular Ecology* **5,** 459–461.

*Strassman, J. E., Barefield, K., Solís, C. R., Hughes, C. R. & Queller, D. C. (1997). Trinucleotide microsatellite loci for a social wasp, *Polistes*. *Molecular Ecology* **6,** 97–100.

*Tagaki, M., Taniguchi, N., Cook, D. & Doyle, R. W. (1997). Isolation and characterization of microsatellite loci from Red Sea bream *Pagrus major* and detection in closely related species. *Fisheries Science* **63,** 199–204.

Tautz, D. (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research* **17,** 6463–6471.

*Taylor, A. C., Sherwin, W. B. & Wayne, R. K. (1994). Genetic variation of microsatellite loci in a bottlenecked species: the northern hairy-nosed wombat *Lasiorhinus krefftii*. *Molecular Ecology* **3,** 277–290.

*Taylor, E. B. (1998). Microsatellites isolated from the threespine stickleback *Gasterosteus aculeatus*. *Molecular Ecology* **7,** 930–931.

*van Oppen, M. J. H., Rico, C., Deutsch, J. C., Turner, G. F. & Hewitt, G. M. (1997). Isolation and characterization of microsatellite loci in the cichlid fish *Pseudotropheus zebra*. *Molecular Ecology* **6,** 387–389.

Ward, R. D., Woodwark, M. & Skibinski, D. O. F. (1994). A comparison of genetic diversity levels in marine, freshwater, and anadramous fishes. *Journal of Fish Biology* **44,** 213–232.

Weber, J. L. & May, P. E. (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics* **44,** 388–396.

Weber, J. L. & Wong, C. (1993). Mutation of human short tandem repeats. *Human Molecular Genetics* **2,** 1123–1128.

*Wooninck, L., Strassman, J. E., Fleischer, R. C. & Warner, R. R. (1998). Characterisation of microsatellite loci in a pelagic spawner: the bluehead wrasse, *Thalassoma bifasciatum*. *Molecular Ecology* **7,** 1613–1614.

*Zane, L., Nelson, W. S., Jones, A. G. & Avise, J. C. (1999). Microsatellite assessment of multiple paternity in natural populations of a live-bearing fish, *Gambusia holbrooki*. *Journal of Evolutionary Biology* **12,** 61–69.

*Zheng, W., Stacey, N. E., Coffin, J. & Strobeck, C. (1995). Isolation and characterization of microsatellite loci in the goldfish *Carassius auratus*. *Molecular Ecology* **4,** 791–792.