An Empirical Evaluation of Genetic Distance Statistics Using Microsatellite Data From Bear (Ursidae) Populations

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ABSTRACT

A large microsatellite data set from three species of bear (Ursidae) was used to empirically test the performance of six genetic distance measures in resolving relationships at a variety of scales ranging from adjacent areas in a continuous distribution to species that diverged several million years ago. At the finest scale, while some distance measures performed extremely well, statistics developed specifically to accommodate the mutational processes of microsatellites performed relatively poorly, presumably because of the relatively higher variance of these statistics. At the other extreme, no statistic was able to resolve the close sister relationship of polar bears and brown bears from more distantly related pairs of species. This failure is most likely due to constraints on allele distributions at microsatellite loci. At intermediate scales, both within continuous distributions and in comparisons to insular populations of late Pleistocene origin, it was not possible to define the point where linearity was lost for each of the statistics, except that it is clearly lost after relatively short periods of independent evolution. All of the statistics were affected by the amount of genetic diversity within the populations being compared, significantly complicating the interpretation of genetic distance data.

MICROSATELLITES are a class of genetic markers that are widely distributed in eukaryotic genomes (TAUTZ and RENZ 1984) and are characterized by high variability. These qualities make microsatellites ideal for studies of ecological genetics and population genetics (BRUFORD and WAYNE 1993; QUELLER *et al.* 1993). It has also been suggested that microsatellites may be used to study the evolutionary relationships between groups that have evolved independently for up to several million years (GOLDSTEIN *et al.* 1995a).

The identification of statistical methods that make maximum use of the information contained in microsatellite data sets will play an important role in determining the range of questions to which these markers may usefully be applied. Two factors that will affect the performance of statistical methods are the mutational dynamics of the markers being employed and the nature of the problem being studied—for example, populations at equilibrium for drift and migration *vs.* populations accumulating mutations during independent evolution.

Several workers have been prompted to develop measures of genetic distance specifically for microsatellites because of the observation that microsatellites conform more closely to the stepwise mutation model (SMM; KIMURA and CROW 1964) than to the infinite alleles model (IAM; OHTA and KIMURA 1973) on which many older statistics were based (GOLDSTEIN *et al.* 1995a,b; SHRIVER *et al.* 1995; SLATKIN 1995). Computer simulations indicate that these new statistics may outperform traditional statistics in some situations, particularly over long periods of independent evolution (GOLDSTEIN *et al.* 1995a; SHRIVER *et al.* 1995; TAKEZAKI and NEI 1996).

It is, however, becoming increasingly clear that the mutational models used in computer simulations oversimplify the dynamics of microsatellite mutations. For example, the mutation rate for one $(CAG)_n$ microsatellite was found to be dramatically higher—with a strong bias towards loss of repeats—in alleles containing 28–30 repeats than in alleles with 20–22 repeats, and as many as 16 repeat units were lost in single mutation events (ZHANG *et al.* 1994). On the other hand, analysis of mutations at the $(CGG)_n$ repeat implicated in fragile X syndrome identified a stability threshold of 34–38 uninterrupted repeats above which dramatic expansions of repeat number become likely (EICHLER *et al.* 1994). Observations of mutation at $(CA)_n$ microsatellites in humans indicate that the majority of mutations involve gain or loss of single repeat units and suggest a bias toward expansion (WEBER and WONG 1993), but the frequency of mutations of larger magnitude remains unknown. Other complicating factors include the suggestion that mutation rate is a function of the difference in size between the two alleles in a given individual (AMOS *et al.* 1996) and the, albeit controversial (ELLEGREN *et al.* 1995; AMOS and RUBINSZTEIN 1996; AMOS *et al.* 1996), contention that the rate and direction of mutation can vary between closely related species (RUBINSZTEIN *et al.* 1995).

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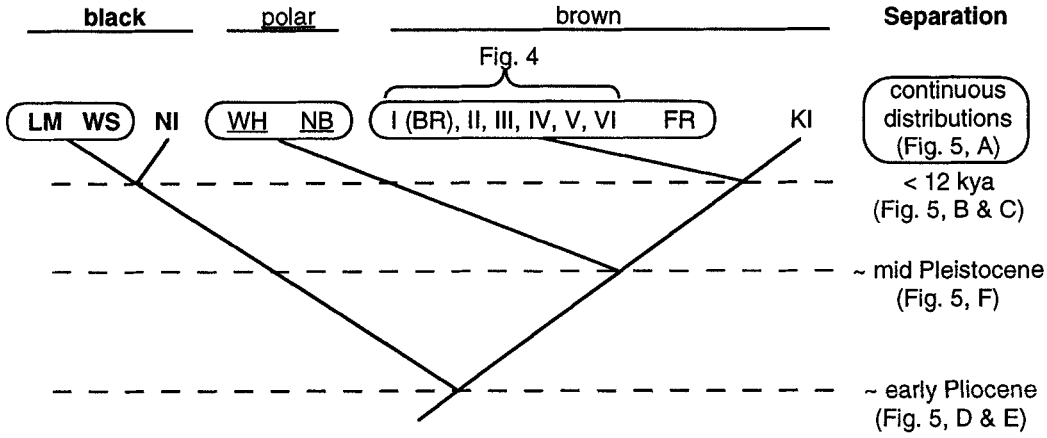

FIGURE 1.—A schematic representation of the evolutionary relationships between the study areas included in this study.

While these mutational dynamics are sufficiently complex and poorly understood to elude precise computer simulations, an even larger concern is that of constraints on allele size. The SMM holds that allele sizes are free to vary over an infinite size range, but it is clear that the number of allele states at microsatellite loci are finite and possibly highly constrained (OSTRANDER *et al.* 1993; BOWCOCK *et al.* 1994; GARZA *et al.* 1995). Constraints on allele size will clearly cause genetic distance measures to plateau, with the level of the plateau being determined by the degree of constraint, the mutation rate, and population size (NAUTA and WEISSING 1996; FELDMAN *et al.* 1997).

Given the presence of the complicating factors mentioned above, it is important to evaluate the performance of genetic distance statistics on microsatellite data sets from groups of organisms with known evolutionary relationships (*e.g.*, FORBES *et al.* 1995). A suite of eight $(CA)_n$ microsatellites has been used extensively (PAETKAU and STROBECK 1994; CRAIGHEAD *et al.* 1995; PAETKAU *et al.* 1995, 1997) to study the ecological and population genetics of the three species of bear that occur in North America: the black bear (*Ursus americanus*), brown bear (*U. arctos*; including grizzly bears), and polar bear (*U. maritimus*). This data set provides an excellent opportunity for such empirical evaluation.

The ursine data allow statistics to be tested at four distinct levels of relationship (Figure 1). Six brown bear study areas arranged linearly across a 2000-km stretch of Arctic tundra in Alaska, Yukon, and the Northwest Territories (Figure 2) provide an opportunity to study isolation-by-distance in a continuous distribution. Next, pairs of study areas from the most extremely separated regions (for which data are available) of the continuous distributions of each of the three species (Figure 3) can provide insight on the maximum distances that may be observed within continuous distributions. Third, the insular brown bear population from the Kodiak Archipelago and the black bear population from insular Newfoundland, both of which have probably been isolated since the end of the Pleistocene, provide an opportunity to evaluate whether genetic distance statistics plateau after periods of less than 20,000 years. Finally, brown bears and polar bears are very recently (mid-Pleistocene) derived sister taxa whereas their lineage diverged from the lineage that gave rise to modern black bears in the late Miocene or early Pliocene (MCLELLAN and REINER 1994; TALBOT and SHIELDS 1996a; WAITS 1996). This clearly defined relationship provides a definitive test of the ability to detect relationships between closely related species with microsatellites.

Six different measures of genetic distance were chosen to evaluate using these data. NEI's (1972) standard distance (D_S) is very popular and has relatively low variance. NEI *et al's* (1983) D_A was chosen because of its superior performance in reconstructing phylogenetic trees from simulated microsatellite data (TAKEZAKI and

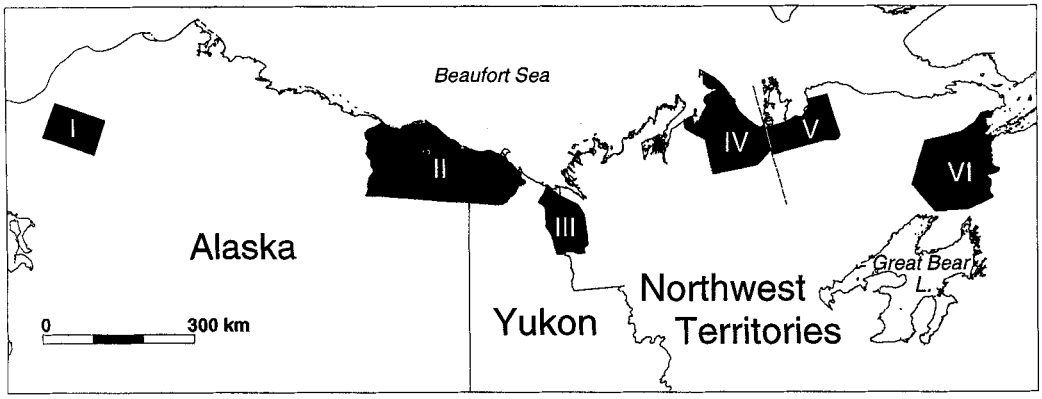

FIGURE 2.—Location of six Arctic brown bear study areas. See Figure 3 for larger context.

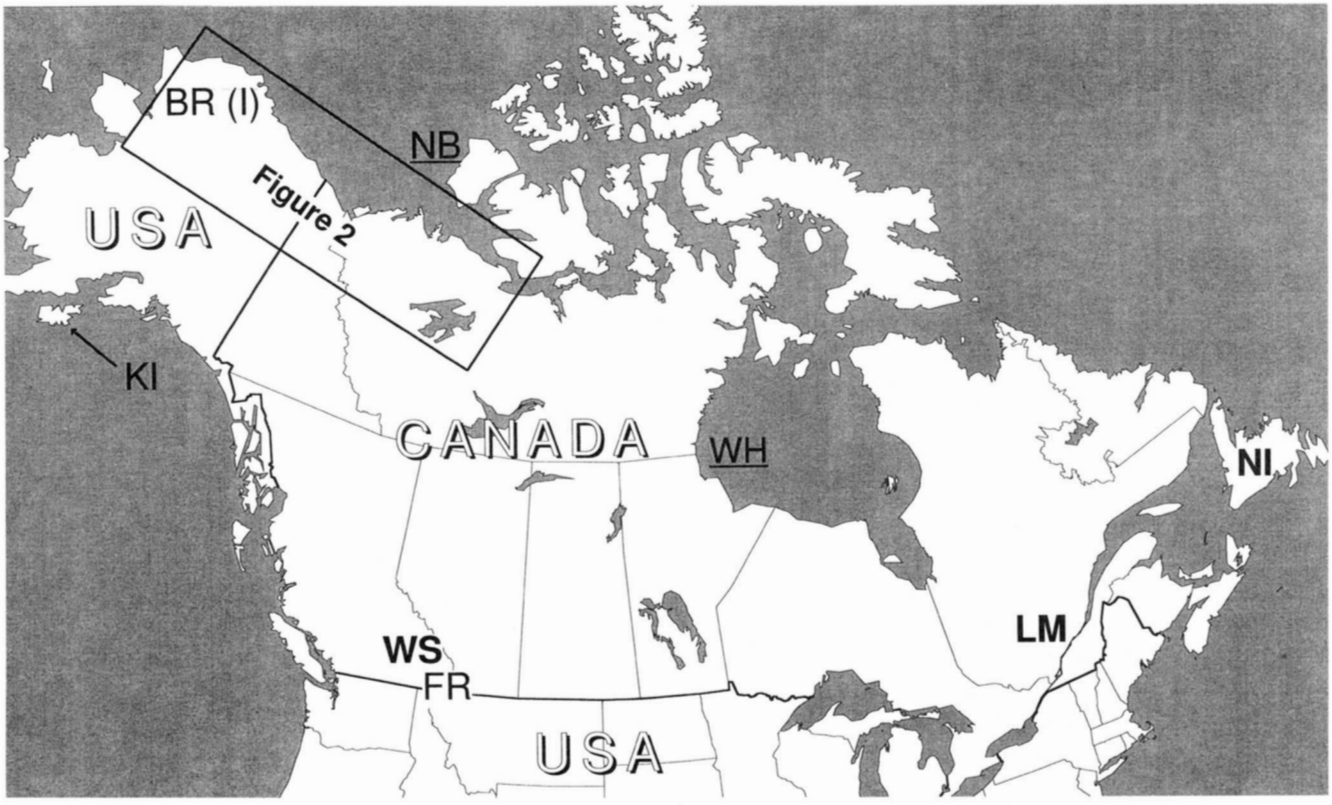

FIGURE 3.—Approximate locations of study areas used in long-distance and interspecific comparisons. The three brown bear study areas are from the western Brooks Range of Alaska (BR, same as I in Figure 2), the Flathead River drainage near the British Columbia/Montana Border (FR), and the Kodiak Archipelago (KI). The black bear study areas are from the West Slopes Bear Project centered around Golden, British Columbia (**WS**), La Mauricie National Park in Quebec (**LM**), and insular Newfoundland (**NI**). The polar bear study areas are from the Western Hudson Bay (WH) and Northern Beaufort Sea (NB) populations.

NEI 1996). SHRIVER *et al.*'s (1995) D_{SW} is a modification of NEI's (1973 in TAKEZAKI and NEI 1996) minimum (D_m) that includes the distance between each pair of alleles being considered. D_m is included for comparison to D_{SW}. GOLDSTEIN *et al's* (1995b) $(\delta\mu)^2$ is based on the difference in mean allele size between populations. This statistic was developed from, and is highly related to, *ASD* (GOLDSTEIN *et al.* 1995a; SLATKIN 1995), which has higher variance and is not considered here. Finally, we introduce a new statistic, D_{LR}, in which the likelihoods of complete multilocus genotypes are compared in two populations.

MATERIALS AND METHODS

Study areas: The study areas are shown in Figures 2 and 3. Twelve polar bear populations have been identified in Canada[1] (TAYLOR and LEE 1995), and the samples used here come from the Western Hudson Bay (WH) and Northern Beaufort Sea (NB) populations. The samples of brown bears from Kodiak Island (KI) and black bears from insular Newfoundland (**NI**) are also from discrete populations. By contrast, the brown bear samples from the western Brooks Range (BR; I) and Flathead River drainage (FR), the remaining five Arctic brown bear samples (II–VI), and the black bear samples from La Mauricie National Park (**LM**) and the West Slope Bear Project (**WS**), are from continuous distributions where discrete populations do not exist.

Two animals were captured at different times in both of study areas IV and V, and one animal was captured in both of study areas V and VI. These animals were included in both study areas where they were sampled.

Microsatellite analysis: Microsatellite analysis was performed with eight microsatellite markers isolated from a black bear genomic library and used Applied Biosystems' four-color fluorescence-based detection system as described previously (PAETKAU *et al.* 1995). Much of the data analyzed here have been published previously, and Table 1 shows sample sizes and references. The data from the Arctic brown bear study areas II and IV have not been published before, and the data from **LM** have been updated to eight loci from the four originally published. Individual genotypes are available on request.

Statistical analysis: The data from each study area were tested for conformity with Hardy-Weinberg (H-W) expectations using the methods of GUO and THOMPSON (1992). Unbiased estimates of expected heterozygosity (NEI and ROYCHOUDHURY 1975) were calculated for each study area.

The formulas for the six genetic distances follow. For populations X and Y, with r loci and m alleles at each locus, and where x_{ij} and y_{ij} are the frequencies of the ith allele at the jth locus in populations X and Y, respectively, define $J_X = \sum_j^r \sum_i^{m_j} x_{ij}^2/r$, $J_Y = \sum_j^r \sum_i^{m_j} y_{ij}^2/r$, and $J_{XY} = \sum_j^r \sum_i^{m_j} x_{ij}y_{ij}/r$. Then

$$D_S = -\ln\left[J_{XY}/\sqrt{J_X J_Y}\right] \quad \text{(NEI 1972)}, \tag{1}$$

$$D_m = (J_X + J_Y)/2 - J_{XY} \tag{2}$$

(NEI 1973 in TAKEZAKI and NEI 1996), and

[1] For population abbreviations in text, underscore denotes polar bears, bold face denotes black bears, and regular type denotes brown bears.

TABLE 1

Sample size (2*N*), mean observed number of alleles (*A*) and mean expected heterozygosity (H_e) for 13 populations of brown, black, and polar bears typed with eight $(CA)_n$ microsatellites

Study area (2*N*)	*A*	H_e
I/BR (296)[a,b]	7.63	0.749
II (48)	6.63	0.764
III (238)[b]	7.50	0.755
IV (46)	5.38	0.670
V (116)[b]	5.75	0.650
VI (72)[b]	5.75	0.605
FR (80)[b]	6.50	0.694
KI (68)[b]	2.13	0.265
WS (232)[b]	9.50	0.806
LM (64)[c]	8.75	0.820
NI (64)[b]	3.00	0.414
WH (60)[d]	5.38	0.626
NB (60)[d]	6.38	0.643

References: [a] CRAIGHEAD *et al.* (1995); [b] PAETKAU *et al.* (1997); [c] PAETKAU and STROBECK (1994); [d] PAETKAU *et al.* (1995).

$$D_A = 1 - \sum_j^r \sum_i^{m_j} \sqrt{x_{ij} y_{ij}}/r \quad \text{(NEI } et\ al.\ 1983\text{)}. \qquad (3)$$

Next define $W_X = \Sigma_k^r \Sigma_{i \neq j} |i - j|\, x_{ik} x_{jk}/r$, $W_Y = \Sigma_k^r \Sigma_{i \neq j} |i - j|\, y_{ik} y_{jk}/r$, and $W_{XY} = \Sigma_k^r \Sigma_{i \neq j} |i - j|\, x_{ik} y_{jk}/r$, where $|i - j|$ is the difference in state (size difference in base pairs divided by 2) between alleles i and j. Then

$$D_{SW} = W_{XY} - (W_X + W_Y)/2 \quad \text{(SHRIVER } et\ al.\ 1995\text{)}. \qquad (4)$$

Next, if $\mu_{x_j} = \Sigma_i\, i x_{ij}$ *and* $\mu_{y_j} = \Sigma_i i y_{ij}$, then

$$(\delta\mu)^2 = \sum_j^r (\mu_{x_j} - \mu_{y_j})^2/r \quad \text{(GOLDSTEIN } et\ al.\ 1995b\text{)}. \qquad (5)$$

Note that for locus G1D, where alleles in brown bears occur every base pair instead of every two base pairs (PAETKAU *et al.* 1997), the difference in state between alleles could take values of whole or half repeat units. This ignores the fact that all odd alleles appear to be derived from a single point deletion event and are therefore more closely related to each other than to any even allele, but does not violate the assumptions of the SMM where alleles that are identical in state are not assumed to be identical by descent. It is generally assumed that polymorphism in the flanking sequence of microsatellites occurs and goes unrecognized. Indeed, flanking sequence polymorphism has been identified at locus G10P in individuals from the **LM** and **NI** study areas (PAETKAU and STROBECK 1995), and this variation is also ignored.

The genotype likelihood ratio distance (D_{LR}) was developed from an assignment test that was used in a study of the genetic structure of Canadian polar bear populations (PAETKAU *et al.* 1995). The probability of each individual's genotype at a particular locus being drawn at random from a population was calculated as $p = x_i^2$ *for homozygotes and* $p = 2x_i x_j$ for heterozygotes. These values were multiplied across all loci to give the likelihood of each individual's eight-locus genotype. When genotype likelihoods were calculated for an individual in its own population, the individual's alleles were subtracted from the allele distributions first to eliminate bias. With this correction there is always the possibility of an allele frequency being zero, and where this occurred a value of 0.01 was used instead.

Consider populations X and Y, with n_X and n_Y individuals sampled, respectively. We can then define L_{iXX} and L_{iXY} as the likelihood of the genotype of individual i—from population X—in population X and the likelihood of the same genotype in population Y, respectively. Then

$$D_{LR} = \left(\frac{1}{n_x} \sum_i^{n_x} \log \frac{L_{iXX}}{L_{iXY}} + \frac{1}{n_Y} \sum_i^{n_Y} \log \frac{L_{iYY}}{L_{iYX}} \right) \div 2. \qquad (6)$$

Thus, if $D_{LR} = 2$, this means that the genotypes of individuals from the two populations being compared are, on average, two orders of magnitude more likely to occur in the individuals' own population than in the other population. While this statistic was developed independently, it turns out to be very closely related to the Kullback-Leibler measure of discriminatory information (MCLACHLAN 1992).

Each genetic distance (1–6) was calculated for 42 pairs of populations (Tables 2 and 3). A calculator that performs the distance calculations can be found at ⟨http://www.biology.ualberta.ca/jbrzusto⟩. For the Arctic brown bear populations, MapInfo 3.0 (MapInfo Corp.) was used to calculate geographic distance (to the nearest 10 km) between the centroids of each pair of populations following the straightest land-based path. Linear regressions and regression and correlation statistics were calculated using Statview 4.51 (Abacus Concepts Inc.).

The data set: This study used data from 479 brown bears, 180 black bears and 60 polar bears (Table 1). Complete eight-locus genotypes were obtained for all individuals. Observed allele distributions are given in APPENDIX A, and genetic distances are shown in Tables 2 and 3, and graphed in Figures 4 and 5.

All pairs of loci were checked for linkage (28 tests) by examining genotypes from known pedigrees (*e.g.*, CRAIGHEAD *et al.* 1995) where at least two offspring were available from a given parent, and the alleles inherited from that parent could be unambiguously identified (n = 14–47 pairs of offspring). One pair of loci (G1A and G10M) had fewer recombinants than would be expected by chance (binomial probability = 0.039), but this was not significant given the number of tests. In every case the data were sufficient to reject the hypothesis that recombination only occurred 10% of the time. This suggests that none of the loci used are tightly linked and that linkage disequilibrium is unlikely to be significant if mating is reasonably random.

Testing for conformity of genotype distributions to Hardy-Weinberg (random mating; H-W) expectations is extremely important with microsatellite data sets, both to confirm that nonamplifying alleles are not present at high frequency (CALLEN *et al.* 1993; PAETKAU and STROBECK 1995; PEMBERTON *et al.* 1995) and to demonstrate that the study areas from which samples are being drawn are not so large that they contain sufficient internal genetic structure to cause a WAHLUND (1928) effect. With a total of 13 populations typed at eight loci each, there were 104 genotype distributions that could be tested for H-W equilibrium. For four loci in the KI study area, however, there were not two or more alleles with more than a single copy observed, so only 100 tests were performed. Of these, 11 deviated from H-W expectations at the 10% level, two were significant at the 5% level, and the same two were significant at the 1% level. There were no significant deviations from H-W at the 10% level when the Dunn-Sidák experimentwise error rate was used (SOKAL and ROHLF 1995).

For each study area, the individual H-W tests were combined across the eight loci. Only the KI sample deviated significantly at the 5% level, and this was due to a dramatic excess of heterozygotes at locus G1D. This indicates that study areas were not large enough to have excessive internal genetic structure.

TABLE 2

Genetic distances used to generate Figure 4

Study areas	Geographic distance (km)	Genetic distance					
		D_S	D_A	D_m	D_{SW}	D_{LR}	$(\delta\mu)^2$
I–II	740	0.124	0.093	0.029	0.072	1.632	0.18
I–III	1010	0.145	0.092	0.034	0.079	2.200	0.24
I–IV	1360	0.319	0.200	0.084	0.212	4.158	0.71
I–V	1510	0.342	0.175	0.091	0.289	3.852	1.42
I–VI	1790	0.431	0.207	0.120	0.256	4.708	0.61
II–III	270	0.091	0.065	0.022	0.049	1.024	0.14
II–IV	620	0.191	0.160	0.055	0.150	2.369	0.67
II–V	770	0.210	0.151	0.061	0.207	2.431	1.26
II–VI	1040	0.299	0.198	0.091	0.206	3.683	0.73
III–IV	370	0.140	0.123	0.042	0.145	1.811	0.66
III–V	520	0.142	0.105	0.044	0.176	1.687	1.02
III–VI	790	0.237	0.155	0.076	0.185	3.064	0.51
IV–V	160	0.045	0.040	0.016	0.067	0.314	0.49
IV–VI	460	0.078	0.086	0.029	0.049	1.193	0.16
V–VI	310	0.053	0.056	0.020	0.074	0.966	0.48

Results were also combined for each locus across all populations, and only locus G1D had a significant departure from H-W at the 5% level. Again, this result was not significant if the experimentwise error rate was used and was due to the excess of heterozygotes in the KI population. These data, combined with the fact that complete genotypes were obtained for all individuals and the congruity of pedigree data in the BR sample (CRAIGHEAD *et al.* 1995), indicate that all alleles were generally amplified successfully.

Isolation by distance: The data set used here includes six Arctic brown bear study areas arranged linearly across a 2000-km stretch of the northern coast of North America (Figure 2). Although habitat and density are obviously not uniform across this strip, there are no major barriers to movement in

TABLE 3

Genetic distances used to generate Figure 5

Section	Study areas	Genetic distance					
		D_S	D_A	D_m	D_{SW}	D_{LR}	$(\delta\mu)^2$
A	BR-FR	0.567	0.312	0.124	0.284	7.26	1.36
A	WS-LM	0.464	0.248	0.073	0.391	5.28	2.59
A	WH-NB	0.302	0.191	0.099	0.211	3.76	0.54
B	KI-BR	0.429	0.383	0.215	0.444	8.76	0.61
B	KI-FR	1.498	0.646	0.419	0.717	16.63	1.72
B	KI-WS	1.463	0.653	0.380	1.368	15.82	5.12
B	KI-LM	1.546	0.682	0.386	1.551	15.81	8.00
B	KI-WH	1.376	0.677	0.428	1.221	17.19	4.93
B	KI-NB	1.110	0.572	0.382	1.339	14.39	5.50
C	NI-BR	1.562	0.674	0.342	2.262	16.47	20.02
C	NI-FR	1.386	0.687	0.346	2.307	16.80	19.69
C	NI-WS	1.276	0.640	0.300	1.565	14.89	11.24
C	NI-LM	0.751	0.450	0.233	0.744	9.51	4.54
C	NI-WH	1.401	0.715	0.373	1.529	18.03	8.67
C	NI-NB	1.102	0.579	0.325	1.708	14.59	10.04
D	BR-WS	0.625	0.356	0.106	0.650	7.49	4.31
D	FR-WS	0.831	0.441	0.148	0.664	9.43	3.15
D	BR-LM	0.744	0.398	0.118	1.000	8.01	8.24
D	FR-LM	0.917	0.481	0.155	1.015	9.78	7.06
E	WS-WH	0.915	0.517	0.182	1.093	10.97	5.62
E	LM-WH	0.750	0.463	0.161	0.953	9.29	5.07
E	WS-NB	1.023	0.475	0.186	1.160	10.45	6.35
E	LM-NB	1.041	0.456	0.187	1.171	9.66	6.76
F	BR-WH	0.914	0.510	0.195	1.088	11.40	6.42
F	FR-WH	1.266	0.568	0.253	1.113	13.55	5.96
F	BR-NB	0.998	0.463	0.198	1.223	10.77	7.28
F	FR-NB	1.353	0.503	0.253	1.163	13.62	6.57

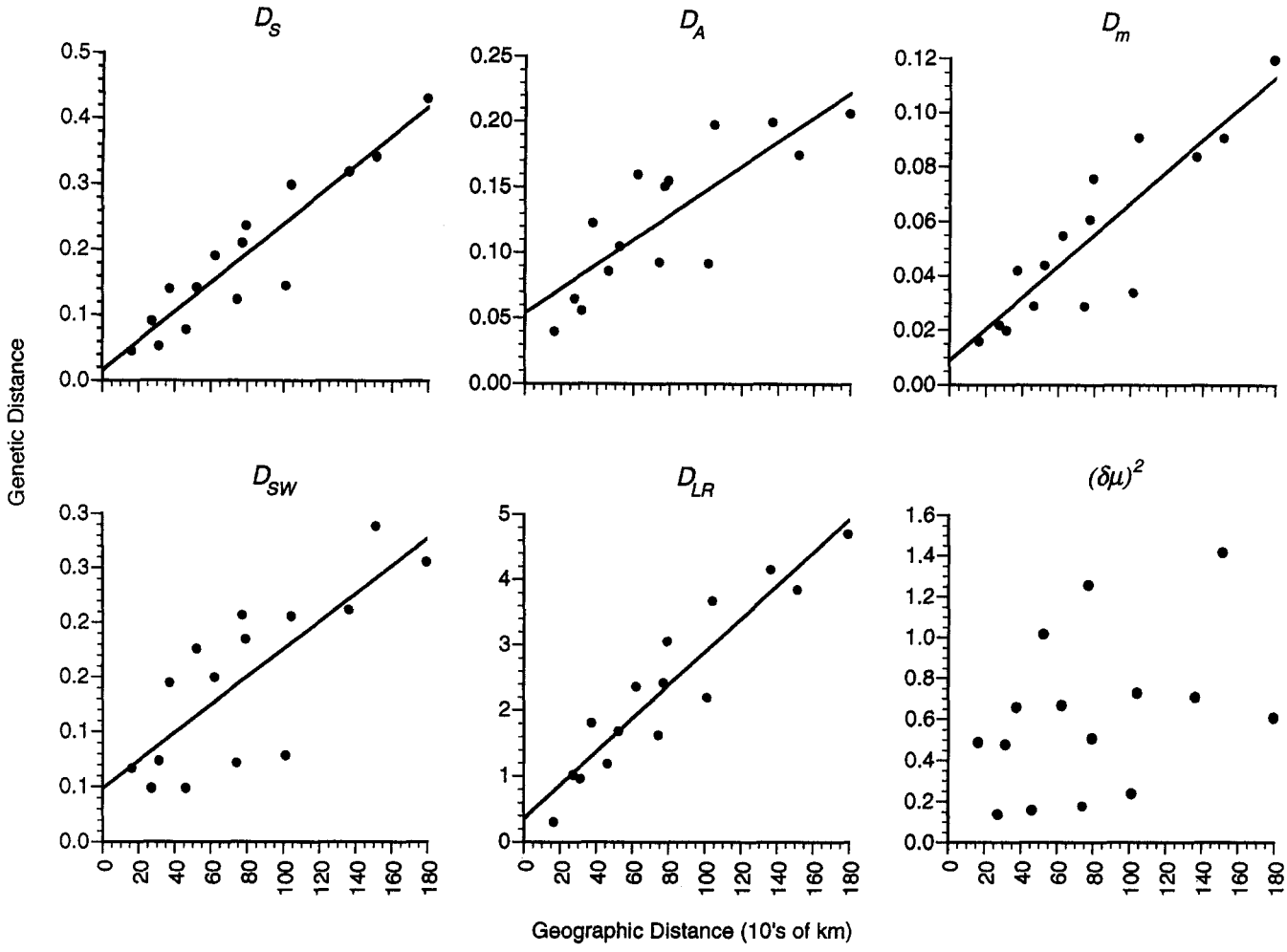

FIGURE 4.—A comparison of genetic and geographic distances for six different statistics using brown bears from six linearly arranged study areas (Figure 2). No regression line is shown for $(\delta\mu)^2$ because the regression explained very little of the variance, and the slope was not significantly different from zero (Table 5). Actual values shown in Table 2.

the region, the latitude of all the study areas is very similar, and the habitat in all areas is dominated by Arctic tundra. Therefore, this region provides a very high level of uniformity relative to most regions of similar size where large mammals with low-density distributions are found. The evaluation of genetic distance statistics within continuous distributions is based on an assumption of isolation-by-distance within the region of study, and this assumption is as reasonable in the Arctic brown bear study region as it is ever likely to be in such a wide-ranging species.

For each genetic distance statistic, measures of geographic and genetic distance were made for the 15 possible pairs of populations (Table 2). The results were plotted (Figure 4) and the linear increase in genetic distance as a function of geographic distance was evaluated using linear regression (Table 4). It may be noted that the physical distance separating populations can only approximate actual ecological distance, with the latter including all the factors that might affect the movements, mating patterns and survival of bears, but this does not violate the assumptions of the regression model since no (known) bias is introduced. More important than lack of precision in measuring the independent variable is the fact that, since each population was used in five of the 15 data points, the assumption of independence of data points is not met. The results should not, therefore, be regarded as providing actual estimates of regression statistics, but as a way to qualitatively discriminate between the performance of the various genetic distance measures.

Both the imprecision with which the independent variable (ecological distance) is known and the relatively small number of loci used would be expected to contribute considerable variance to the measurements of genetic distance, so it is perhaps surprising that approximately 87% of the variance in both D_S and D_{LR} was explained by linear regression on geographic distance (Table 4). Even more surprising, the values of D_S and D_{LR} for each pair of populations have a correlation coefficient in excess of 0.98. These two statistics treat the data in radically different ways—as opposed to distances like D_S, D_m and D_{SW}, which differ only in the arrangement and qualification of terms—yet perform in an manner that is indistinguishable in this data set and yield extremely similar results.

With the exception of $(\delta\mu)^2$ all of the statistical measures had highly significant linear regressions on geographic distance ($P < 0.001$). In fairness to its developers, $(\delta\mu)^2$ was never intended for studying distances at the fine scale used here because it has relatively high variance and because statistics based on the IAM are expected to remain linear over short periods of time (GOLDSTEIN *et al.* 1995a,b). For example, assuming constant population size, D_S is expected to remain relatively linear under the SMM up to values of approximately 0.5 (NEI 1987). It should also be noted that, in the continuous distribution studied here, genetic drift is primarily responsible for the genetic differentiation of study areas so the use of accurate mutational models is not of critical importance. The other statistic that was developed specifically to accommo-

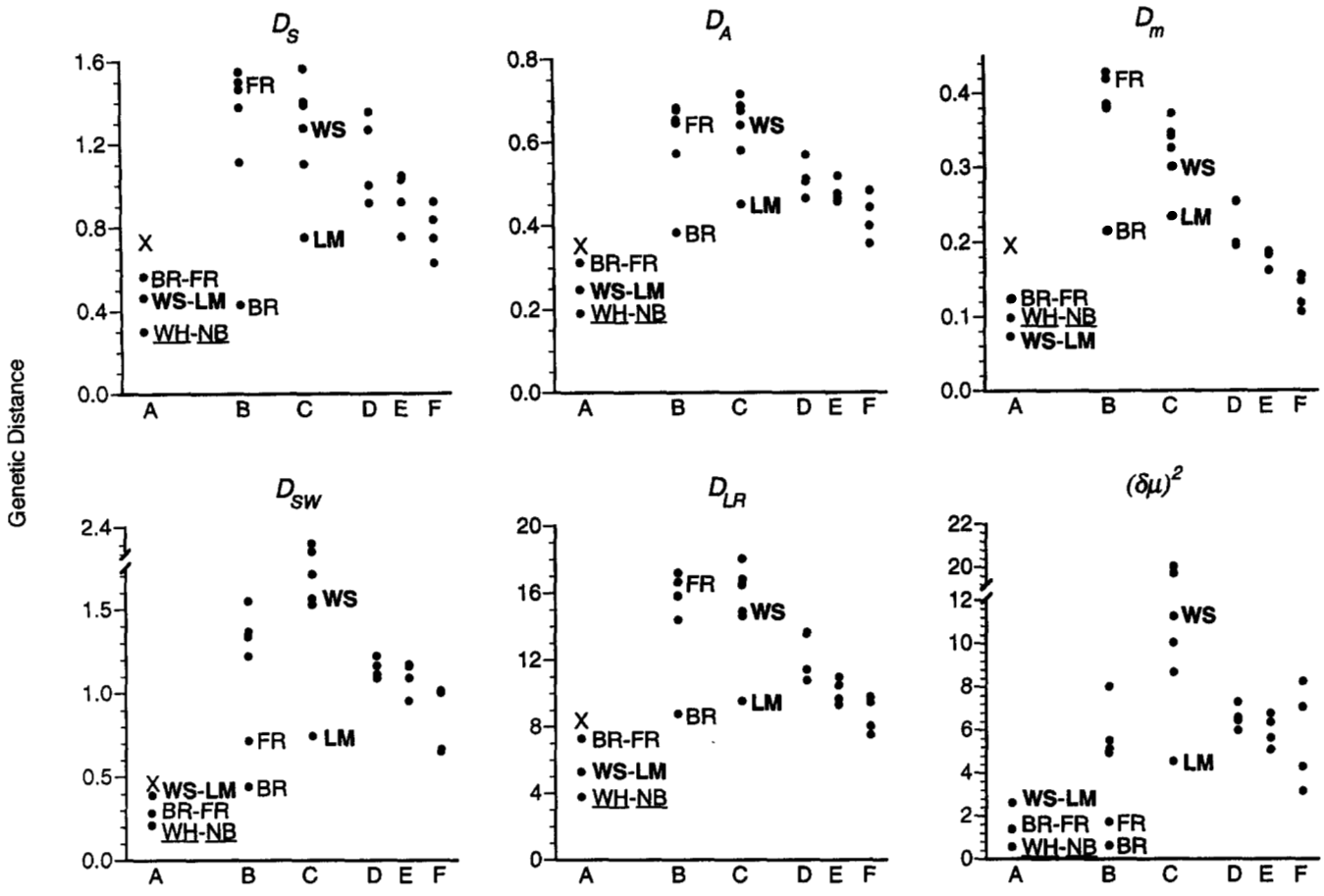

FIGURE 5.—Genetic distances involving two widely separated populations from each of three species of bears (BR, FR, **WS**, **LM**, WH, NB). The letters A–F in this figure indicate specific comparisons (points on the *X*-axis) undertaken with all six statistics and do not refer to the individual graphs. The time scales involved are illustrated in Figure 1. (A) The three intraspecific distances. The × represents the expected value of BR-FR based on an extrapolation of the linear regression shown in Figure 4. (B and C) Genetic distances from each of the six study areas in A to Kodiak Island (KI) and the island of Newfoundland (**NI**), respectively. Populations are identified for intraspecific comparisons. (D) Genetic distances between the four possible pairs of polar bear and brown bear study areas used in A. (E and F) Identical to D, but for polar bears-black bears and brown bears-black bears, respectively. Actual values shown in Table 3.

date the pseudo-stepwise mutation process of microsatellites was D_{SW}, and this measure also clearly under performs D_S, D_m, and D_{LR}.

In addition to low variance and linearity, another quality that is desirable in genetic distance statistics is that the value goes to zero as the allele distributions being compared become identical. In this respect, D_A performs poorly on the brown bear data set because the *Y*-intercept predicted by the linear regression differs significantly from zero (Table 4). Actually, the only circumstance under which D_A could be zero is if the populations being compared are fixed for the same allele at all loci. Both D_{SW} and $(\delta\mu)^2$ gave values for the *Y*-

TABLE 4

Regression statistics for Figure 4

		Regression		*Y*-Intercept		Correlation matrix				
	R^2	F	P	t	P	D_S	D_A	D_m	D_{SW}	D_{LR}
D_S	0.870	87.12	<0.0001	0.706	0.492					
D_A	0.671	26.50	0.0002	3.243	0.006	0.942				
D_m	0.780	46.16	<0.0001	1.135	0.277	0.987	0.951			
D_{SW}	0.603	19.76	0.0007	1.862	0.085	0.903	0.882	0.914		
D_{LR}	0.877	92.43	<0.0001	1.487	0.161	0.984	0.955	0.970	0.871	
$(\delta\mu)^2$	0.146	2.22	0.1601	2.047	0.061	0.505	0.524	0.520	0.809	0.453

The proportion of variance in genetic distance values explained by the linear regression on geographic distance (R^2); the significance of the regression (*F*-value and probability); the significance of the deviation from zero of the *Y*-value predicted by the regression at $X = 0$ (*t*-value and probability); correlation coefficients of all pairs of distance measures.

intercept that differ from zero at a marginally significant level ($P < 0.1$).

Genetics of Arctic brown bears: Notwithstanding a certain degree of circularity, the strong relationship between geographic and certain genetic distances can also be used to reflect on the genetic structure of the distribution of brown bears across the Arctic coast. Although landscape considerations, combined with knowledge of local brown bear movements, led us to assume that no significant genetic discontinuities existed in this distribution, the six study areas span the distributions of two mitochondrial DNA (mtDNA) clades that are approximately as divergent as some brown bear mtDNA lineages and polar bear mtDNA lineages (TALBOT and SHIELDS 1996b; WAITS *et al.* 1997). Furthermore, ecological considerations and the peninsular distribution of brown bears on the barren grounds of the Northwest Territories have led to the identification of a "barren ground grizzly bear" population (BANFIELD 1987); a population that would include three of the six study areas sampled here (IV–VI).

Although the mtDNA data may reflect interesting evolutionary events, these data have the potential to be inappropriate for studying contemporary distributions of bears because mtDNA is maternally inherited and, given the generally much larger movements of male bears (CANFIELD and HARTING 1987), gene flow is presumably effected disproportionately by males. The microsatellite data presented here show that the boundaries suggested by ecological or mtDNA data do not reflect actual genetic divisions in the current distribution of Arctic brown bears. The results also confirm the power of microsatellites in studying fine-scale population structure, even in species that are characterized by small numbers of individuals distributed over vast tracts of land.

Widely separated populations: For this level of comparison two widely separated study areas within the continuous distributions of each of the three North American bear species were used (Figure 3). The BR and FR brown bear study areas are approximately 3200 km apart and represent the extreme northwestern and southern regions of the continuous brown bear distribution (Figure 5A). The WH and NB populations are among the most genetically distinct Canadian polar bear populations and, assuming that gene flow follows a maritime route via the east coast of Baffin Island (PAETKAU *et al.* 1995), are separated by over 4500 km. The **WS** and **LM** black bear populations do not represent extremes of the North American distribution, but are separated by approximately 3400 km, assuming a slight detour north of the prairies where black bears are not found.

Predicting the expected genetic distances between these three pairs of populations is clearly a treacherous task. Nonetheless, polar bears undertake dramatically larger movements than brown bears, and brown bears generally have considerably larger movements and home ranges than black bears (STIRLING 1993), so one might predict that the genetic distances would reflect these differences. Certainly the distance between BR and FR should be greater than the largest distances observed between pairs of Arctic brown bear study areas.

In fact, all the measures except D_m show the two polar bear populations as the most similar, but the black bear study areas only come out as being most distinct with D_{SW} and $(\delta\mu)^2$. The BR–FR distance is greater than any distance calculated for Arctic brown bear populations for all but D_{SW} and $(\delta\mu)^2$, although only just for D_m. Extrapolation of the regression formulas calculated from the linear study areas, however, shows that the BR-FR distance is lower in all cases than would be predicted. This may indicate that some or all of the distance measures are losing linearity at this level of separation, but this explanation cannot be presented with confidence given the diversity of habitat between the BR and FR study areas.

Insular populations: For this section the six populations used in long-distance intraspecific comparisons (Figure 5A) were compared to each of two insular populations: KI and **NI** (Figure 5, B and C). For each island population these comparisons included two conspecific study areas and four study areas from different species.

Several lines of evidence point to very a very similar evolutionary history for the KI and **NI** populations: both islands were glaciated as recently as 14,000 years ago (FLINT 1971; DYKE and PREST 1987); extremely reduced genetic diversity (PAETKAU and STROBECK 1994; PAETKAU *et al.* 1997) and large distances of ocean (>17 km) separating them from the continent suggest extensive periods of isolation; very similar or identical mtDNA haplotypes have been found in nearby continental populations (BR and **LM**, respectively) (PAETKAU and STROBECK 1996; TALBOT and SHIELDS 1996b; WAITS *et al.* 1997). In short, the most parsimonious evolutionary hypothesis for these populations is that they have existed in isolation since the rise of ocean levels at the end of the Pleistocene, but were not previously isolated in glacial refugia. This would date their period of isolation at under 12,000 years.

If microsatellite data are even modestly useful in studying evolutionary relationships between species, then the intraspecific genetic distances to insular populations should consistently be well below interspecific genetic distances. All six measures used here fail to pass this test, with some intraspecific values consistently exceeding interspecific values.

Despite this failure, it is not clear that there is no signal in the comparisons made to insular populations. All six measures show that the BR study area has the closest genetic relationship to KI, and that **LM** comes out closest to **NI**. This is in agreement with mtDNA data and may actually reflect the region of the species distributions from which the insular populations were founded. Still, the fact that the FR-KI distances are on par with interspecies distances for all but D_{SW} and $(\delta\mu)^2$, and that the **WS-NI** distances are on par with interspecific distances in every case, indicates that all the genetic distances are reaching a plateau at the intraspecific level.

It should be noted that the exaggerated values for D_{SW} and $(\delta\mu)^2$ in Figure 5C relative to Figure 5B are due to the fixation of extremely short alleles at locus G10L in the **NI** population (APPENDIX A). Obviously the use of very large numbers of loci would reduce the impact of such fortuitous events, but it seems unlikely that the general conclusions would be altered significantly.

Interspecific comparisons: The most powerful aspect of this data set for testing the performance of genetic distances in addressing evolutionary questions with microsatellites is the fact that it contains a pair of sister species (polar bears and brown bears) that diverged in the mid-Pleistocene and an outgroup species (black bears) that diverged from the polar bear-brown bear lineage at least several times as long ago (MCLELLAN and REINER 1994; TALBOT and SHIELDS 1996a; WAITS 1996) (Figure 5, D–F). If microsatellites are to have any potential in addressing difficult relationships such as the human-chimpanzee-gorilla tricotomy (BOWCOCK *et al.* 1994; GOLDSTEIN *et al.* 1995b), then they should easily resolve these clearly separated levels of relationship in bears.

None of the distance measures shows any sign of being able to resolve the sister relationship of polar bears and brown bears. In fact, the distances between the polar bear and brown bear study areas (Figure 5D) are generally larger than for the other two pairs of species (Figure 5, E and F). Furthermore, the smallest interspecific distances are never more than 1.7 times greater than the largest distances calculated within continuous distributions (Figure 5A). The greatest separation in this regard is for D_{SW} and $(\delta\mu)^2$, suggesting that these statistics achieve the greater period of linearity expected theo-

retically, but linearity is still clearly lost for these statistics well below the interspecific level.

The expectation of $(\delta\mu)^2$ is known (GOLDSTEIN *et al.* 1995b) so, by assuming that the mutation rates found at $(CA)_n$ repeats in humans (WEBER and WONG 1993; AMOS *et al.* 1996) can be applied to bears, it is possible to estimate the level at which this statistic is reaching a plateau. Using reasonable estimates of mutation rate ($v = 0.001$) and generation time ($t = 10$ years), the mean value of $(\delta\mu)^2$ observed between noninsular populations from different species (mean of 12 values; Figure 5, D–F) corresponds to a period of 30,400 years. Even conservative estimates of $v = 0.0001$ and $t = 15$ years yield an estimated time since divergence of 456,000 years, still an order of magnitude less than the estimated 5 million years (MCLELLAN and REINER 1994; TALBOT and SHIELDS 1996a; WAITS 1996) since the divergence of black bears and the other two species. We conclude that even $(\delta\mu)^2$ is reaching a plateau at a level that corresponds to 3000 to 30,000 generations since divergence, with the former value likely to be closer to reality.

The data from bears suggest that microsatellites may not be nearly as useful for addressing evolutionary problems as had previously been hoped. It is now very important that the existence and magnitude of this limitation be confirmed in other data sets.

Constraints on allele distributions: Genetic distance measures based on the SMM, such as D_{SW} and $(\delta\mu)^2$, will remain linear for millions of years if the mutational dynamics of the markers used conform to this model. It is, therefore, very clear that microsatellites depart from the SMM sufficiently to cause a tremendous gap between the theoretical capabilities and the actual performance of these statistics. The best explanation for this gap between theory and practice is that constraints on allele sizes at microsatellite loci (OSTRANDER *et al.* 1993; BOWCOCK *et al.* 1994; GARZA *et al.* 1995) cause all genetic distance measures to plateau well below levels predicted under the assumption that allele distributions are unconstrained (NAUTA and WEISSING 1996; FELDMAN *et al.* 1997).

There is growing evidence that microsatellite allele distributions are constrained, perhaps very tightly constrained. For example, of 101 $(CA)_n$ microsatellite clones sequenced from canine genomic libraries, 96 had between 11 and 22 uninterrupted (CA) repeats (range, 8–25 repeats; OSTRANDER *et al.* 1993). Of the eight loci used in this study, seven had cloned alleles with between 17 and 21 uninterrupted repeats, although locus G10L appears to be quite unusual in this respect, having a cloned allele with 34 repeats (PAETKAU *et al.* 1995). It should be noted, however, that the methods used to isolate markers confound these data insofar as libraries are typically made with small inserts, selecting against large repeats, and clones with very small numbers of repeats are generally discarded.

Evidence for constraints in bears: The allele distributions given in APPENDIX A can be expressed in terms of number of $(CA)_n$ repeats assuming that differences in length between alleles are due entirely to changes in the number of repeats and not to changes in the length of sequences flanking the repeat region. This assumption appears to generally hold true as the flanking regions have been sequenced for at least two alleles from each of the eight species of bears for loci G1D and G10P, and the only flanking sequence length polymorphism found was the point deletion that is responsible for the odd-sized alleles at locus G1D in brown bears.

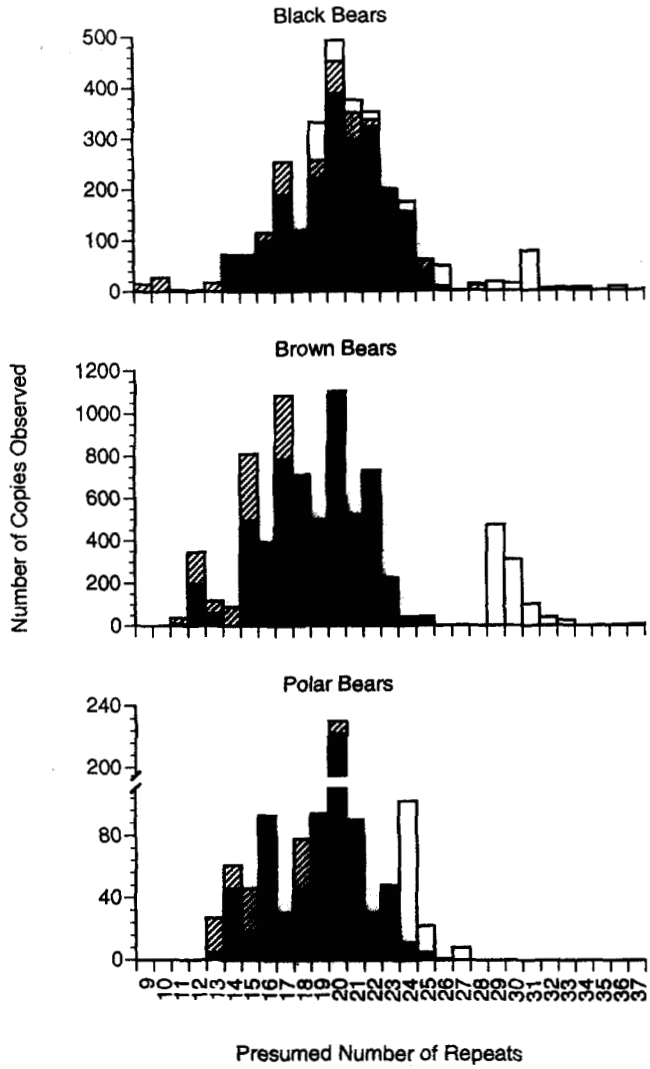

FIGURE 6.—Observed distributions of alleles (APPENDIX A) graphed based on presumed number of uninterrupted (CA) repeats. Loci G10X and G10L, which were relatively skewed to the left and right, respectively, are show as hatched bars and open bars, respectively. The other six loci are shown with black bars.

All the alleles from each of the three species of bears in this study—a total of 11,552 observations—were combined and graphed based on the presumed number of repeats (Figure 6), and it is apparent that the allele distributions are very similar, both between loci and between species. For example, combining all loci except G10L, 98.97% of alleles have between 12 and 25 repeats. Even with G10L, the total range is only 9–37 repeats. Furthermore, when data are combined from all eight loci, the modal number of repeats in each of the three species is the same: 20. The mean allele size ranges from 19.4 in polar bears to 20.5 in black bears, standing in contrast to previous findings that mean allele size tends to be considerably larger in the species that was used as the source for microsatellites markers (black bears in this case) than in nonsource species (BOWCOCK *et al.* 1994; FITZSIMMONS *et al.* 1995; FORBES *et al.* 1995).

One way in which constraints on allele size have been evaluated is by comparing expected and observed differences in mean allele size ($\delta\mu$). Since we have already calculated $(\delta\mu)^2$ for many pairs of populations, we can compare expected and observed values of this statistic. Using the same reasonable ($v = 0.001$, $t = 10$ years) and conservative ($v = 0.0001$, $t = 15$ years) estimates of mutation rate and generation time, we determine that the expected values for either black bear-brown bear or black bear-polar bear distances (~5 my divergence; McLellan and Reiner 1994; Talbot and Shields 1996a; Waits 1996) would be 1000 and 67, respectively. For polar bear-brown bear comparisons (~1 my divergence) the expected values are 200 and 13.3, respectively.

Four interspecific calculations of $(\delta\mu)^2$ were made for each pair of species (Figure 5, D–F, Table 3) with the following average values: black bear-brown bear, 5.7; black bear-polar bear, 5.9; brown bear-polar bear, 6.6. The $(\delta\mu)^2$ values were also examined on a locus by locus basis. In this case, all 32 black bear-brown bear distances and all 32 black bear-polar bear distances were below the conservative estimate of an expected value of 30. Similarly, only the four values for locus G10L were above the conservative estimate of 13.3 for brown bear-polar bear distances. Simple binomial probability indicates that these data are strong evidence for constraints on allele size in this microsatellite data set.

Even if the estimates of mutation rate used here were overestimates for the loci we used, the simple fact is that populations with something like 10^4 years of isolation have similar genetic distances to species with over 10^6 years of isolation, an indication that the distributions of alleles at these loci are constrained.

Impact of diversity within populations: Figure 5 includes interspecific comparisons between populations that span a wide range of within-population genetic diversity; from $H_e = 0.8$ for **WS** and **LM** to $H_e = 0.26$ for KI (Table 1). An explanation for some of the patterns observed in Figures 4 and 5 is that the magnitude of genetic distance values is exaggerated for populations with lower diversity.

Chakraborty and Nei (1976) showed that population bottlenecks cause a marked, if reversible, increase in D_S, but concluded that diversity within populations was not generally of concern as long as values of $\theta = 4N_e v$ (where N_e is the genetic effective population size) remain well below 1. This condition is easily met with most allozyme markers, but for microsatellites it would be unusual for θ to be as low as 1—for example, the estimated θ for **WS** under the SMM (Kimura and Crow 1964) is 12! Thus, the same data reviewed with microsatellite markers in mind would lead to the conclusion that within-population genetic diversity has a large impact on D_S.

Although the other distance measures used here have not been studied as thoroughly as D_S, it is easy to see that, with constraints on allele distribution, all the genetic distances except D_A will drop toward zero as population size increases: in infinitely large populations allele distributions would be identical, conforming to the probability distribution of allele states (the shape of this distribution depends on mutational dynamics, but the similarity of the distributions observed in the three species studied here suggests that it is approximated by Figure 6). Reference to APPENDIX A shows that some of the populations studied here contain most of the alleles that have ever been observed at some loci demonstrating that this issue may be of practical concern.

TABLE 5

Correlation coefficients between 20 interspecific genetic distances (Table 3) and mean H_e (Table 1) in the two study areas being compared

		95% Confidence interval	
	r	Lower	Upper
D_S	−0.760	−0.900	−0.478
D_A	−0.871	−0.948	−0.697
D_m	−0.977	−0.991	−0.942
D_{SW}	−0.626	−0.837	−0.253
D_{LR}	−0.912	−0.965	−0.787
$(\delta\mu)^2$	−0.283	−0.645	0.182

The relationship of D_A to diversity is more complicated. In populations with identical allele distributions the genetic distance can be as low as zero—for populations fixed for identical alleles at each locus—but may actually be quite large in populations with many alleles. However, whereas J_{XY} is independent of population size (Chakraborty and Nei 1976), when the square root is introduced in calculating D_A, this term will be biased upwards as the diversity of the populations being compared increases and the frequency of each allele decreases. The net result is that D_A should generally be biased down with increasing diversity, similar to the other distance measures.

To test the response of the genetic distance measures to within-population genetic diversity, the correlation coefficients between genetic distance and the average H_e of the two populations being compared were calculated for each of 20 interspecific genetic distances (Figure 5, B–F; Table 5). This test is underpinned by the assumption that all the distance values being compared are at the equilibrium level dictated by constraints on allele distributions; this way the values are not confounded by biologically driven differences in genetic distance or by high rates of genetic drift in smaller populations. All of the genetic distance measures except $(\delta\mu)^2$ were significantly negatively correlated with H_e. One measure, D_m, had a correlation coefficient of −0.977 indicating that essentially all of the variation in distance values observed in interspecific comparisons with this statistic was due to the genetic diversity of the populations being compared (this is expected if J_{XY} is at equilibrium since J_X and J_Y are simply the expected

homozygosity of the populations being compared). The failure to detect a significant negative correlation with $(\delta\mu)^2$ almost certainly has more to do with its high variance than any immunity to diversity effects, although this distance measure is clearly affected to a lesser degree than some of the others.

The strong effect of diversity on genetic distance measures justifies a re-examination of the data presented in Figures 4 and 5. For example, excluding D_{SW} and $(\delta\mu)^2$, the FR-KI and **WS-NI** distances (Figure 5, B and C), both of which compare a continental population to a conspecific insular population with very low genetic diversity, are higher than every interspecies distance in which both populations being compared are part of large continuous distributions (Figure 5, D–F; with the single exception of FR-NB which is larger than **WS-NI** for D_S). It appears that diversity effects are dramatically exaggerating distances to insular populations for these statistics. In addition, there is a general tendency, quite strong for D_S, D_m, and D_{LR}, for interspecific values to decrease with increasing mean H_e in the study areas being compared (Figure 5D through Figure 5F).

We can also revisit the long-distance intrapsecific comparisons (Figure 5A). Recall that, contrary to prediction, the WH-NB distance was not the smallest for D_m and that the **WS-LM** distance only came out as largest for D_{SW} and $(\delta\mu)^2$. These deviations from prediction may result from diversity effects. For example, it is not surprising that for D_m, the statistic most affected by diversity (Table 5), the polar bear distance (low H_e) is shifted up relative to expectation, and the black bear distance (high H_e) is shifted down relative to expectation.

The results from the linear Arctic brown bear study areas may also have been affected by diversity. H_e for these populations ranges from 0.74 to 0.76 in Alaska and the Yukon (study areas I–III), but drops to 0.60 in the most easterly study area in the Northwest Territories (VI; Table 1). Looking at the plots for D_S, D_m, and D_{LR} (Figure 4), the two points that lie the greatest distance above the regression line are the distances for II–VI and III–VI, whereas the two points furthest below the regression line are for I–II and I–III. These outliers may be due to chance, or may have a natural explanation—for example, subtly reduced gene flow across the MacKenzie River, which separates study areas I–III from IV–VI—but the effects of diversity certainly cannot be discounted.

Summary: The results from the six linear Arctic brown bear study areas confirm the power of microsatellites for studying fine-scaled population structure. Three statistics—D_S, D_m, and D_{LR}—performed particularly well at this scale. Since genetic drift is the primary force driving genetic distances at this scale, the variance of the measures used is a more important consideration than accurate mutational models. Given the intimate relationship between D_S and D_m, we recommend the use of D_S and D_{LR} to provide relatively independent estimates of genetic distance in studies working at a similar scale.

The data suggest that some of the genetic distance statistics are beginning to plateau at the level represented by the most geographically separated regions of the continuous North American brown bear distribution. Studies in other organisms, where larger numbers of populations can be used and where habitat is fairly homogenous over larger relative distances, will be required to more precisely define the point at which significant departures from linearity begin to occur.

The fact that the genetic distances between some continental populations and conspecific insular populations are on par with, if not greater than, interspecific genetic distances suggests that most of the distance statistics are reaching a plateau level after less than 20,000 years of separation. D_{SW} and $(\delta\mu)^2$ may still be relatively linear at this level, but the variance of these statistics makes it difficult to draw strong conclusions when using only eight loci.

All the genetic distance measures used here were completely unable to identify the very close sister relationship of polar bears and brown bears. If these data are typical for microsatellite markers—an assumption that must now be evaluated with other data sets—it appears that even $(\delta\mu)^2$ plateaus after periods of time that are very short in evolutionary terms and that microsatellites are unlikely to be useful for resolving relationships between species. Presumably constraints on allele distributions are responsible for this limitation. It is likely that there is a window between the point where some statistics lose linearity because of inappropriate mutational model and the point where $(\delta\mu)^2$ loses linearity because of constraints on allele distributions, but it remains to be demonstrated whether this window is large enough to compensate for the relatively large variance of this statistic.

The effect of genetic diversity on genetic distance statistics complicates their interpretation, and this effect must be considered when analyzing microsatellite data sets.

We thank JOHN BRZUSTOWSKI for developing genetic distance programs on the World Wide Web. This work would be impossible without the large number of people who shared samples, information and advice. For samples from study areas II and IV, which have not been published before, this help came from HARRY V. REYNOLDS, GERALD W. GARNER, TOM MCCABE, DON YOUNG, ERNIE VYSE, MARSHA BRANIGAN, GRANT MCHUTCHEON, Parks Canada, Northwest Territories Department of Renewable Resources, U.S. Fish and Wildlife Service, Alaska Department of Fish and Game, U.S. National Biological Service, and Federal Aid for Wildlife Restoration. C. STROBECK received funding for this research from Parks Canada and the Natural Sciences and Engineering Research Council.

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Communicating editor: R. R. HUDSON

Appendix A

Observed allele frequency distributions

Study Area	Alleles at locus G1A										
	180	*184*	*186*	*188*	*190*	*192*	*194*	*196*	*198*	*200*	*202*
LM					5	3	17	7	23	8	1
WS		36		9	8	59	94	8	18		
NI						35		1		28	
II	2	12	4			6	13	4	1	6	
FR		4	20		6	2	41		7		
KI		25			9	34					
IV	2	26					18				
VI		50					21		1		
V	3	78					34		1		
III	28	61	8		9	13	95	2	8	14	
I (BR)	23	72	1	2	11	63	119			5	
NB					21	9	14	6	3	7	
WH					5		43	1	10		1

	Alleles at locus G1D															
	172	*174*	*175*	*176*	*177*	*178*	*179*	*180*	*181*	*182*	*183*	*184*	*185*	*186*	*188*	*190*
LM	2	1		23		6				8		11		6	7	
WS	33	24		109		26		3		15		1		6	12	3
NI										62		1			1	
II	6	9		6	6	1			9	5				6		
FR			13		1	5	19	21	2	4	5	8	1		1	
KI						43				23				2		
IV	3	5		14	2	7				1	1	6		7		
VI	12	7		9	2	15		1		1	1	5		19		
V	18	18		35	5	12		1		11	5	8		3		
III	19	33		26	36	31		3	24	35		11		20		
I (BR)	72	11		10	42	63		3	31	25		13		26		
NB								1		33		12		3	9	2
WH								9		36		8			7	

	Alleles at locus G10B											
	140	*142*	*148*	*150*	*152*	*154*	*156*	*158*	*160*	*162*	*164*	*166*
LM						4	22	2	10	1	25	
WS							34	44	61	50	42	1
NI					5	41					18	
II	5		8	3	5	6		2	17		2	
FR					9	3	2	21	12	33		
KI						67		1				
IV	10		1	2	11	1	2		19			
VI	19			2	12	2	10	1	23	2	1	
V	46		2	9	15	5	9		30			
III	53		2	24	40	8		7	85		19	
I (BR)	49		27	12	7	4	13	47	121		16	
NB		5		1	9	25	9	11				
WH				4		9	43	4				

	Alleles at locus G10C									
	99	*101*	*103*	*105*	*107*	*109*	*111*	*113*	*115*	*117*
LM	1		11	4		10	7	8	20	3
WS	71		57	29	22	4	33	9	7	
NI								39	23	2
II		1	13	23	1	5	4	1		
FR		11	4	44	16		5			
KI				31			36	1		

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Continued

	Alleles at locus G10C									
	99	*101*	*103*	*105*	*107*	*109*	*111*	*113*	*115*	*117*
IV			14	29		3				
VI			2	62	8					
V			26	89	1					
III		23	67	110	9	6	2	21		
I (BR)		2	78	101	12	1	74	28		
NB			46	8	1	3	1		1	
WH		1	30		2	9	6	12		

	Alleles at locus G10L																		
	133	*135*	*137*	*139*	*141*	*143*	*145*	*147*	*149*	*151*	*153*	*155*	*157*	*159*	*161*	*163*	*165*	*169*	*171*
LM	1	7	7	9	3	5		1	5		3	13	3	2	3	2			
WS		18	35	2	12		20		35			4	12	75	1	4	6	8	
NI		50		14															
II												25	21			2			
FR												18	46	12	1			3	
KI												68							
IV												17	21	8					
VI												24	36	12					
V												54	44	18					
III										1		129	61	25		22			
I (BR)												141	83	27	39	2			4
NB							48	10	1	1									
WH					1	2	43	7		7									

	Alleles at locus G10M											
	196	*200*	*204*	*206*	*208*	*210*	*121*	*214*	*216*	*218*	*220*	*222*
LM				3	6	13	15	16	1	9		1
WS		2	4	10	26	33	56	24	54	15	8	
NI						15	46		3			
II				2	15	6	6	18		1		
FR	1			34		23	19	3				
KI					68							
IV					11	25	1	6	1	2		
VI					6	52	1	13				
V					15	66	9	19		7		
III				23	68	67	33	23		24		
I (BR)				24	134	16	32	81		8		1
NB		2		2	23	15	7	5	6			
WH		5			7	19	4	18	2	5		

	Alleles at locus G10P													
	139	*141*	*145*	*147*	*149*	*151*	*153*	*155*	*157*	*159*	*161*	*163*	*165*	*167*
LM						4		11	13	11	12	4	8	1
WS					1	11	38	37	30	56	20	26		13
NI					1			1	22	39	1			
II	1					7	6	10	6	6	12			
FR					3	33	19	1	11	10	3			
KI							45	21	2					
IV		1				5		8	6	2	23	1		
VI	4	4				1	15	11	2	2	33			
V		4				10	16	17	12	10	45		2	
III	2	6			2	48	51	42	20	15	52			
I (BR)	2				11	26	102	21	58	23	53			
NB			31	1	3	2	7	7	7	1	1			
WH			15	12	7	7		19						

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Continued

	Alleles at locus G10X																	
	125	*127*	*129*	*131*	*133*	*135*	*137*	*139*	*141*	*143*	*145*	*147*	*149*	*151*	*153*	*157*	*159*	*163*
LM	7	1						3	19	3		4	8	6	11	1	1	
WS	8	27	3	2	17			15			36	59	45	7	2	1		10
NI									46							18		
II				7	2	6	16	1	16									
FR			1		5	5	4	15	45	5								
KI							68											
IV			5	17		1	12		11									
VI			3	28	10	4	5		22									
V			11	37	5	2	22		39									
III				44	5	9	71		103		6							
I (BR)			8	16	31	62	117		62									
NB					16	3	5		7	24	1	4						
WH					6	12	27		1	9		4	1					

Allele designations are the size in the base pairs relative to the GS2500 (ABI) internal lane standard. All genotype designations were also checked visually against adjacent lanes.