EVOLUTION

INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

PUBLISHED BY

THE SOCIETY FOR THE STUDY OF EVOLUTION

Vol. 55 **September 2001** **No. 9**

Evolution, 55(9), 2001, pp. 1717–1733

MICROSATELLITE EVOLUTION IN VERTEBRATES: INFERENCE FROM AC DINUCLEOTIDE REPEATS

BRYAN D. NEFF[1,2] AND MART R. GROSS[3,4]

[1]*Department of Zoology, University of Western Ontario, London, Ontario N6A 5B7, Canada*

[2]*E-mail: bneff@uwo.ca*

[3]*Department of Zoology, University of Toronto, 25 Harbord Street, Toronto, Ontario M5S 3G5, Canada*

[4]*E-mail: mgross@zoo.utoronto.ca*

Abstract.—We analyze published data from 592 AC microsatellite loci from 98 species in five vertebrate classes including fish, reptiles, amphibians, birds, and mammals. We use these data to address nine major questions about microsatellite evolution. First, we find that larger genomes do not have more microsatellite loci and therefore reject the hypothesis that microsatellites function primarily to package DNA into chromosomes. Second, we confirm that microsatellite loci are relatively rare in avian genomes, but reject the hypothesis that this is due to physical constraints imposed by flight. Third, we find that microsatellite variation differs among species within classes, possibly relating to population dynamics. Fourth, we reject the hypothesis that microsatellite structure (length, number of alleles, allele dispersion, range in allele sizes) differs between poikilotherms and homeotherms. The difference is found only in fish, which have longer microsatellites and more alleles than the other classes. Fifth, we find that the range in microsatellite allele size at a locus is largely due to the number of alleles and secondarily to allele dispersion. Sixth, length is a major factor influencing mutation rate. Seventh, there is a directional mutation toward an increase in microsatellite length. Eighth, at the species level, microsatellite and allozyme heterozygosity covary and therefore inferences based on large-scale studies of allozyme variation may also reflect microsatellite genetic diversity. Finally, published microsatellite loci (isolated using conventional hybridization methods) provide a biased estimate of the actual mean repeat length of microsatellites in the genome.

Key words.—Ascertainment bias, directionality, evolution, genome size, microsatellite, mutation, vertebrates.

Received November 30, 2000. Accepted May 24, 2001.

Microsatellites are a widespread and important component of vertebrate genomes (Jarne and Lagoda 1996; Goldstein and Schlötterer 1999), and more is becoming known about their evolution and molecular dynamics, particularly in focal species such as humans. In this paper we analyze published data on fish, reptiles, amphibians, birds, and mammals to address the following major questions: Do microsatellites have a functional role in the genome? Why are microsatellites rare in avian genomes? Do classes or species differ in microsatellite structure (dispersion, length, polymorphism, etc.)? Does microsatellite structure differ between poikilotherms and homeotherms? Is variation in the range of allele size (the difference in the number of repeats between the smallest and largest allele) at a locus due to the number of alleles or allele dispersion (the average number of bases between adjacent alleles)? Do longer microsatellite loci have higher mutation rates? Is there directional mutation toward an increased length? Does microsatellite polymorphism correlate with allozyme polymorphism? Finally, we also address a question regarding sampling bias: Does screening methodology bias isolation toward longer microsatellite loci? We present the results of our analysis of 592 AC microsatellite loci, one of the most abundant microsatellite motifs in vertebrates (Jarne and Lagoda 1996; Hancock 1999), from 98 species of fish, reptiles, amphibians, birds, and mammals.

Researchers have proposed several functional roles for AC microsatellite loci (reviewed by Kashi and Soller 1999). For example, microsatellites may facilitate recombination (Pardue et al. 1987; Stallings et al. 1991), enhance transcriptional activity of genes (Hamada et al. 1982, 1984; but see Stallings et al. 1991), or contribute to chromosomal structure through DNA packaging and condensing (Stallings et al. 1991; Gaillard and Strauss 1994). These hypotheses generate several predictions. If microsatellites are in fact involved in the packaging and condensing of DNA, then larger genomes should contain a greater number, or possibly longer, microsatellite loci. However, if they are involved in recombination or transcription, then no relationship should exist between genome size and the number of microsatellite loci or their average length because most variation in genome size is attributed to differences in the amount of noncoding DNA, which is not transcribed (e.g., Venturini et al. 1987; Vinogradov 1995).

To infer the potential function of microsatellites, we examine the relationship between genome size and AC microsatellite dispersion, number, and length.

Microsatellite loci are thought to be less common in birds as compared to other animals. Primmer et al. (1997) speculated that a lower frequency of microsatellite loci in avian genomes is a result of a reduction in genome size imposed by the evolution of flight, which in turn results in a lower proportion of noncoding DNA. Alternatively, they suggest avian genomes lack evolutionary precursors to microsatellites, especially SINEs, LINEs, and poly(A) tails. The first hypothesis suggests that larger genomes should have proportionately more microsatellite loci. That is, there should be a positive relationship between the number of microsatellite loci and genome size and a negative relationship between microsatellite dispersion, the average number of DNA bases separating adjacent microsatellite loci, and genome size. The latter relationship would exist because the loss of noncoding DNA would result in a greater proportion of coding DNA and therefore fewer microsatellite loci with a greater dispersion. Bats have smaller genomes and fewer microsatellite loci as compared to many other mammals and apparently provide support for this flight hypothesis (Van Den Bussche et al. 1995). The second hypothesis, by contrast, suggests that there will be class-specific differences in the frequency of microsatellite loci attributable to variation in the abundance of microsatellite evolutionary precursors. However, a negative relationship need not exist between microsatellite dispersion and genome size. To address these two hypotheses, we look for class-specific differences in AC microsatellite dispersion and examine its relationship to genome size.

Researchers have hypothesized that structural differences exist between the microsatellite loci of poikilotherms and homeotherms. For example, it has been observed that microsatellite loci are longer, have a greater range in allele size, and are more degenerate (i.e., contain more base substitutions or deletions) in fish as compared to mammals (e.g., Brooker et al. 1994; Colbourne et al. 1996; O'Reilly et al. 1996). Brooker et al. (1994) suggest that the wider range and lower temperatures experienced by fish, and poikilotherms in general, may increase the rate of mutation by DNA polmerases at microsatellite loci, generating the longer and more degenerate microsatellites with the greater range in allele size. To date, however, the structural differences hypothesized between poikilotherms and homeotherms have not been statistically examined. It is also unknown whether such differences exist only between fish and mammals or more generally between poikilotherms and homeotherms. To address these questions we quantify the differences in AC microsatellite length, allele size range, microsatellite dispersion, number of alleles, and allele dispersion among vertebrate species and classes. Then we quantify the differences between the poikilotherms (fish, reptiles, and amphibians) and homeotherms (mammals and birds). We also looked within the fish for differences between those living in temperate versus tropical environments. Finally, we quantify the independent contributions of allele number and allele dispersion to allele size range.

Mutation rates vary considerable among microsatellite loci, and slipped strand mispairing is considered to be the predominant mutational mechanism by which microsatellites mutate and form new alleles (e.g., Schlötterer and Tautz 1992; Strand et al. 1993; reviewed by Eisen 1999). Researchers have proposed that longer and less-degenerate microsatellite loci are more polymorphic, inferring a higher mutation rate (e.g., Weber 1990; Rose and Falush 1998). Longer microsatellite loci may be more susceptible to slipped strand mispairing, whereas point mutations may interrupt repeat sequences and thereby decrease microsatellite length and reduce slippage (Chung et al. 1993; Pépin et al. 1995; Kruglyak et al. 1998). Thus, the large variation in mutation rate across loci may be largely attributed to variation in the length of microsatellite loci and in the degree of degeneracy of the repeat sequence. Although this has been well studied in a select few organism including humans (e.g., Weber 1990), yeast (Wierdl et al. 1997), and swallows (Primmer et al. 1998), its generalization across species and classes has not been established. We therefore examine the relationship between the number of alleles and heterozygosity and the average microsatellite length in each of the five classes.

A particularly interesting finding is that allele size variance in heterozygotes (heterozygote instability) may increase the mutation rate at microsatellite loci. Amos et al. (1996) showed that heterozygotes that differ greatly in allele length are less stable and have a greater tendency to mutate. The difference in length between the two homologues is thought to destabilize the microsatellite and increase its potential for strand slippage mutations during meiosis (see also Rubinsztein et al. 1995; Amos 1999 and references within). They also propose that microsatellite loci have a mutational bias, increasing rather than decreasing in length (see also Amos and Rubinsztein 1996; Primmer et al. 1996a; Hutter et al. 1998; but see Ellegren et al. 1995; Zhu et al. 2000). Such directional mutation has not been examined in microsatellites from a large taxonomic group. We therefore use heterozygote instability as a measure of mutation rate and determine if this measure is correlated with microsatellite length.

A potential relationship between variation in microsatellites and allozymes has not been studied. Nevo et al. (1984) provided a detailed analysis of genetic variation at allozyme loci among classes of organisms. They show that there is no significant difference in heterozygosity among birds, mammals, fish, and reptiles (excluding parthenogenetic species), but the proportion of loci that are polymorphic is significantly higher in birds as compared to either mammals or fishes. Generally, across all classes, ecological and demographic parameters in addition to life-history characteristics contribute to the observed levels of genetic diversity at allozyme loci. If microsatellite variation is correlated with allozyme variation, then it is possible that inferences based on large-scale studies of allozymes will also pertain to microsatellite genetic diversity. We therefore investigate the relationship between microsatellite and allozyme variation.

Finally, it has been suggested that the screening and development process for microsatellites selects for longer microsatellite loci—the ascertainment bias hypothesis (e.g., Ellegren et al. 1995, 1997; FitzSimmons et al. 1995; Forbes et al. 1995; Primmer et al. 1996b; but see Crawford et al. 1998; Zhu et al. 2000). Microsatellites are typically isolated from clones of DNA fragments from the target species using a

TABLE 1.
Microsatellite characteristics.

Characteristic	Definition
Dispersion	Average number of bases between adjacent microsatellites within a genome. Calculated as: average clone insert size (kb)/[(proportion of positive clones − proportion of false positives − proportion of duplicate clones) × proportion of sequences of AC motif].
Length	Average number of repeats at a locus. Calculated as: 0.5 × (longest repeat + smallest repeat). The number of repeats in the sequenced clone was used for length when allele data were unavailable or the length of the cloned microsatellite allele was not presented in the paper or in GenBank.
Alleles	Number of alleles at a locus.
Range	Difference in length of the shortest and longest allele at a locus expressed in number of bases. Calculated as: (longest allele − shortest allele).
Allele dispersion	Average number of bases separating adjacent alleles at a locus. Calculated as: range/(alleles − 1).
Heterozygosity	Proportion of individuals heterozygous at a locus. Where possible, the expected heterozygosity was used.

probe and hybridization technique. This process itself may directly select for longer repeats because the probe may not hybridize as well to short repeat sequences (e.g., < 12 repeats; see Forbes et al. 1995 and references within). Alternatively, because researchers are generally in search of polymorphic genetic markers, there may be a bias to select the longest microsatellite loci from a set of sequences (a form of publication bias). The average length of microsatellite loci from which primers are developed would therefore be an overestimate of the average length of all microsatellite loci within the genome. This could lead to inaccurate inference about the mutational dynamics when comparing these estimates to estimates of homologous loci in other species (see Ellegren et al. 1995) or simply to inconsistencies when comparing to lengths obtained from other methods such as random sequencing of DNA (see Weber 1990; Beckman and Weber 1992). Evidence for the ascertainment bias hypothesis is mixed. For example, Ellegren et al. (1997) analyzed 27 randomly selected microsatellite loci developed from either cattle (13 loci) or sheep (14 loci) and amplifiable in both species. They showed that the microsatellite loci (or at least the polymerase-chain-reaction-amplified products) are longer in the species from which they originated as compared to the homologues in the other species. Forbes et al. (1995) also provided support, finding that eight microsatellites isolated in domestic sheep are longer as compared to their homologues in wild sheep. However, Crawford et al. (1998) provided a detailed analysis of 472 cattle and sheep microsatellites and found no support for the ascertainment hypothesis. Zhu et al. (2000) used sequence data for three microsatellites conserved across 58 species of wasps and a phylogenetic approach to show that the longest stretch of uninterrupted repeats decreases with phylogenetic distance from the species in which the microsatellites were isolated, but the overall repeat length does not. Thus, testing the ascertainment bias hypothesis can be quite complex.

Here we develop and test an analogous hypothesis, the intraspecific ascertainment bias hypothesis. If the cloning process selects for longer microsatellite loci, either indirectly through the efficiency of hybridization or researcher propensity to select the longest subset of repeats, then it should also be true that the number of repeats in the cloned microsatellite allele should be greater than the number of repeats averaged across all alleles at the locus in the population as a whole. To demonstrate the intraspecific hypothesis, consider the following example. Cloning typically involves collecting segments of DNA sequences from an individual. Each of these segments most commonly represents a unique microsatellite locus and more importantly, a single allele (the cloned allele) from the population of alleles. Initially, ignoring a hybridization bias, the length of the cloned allele will just as often be the shortest allele in the population as the longest allele. Therefore, across all of the sequence segments representing unique microsatellite loci that a researcher has, some will be short alleles and others will be long alleles as compared to the mean allele lengths in the population. If researchers bias their selection to the cloned alleles with the greatest number of repeats—the long alleles—then the reported cloned alleles should, on average, be longer than the alleles within the population. The difference would be further exaggerated if there is also a hybridization bias for longer alleles. We therefore compare the length of each AC microsatellite locus in the cloned insert to its average length in the population.

METHODS

Data Survey

AC microsatellite loci were characterized using similar criteria (Table 1) for the five vertebrate classes. We focused on

TABLE 2. Summary of the microsatellite characteristics for the five vertebrate classes.

Class	No. species	Dispersion	Length	Alleles	Range	Allele dispersion	Heterozygous
Fish	27	37 ± 51	23 ± 6	13.7 ± 9.1	36 ± 22	3.7 ± 1.3	0.68 ± 0.20
Reptiles	10	93 ± 62	17 ± 3	7.7 ± 4.0	24 ± 12	3.6 ± 0.7	0.60 ± 0.21
Amphibians	8	—	14 ± 4	6.9 ± 3.0	21 ± 11	4.0 ± 1.0	0.54 ± 0.16
Birds	20	382 ± 304	16 ± 4	9.5 ± 4.6	26 ± 13	3.4 ± 1.2	0.69 ± 0.16
Mammals	33	58 ± 40	19 ± 4	8.6 ± 3.8	22 ± 11	3.0 ± 0.9	0.70 ± 0.09

TABLE 3. Summary of variation in the microsatellite characteristics among all species and species within each of the five classes. Regression coefficients (r^2) are presented for statistically significant results. Nonsignificant results are indicated by ns.

Species	Length	Alleles	Range	Allele dispersion	Heterozygous
All	0.40***	0.58***	0.50***	0.30***	0.46***
Fish	ns	0.58***	0.46***	0.34**	0.57***
Reptiles	ns	0.56***	0.39**	ns	0.55***
Amphibians	0.32**	0.57***	0.55***	ns	0.41*
Birds	0.52***	0.45***	0.45***	0.30*	0.48***
Mammals	0.38***	0.52***	0.46***	0.20*	0.22**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

studies reporting on both microsatellites and polymorphism data. To avoid potential errors introduced by publication biases, only polymorphic loci were included. Studies that did not have data for at least three of the first six characteristics in Table 1 or did not derive polymorphism estimates from at least 10 individuals were omitted. Exceptions were made for the dispersion-genome size analysis, in which three additional species were included because of the quality of their dispersion and genome size data, and for amphibians, which had the smallest sample size. Haploid genome sizes, measured in picograms, were obtained where possible (Appendices 1, 2). When unavailable, genome size was estimated from the average of phylogenetically similar species. Only one estimate of genome size could be made for the three snakes; we therefore averaged the microsatellite characteristics from each of these species in analyses with genome size.

Microsatellite dispersion (Table 1) was calculated from studies that did not use enrichment protocols. Where possible, the proportion of false positives and duplicate clones were accounted for in the calculation. When multiple probes were used, the proportion of microsatellite loci reported that were AC repeats was assumed to represent the same proportion in the positive clones. Provided that researchers are not more or less likely to report AC repeats when probing with several motifs, this assumption should not bias our calculation. The average clone insert size was assumed to be an average of the digested fragment size range used in the ligation process. For example, it was common that fragments ranging from 200 to 700 bases were used in the ligation, resulting in an average clone insert of 450 bases. Although it would have been more reliable to include the actual average clone insert size as determined from sequence analysis, these data were rarely presented. If the clone inserts are smaller than the average fragment size as calculated here, then the dispersion calculation will be overestimated. However, this potential bias is independent of species or class, and therefore does not confound our comparative analysis. Overall, our approach to calculating dispersion is common in the literature and generally provides robust calculations, as verified from alternative methods (e.g., Primmer et al. 1997).

Microsatellite length (number of AC repeats) was determined from the length of the cloned allele and the range in allele sizes in the population studied (see Table 1). Compound repeats (see Weber 1990) were excluded unless the AC repeat was the dominant motif (i.e., had more repeats). The number of repeats in the cloned allele was calculated as the longest stretch of AC repeats not interrupted by more than two bases. Thus, our microsatellite data included both perfect and imperfect repeat sequences (see Weber 1990). Two bases was selected as our criterion because we felt that

TABLE 4. Summary of variation in the microsatellite characteristics among the five classes. The total variation explained by class differences (r^2) and the *P*-values associated with the multiple comparisons are presented. Nonsignificant results are indicated by ns. Amphibians were not included in analysis of dispersion because there was only a single estimate.

	Dispersion	Length	Alleles	Range	Allele dispersion	Heterozygous
Total	0.50***	0.28***	0.15**	0.14*	ns	ns
Fish						
Reptiles	ns	*	*	ns	ns	ns
Amphibians	—	***	*	ns	ns	ns
Birds	***	***	ns	ns	ns	ns
Mammals	ns	*	*	*	ns	ns
Reptiles						
Amphibians	—	ns	ns	ns	ns	ns
Birds	***	ns	ns	ns	ns	ns
Mammals	ns	ns	ns	ns	ns	ns
Amphibians						
Birds	—	ns	ns	ns	ns	ns
Mammals	—	*	ns	ns	ns	ns
Birds						
Mammals	***	ns	ns	ns	ns	ns

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

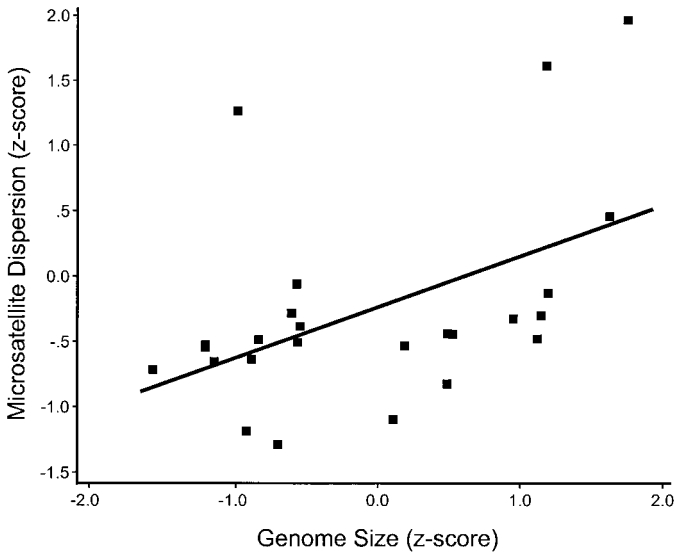

FIG. 1. The relationship between microsatellite dispersion (average number of bases between adjacent loci) and genome size (*dispersion* = 0.47 × *genome size*; $r^2 = 0.22$, df = 24, $P = 0.019$). The relationship is also significant when nonparametric correlation is used (Spearman: $\rho = 0.56$, $P = 0.003$, $n = 25$).

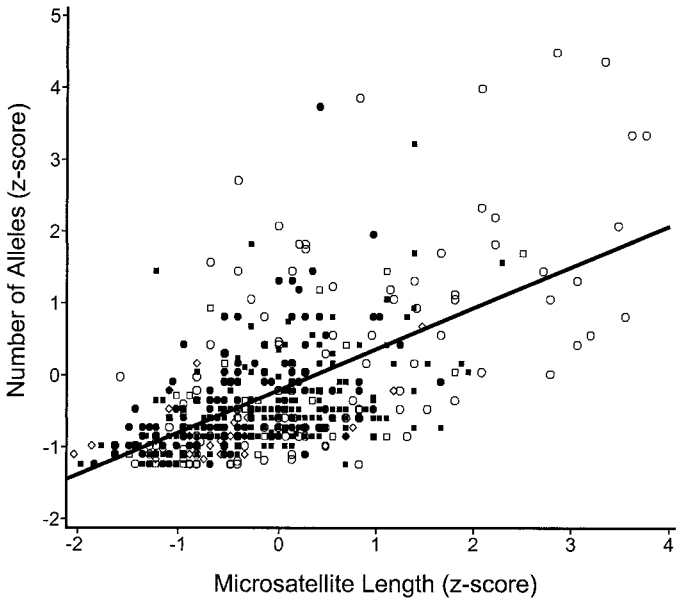

FIG. 2. The relationship between the number of alleles and microsatellite length (average number of repeats; *alleles* = 0.584 × *length*; $r^2 = 0.34$, df = 520, $P < 0.001$). The data are shown by class: fish (open circle; $r^2 = 0.41$, df = 114, $P < 0.001$); reptiles (open square; $r^2 = 0.29$, df = 56, $P < 0.001$); amphibians (diamond; $r^2 = 0.28$, df = 31, $P = 0.002$); birds (filled circle; $r^2 = 0.25$, df = 110, $P < 0.001$); and mammals (filled square; $r^2 = 0.26$, df = 205, $P < 0.001$). There was no significant difference in the slopes of the regressions among classes (Tukey post hoc: $F_{4,512} = 0.31$, $P > 0.10$; regressions lines are not shown, for clarity). Variables are expressed as *Z*-scores within species to control for absolute differences among species, and number of alleles is controlled for the number of individuals sampled ($r^2 = 0.08$, df = 568, P 0.001). There was no effect of the number of populations ($P > 0.10$).

it captured the potential length-mutational dynamics of microsatellites (see also Weber and Wong 1993). For example, many long repeat sequences were interrupted by a single point or insertion/deletion mutation. We felt that a microsatellite of sequence $(AC)_{15}A(AC)_{10}$ or $(AC)_{15}AA(AC)_{10}$ was longer than a microsatellite of $(AC)_{15}$. By our criteria, the first two microsatellites have a length of 25 repeats, whereas the second has a length of 15 repeats. Although the degree of degeneracy of the repeat motif can affect the strand slippage mutation rate (Weber and Wong 1993), similar proportions of perfect and imperfect repeats were characterized among the classes ($P > 0.10$; data not shown). Therefore, our criteria does not bias the comparative analysis.

Polymorphism data were based on a variable number of individuals sampled from one or more populations. We report the mean number of individuals and populations sampled for each species. When the number of populations was not explicitly reported, but it was indicated that individuals were sampled from geographically distinct areas, each area was considered a population. When no indication was given, it was assumed that a single population was sampled. Where possible, when multiple populations were sampled, polymorphism values were calculated within each population separately and then averaged to avoid introducing potential error from mixing data from multiple populations. Where appropriate, the number of individuals sampled in each study was statistically controlled using the natural log of the value. Overall, there was no significant difference in the number of individuals or populations sampled across the five classes.

Genome Size

For each species, the number of microsatellite loci was calculated from genome size divided by dispersion. Results from analyses involving this variable and genome size must be interpreted with caution because the independent variable (genome size) appears in the calculation of the dependent variable (number of microsatellite loci) and could lead to a spurious correlation (Zar 1999). Specifically, because genome size appears in the numerator of the dependent variable, the regression coefficient will be overestimated, and therefore only positive regressions could be spurious. All variables were *Z*-scored within classes to control for differences among the classes (see below; Zar 1999). In addition to the species in Appendix 1, we included the brown trout (*Salmo trutta*): genome size = 2.48 pg, microsatellite dispersion = 23 kb (Estoup et al. 1993); the white-footed mouse (*Peromyscus leucopus*): genome size = 3.5 pg, microsatellite dispersion = 40 kb; and a New World bat (*Macrotus waterhousii*): genome size = 2.8 pg, microsatellite dispersion = 106 kb (Van Den Bussche et al. 1995). The mean microsatellite length for each species was used in analyses of length and genome size.

Species and Classes

Species-specific differences in microsatellite loci were examined among all classes as well as within each of the five classes separately using the data from all loci. Class-specific differences in microsatellite loci were controlled for differences in the number of loci examined among species by av-

eraging the data from multiple loci prior to analysis (see Appendix 1).

Mutation

In all mutational analyses, microsatellite characteristics were *Z*-scored within species to control for differences among the species. To test for directional mutation, we first calculated an index of heterozygote instability. This was done using the first axis of the principal component analysis (PCA) of range and allele dispersion. Computer simulations were used to ensure that the PCA variable was highly correlated with the average difference in allele length in heterozygotes ($r^2 > 0.9$; data not shown). The PCA variable was used as a measure of mutation rate (see Amos et al. 1996). We used linear regression of length, controlled for alleles and heterozygosity, versus mutation rate (the PCA variable). Length was controlled for the number of alleles and heterozygosity to prevent a potential spurious correlation because range (and therefore the PCA variable) and length were both correlated with alleles.

The average allozyme heterozygosity was collected for each species from Nevo et al. (1984), or in some cases from the same paper reporting the microsatellites.

Statistical Analyses

Statistics were performed using SPSS for Windows (ver. 10.0). Analysis of variance (ANOVA) tests assumed an unbalanced design and used Type III sum of squares. Post hoc tests used Tukey's analysis. All proportional data were arcsine transformed (Zar 1999). Averages are reported plus or minus one standard deviation. All *P*-values are from two-tailed tests, unless otherwise indicated.

RESULTS

It total we analyzed data on 592 AC microsatellite loci from 98 species of fish, reptiles, amphibians, birds, and mammals. These data are summarized by species in Appendix 1 and by class in Table 2.

Genome Size

AC microsatellite dispersion was positively related to genome size (Fig. 1). This implies that as genome size increases, the distance between adjacent microsatellite loci also increases. The significance of this relationship did not change when only genome size estimates based on the actual species or an average of multiple species within the same genus were used ($P < 0.05$, $n = 20$). There was no relationship between the number of microsatellite loci and genome size (linear regression: $r^2 = 0.01$, df = 24, $P = 0.71$). In this latter analysis, a positive relationship was not masked by potential spurious effects because the regression coefficient is likely to be overestimated and not underestimated (see Methods). There was also no relationship between the average microsatellite length and genome size ($r^2 = 0.02$, df = 21, $P = 0.54$). Therefore, larger genomes do not appear to have proportionately more or longer microsatellite loci.

Species

AC microsatellite loci were highly variable among species within classes. This includes variation in length, number of alleles, allele range, allele dispersion, and heterozygosity (Table 3). Allele dispersion had the least variation, and number of alleles had the most. Variation in the size range of microsatellite alleles was primarily due to variation in the number of alleles ($\beta = 0.93$) and secondarily to allele dispersion ($\beta = 0.38$; multiple linear regression: $r^2 = 0.88$, $F_{2,448} = 1683$, $P < 0.001$). In birds and mammals, all characters differed significantly among species. In fish, number of alleles, allele range, allele dispersion, and heterozygosity, but not length, differed among species. In reptiles, number of alleles, allele range, and heterozygosity, but not length or allele dispersion, differed among species. In amphibians, length, number of alleles, allele range, and heterozygosity, but not allele dispersion, differed among species. Thus, considerably structural differences exist in microsatellite loci among species within the classes.

Classes

AC microsatellite dispersion was highly variable among the classes (ANOVA: $r^2 = 0.50$, $F_{3,50} = 11.3$, $P < 0.001$; Table 4). Microsatellites were least common in birds (dispersion = 382 kb) and most common in fish (dispersion = 37 kb; Table 2). There was a positive relationship between microsatellite dispersion and genome size (see above). However, there was no relationship between the number of microsatellite loci and genome size. Therefore, it is unlikely that a reduction in genome size explains the deficiency of microsatellite loci in birds.

There was significant variation in other microsatellite characters (Table 4). This included length ($r^2 = 0.28$, $F_{4,90} = 8.35$, $P < 0.001$), number of alleles ($r^2 = 0.15$, $F_{4,97} = 3.94$, $P = 0.005$), and allele range ($r^2 = 0.14$, $F_{4,87} = 2.87$, $P = 0.029$). There was marginally significant variation among the classes in allele dispersion ($r^2 = 0.10$, $F_{4,77} = 2.05$, $P = 0.092$), but no significant variation in heterozygosity ($r^2 = 0.08$, $F_{4,94} = 1.87$, $P = 0.12$).

There was no significant difference in the number of individuals or the number of populations analyzed in each study among the five classes ($P > 0.10$; see Table 2).

Poikilotherms and Homeotherms

AC microsatellite characters did not differ between the poikilotherms and homeotherms. This included microsatellite dispersion, length, number of alleles, range, allele dispersion, and heterozygosity (Tables 2, 4). There were significant differences between fish and mammals in length, alleles, and range (Table 4). Fish had longer microsatellite loci with more alleles that spanned a larger range as compared to mammals. Within the fish, there were no differences between those in temperate environments as compared to those in tropical environments ($P > 0.05$ for all comparisons). Therefore, although differences exist between the microsatellite loci of fish and mammals, it is not strictly a poikilotherm-homeotherm difference and it does not seem to be related to in vivo temperature.

Mutation

There was a positive linear relationship between microsatellite length (i.e., average number of repeats at a locus) and the number of alleles across all classes as well as within each class (Fig. 2). These relationships were controlled for the number of individuals analyzed at each locus (see Fig. 2). The slope of the regressions was greatest in fish, followed by reptiles, amphibians, mammals, and lastly birds, but the slopes were not significantly different among classes (see Fig. 2). Similar to the number of alleles, there was a positive linear relationship between microsatellite length and heterozygosity across all classes ($r^2 = 0.18$, $F_{1,508} = 112.1$, $P < 0.001$) as well as within each class (fish: $r^2 = 0.25$, $F_{1,107} = 34.8$, $P < 0.001$; reptiles: $r^2 = 0.17$, $F_{1,56} = 11.0$, $P = 0.002$; amphibians: $r^2 = 0.10$, $F_{1,31} = 3.3$, $P = 0.080$; birds: $r^2 = 0.21$, $F_{1,105} = 27.1$, $P < 0.001$; mammals: $r^2 = 0.17$, $F_{1,205} = 41.7$, $P < 0.001$), again controlling for the number of individuals analyzed. The slopes of the regressions within each class were not significantly different ($F_{4,500} = 0.53$, $P > 0.10$).

Across all classes, there was a positive correlation between the range-allele dispersion PCA variable (representing the average length difference of alleles in heterozygous genotypes) and length, independent of the number of alleles and heterozygosity (multiple linear regression: $\beta = 0.241$, $t = 5.36$, df = 386, $P < 0.001$). Within each class there was also a positive correlation, but it was significant in only fish ($\beta = 0.248$, $t = 2.59$, df = 84, $P = 0.011$), mammals ($\beta = 0.357$, $t = 4.79$, df = 171, $P < 0.001$), and birds ($\beta = 0.242$, $t = 2.42$, df = 77, $P = 0.018$). The lack of a significant correlation in reptiles and amphibians may reflect the smaller sample sizes in these two classes (see Table 2). There was no difference in the slope of the regressions in fish, birds, and mammals ($F_{2,330} = 0.21$, $P > 0.10$), which suggests that microsatellite loci with higher mutation rates, as measured by heterozygote instability, are also longer. As such, microsatellite loci show a propensity to increase in length.

Allozyme heterozygosity data for 39 of our species (40% of our sample) were obtained. There was a significant positive correlation between allozyme and microsatellite heterozygosity (Spearman's nonparametric correlation: $\rho = 0.35$, $n = 39$, $P = 0.031$).

Intraspecific Ascertainment Bias Hypothesis

Both the clone length and average length (within a population) were available for 290 microsatellite loci from 51 species. The average clone length was 18.9 ± 8.0 (SD) and ranged from four to 59 repeats. The average microsatellite length was 18.3 ± 7.5 and ranged from four to 65 repeats. These lengths were significantly different (paired *t*-test: $t = 1.97$, df = 289, $P = 0.025$; one-tailed). These data provide support for the intraspecific ascertainment hypothesis, namely that the isolation process selects for longer microsatellite loci.

Discussion

We have analyzed data from 592 AC microsatellite loci from 98 species in five vertebrate classes (fish, reptiles, amphibians, birds, and mammals) to address nine questions about microsatellite evolution. First, we found that larger genomes do not have proportionately more AC microsatellite loci, as might be predicted if microsatellites play a major role in the folding and condensing of DNA into chromosomes (cf. Stallings et al. 1991; Gaillard and Strauss 1994). However, we have analyzed only a single repeat motif and, although it is one of the most common in vertebrate genomes, it is possible that the number of other microsatellite loci increases with genome size. Alternatively, the packaging role of AC microsatellites may be more limited within the genome, for example, it may be restricted to only coding regions (e.g., Hayes and Dixon 1985; Braaten et al. 1988). Further, we have not considered the position of the AC microsatellite loci within the genome and its potential influence on packaging and condensing DNA (but see Hancock 1999). Nevertheless, accumulating evidence shows that microsatellite loci have specific protein-binding properties and play an important role in transcriptional activity (e.g., Hamada et al. 1984; Vashakidze et al. 1988; reviewed by Kashi and Soller 1999). In this case, it is not expected that genome size and the number of microsatellite loci should be related because the amount of coding DNA is generally independent of genome size. Instead, this latter function predicts a positive relationship between genome size and microsatellite dispersion (the average number of bases separating adjacent microsatellite loci), which is consistent with our data. A more accurate test of this latter hypothesis would compare the number of microsatellite loci in a genome with the amount of coding DNA or the number of genes.

The positive correlation between genome size and microsatellite dispersion cannot be explained by noise in our calculation of dispersion. This explanation would require that screening of larger genomes result in relatively fewer positive clones independent of the actual frequency of AC microsatellite loci in the genome. It seems unlikely (given the isolation process) that starting with a small or large genome should a priori affect the ratio of positive clones to total clones screened. In addition, our calculations of microsatellite dispersion are consistent with estimates from alternative methods. For example, in birds Primmer et al. (1997) used an alternative method to determine the average length and frequency of microsatellites in three divergent avian lineages including chicken, woodpecker, and swallow, and found that there was one AC microsatellite that was at least 14 repeats in length every 350–450 kb. In the swallow we found that there was one AC microsatellite, averaging 14 repeats, every 350 kb, and across all the birds, we found that there was one AC microsatellite, averaging 16 repeats, every 382 kb. Therefore, our dispersion estimates seem reasonably accurate.

Second, we confirmed that AC microsatellite loci are relatively rare in avian genomes. We were able to reject the hypothesis that their rareness results from constraints imposed by flight (see Primmer et al. 1997). This hypothesis predicts a positive relationship between genome size and the number of microsatellite loci and a negative relationship between genome size and microsatellite dispersion, neither of which we found. In addition, several fish species also have very small genomes, some smaller than birds, yet have considerably more microsatellite loci. Therefore, the reduction

of microsatellite frequency in birds (and bats) is not likely due to a reduction in the size of their genome.

Primmer et al. (1997) raised a second hypothesis to explain the reduction of microsatellite loci in avian genomes. They suggest that the avian lack of SINEs, LINEs, and poly(A) tails, sources for the evolution of microsatellites, constrains their frequency. The class-specific constraints received some support in our analysis because a large portion of the variation in microsatellite dispersion was due to differences among classes. Additional support for phylogenetic influence may come from plants. The frequency of microsatellite loci, particularly the AC motif, within plant genomes appears to be lower than in mammals (Lagercrantz et al. 1993), even though some plants have very large genomes (Wachtel and Tiersch 1993). Similar to birds, some plants show a reduction in the evolutionary precursors to microsatellites (but see Lagercrantz et al. 1993). Therefore, the reduction of microsatellite frequency in birds, and more generally class-specific differences in microsatellite frequency, may reflect differences in the abundance of evolutionary precursors.

Third, we found that microsatellite loci differ in structure and polymorphism among classes and species. Although considerable allozyme variation has been explained by ecological, demographic, and life-history correlates (e.g., Nevo et al. 1984), less attention has been given to microsatellites. However, the correlation between allozyme and microsatellite variation indicates that the mechanisms underlying the variation at both types of loci may be similar. For example, northern pike have the fewest alleles and the lowest degree of heterozygosity among the fish surveyed. Northern pike populations are often founded by only a few individuals and are characterized by small population sizes (Miller and Kapuscinski 1996), both demographic characteristics of species with low allozyme variability. DeWoody and Avise (2000) showed more broadly that marine fish species have greater microsatellite allele variation as compared to freshwater species and that this is consistent with the increased evolutionary effective population sizes of marine species. It is likely that much of the variation in polymorphism at microsatellite loci that exist between species and, more generally, classes can be attributed to differences in population biology and to a lesser extent to differences in natural selection (or linkage to loci under selection) pertaining to the function of the microsatellite loci.

Fourth, we rejected the hypothesis that there are general differences in AC microsatellite structure between poikilotherms and homeotherms. Several authors have speculated that the greater in vivo temperature variation experienced by poikilotherms could increase mutation rates and consequently explain the apparent differences in microsatellite length, allele dispersion, and range in allele size between poikilotherms and homeotherms (e.g., Brooker et al. 1994; Colbourne et al. 1996; O'Reilly et al. 1996). However, these speculations have been largely based on comparisons of fish, living in a colder water environment, to mammals. Our analysis comparing poikilotherms (fish, reptiles, and amphibians) to homeotherms (birds and mammals) revealed no difference in their microsatellite characters. Furthermore, we found no difference between the microsatellites of temperate versus tropical fish. Therefore, the differences are between two specific vertebrate classes, fish and mammals, rather than between two metabolic types. Indeed, fish have significantly longer AC microsatellite loci than reptiles, amphibians, birds, and mammals.

Brooker et al. (1994) also suggested that the colder water environment of fish may select for longer microsatellite loci, as compared to mammals. For example, if microsatellite sequences are involved in the melting apart of double-stranded DNA and increased length facilitates melting (Hamada et al. 1984), then longer microsatellite loci may be favored in fish as compared to homeotherms or poikilotherms living in warmer environments. However, it should also be expected that fish living in temperate environments should have longer microsatellite loci as compared to fish in tropical environments, a relationship we did not find (although our sample size was small for this comparison). Thus, it seems unlikely that in vivo temperature can account for the longer microsatellites in fish.

Recent models that examine the effects of the per repeat mutation rate at microsatellite loci have been able to explain differences in length distributions of microsatellites within and among genomes (Kruglyak et al. 1998, 2000; Falush and Iwasa 1999). These models assume that the rate of slipped strand mispairing (the predominant mutational mechanism at microsatellites; e.g., Schlötterer and Tautz 1992; Strand et al. 1993) is equal to the product of the length of the microsatellite and the per repeat mutation rate. Thus, the probability of a mutation at a microsatellite locus increases as either its length (number of repeats) or per repeat mutation rate increases. These models also assume that rare point mutations divide a continuous repeat into two smaller loci. They show that microsatellite loci with higher per repeat mutation rates are longer, on average. We found that fish had the highest per repeat mutation rate (as measured by the number of alleles controlled for microsatellite length and number of individuals sampled; see Fig. 2) and the longest microsatellites of the five classes. Moreover, within the poikilotherms, we found that the per repeat mutation rate was highest in fish, followed by reptiles and amphibians, and this corresponded to the mean microsatellite length within these classes. Similarly, within the homeotherms, the per repeat mutation rate was higher in mammals than birds, and mammals had longer microsatellite loci. Thus, differences in the per repeat mutation rate may explain much of the differences in microsatellite length among the classes (also see below).

Fifth, we found that variation in the range of AC microsatellite allele sizes across classes was primarily due to differences in the number of alleles and secondly to allele dispersion. O'Reilly et al. (1996) have proposed that the range in allele size of dinucleotide, and possibly tetranucleotide, microsatellites are greater in fish than in mammals and that this may be attributed to an increase in the number of alleles or allele dispersion in fish. Our analysis, based on AC dinucleotide microsatellites, supports some of their observations: We found that fish microsatellites have a greater range in allele size and more alleles, but no difference in allele dispersion. Moreover, across all classes, variation in the range in allele size can be attributed about two and a half times more to differences in the number of alleles as compared to allele dispersion. These results are also consistent with a stepwise mutational process in which the predominate

mutation is of a single repeat unit (Kimura and Ohta 1978; Shriver et al. 1993; Valdes et al. 1993).

Sixth, we found that microsatellite length is a major factor influencing mutation rate. Within a few species there has been considerable support that its rate increases with the length of the repeat array (e.g., Weber 1990; Wierdl et al. 1997; Primmer et al. 1998). Our study supports the generality of this hypothesis across five vertebrate classes. More than one-third of the variation in AC microsatellite polymorphism, and thus mutation rate, was explained by length. Within fish, this value was nearly one-half. Therefore, microsatellite length appears to be a major factor influencing the mutation rate at AC microsatellite loci.

Seventh, we found evidence that microsatellite loci show directional mutation. Moreover, the mutations are related to heterozygote instability. Amos et al. (1996) recently examined microsatellite germline mutations in human families and showed that mutations occur disproportionately in individuals that are heterozygous and have a greater difference in allele size. They also showed that human microsatellite mutations may show directionality toward increased length. Primmer et al. (1996a) showed directional mutation in a bird microsatellite (also see Primmer and Ellegren 1998; Primmer et al. 1998), and Zhu et al. (2000) used a phylogenetic approach to show directional mutation in three wasp microsatellites. Based on a large number of AC microsatellite loci, we found that loci with a higher mutation rate, as measured by heterozygote instability, were longer. As such, microsatellites in general may show a mutational bias to increase in length. These results cannot be attributed to a spurious correlation with allele number because we controlled for both allele number and heterozygosity in the analysis. Therefore, the increased heterozygote instability in our analysis is not a result of longer microsatellite loci having more alleles and consequently a greater average span in allele size. Given that microsatellite lengths may be constrained (e.g., Nauta and Weissing 1996; but see Kruglyak et al. 1998), the directional mutation must be countered at some point, possibly by occasional mutations that substantially decrease microsatellite length (e.g., Primmer et al. 1998). Alternatively, large microsatellite loci may degrade by point mutations or may be broken apart (e.g., Kruglyak et al. 1998; Falush and Iwasa 1999). Given that microsatellites in fish have more alleles and a greater range in allele sizes, they should also have increased heterozygote instability, which in turn could explain their increased length as compared to the other vertebrates.

Eighth, at the species level, we found that microsatellite variation was significantly correlated with allozyme variation. If both allozymes and microsatellites are neutral markers in a mutation-drift balance, then it is expected that genetic variation at these two marker types should be highly correlated. However, we found only a small correlation. It is possible that the correlation is weakened by various demographic factors. For example, populations that are highly inbred or have low effective size are more likely to have lower degrees of heterozygosity, and it is unlikely that the microsatellite and allozyme datasets are based on the same populations within each species. Furthermore, microsatellites or allozymes may not be in a mutation-drift balance. For example, it is likely that selection influences allele variants at these loci (e.g., Nevo et al. 1984; Garza et al. 1997). Differential selection intensities can affect the degree of heterozygosity and may obscure the correlation between microsatellite and allozyme variation. Alternatively, founding and bottleneck events may perturb the loci from a mutation-drift balance. Given that mutation rates at microsatellite loci are orders of magnitude higher than at allozymes (for a review, see Jarne and Lagoda 1996), microsatellite loci will replenish allelic variation and return to a mutation-drift balance sooner than allozymes (e.g., Tessier et al. 1997). Therefore, it seems likely that allozyme and microsatellite variation are correlated, and similar inferences from large-scale studies of allozymes, such as Nevo et al. (1984), can be made for microsatellites.

Finally, we found support for the intraspecific ascertainment bias hypothesis. The microsatellite isolation process appears to select for marginally longer microsatellite loci. Our hypothesis predicts that if an ascertainment bias exists, then the length of the microsatellite allele (i.e., the number of repeats) in the clone should on average be longer than the average length of the microsatellite locus in the population. We found a significant difference between the clone length and average length, suggesting that either the probing and hybridization process involved in screening microsatellites directly selects or researchers report longer microsatellite loci. Interestingly, the difference between the clone length and mean length was just over half a repeat ($18.9 - 18.3 = 0.6$). We were able to detect this small difference because of our large sample size based on almost 300 loci from 51 species. Therefore, the bias appears to be quite small and may explain conflicting results from tests of the cross-species ascertainment bias hypothesis (e.g., Ellegren et al. 1995, 1997; Crawford et al. 1998; Zhu et al. 2000).

Acknowledgments

This work was supported by the Natural Science and Engineering Research Council of Canada. We thank the authors of the published studies from which we retrieved the data and four anonymous reviewers for their helpful comments.

Literature Cited

Alderson, G. W., H. L. Gibbs, and S. G. Sealy. 1999. Parentage and kinship studies in an obligate brood parasitic bird, the brown-headed cowbird (*Molothrus ater*) using microsatellite DNA markers. J. Hered. 90:182–190.

Amos, W. 1999. A comparative approach to the study of microsatellite evolution. Pp. 66–79 *in* D. B. Goldstein and C. Schlötterer, eds. Microsatellites: evolution and applications. Oxford Univ. Press, Oxford, U.K.

Amos, W., and D. C. Rubinsztein. 1996. Microsatellite are subject to directional evolution. Nat. Genet. 12:13–14.

Amos, W., S. J. Sawcer, R. W. Feakes, and D. C. Rubinsztein. 1996. Microsatellites show mutational bias and heterozygote instability. Nat. Genet. 13:390–391.

Angers, B., L. Bernatchez, A. Angers, and L. Desgroseillers. 1995. Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographic scale. J. Fish. Biol. 47(Suppl. A.):177–185.

Arens, P., W. Van't Westende, R. Bugter, M. J. M. Smulders, and B. Vosman. 2000. Microsatellite markers for the European tree frog *Hyla arborea*. Mol. Ecol. 9:1944–1946.

Asai, S., C. Shimoda, I. Nishiumi, K. Eguchi, and S. Yamagishi.

1999. Isolation of microsatellite loci for paternity testing in the *Rufous vanga* Schetba rufa. Mol. Ecol. 8:513–514.
Bachmann, K., B. A. Harrington, and J. P. Craig. 1972. Genome size in birds. Chromosoma 37:405–416.
Bagley, M. J., and J. B. Geller. 1998. Characterization of microsatellite loci in the vermilion snapper *Rhomboplites aurorubens* (Percoidei: Lutjanidae). Mol. Ecol. 7:1089–1090.
Batargias, C., E. Dermitzakis, A. Magoulas, and E. Zouros. 1999. Characterization of six polymorphic microsatellite markers in gilthead seabream, *Sparus aurata* (Linnaeus 1758). Mol. Ecol. 8:897–898.
Becher, S. A., and R. Griffiths. 1997. Isolation and characterization of six polymorphic microsatellite loci in the European hedgehog *Erinaceus europaeus.* Mol. Ecol. 6:89–90.
Beck, S., F. Badbanchi, M. Otto, N. Grzeschik, J. Kunz, N. Speich, M. Gessler, and K.-H. Grzeschik. 1996. Six new polymorphic microsatellite markers used for the integration of genetic and physical maps of human chromosome 7. Hum. Genet. 97: 842–844.
Beckman, J. S., and J. L. Weber. 1992. Survey of human and rat microsatellites. Genomics 12:627–631.
Bensch, S., T. Price, and J. Kohn. 1997. Isolation and characterization of microsatellite loci in the *Phyloscopus* warbler. Mol. Ecol. 6:91–92.
Berlin, S., J. Merilä, and H. Ellegren. 2000. Isolation and characterization of polymorphic microsatellite loci in the common frog, *Rana temporaria.* Mol. Ecol. 9:1938–1939.
Borer, S. O., L. M. Miller, and A. R. Kapuscinski. 1999. Microsatellites in walleye *Stizostedion vitreum.* Mol. Ecol. 8:336–338.
Boudjemadi, K., O. Martin, J. C. Simon, and A. Estoup. 1999. Development and cross-species comparison of microsatellite markers in two lizard species, *Lacerta vivipara* and *Podarcis muralis.* Mol. Ecol. 8:518–520.
Braaten, D. C., J. R. Thomas, R. D. Little, K. R. Dickson, I. Goldberg, D. Schlessinger, A. Ciccodicola, and M. D'Urso. 1988. Locations and contexts of sequences that hybridize to poly(dG-dT) (dC-dA) in mammalian ribosomal DNAs and two X-linked genes. Nucleic Acids Res. 16:865–881.
Brooker, A. L., D. Cook, P. Bentzen, J. M. Wright, and R. W. Doyle. 1994. Organization of microsatellites differs between mammals and cold-water teleost fishes. Can. J. Fish. Aquat. Sci. 51:1959–1966.
Buchanan, F. C., M. K. Friesen, R. P. Littlejohn, and J. W. Clayton. 1996. Microsatellites from the beluga whale *Delphinapterus leucas.* Mol. Ecol. 5:571–575.
Buchanan, F. C., L. D. Maiers, T. D. Thue, B. G. E. De March, and R. E. A. Stewart. 1998. Microsatellites from the Atlantic walrus *Odobenus rosmarus rosmarus.* Mol. Ecol. 7:1083–1085.
Buchholz, W. G., J. M. Pearce, B. J. Pierson, and K. T. Scribner. 1998. Dinucleotide repeat polymorphisms in waterfowl (family Anatidae): characterization of a sex-linked (Z-specific) and 14 autosomal loci. Anim. Genet. 29:322–332.
Burg, T. M. 1999. Isolation and characterization of microsatellites in albatrosses. Mol. Ecol. 8:338–341.
Burland, T. M., E. M. Barratt, and P. A. Racey. 1998. Isolation and characterization of microsatellite loci in the brown long-eared bat *Plecotus auritus,* and cross-species amplification within the family Vespertilionidae. Mol. Ecol. 7:136–138.
Burns, E. L., and B. A. Houlden. 1999. Isolation and characterization of microsatellite markers in the broad-headed snake *Hoplocephalus bungaroides.* Mol. Ecol. 8:520–521.
Chung, M.-Y., L. P. W. Ranum, L. A. Duvick, A. Servadio, H. Y. Zoghbi, and H. T. Orr. 1993. Evidence for a mechanism predisposing to intergenerational CAG repeat instability in spinocerebellar ataxia type I. Nat. Genet. 5:254–258.
Ciofi, C., and M. W. Bruford. 1998. Isolation and characterization of microsatellite loci in the Komodo dragon *Varanus komodoensis.* Mol. Ecol. 7:134–136.
Colbourne, J. K., B. D. Neff, J. M. Wright, and M. R. Gross. 1996. DNA fingerprinting of bluegill sunfish (*Lepomis macrochirus*) using $(AC)_n$ microsatellites and its potential for assessment of mating success. Can. J. Fish. Aquat. Sci. 53:342–349.
Condrey, M. J., and P. Bentzen. 1998. Characterization of coastal cutthroat trout (*Oncorhynchus clarki clarki*) microsatellites and their conservation in other salmonids. Mol. Ecol. 7:787–789.
Crawford, A. M., S. M. Kappes, K. A. Paterson, M. J. deGotari, K. G. Dodds, B. A. Freking, R. T. Stone, and C. W. Beattie. 1998. Microsatellite evolution: testing the ascertainment bias hypothesis. J. Mol. Evol. 46:256–260.
Curtis, J. M., and E. B. Taylor. 2000. Isolation and characterization of microsatellite loci in the Pacific giant salamander *Dicamptodon tenebrosus.* Mol. Ecol. 9:116–118.
Daemen, E., F. Volckaert, T. Cross, and F. Ollevier. 1997. Four polymorphic microsatellite markers in the European eel *Anguilla anguilla* (L.). Anim. Genet. 28:68.
Dallas, J. F., and S. B. Piertney. 1998. Microsatellite primers for the Eurasian otter. Mol. Ecol. 7:1248–1251.
Davis, C. S., and C. Strobeck. 1998. Isolation, variability, and cross-species amplification of polymorphic microsatellite loci in the family Mustelidae. Mol. Ecol. 7:1776–1778.
Dawson, R. J. G., H. L. Gibbs, K. A. Hobson, and S. M. Yezerinac. 1997. Isolation of microsatellite DNA markers from a passerine bird, *Dendroica petechia* (the yellow warbler), and their use in population studies. Heredity 79:506–514.
Degnan, S. M., B. C. Robertson, S. M. Clegg, and C. C. Moritz. 1999. Microsatellite primers for studies of gene flow and mating systems in white-eyes (*Zosterops*). Mol. Ecol. 8:159–160.
DeWoody, J. A., and J. C. Avise. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. J. Fish Biol. 56:461–473.
DeWoody, J. A., R. L. Honeycutt, and L. C. Skow. 1995. Microsatellite markers in white-tailed deer. J. Hered. 86:317–319.
Double, M. C., D. Dawson, T. Burke, and A. Cockburn. 1997. Finding the fathers in the least faithful bird: a microsatellite-based genotyping system for the superb fairy-wren *Malurus cyaneus.* Mol. Ecol. 6:691–693.
Eisen, J. A. 1999. Mechanistic basis for microsatellite instability. Pp. 34–48 *in* D. B. Goldstein and C. Schlötterer, eds. Microsatellites: evolution and applications. Oxford Univ. Press, Oxford, U.K.
Ellegren, H. 1992. Polymerase-chain-reaction (PCR) analysis of microsatellites—a new approach to studies of genetic relationships in birds. AUK. 109:886–895.
Ellegren, H., C. Primmer, and B. C. Sheldon. 1995. Microsatellite 'evolution': directionality or bias. Nat. Genet. 11:360–362.
Ellegren, H., S. Moore, N. Robinson, K. Byrne, W. Ward, and B. C. Sheldon. 1997. Microsatellite evolution: a reciprocal study of repeat lengths at homologous loci in cattle and sheep. Mol. Biol. Evol. 14:854–860.
Estoup, A., P. Presa, F. Krieg, D. Vaiman, and R. Guyomard. 1993. $(CT)_n$ and $(AC)_n$ microsatellites: a new class of genetic markers for *Salmo trutta* L. (brown trout). Heredity 71:488–496.
Falush, D., and Y. Iwasa. 1999. Size-dependent mutability and microsatellite constraints. Mol. Biol. Evol. 16:960–966.
Favre, L., and F. Balloux. 1997. Characterization of microsatellite loci in the greater white-toothed shrew *Crocidura russula.* Mol. Ecol. 6:595–596.
FitzSimmons, N. N., C. Moritz, and S. S. Moore. 1995. Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. Mol. Biol. Evol. 12:432–440.
Forbes, S. H., J. T. Hogg, F. C. Buchanan, A. M. Crawford, and F. W. Allendorf. 1995. Microsatellite evolution in congeneric mammals: domestic and bighorn sheep. Mol. Biol. Evol. 12: 1106–1113.
Galbusera, P., F. A. Volckaert, B. Hellemans, and F. Ollevier. 1996. Isolation and characterization of microsatellite markers in the African catfish *Clarias gariepinus* (Burchell, 1822). Mol. Ecol. 5:703–705.
Gaillard, C., and F. Strauss. 1994. Association of poly(CA)-poly(TG) DNA fragments into four-stranded complexes bound by HMG1 and 2. Science 264:433–436.
Garcia de Leon, F. J., L. Chikhi, and F. Bonhomme. 1997. Microsatellite polymorphism and population subdivision in natural populations of European sea bass *Dicentrarchus labrax* (Linnaeus, 1758). Mol. Ecol. 6:51–62.
Garza, J. C., J. Dallas, D. Duryadi, S. Gerasimov, H. Croset, and

P. Boursot. 1997. Social structure of the mound-building mouse *Mus spicilegus* revealed by genetic analysis with microsatellites. Mol. Ecol. 6:1009–1017.

Gibbs, H. L., K. A. Prior, P. J. Weatherhead, and G. Johnson. 1997. Genetic structure of populations of the threatened eastern massasauga rattlesnake, *Sistrurus catenatus*: evidence from microsatellite DNA markers. Mol. Ecol. 6:1123–1132.

Gibbs, H. L., L. De Sousa, K. Marchetti, and H. Nakamura. 1998. Isolation and characterization of microsatellite DNA loci for an obligate brood parasitic bird, the common cuckoo (*Cuculus canorus*). Mol. Ecol. 7:1437–1439.

Glenn, T. C., H. C. Dessauer, and M. J. Braun. 1998. Characterization of microsatellite DNA loci in American alligators. Copeia 3:591–601.

Gockel, J., B. Harr, C. Schlötterer, W. Arnold, G. Gerlach, and D. Tautz. 1997. Isolation and characterization of microsatellite loci from *Apodemus flavicollis* (Rodentia, Muridae) and *Clethrionomys glareolus* (Rodentia, Cricetidae). Mol. Ecol. 6:597–599.

Goldstein, D. B., and C. Schlötterer. 1999. Microsatellites: evolution and applications. Oxford Univ. Press, Oxford, U.K.

Hamada, H., M. G. Petrino, and T. Takunaga. 1982. A novel repeated element with Z-DNA forming potential is widely found in evolutionarily diverse eukaryotic genomes. Proc. Natl. Acad. Sci. USA 79:6465–6469.

Hamada, H., M. Seidman, B. Howard, and C. M. Gorman. 1984. Enhanced gene expression by the poly(dT-dG) multiplied by poly(dC-dA) sequence. Mol. Cell. Biol. 4:2622–2630.

Hancock, J. M. 1999. Microsatellites and other simple sequences: genomic context and mutational mechanisms. Pp. 1–9 *in* D. B. Goldstein and C. Schlötterer, eds. Microsatellites: evolution and applications. Oxford Univ. Press, Oxford, U.K.

Hayes, T. E., and J. E. Dixon. 1985. Z-DNA in the rat somatostatin gene. J. Biol. Chem. 260:8145–8156.

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

Hinegardner, R., and D. E. Rosen. 1972. Cellular DNA content and the evolution of teleostean fishes. Am. Nat. 106:621–644.

Houlden, B. A., P. England, and W. B. Sherwin. 1996. Paternity exclusion in koalas using hypervariable microsatellites. J. Hered. 87:149–152.

Huang, D. P., K. W. Lo, C. A. Van Hasselt, J. K. S. Woo, P. H. K. Choi, S-F. Leung, S-T. Cheung, P. Cairns, D. Sidransky, and J. C. K. Lee. 1994. A region of homozygous deletion on chromosome 9p21–22 in primary nasopharyngeal carcinoma. Cancer Res. 54:4003–4006.

Hutter, C. M., M. D. Schug, and C. F. Aquadro. 1998. Microsatellite variation in *Drosophila melanogaster* and *Drosophila simulans:* a reciprocal test of the ascertainment hypothesis. Mol. Biol. Evol. 15:1620–1636.

Ishibashi, Y., T. Saitoh, S. Abe, and M. C. Yoshida. 1996. Null microsatellite alleles due to nucleotide sequence variation in the grey-sided vole *Clethrionomys rufocanus.* Mol. Ecol. 5:589–590.

Jarne, P., and J. L. Lagoda. 1996. Microsatellites, from molecules to populations and back. Trends Ecol. Evol. 8:285–288.

Jekielek, J., and C. Strobeck. 1999. Characterization of polymorphic brown lemur (*Eulemur fulvus*) microsatellite loci and their amplification in the family Lemuridae. Mol. Ecol. 8:901–903.

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

Jones, A. G., S. Ostlund-Nilsson, and J. C. Avise. 1998. A microsatellite assessment of sneaked fertilizations and egg thievery in the fifteenspine stickleback. Evolution 52:848–858.

Kashi, Y., and M. Soller. 1999. Functional roles of microsatellites and minisatellites. Pp. 10–23 in D. B. Goldstein and C. Schlötterer, eds. Microsatellites: evolution and applications. Oxford Univ. Press, Oxford, U.K.

Kichler, K., M. T. Holder, S. K. Davis, R. Márquez-M, and D. W. Owens. 1999. Detection of multiple paternity in the Kemp's ridley sea turtle with limited sampling. Mol. Ecol. 8:819–830.

Kimura, M., and T. Ohta. 1978. Stepwise mutation model and distribution of allelic frequencies in a finite population. Proc. Natl. Acad. Sci. USA. 75:2868–2872.

Kimwele, C. N., J. A. Graves, T. Burke, and O. Hanotte. 1998. Development of microsatellite markers for parentage typing of chicks in the ostrich *Struthio camelus.* Mol. Ecol. 7:249–251.

Krenz, J. D., R. D. Semlitsch, H. C. Gerhardt, and P. A. Mahoney. 1999. Isolation and characterization of simple sequence repeat loci in the gray tree frog, *Hyla chrysoscelis.* Genome 42: 676–680.

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.

Kruglyak, S., R. T. Durrett, M. D. Schug, and C. F. Aquadro. 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.

Lagercrantz, U., H. Ellegren, and L. Andersson. 1993. The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Res. 21:1111–1115.

Largiadèr, C. R., V. Fries, B. Kobler, and T. C. M. Bakker. 1999. Isolation and characterization of microsatellite loci from the three-spined stickleback (*Gasterosteus aculeatus* L.). Mol. Ecol. 8:342–344.

Lockwood, S. F., and J. N. Derr. 1992. Intra- and interspecific genome-size variation in the Salmonidae. Cytogenet. Cell Genet. 59:303–306.

Lockwood, S. F., B. S. Holland, J. W. Bickham, B. G. Hanks, and J. J. Bull. 1991. Intraspecific genome size variation in a turtle (*Trachemys scripta*) exhibiting temperature-dependent sex determination. Can. J. Zool. 69:2306–2310.

McConnell, S. K., P. O'Reilly, L. Hamilton, J. M. Wright, and P. Bentzen. 1995. Polymorphic microsatellite loci from Atlantic salmon (*Salmo salar*): genetic differentiation of North American and European populations. Can. J. Fish. Aquat. Sci. 52: 1863–1872.

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

McDonald, D. B., and W. K. Potts. 1994. Cooperative display and relatedness among males in a lek-mating bird. Science 266: 1030–1032.

McRae, S. B., and W. Amos. 1999. Characterization of hypervariable microsatellites in the cooperatively breeding white-browed sparrow weaver *Plocepasser mahali.* Mol. Ecol. 8:903–904.

Menotti-Raymond, M. A., and S. J. O'Brien. 1995. Evolutionary conservation of ten microsatellite loci in four species of felidae. J. Hered. 86:319–322.

Miller, L. M., and A. R. Kapuscinski. 1996. Microsatellite DNA markers reveal new levels of genetic variation in northern pike. Trans. Am. Fish. Soc. 125:971–977.

Morin, P. A., J. J. Moore, R. Chakraborty, L. Jin, J. Goodall, and D. S. Woodruff. 1994. Kin selection, social structure, gene flow, and the evolution of chimpanzees. Science 265:1193–1201.

Morris, D. B., K. R. Richard, and J. M. Wright. 1996. Microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic study of salmonids. Can. J. Fish. Aquat. Sci. 53:120–126.

Nauta, M. J., and F. J. Weissing. 1996. Constraints on allele size at microsatellite loci: implications for genetic differentiation. Genetics 143:1021–1032.

Neff, B. D., P. Fu, and M. R. Gross. 1999. Microsatellite evolution in sunfish (Centrarchidae). Can. J. Fish. Aquat. Sci. 56: 1198–1205.

Neumann, K., and J. H. Wetton. 1996. Highly polymorphic microsatellites in the house sparrow *Passer domesticus.* Mol. Ecol. 5: 307–309.

Nevo, E., A. Beiles, and R. Ben-Shlomo. 1984. The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. *In* G. S. Mani, ed. Evolutionary dynamics of genetic diversity: Lecture notes in biomathematics. Springer, Berlin.

Nievergelt, C. M., N. I. Mundy, and D. S. Woodruff. 1998. Microsatellite primers for genotyping common marmosets (*Callithrix jacchus*) and other callitrichids. Mol. Ecol. 7:1432–1434.
Nyakaana, S., and P. Arctander. 1998. Isolation and characterization of microsatellite loci in the African elephant, *Loxodonta africana*. Mol. Ecol. 7:1436–1437.
O'Connell, M., J. M. Wright, and A. Farid. 1996. Development of PCR primers for nine polymorphic American mink *Mustela vison* microsatellite loci. Mol. Ecol. 5:311–312.
O'Connell, M., M. C. Dillon, J. M. Wright, P. Bentzen, S. Merkouris, and J. Seeb. 1998. Genetic structuring among Alaskan Pacific herring populations identified using microsatellite variation. J. Fish Biol. 53:150–163.
Ohnishi, N., Y. Ishibashi, T. Saitoh, S. Abe, and M. C. Yoshida. 1998. Polymorphic microsatellite DNA markers in the Japanese wood mouse *Apodemus argenteus.* Mol. Ecol. 7:1431–1432.
Olmo, E. 1991. Genome variations in the transition from amphibians to reptiles. J. Mol. Evol. 33:68–75.
Olmo, E., G. Odierna, T. Capriglione, and O. Cobror. 1985. Different trends in the variations of the main genomic components in turtles and scaly-reptiles. Comp. Biochem. Physiol., B. 80: 441–446.
Olsen, J. B., P. Bentzen, and J. S. Seeb. 1998. Characterization of seven microsatellite loci derived from pink salmon. Mol. Ecol. 7:1087–1089.
O'Reilly, P. T., L. C. Hamilton, S. K. McConnell, and J. M. Wright. 1996. Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Can. J. Fish. Aquat. Sci. 53: 2292–2298.
Paetkau, D., and C. Strobeck. 1994. Microsatellite analysis of genetic variation in black bear populations. Mol. Ecol. 3:489–495.
Paetkau, D., W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite analysis of population structure in Canadian polar bears. Mol. Ecol. 4:347–354.
Painter, J., R. H. Crozier, Y. C. Crozier, and M. F. Clarke. 1997. Characterization of microsatellite loci for a co-operatively breeding honeyeater. Mol. Ecol. 6:1103–1105.
Pardue, M. L., K. Lowenhaupt, A. Rich, and A. Nordheim. 1987. (dC-dA)n (dG-dT)n sequences have evolutionarily conserved chromosomal locations in *Drosophila* with implications for roles in chromosome structure and function. EMBO J. 6:1781–1789.
Parker, K. M., K. Hughes, T. J. Kim, and P. W. Hedrick. 1998. Isolation and characterization of microsatellite loci from the Gila topminnow (*Poeciliopsis o. occidentalis*) and their utility in guppies (*Poecilia reticulata*). Mol. Ecol. 7:361–363.
Pépin, L., Y. Amigues, A. Lépingle, J. Berthier, A. Bensaid, and D. Vaiman. 1995. Sequence conservation of microsatellites between *Bos taurus* (cattle), *Capra hircus* (goat) and related species: examples of use in parentage testing and phylogeny analysis. Heredity 74:53–61.
Petren, K. 1998. Microsatellite primers from *Geospiza fortis* and cross-species amplification in Darwin's finches. Mol. Ecol. 7: 1782–1784.
Piertney, S. B., and J. F. Dallas. 1997. Isolation and characterization of hypervariable microsatellites in the red grouse *Lagopus lagopus scoticus.* Mol. Ecol. 6:93–95.
Piertney, S. B., A. Goostrey, J. F. Dallas, and D. N. Carss. 1998. Highly polymorphic microsatellite markers in the great cormorant *Phalacrocorax carbo.* Mol. Ecol. 7:138–140.
Primmer, C. R., and H. Ellegren. 1998. Patterns of molecular evolution in avian microsatellites. Mol. Biol. Evol. 15:997–1008.
Primmer, C. R., A. P. Møller, and H. Ellegren. 1995. Resolving genetic relationships with microsatellite markers: a parentage testing system for the swallow *Hirundo rustica.* Mol. Ecol. 4: 493–498.
Primmer, C. R., H. Ellegren, N. Saino, and A. P. Møller. 1996a. Directional evolution in germline microsatellite mutations. Nat. Genet. 13:391–393.
Primmer, C. R., A. P. Møller, and H. Ellegren. 1996b. A wide-range survey of cross-species microsatellite amplification in birds. Mol. Ecol. 5:365–378.
———. 1996c. New microsatellites from the pied flycatcher *Ficedula hypoleuca* and the swallow *Hirundo rustica* genomes. Hereditas 124:281–283.
Primmer, C. R., T. Raudsepp, B. P. Chowdhary, A. P. Møller, and H. Ellegren. 1997. Low frequency of microsatellites in the avian genome. Genome Res. 7:471–482.
Primmer, C. R., N. Saino, A. P. Møller, and H. Ellegren. 1998. Unraveling the process of microsatellite evolution through analysis of germ line mutations in barn swallows *Hirundo rustica.* Mol. Biol. Evol. 15:1047–1054.
Prosser, M. R., H. L. Gibbs, and P. J. Weatherhead. 1999. Microgeographic population genetic structure in the northern water snake, *Nerodia sipedon sipedon* detected using microsatellite DNA loci. Mol. Ecol. 8:329–333.
Ragland, C. J., and J. R. Gold. 1989. Genome size variation in North American sunfish genus *Lepomis* (Pisces: Centrarchidae). Genet. Res. 53:173–182.
Richard, K. R., H. Whitehead, and J. M. Wright. 1996. Polymorphic microsatellites from sperm whales and their use in the genetic identification of individuals from naturally sloughed pieces of skin. Mol. Ecol. 5:313–315.
Rico, C., I. Rico, and G. Hewitt. 1996. 470 million years of conservation of microsatellite loci among fish species. Proc. R. Soc. Lond. B. 263:549–557.
Røed, K. H., and L. Midthjell. 1998. Microsatellites in reindeer, *Rangifer tarandus*, and their use in other cervids. Mol. Ecol. 7: 1773–1776.
Roques, S., D. Pallotta, J. M. Sevigny, and L. Bernatchez. 1999. Isolation and characterization of polymorphic microsatellite markers in the North Atlantic redfish (Teleostei: Scorpaenidae, genus *Sebastes*). Mol. Ecol. 8:685–687.
Rose, O., and D. Falush. 1998. A threshold size for microsatellite expansion. Mol. Biol. Evol. 15:613–615.
Roth, M.-P., L. Dolbois, N. Borot, C. Amadou, M. Clanet, P. Pontarotti, and H. Coppin. 1995. Three highly polymorphic microsatellites at the human myelin oligodendrocyte glycoprotein locus, 100 kb telomeric to HLA-F: characterization and Relation to HLA haplotypes. Hum. Immunol. 43:276–282.
Rowe, G., T. J. C. Beebee, and T. Burke. 1997. PCR primers for polymorphic microsatellite loci in the anuran amphibian *Bufo calamita.* Mol. Ecol. 6:401–402.
Rubinsztein, D. C., W. Amos, J. Leggo, S. Goodburn, S. Jain, S. H. Li, R. L. Margolis, C. A. Ross, and M. A. Ferguson-Smith. 1995. Microsatellite evolution: evidence for directionality and variation in rate between species. Nat. Genet. 10:337–343.
Schlötterer, C., and D. Tautz. 1992. Slippage synthesis of simple sequences DNA. Nucleic Acids Res. 20:211–215.
Scribner, K. T., J. R. Gust, and R. L. Fields. 1996. Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. Can. J. Fish. Aquat. Sci. 53:833–841.
Shinohara, M., X. DomingoRoura, and O. Takenaka. 1997. Microsatellites in the bottlenose dolphin *Tursiops truncatus.* Mol. Ecol. 6:695–696.
Shriver, M. D., L. Jin, R. Chakraborty, and E. Boerwinkle. 1993. VNTR allele frequency distribution under the stepwise mutation model. Genetics 134:983–993.
Spencer, C. C., C. A. Chlan, J. E. Neigel, K. T. Scribner, M. C. Wooten, and P. L. Leberg. 1999. Polymorphic microsatellite markers in the western mosquitofish, *Gambusia affinis.* Mol. Ecol. 8:157–158.
Stallings, R. L., A. F. Ford, D. Nelson, D. C. Torney, C. E. Hildebrand, and R. K. Moyzis. 1991. Evolution and distribution of (AC)n repetitive sequences in mammalian genomes. Genomics 10:807–815.
Stevens, S., J. Coffin, and C. Strobeck. 1997. Microsatellite loci in Columbian ground squirrels *Spermophilus columbianus.* Mol. Ecol. 6:493–495.
Strand, M., T. A. Prolla, R. M. Liskay, and T. D. Petes. 1993. Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. Nature 365:274–276.
Taylor, A. C., and D. W. Cooper. 1998. Microsatellite markers for the Phalangerid marsupial, the common brushtail possum (*Trichosurus vulpecula*). Mol. Ecol. 7:1780–1782.

Tessier, N., L. Bernatchez, and J. M. Wright. 1997. Population structure and impact of supportive breeding inferred from mitochondrial and microsatellite DNA analyses in land-locked Atlantic salmon *Salmo salar* L. Mol. Ecol. 6:735–750.

Tikel, D., D. Paetkau, M. N. Cortinas, R. Leblois, C. Moritz, and A. Estoup. 2000. Polymerase chain reaction primers for polymorphic microsatellite loci in the invasive toad species *Bufo marinus.* Mol. Ecol. 9:1927–1929.

Valdes, A. M., M. Slatkin, and N. B. Freimer. 1993. Allele frequencies at microsatellite loci:the stepwise mutation model revisited. Genetics 133:737–749.

Van Den Bussche, R. A., J. L. Longmire, and R. J. Baker. 1995. How bats achieve a small C-value: frequency of repetitive DNA in *Macrotus.* Mamm. Genome 6:521–525.

van Oppen, M. J. H., C. Rico, J. C. Deutsch, G. F. Turner, and G. M. Hewitt. 1997. Isolation and characterization of microsatellite loci in the cichlid fish *Pseudotropheus zebra.* Mol. Ecol. 6: 387–388.

Vashakidze, R. P., M. G. Chelidze, N. A. Mamulashvili, K. G. Kalandarishvili, and N. V. Tsalkalamanidze. 1988. Nuclear proteins from *Drosophila melanogaster* embryos which specifically bind to simple homopolymeric sequences poly(dT-dG)-(dC-dA). Nucleic Acids Res. 16:4989–4994.

Venturini, G., E. Capanna, and B. Fontana. 1987. Size and structure of the bird genome. II. Repetitive DNA and sequence organization. Comp. Biochem. Physiol. 87B:975–979.

Villarreal, X., J. Bricker, H. K. Reinert, L. Gelbert, and L. M. Bushar. 1996. Isolation and characterization of microsatellite loci for use in population genetic analysis in the timber rattlesnake, *Crotalus horridus.* J. Hered. 87:152–154.

Vinogradov, A. E. 1995. Nucleotypic effect in homeotherms: body-mass-correlated basal metabolic rate of mammals is related to genome size. Evolution 49:1249–1259.

———. 1997. Nucleotypic effect in homeotherms: body-mass independent resting metabolic rate of passerine birds is related to genome size. Evolution 51:220–225.

Wachtel, S. S, and T. R. Tiersch. 1993. Variations in genome mass. Comp. Biochem. Physiol. 104B:207–213.

Ward, W. K., H. C. McPartlan, M. E. Matthews, and N. A. Robinson. 1998. Ostrich microsatellite polymorphisms at the VIAS-OS4, VIAS-OS8, VIAS-OS14, VIAS-OS22, and VIAS-OS29 loci. Anim. Genet. 29:331.

Weber, J. L. 1990. Informativeness of human (dC-dA)n. (dG-dT)n polymorphisms. Genomics 7:524–530.

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

Wierdl, M., M. Dominska, and T. D. Petes. 1997. Microsatellite instability in yeast: dependence on the length of the microsatellite. Genetics 146:769–779.

Wilson, G. A., C. Strobeck, L. Wu, and J. W. Coffin. 1997. Characterization of microsatellite loci in caribou *Rangifer tarandus,* and their use in other artiodactyls. Mol. Ecol. 6:697–699.

Wooninck, L., J. E. Strassmann, R. C. Fleischer, and R. R. Warner. 1998. Characterization of microsatellite loci in a pelagic spawner: the bluehead wrasse, *Thalassoma bifasciatum.* Mol. Ecol. 7: 1613–1614.

Wooten, M. C., K. T. Scribner, and J. T. Krehling. 1999. Isolation and characterization of microsatellite loci from the endangered beach mouse *Peromyscus polionotus.* Mol. Ecol. 8:167–168.

Wu, L., L. Kaufman, and P. A. Fuerst. 1999. Isolation of microsatellite markers in *Astatoreochromis alluaudi* and their cross-species amplifications in other African cichlids. Mol. Ecol. 8: 895–897.

Wyttenbach, A., L. Favre, and J. Hausser. 1997. Isolation and characterization of simple sequence repeats in the genome of the common shrew. Mol. Ecol. 6:797–800.

Zamudio, K. R., and A. M. Wieczorek. 2000. Microsatellites for studies of ecology, behaviour, and evolution in Yarrow's spiny lizard (*Sceloporus jarrovii*). Mol. Ecol. 9:1667–1669.

Zar, J. H. 1999. Biostatistical analysis. 4th ed. Prentice-Hall, Upper Saddle River, NJ.

Zeisset, I., G. Rowe, and T. J. C. Beebee. 2000. Polymerase chain reaction primers for microsatellite loci in the northern European water frogs *Rana ridibunda* and *R. Lessonae.* Mol. Ecol. 9: 1173–1174.

Zheng, W., N. E. Stacey, J. Coffin, and C. Strobeck. 1995. Isolation and characterization of microsatellite loci in the goldfish *Carassius auratus.* Mol. Ecol. 4:791–792.

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.

Corresponding Editor: B. Bowen

APPENDIX 1

Characterization of polymorphic AC microsatellites in fish, reptiles, amphibians, birds, and mammals. All characters are defined in Table 1.

Species	Dispersion	Length	Alleles	Range	Allele dispersion	Heterozygous	No. loci surveyed	Ind.[1]	Pop.[2]	Source
Fish (n = 27)										
African catfish (*Clarius gariepinus*)	—	17.1 ± 3.5	7.7 ± 4.0	16.3 ± 10.8	2.4 ± 0.4	—	7	38	1	Galbusera et al. 1996
Atlantic cod (*Gadus morhua*)	7	30.5 ± 16.3	28.5 ± 15.9	78.0 ± 45.6	2.9 ± 0.6	0.87 ± 0.04	4	127	1	Brooker et al. 1994
Atlantic salmon (*Salmo salar*)	60	21.0 ± 15.6	6.0 ± 5.0	20.9 ± 25.5	3.6 ± 1.1	0.54 ± 0.15	3	29	5	McConnell et al. 1995
Bluegill sunfish (*Lepomis macrochirus*)	14	22.4 ± 6.6	6.8 ± 2.0	30.8 ± 12.1	5.6 ± 2.1	0.62 ± 0.08	9	100	1	Colbourne et al. 1996 Neff et al. 1999
Bluehead wrasse (*Thalassoma bifasciatum*)	—	30.0 ± 19.8	30.5 ± 3.5	—	—	0.96 ± 0.01	2	30	1	Wooninck et al. 1998
Brook charr (*Salvelinus fontinalis*)	—	32.8 ± 13.4	3.4 ± 1.5	—	—	0.39 ± 0.22	4	20	5	Angers et al. 1995
Chinook salmon (*Oncorhynchus tshawytscha*)[3]	—	—	4.3 ± 3.2	18.3 ± 21.8	4.6 ± 1.7	0.47 ± 0.25	6	28	1–3	Scribner et al. 1996
Cichlid (*Astatoreochromis alluaudi*)	24	26.7 ± 14.6	15.3 ± 10.3	39.3 ± 26.1	3.8 ± 3.1	0.67 ± 0.41	6	31	1	Wu et al. 1999
Cichlid (*Pseudotropheus zebra*)	35	25.8 ± 13.4	24.2 ± 17.5	59.0 ± 42.9	2.5 ± 0.3	0.86 ± 0.12	4	96	1	van Oppen et al. 1997
Cutthroat trout (*Oncorhynchus c. clarki*)	—	22.2 ± 5.7	9.0 ± 2.8	39.0 ± 15.5	5.0 ± 1.9	0.85 ± 0.04	4	20	2	Condrey and Bentzen 1998
European eel (*Anguilla anguilla*)	—	12.1 ± 6.5	13.1 ± 8.9	27.5 ± 23.7	2.3 ± 0.8	0.70 ± 0.28	4	46	2	Daemen et al. 1997
European sea bass (*Dicentrarchus labrax*)	—	23.5 ± 7.7	16.9 ± 4.8	—	—	0.84 ± 0.08	6	43	4	Garcia de Leon et al. 1997
Gila topminnow (*Poeciliopsis o. occidentalis*)	105	29.8 ± 19.9	6.8 ± 5.7	21.0 ± 16.7	4.1 ± 1.4	0.26 ± 0.17	4	140	4	Parker et al. 1998
Gilthead seabream (*Sparus aurata*)	—	25.4 ± 9.3	16.5 ± 7.6	40.0 ± 21.1	2.5 ± 0.3	0.86 ± 0.18	6	32	1	Batargias et al. 1999
Goldfish (*Carassius auratus*)	16	11.4 ± 2.0	3.8 ± 1.0	13.5 ± 7.1	5.6 ± 4.3	0.57 ± 0.08	4	20	1	Zheng et al. 1995
Gulf pipefish (*Syngnathus scovelli*)	12	—	24.5 ± 4.8	65.5 ± 23.7	2.8 ± 0.7	0.92 ± 0.02	4	61	1	Jones and Avise 1997
North Atlantic redfish (*Sebastes* spp.)	33	19.7 ± 4.5	10.3 ± 3.5	—	—	0.73 ± 0.21	3	30	1	Roques et al. 1999
Northern pike (*Esox lucius*)	—	16.2 ± 2.9	2.2 ± 0.2	6.0 ± 2.8	5.3 ± 3.2	0.29 ± 0.10	4	36	4	Miller and Kapuscinski 1996
Pacific herring (*Clupea harengus pallasi*)	5	24.2 ± 14.1	27.0 ± 7.7	55.0 ± 14.3	2.1 ± 0.1	0.90 ± 0.02	4	197	1	O'Connell et al. 1998
Pink salmon (*Oncorhynchus gorbuscha*)	14	29.3 ± 23.6	8.8 ± 6.7	31.2 ± 19.9	6.2 ± 4.6	0.64 ± 0.31	5	20	1	Olsen et al. 1998
Rainbow trout (*Oncorhynchus mykiss*)	22	—	24.7 ± 19.1	74.0 ± 46.9	3.7 ± 1.6	0.68 ± 0.14	3	500	1	Morris et al. 1996 McConnell et al. 1995 McConnell et al. 1997
Sandbar shark (*Carcharhinus plumbeus*)	202	11.0 ± 2.1	2.5 ± 0.7	4.0 ± 2.8	2.5 ± 0.7	0.30 ± 0.33	2	72	3	Heist and Gold 1999
Stickleback (*Gasterosteus aculeatus*)	6	24.2 ± 5.4	6.2 ± 2.4	24.6 ± 19.9	4.4 ± 1.9	0.60 ± 0.15	10	50	1	Largiadèr et al. 1999
Stickleback (*Spinachia spinachia*)	11	23.5 ± 8.0	20.3 ± 14.4	45.3 ± 33.4	2.3 ± 0.5	0.80 ± 0.20	6	49	1	Jones et al. 1998
Vermilion snapper (*Rhomboplites aurorubens*)	—	23.8 ± 10.4	23.4 ± 11.2	—	—	0.90 ± 0.08	7	60	1	Bagley and Geller 1998
Walleye (*Stizostedion vitreum*)	—	14.5 ± 4.0	6.2 ± 2.3	16.0 ± 10.7	2.9 ± 0.7	0.65 ± 0.13	5	186	1	Borer et al. 1999
Western mosquitofish (*Gambusia affinis*)	—	28.2 ± 1.4	20.0 ± 1.4	68.0 ± 24.0	3.5 ± 1.0	0.73 ± 0.03	2	20	1	Spencer et al. 1999
Reptiles (n = 10)										
American alligator (*Alligator mississippiensis*)	—	20.6 ± 4.3	6.6 ± 1.9	19.0 ± 7.6	3.4 ± 0.7	0.65 ± 0.14	8	28	2	Glenn et al. 1998
Broad-headed snake (*Hoplocephalus bungaroides*)	69	13.4 ± 2.3	4.0 ± 2.0	—	—	0.48 ± 0.32	5	16	2	Burns and Houldern 1999
Common lizard (*Lacerta vivipara*)	18	19.0 ± 2.2	9.4 ± 1.7	24.8 ± 4.1	3.1 ± 1.0	0.87 ± 0.03	5	16	1	Boudjemadi et al. 1999
Kemp's ridley sea turtle (*Lepidochelys kempi*)	75	15.0 ± 9.9	6.5 ± 5.0	—	—	0.44 ± 0.52	2	98	1	Kichler et al. 1999
Komodo dragon (*Varanus komodoensis*)	200	16.1 ± 5.1	3.0 ± 1.4	5.9 ± 4.8	2.8 ± 1.1	0.27 ± 0.18	7	20	4	Ciofi and Bruford 1998
Massasauga rattlesnake (*Sistrusus c. catenatus*)	96	19.2 ± 3.4	6.0 ± 1.7	20.3 ± 9.5	4.0 ± 1.1	0.50 ± 0.28	3	73	1	Gibbs et al. 1997
Northern water snake (*Nerodia sipedon sipedon*)	81	20.0 ± 7.4	11.4 ± 7.0	35.7 ± 30.3	3.8 ± 2.5	0.72 ± 0.22	8	50	3	Prosser et al. 1999

APPENDIX 1
Continued.

Species	Dispersion	Length	Alleles	Range	Allele dispersion	Heterozygous	No. loci surveyed	Ind.[1]	Pop.[2]	Source
Timber rattlesnake (*Crotalus horridus*)	40	13.5 ± 2.2	4.8 ± 2.3	15.0 ± 7.8	4.4 ± 2.6	0.38 ± 0.19	6	24	5	Villarreal et al. 1996
Turtles[4]	190	19.5 ± 9.1	16.3 ± 6.3	39.7 ± 18.0	2.6 ± 0.4	0.80 ± 0.09	6	41	2	FitzSimmons et al. 1995
Yarrow's spiny lizard (*Sceloporus jarrovii*)	—	15.4 ± 3.5	8.6 ± 1.6	34.6 ± 10.3	4.6 ± 1.0	0.84 ± 0.07	7	19	2	Zamudio and Wieczorek 2000
Amphibians (n = 8)										
Cane toad (*Bufo marinus*)	—	14.3 ± 3.1	4.2 ± 1.7	15.6 ± 7.2	5.1 ± 1.8	0.56 ± 0.20	9	26	2	Tikel et al. 2000
Common frog (*Rana temporaria*)	—	11.0 ± 5.8	7.0 ± 3.0	27.7 ± 9.2	4.9 ± 1.1	0.72 ± 0.02	3	31	1	Berlin et al. 2000
European tree frog (*Hyla arborea*)	—	19.4 ± 4.1	5.0 ± 1.3	15.2 ± 8.5	3.7 ± 1.7	0.75 ± 0.08	8	12	2	Arens et al. 2000
Gray tree frog (*Hyla chrysoscelis*)	83	12.2 ± 5.6	—	—	—	—	23	—	—	Krenz et al. 1999
Marsh frog (*Rana ridibunda*)	—	19.3 ± 5.0	5.0 ± 1.0	16.0 ± 3.5	4.0 ± 0.4	0.56 ± 0.08	3	31	1	Zeisset et al. 2000
Natterjack toad (*Bufo calamita*)	—	7.5 ± 5.0	5.5 ± 3.5	15.0 ± 1.4	4.6 ± 3.3	0.36 ± 16	2	312	4	Rowe et al. 1997
Pacific giant salamander (*Dicamptodon tenebrosus*)	—	18.3 ± 8.2	13.0 ± 3.6	44.0 ± 19.7	3.6 ± 0.9	0.34 ± 0.18	3	72	11	Curtis and Taylor 2000
Pool frog (*Rana lessonae*)	—	13.2 ± 2.4	8.4 ± 4.8	13.8 ± 6.6	2.2 ± 0.9	0.50 ± 0.33	4	31	1	Zeisset et al. 2000
Birds (n = 20)										
Bell miner (*Manorina melanophrys*)	—	21.2 ± 6.3	7.2 ± 2.9	24.4 ± 9.2	4.4 ± 1.7	0.61 ± 0.21	5	361	1	Painter et al. 1997
Brown-headed cowbird (*Molothrus ater*)	231	18.9 ± 6.3	16.6 ± 9.0	40.0 ± 24.1	2.5 ± 0.7	0.85 ± 0.10	5	65	1	Alderson et al. 1999
Canada goose (*Branta canadensis*)	—	10.2 ± 2.6	5.5 ± 2.6	—	—	0.75 ± 0.10	4	10	1	Buchholz et al. 1998
Cuckoo (*Cuculus canorus*)	467	16.8 ± 3.1	9.2 ± 3.9	36.0 ± 18.6	5.2 ± 4.1	0.78 ± 0.07	4	159	1	Gibbs et al. 1998
Darwin's finch (*Geospiza fortis*)	—	18.6 ± 4.5	7.1 ± 2.9	25.5 ± 13.0	4.4 ± 1.6	0.67 ± 0.18	13	36	1	Petren 1998
Great cormorant (*Phalacrocorax carbo*)	—	16.0 ± 3.5	13.7 ± 7.2	—	—	0.63 ± 0.13	3	100	3	Piertney et al. 1998
Grey-headed albatross (*Diomedea chrysostoma*)	1100	11.6 ± 2.5	3.9 ± 2.2	5.8 ± 4.3	2.0 ± 0.0	0.46 ± 0.23	8	50	1	Burg 1999
House sparrow (*Passer domesticus*)	178	23.0 ± 0.0	8.5 ± 2.1	31.0 ± 18.4	3.9 ± 1.3	0.77 ± 0.03	2	40	1	Neumann and Wetton 1996
Leaf warbler (*Phylloscopus occipitalis*)	—	10.4 ± 2.8	6.8 ± 1.1	13.4 ± 5.4	2.3 ± 0.7	—	5	16	1	Bensch et al. 1997
Long-tailed manakin (*Chiroxiphia linearis*)	—	—	2.3 ± 0.6	7.3 ± 5.0	6.0 ± 5.3	0.38 ± 0.15	3	68	1	McDonald and Potts 1994
Ostrich (*Struthio camelus*)	—	18.0 ± 5.3	8.6 ± 5.1	32.4 ± 19.8	3.1 ± 1.2	0.68 ± 0.18	11	29	1	Kimwele et al. 1998 Ward et al. 1998
Pied flycatcher (*Ficedula hypoleuca*)	—	19.8 ± 1.1	12.8 ± 6.6	39.2 ± 21.1	3.2 ± 0.8	0.82 ± 0.10	4	20	1	Ellegren 1992 Primmer et al. 1996c
Red grouse (*Lagopus l. scoticus*)	224	14.5 ± 1.9	8.4 ± 2.1	—	—	0.76 ± 0.10	8	50	1	Piertney and Dallas 1997
Rufous vanga (*Schetba rufa*)	—	15.9 ± 2.5	10.0 ± 2.6	26.8 ± 10.0	2.9 ± 0.4	0.79 ± 0.08	5	62	1	Asai et al. 1999
Silvereye (*Zosterops lateralis*)	246	13.2 ± 3.3	6.4 ± 4.2	13.1 ± 7.8	3.1 ± 2.2	0.56 ± 0.29	7	20	1	Degnan et al. 1999
Superb fairy-wren (*Malurus cyaneus*)	—	22.2 ± 4.0	12.1 ± 3.5	—	—	0.79 ± 0.12	7	17	1	Double et al. 1997
Swallow (*Hirundo rustica*)	350	14.0 ± 6.7	16.2 ± 15.3	48.8 ± 39.6	3.2 ± 1.4	0.72 ± 0.24	5	38	1	Primmer et al. 1995 Ellegren 1992
Wandering albatross (*Diomedea exulans*)	1100[5]	9.2 ± 2.2	3.3 ± 1.2	6.0 ± 2.8	2.9 ± 1.6	0.37 ± 0.15	6	90	5	Burg 1999
White-browed sparrow weaver (*Plocepasser mahali*)	—	17.4 ± 3.0	12.5 ± 3.6	27.0 ± 10.4	2.4 ± 0.4	0.91 ± 0.04	6	375	1	McRae and Amos 1999
Yellow warbler (*Dendroica petechia*)	259	16.7 ± 2.1	19.3 ± 2.2	38.7 ± 8.1	2.1 ± 0.3	0.82 ± 0.12	3	48	2	Dawson et al. 1997
Mammals (n = 33)										
African elephant (*Loxodonta africana*)	—	14.4 ± 2.1	8.0 ± 4.0	—	—	0.73 ± 0.12	5	146	11	Nyakaana and Arctander 1998
American badger (*Taxidea taxus*)	58	16.9 ± 4.0	8.2 ± 2.5	15.5 ± 5.3	2.2 ± 0.5	0.83 ± 0.07	4	19	1	Davis and Strobeck 1998
American marten (*Martes americana*)	44	15.5 ± 4.1	6.2 ± 2.8	9.0 ± 4.4	1.8 ± 0.7	0.65 ± 0.25	11	30	1	Davis and Strobeck 1998
American mink (*Mustela vison*)	97	—	5.3 ± 1.8	14.0 ± 9.6	3.2 ± 2.2	0.62 ± 0.13	9	47	4	O'Connell et al. 1996
Atlantic walrus (*Odobenus rosmarus rosmarus*)	—	19.3 ± 6.4	8.0 ± 4.4	15.5 ± 9.9	2.2 ± 0.2	0.70 ± 0.24	11	75	2	Buchanan et al. 1998

APPENDIX 1
Continued.

Species	Dispersion	Length	Alleles	Range	Allele dispersion	Heterozygous	No. loci surveyed	Ind.[1]	Pop.[2]	Source
Beach mouse (*Peromyscus polionotus*)	11	23.0 ± 1.7	3.0 ± 1.7	—	—	0.57 ± 0.16	3	10	1	Wooten et al. 1999
Beluga whale (*Delphinapterus leucas*)	—	17.3 ± 5.0	8.8 ± 4.0	—	—	0.65 ± 0.20	13	100	2	Buchanan et al. 1996
Bighorn sheep (*Ovis canadensis*)	65	—	6.1 ± 2.1	13.0 ± 9.9	2.3 ± 0.7	—	8	20	5	Forbes et al. 1995
Black bear (*Ursus americanus*)	45	22.0 ± 2.9	6.3 ± 2.0	14.0 ± 5.4	2.6 ± 0.3	0.65 ± 0.09	4	29	3	Paetkau and Strobeck 1994
Brown lemur (*Eulemur fulvus*)	38	13 ± 5	5.6 ± 2.2	13 ± 9	2.6 ± 1.0	0.58 ± 0.26	10	33	1	Jekielek and Strobeck 1999
Brown long-eared bat (*Plecotus auritus*)	—	24.4 ± 8.1	13.2 ± 7.4	30.0 ± 18.7	2.5 ± 0.6	0.76 ± 0.15	4	662	1	Burland et al. 1998
Bottlenose dolphin (*Tursiops truncatus*)	—	20.0 ± 3.1	6.4 ± 1.1	—	—	0.77 ± 0.03	5	48	1	Shinohara et al. 1997
Caribou (*Rangifer tarandus*)	—	17.2 ± 4.1	6.2 ± 1.8	16.1 ± 8.8	3.1 ± 1.3	0.70 ± 0.13	29	22	1–2	Wilson et al. 1997 Røed and Midthjell 1998
Chimpanzee (*Pan troglodytes schweinfurthii*)	—	—	6.8 ± 1.3	24.4 ± 15.0	4.0 ± 1.9	0.67 ± 0.17	5	35	1	Morin et al. 1994
Common brushtail possum (*Trichosurus vulpecula*)	—	17.0 ± 4.8	11.7 ± 5.1	41.4 ± 19.4	5.5 ± 7.0	0.72 ± 0.19	8	92	1–2	Taylor and Cooper 1998
Common marmoset (*Callithrix jacchus*)	—	14.7 ± 4.4	4.3 ± 1.8	10.6 ± 9.1	2.8 ± 1.1	0.60 ± 0.11	7	98	1	Nievergelt et al. 1998
Common shrew (*Sorex araneus*)	15	16.4 ± 6.3	19.0 ± 11.2	—	—	0.75 ± 0.20	7	330	1	Wyttenbach et al. 1997
Domestic cat (*Felis catus*)	—	18.8 ± 3.0	6.3 ± 1.3	—	—	0.77 ± 0.10	10	10	1	Menotti-Raymond and O'Brien 1995
Eurasian otter (*Lutra lutra*)	—	26.8 ± 1.3	7.8 ± 1.9	23.6 ± 7.1	3.5 ± 0.4	0.55 ± 0.07	5	32	3	Dallas and Piertney 1998
European hedgehog (*Erinaceus europaeus*)	15	18.8 ± 3.2	7.6 ± 1.1	—	—	0.68 ± 0.15	5	143	8	Becher and Griffiths 1997
Grey red-backed vole (*Clethrionomys rufocanus bedfordiae*)	—	21.9 ± 7.3	16.0 ± 6.6	36.4 ± 18.6	2.4 ± 0.2	0.65 ± 0.17	5	81	1	Ishibashi et al. 1996
Ground squirrel (*Spermophilus columbianus*)	132	18.9 ± 4.4	6.2 ± 2.4	17.6 ± 10.2	3.3 ± 1.2	0.64 ± 0.18	9	42	2	Stevens et al. 1997
Human (*Homo sapiens*)	28	15.0 ± 4.1	7.2 ± 2.5	16.3 ± 9.0	2.5 ± 0.5	0.68 ± 0.10	13	95	1	Stallings et al. 1991 Huang et al. 1994 Roth et al. 1995 Beck et al. 1996
Japanese wood mouse (*Apodemus argenteus*)	—	21.5 ± 4.7	8.7 ± 2.6	26.7 ± 10.6	3.5 ± 0.9	0.73 ± 0.18	6	20	1	Ohnishi et al. 1998
Koala (*Phascolarctos cinereus*)	—	25.6 ± 5.0	6.5 ± 1.5	27.3 ± 10.6	5.1 ± 1.8	0.54 ± 0.17	6	12	1	Houlden et al. 1996
Mound-building mouse (*Mus spicilegus*)	18	—	14.4 ± 6.7	50.8 ± 36.0	3.5 ± 1.3	0.81 ± 0.18	5	130	4	Garza et al. 1997 Stallings et al. 1991
Polar bear (*Ursus maritimus*)	—	18.5 ± 1.5	6.7 ± 0.8	13.6 ± 2.8	2.4 ± 0.3	0.69 ± 0.16	4	27	4	Paetkau et al. 1995
Red vole (*Clethrionomys glareolus*)	120	20.5 ± 1.9	12.0 ± 1.4	25.5 ± 5.3	2.3 ± 0.3	0.88 ± 0.05	4	112	1	Gockel et al. 1997
Sperm whale (*Physeter macrocephalus*)	98	20.7 ± 5.0	13.7 ± 3.8	—	—	0.87 ± 0.07	3	73	1	Richard et al. 1996
White-tailed deer (*Cervid* sp.)	—	19.2 ± 3.2	7.6 ± 1.8	19.2 ± 5.6	2.9 ± 0.4	0.62 ± 0.21	5	41	1	DeWoody et al. 1995
White-toothed shrew (*Crocidura russula*)	—	24.8 ± 5.6	10.8 ± 2.3	—	—	0.84 ± 0.02	6	142	15	Favre and Balloux 1997
Wolverine (*Gulo gulo*)	58	16.7 ± 4.4	4.6 ± 1.7	8.8 ± 5.9	2.3 ± 0.7	0.63 ± 0.14	5	16	1	Davis and Strobeck 1998
Yellow-necked mouse (*Apodemus flavicollis*)	120	24.2 ± 3.2	16.5 ± 2.4	38.5 ± 13.3	2.4 ± 0.5	0.88 ± 0.05	4	94	1	Gockel et al. 1997

[1] Average number of individuals genotyped per locus per population.
[2] Number of populations from which the individuals were sampled.
[3] Microsatellite primers were designed in Sockeye salmon (*Oncorhynchus nerka*).
[4] Data from three marine species were combined in source: green (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*), and loggerhead (*Caretta caretta*).
[5] Statistical analyses used only one estimate of dispersion for the albatrosses.

APPENDIX 2
Genome size estimates for species in Appendix 1.

Species	Method of estimate	Reference[1]
Fish (n = 19)		
African catfish (*Clarius gariepinus*)	based on an average of three species of walking catfish (Clariidae): 1.20 ± 0.00 pg	Hinegardner and Rosen 1972
Chichlid (*Astatoreochromis alluaudi*)	based on the average of five species of haplochromine cichlids: 1.12 ± 0.08 pg	
Cichlid (*Pseudotropheus zebra*)	based on the average of 16 species of Cichlidae: 1.15 ± 0.09 pg	
European eel (*Anguilla anguilla*)	1.4 pg	
European sea bass (*Dicentrarchus labrax*)	based on an average of 10 species of sea bass (Serranidae): 1.24 ± 0.05 pg	
Gila topminnow (*Poeciliopsis o. occidentalis*)	based on an average of four species of Poeciliidae: 0.93 ± 0.05 pg	
Goldfish (*Carassius auratus*)	2.0 pg	
Gulf pipefish (*Syngnathus scovelli*)	0.65 pg	
Three-spined stickleback (*Gasterosteus aculeatus*)	0.70 pg	
Atlantic redfish (*Sebastes fasciatus*)	based on the average of three species of *Sebastodes*: 1.01 ± 0.08 pg	
Fifteen spine stickleback (*Spinachia spinachia*)	based on an average of three species of Gasterosteiformes: 0.65 ± 0.06 pg	
Atlantic cod (*Gadus morhua*)	0.90 pg	Lockwood and Derr 1992
Atlantic salmon (*Salmo salar*)	2.85 pg	
Brook charr (*Salvelinus fontinalis*)	2.74 pg	
Brown trout (*Salmo trutta*)	2.48 pg	
Chinook salmon (*Oncorhynchus tshawytscha*)	2.47 pg	
Pink salmon (*Oncorhynchus gorbuscha*)	2.46 pg	
Rainbow trout (*Oncorhynchus mykiss*)	2.33 pg	
Bluegill sunfish (*Lepomis macrochirus*)	0.94 pg	Ragland and Gold 1989
Reptiles (n = 5)		
Massasauga rattlesnake (*Sistrusus c. catenatus*)	based on an average of four species of snakes: 2.32 ± 1.05 pg	Olmo et al. 1985; Olmo 1991
Northern water snake (*Nerodia sipedon sipedon*)	based on an average of four species of snakes: 2.32 ± 1.05 pg	
Timber rattlesnake (*Crotalus horridus*)	based on an average of four species of snakes: 2.32 ± 1.05 pg	
Common lizard (*Lacerta vivipara*)	based on *Lacerta sicula*: 2.2 pg	Olmo et al. 1985
Turtles	based on an average of eight species of turtle: 3.69 ± 0.99 pg	Olmo et al. 1985; Lockwood et al. 1991
Birds (n = 5)		
Brown-headed cowbird (*Molothrus ater*)	based on an average of two species of Icteridae: 1.55 ± 0.12 pg	Bachmann et al. 1972
Yellow warbler (*Dendroica petechia*)	based on an average of three species of Parulidae: 1.60 ± 0.12 pg	
House sparrow (*Passer domesticus*)	1.25 pg	Vinogradov 1997
Swallow (*Hirundo rustica*)	based on Hirundinidae (*Ptyonoprogne rupestris*): 1.72 pg	Venturini et al. 1987
Pied flycatcher (*Ficedula hypoleuca*)	based on an average of three species of Muscicapidae: 1.45 ± 0.13 pg	Venturini et al. 1987; Vinogradov 1997
Mammals (n = 5)		
Common shrew (*Sorex araneus*)	based on an average of two species of *Sorex*: 2.83 ± 0.10 pg	Vinogradov 1995
Ground squirrel (*Spermophilus columbianus*)	based on an average of two species of Sciuridae: 4.10 ± 1.52 pg	
Human (*Homo sapiens*)	3.50 pg	
Koala (*Phascolarctos cinereus*)	3.82 pg	
Mound-building mouse (*Mus spicilegus*)	based on *Mus musculus*: 3.32 pg	

[1] Data is organized by reference and the reference is not repeated for each entry.