Development and application of single nucleotide polymorphisms (SNPs) for bowhead whale population structure analysis.

Phillip A. Morin, Brittany L. Hancock

Southwest Fisheries Science Center, 8604 La Jolla Shores Dr., La Jolla, CA 92037, USA

J. Craig George

North Slope Borough Dept. of Wildlife Management, PO Box 69, Barrow, AK 99723, USA

Key Words: Genetics, Biomarkers, Breeding grounds, Feeding grounds, Stock identity

ABSTRACT

We present the preliminary results of efforts to develop new class of nuclear genetic markers, single nucleotide polymorphisms (SNPs), for bowhead whale genetic studies. We have screened over 9000 bp of random bowhead DNA sequence, identifying 67 SNPs. Nineteen SNP assays were optimized initially and used to genotype modern and historical bowhead DNA samples from St. Lawrence Island and Barrow, Alaska. We developed novel methods for genotyping multiple nuclear loci from historical and poor quality samples, demonstrating high efficiency and low estimated error rates. Preliminary population analyses with this limited set of loci show no evidence of population structure for various strata analyzed previously with mtDNA and microsatellite analysis. Future application of additional loci and samples should demonstrate the power of SNPs for population structure analysis, providing a strong alternative to microsatellite loci.

Introduction

Genetic analyses of bowhead whales (*Balaena mysticetus*) will benefit from using markers that allow datasets to build over a period of decades and that allow sample size augmentation, both spatially and temporally, through use of bone and baleen. The two most widely used marker types, mitochondrial DNA and microsatellites, while useful and indeed optimal for some applications, suffer from technical and analytical limitations. Analytical issues include the limitations of a single locus and its inheritance patterns (mtDNA, Gagneux et al. 1997; Hare 2001; Harpending et al. 1998), high and variable mutation rates that are difficult to model for appropriate analysis (microsatellites, Balloux & Lugon-Moulin 2002; Excoffier & Yang 1999), limitations due to sample size (B-Rao 2001), technical issues such as nuclear inserts of mitochondrial DNA (Numts, Bensasson *et al.* 2001), and microsatellite stutter bands, null alleles, and allelic dropout (Callen et al. 1993; Gagneux et al. 1997; Morin et al. 2001; Navidi et al. 1992; Taberlet et al. 1996). Microsatellites are particularly limited in long-term studies that require addition of data over time, across multiple labs (Hoffman et al. 2006; LaHood et al. 2002), and from samples of variable or poor quality (Broquet & Petit 2004; Paetkau 2003; Wandeler et al. 2003).

SNP genotyping is rapidly becoming a powerful tool for assessing genetic variation in wild populations (reviewed in Brumfield et al. 2003; Morin et al. 2004). Recent applications show that SNPs are extremely frequent, relatively easy to ascertain in many non-model organism genomes (Aitken et al. 2004; Elfstrom et al. 2006; Morin et al. 2006; Primmer et al. 2002), and can be applied to a wide range of population studies, from individual identification to population structure and taxonomy (Elfstrom et al. 2006; Glaubitz et al. 2003; Kuhner et al. 2000; Seddon et al. 2005; Smith et al. 2004). Some of the benefits of using SNPs relative to other nuclear markers, such as microsatellites, include ease and efficiency of discovery and genotyping (e.g., Elfstrom et al. 2006; Morin et al. 2006), ability to target variation in random genomic regions or known genes (Aitken et al. 2004; Kohn et al. 2006), existence of theoretical treatment (Chakraborty et al. 1999; Hedrick 2005; Kalinowski 2002; Nielsen 2000; Ryman et al. 2006) and analytical tools and methods for assessing power and population parameters (Ryman 2006; Ryman & Palm 2006).

SNPs offer potential benefits over microsatellites in several ways: 1) SNP genotypes are based on detection of DNA sequence nucleotide differences rather than PCR product size differences, so that genotype

data are universally comparable and portable, without the need to include common controls among studies and across time and technologies, as is the case with microsatellites. Thus, SNP studies can be replicated, performed in parallel across several laboratories, and added to as samples become available without the need to calibrate results at each step in the process. Non-portability of microsatellite data has resulted in significant data use limitations in some studies (e.g. Hoffman et al. 2006). 2) SNP genotyping technologies vary widely, allowing a choice of systems to meet the cost, throughput and equipment needs of each laboratory. Technologies can range from simple and standard (such as electrophoresis-based systems) to highly multiplexed for high-throughput genotyping (e.g., microarrays). 3) Although individual SNPs typically provide reduced statistical power compared to individual microsatellites, because they are typically limited to 2 alleles per locus, collections of SNP loci can rival microsatellites in power for most types of studies (reviewed in Morin et al. 2004), and can even provide greater power in some instances (Rosenberg et al. 2003).

Although the potential application of SNPs to degraded, historical, and ancient samples has been discussed in the literature (Asher & Hofreiter 2006; Noonan et al. 2005; Poinar et al. 2006; Römpler et al. 2006; Surridge et al. 2002), there are few examples to date (Römpler et al. 2006). As for all degraded samples, the issues of working with few copies of DNA also pertain to SNPs: contamination, allelic dropout, and PCR failure. For SNPs to be useful for these types of studies, they need to be assayed from relatively small DNA fragments (<150bp), and make use of multiplex PCR to minimize the number of PCR reactions performed from each extract. In a recent publication (Morin & McCarthy 2007), we have presented methods to allow genotyping of historical and low-quality samples by developing multiplex pre-amplification of all SNP loci in one PCR reaction prior to performing individual genotyping assays. Here we present results of our efforts to ascertain SNPs for bowhead whales, and apply them for analysis of population structure in the BCB bowheads, using modern and historical samples. We compare results and power to detect population structure for the two nuclear genetic marker systems: SNPs, and microsatellites

Materials and Methods:

We set out to develop approximately 30 SNP assays for bowhead population structure analysis. To do this, we obtained DNA sequences from previously cloned bowhead DNA from John Bickham. This DNA had been cloned as part of an effort to develop new microsatellite markers for bowhead genetics. Cloned DNA consists of random fragments of DNA inserted into bacterial DNA vectors, followed by enrichment for microsatellite repeats. Many of the cloned fragments, however, do not contain repeats, or have substantial stretches of non-repetitive DNA in addition to microsatellite repeats. We used the sequence information for these non-repetitive regions to design PCR primers to amplify those regions of DNA from multiple bowhead DNA samples, representing geographically widespread samples (Russia, St Lawrence Island, Barrow, and eastern Canada).

We sequenced 22 loci from nine to 17 bowhead DNA samples (average 13), for a total of approximately 9,422 bp of DNA sequence per sample. We detected 67 SNPs distributed among 21 of the loci, for an average of one SNP every 140 bp. This is a relatively high frequency of SNPs compared to other mammals that we have studied (Morin et al. 2006; Smith et al. 2004), though others have found similar SNP frequencies (N. Belfiore (ground squirrels, lizards), S. Roden (green sea turtles), pers comm.).

We initially generated assays for 20 independent SNPs (i.e., only one SNP per locus). Nineteen of these assays were optimized and used for SNP genotyping. Our genotyping methods have been described elsewhere (Morin & McCarthy 2007). We also obtained mtDNA sequence data from all of the samples (details in LeDuc et al. 2007)

We used the allele frequencies from all samples genotyped to simulate genetic power to detect population structure, using the program POWSIM (Ryman & Palm 2006). We simulated population drift to Fst levels of 0.0025, 0.005, 0.01, and 0.02 using effective population size (Ne) of 1000 and varying numbers of generations (t) (Table 1).

Analysis of population structure from SNP data was conducted using AMOVA in the software Arlequin v. 3.0 (Excoffier et al. 2005) and GENEPOP v. 3.4 (Raymond & Rousset 1995).

Results:

Results of this study are preliminary, as we have not completed genotyping of additional SNP loci beyond the 19 completed for data availability to IWC in November, 2006. Ongoing work includes genotyping of 10 additional loci on the samples reported here and additional samples from the Sea of Okhotsk. Use of additional loci will increase power to detect population structure.

We detected a genotyping error rate of < 1% across all samples, but this was heavily biased towards very poor quality historical samples with <10 copies/µl of DNA; the error rate in modern, high-quality samples was 0.1%. Details of our genotyping methods, replication, and error rates are in Morin and McCarthy (2007).

Power simulations indicated that the 19 SNPs used for this study have a 95% probability of detecting an F_{st} of approximately 0.02 when there are two populations, each with an effective size of 1,000, and 55 samples are genotyped from each population (Table 1).

Table 1. POWSIM Power simulation for 19 SNP loci, with 55 samples from each of two simulated populations. t is the simulated number of generations of drift used by the program to obtain the desired Fst and power estimate.

F_{st}	t	Power
0.0025	5	0.21
0.005	10	0.42
0.01	20	0.77
0.02	40	0.95

Our sample sets consisted of 107 samples in total, consisting of 50 samples from Saint Lawrence Island (SLI) and 57 samples from Barrow, after exclusion of duplicate samples and one from each pair of mother/fetus samples. Barrow samples were all from spring annual aboriginal kills. SLI samples were from a combination of tissue from recent aboriginal kills, and bone or baleen from historical aboriginal kills (4 to 40 years before present). See Morin and McCarthy (2007) for details.

Previous analysis of BCB bowhead population structure comparing the Barrow and SLI strata using mtDNA and microsatellites has indicated allele frequency differences in microsatellite loci (Givens et al. 2007), but not in mtDNA (LeDuc et al. 2006). Fst values based on 33 microsatellite loci were not significantly different from zero when all samples from SLI (N=25) were compared with all samples from Barrow (N=213) (Givens et al. 2007). When Barrow samples were compared to subsamples of SLI from Gambell and Savoonga, frequencies were significantly different for Barrow vs. Savoonga, but not Barrow vs. Gambell. The Savoonga difference was limited to fall Savoonga samples. We tested for spatial population structure using the SNP data to compare with the results of the other analyses. Comparison of Barrow (N=57) to all SLI (N=50) samples resulted in an F_{st} not significantly different from zero (Fst = -0.00465, p = 0.96). Comparison of samples from Gambell (N=31) and Savoonga (N=19) within SLI were also non-significant (Table 2). It should be noted that the sample sets used in these analyses were not the same, as microsatellite data could only be generated from modern skin samples, not from historical bone or baleen samples. As such, the SLI sample size is larger for the SNP data set than for the mircosatellite data set. Only a subset of Barrow samples was used for SNP genotyping. These were selected from the whole sample set to include 60 samples that had the highest percentage of completed microsatellite genotypes (i.e., the PCR failure rate was low), and from spring hunt animals only.

Pastene et al. (2007) found a difference in mtDNA haplotype frequencies between fall and spring SLI samples. These results were preliminary, and included at least one sample duplication (96G3), but indicated a significant F_{st} value (0.057, p = .0179). We tested this relationship with SNP data, and found no significant difference between samples collected in spring and fall (Table 2). Our sample set was nearly identical to that used by Pastene et al, differing by two samples. One sample was replaced in our data set by that of it's first order relative (a fetus (05S7f) genotyped instead of its mother (05S7)), and another sample (89S1) was not included in our analysis because we have not yet completed genotyping on it. The remaining 31 samples were the same for both analyses. It should be pointed out that power to detect population structure is further reduced in this analysis because of the small samples sizes for each stratum; we have not estimated expected power for these small sample sizes.

In addition to the seasonal allele frequency differences reported by Pastene et al. (2007), analysis of mtDNA from samples stratified by year of birth has resulted in a finding of significant allele frequency differences between the oldest and youngest age groups (LeDuc et al. 2007). We repeated the birth-year stratification for the SNP loci, and found no indication of nuclear locus differentiation. The sample size for this analysis, however, was significantly reduced compared to the mtDNA analysis, with only 16 samples in total analyzed (7 animals born before 1950; 9 animals born after 1950). Age data are not available for the other 94 samples for which we have SNP genotypes.

Table 2: Population comparison results from AMOVA (F_{st}) as calculated in Arlequin, and Chi Square (χ^2) from Fisher's exact test as calculated in GENEPOP.

Strata	F_{st}	p	χ^2	p	df
Barrow-SLI	-0.005	0.96	22.14	0.98	38
Savoonga-Gambell	0.003	0.40	26.19	.88	36
SLI spring-fall	0.004	0.48	18.60	0.99	34
SLI age groups	-0.023	0.79	15.24	.99	34

Skull and baleen sample matching:

Because many of the skulls and baleen samples could not be assigned with certainty to a whale ID (i.e., a whale that was killed and assigned an ID number at that time), we performed genetic match analysis using the program DROPOUT (McKelvey & Schwartz 2004). This program looks for multilocus genotype matches among samples, and identifies sample pairs with complete or nearly matched genotypes. It also calculates the distribution of matches and estimates the likelihood of a given number of matched genotypes by chance alone.

We confirmed assignment of two samples that were assigned to a whale ID based on interviews with whaling captains and local residents of Gambell (Table 3: C. George, pers comm.).

Table 3: Genetically matched pairs of samples, based on composite SNP genotypes. The mtDNA haplotype for each sample in a matched pair is also shown. Composite genotypes that differ at 1 locus and have the same mtDNA haplotype are putative matches, with mismatched genotypes assumed to be in error, possibly because of allelic dropout.

Sample ID	Matched to sample ID	mtDNA haplotype	WhaleID assigned by C. George	Comments
GBAL5	02G2	BH31	(not assigned)	Possible match to GSK13, but data incomplete
GSK5	96G3	BH51	96G3*	Genotypes differ at 1 locus
GSK2	03G1	BH42	03G1*	
GSK14	GBAL1	BH50	95G4*	95G4 sample not available
GSK14	GBAL2	BH50	95G4*	95G4 sample not available
GSK17	GBAL4	BH42	90G3	90G3 sample not available
SBAL1	SBAL4	BH5	98S3	SBAL4 taken from skull SSK3 (98S3 and SSK3 not sampled)
GSK22	GBAL3	BH42	01G1(92G2)	3 skulls in a pile; 01G1 & 92G2 not available
GSK23	96G1_2	BH22	86G3(88G2)	Genotypes differ at 1 locus; 86G3 & 88G2 not available
GSK27	04G3	BH28	04G1	Genotypes differ at 1 locus
01B12	01B16	BH42		Genotypes differ at 1 locus
02S2	02S4	BH42		Previously matched (02S2_4)

* whale ID 'fairly certain', C. George.

In addition to the samples matched with SNP genotypes and mtDNA haplotypes in Table 3, we were able to refute several tentative whale ID's given to the skull samples based on interviews with whaling captains and residents of Gambell. For one sample, we were able to positively exclude a match because the both the composite SNP genotypes and the mtDNA haplotypes did not match: GSK19 ≠ 96G1. For two other cases, although we didn't have whale tissue samples to compare to skull samples for these pairs, the fact that the skulls had genotypes that matched to one whale ID, but had been assigned to a different whale ID, indicates that they were originally mis-assigned: GSK23 ≠ 86G3 or 88G2, GSK27 ≠ 04G1. The remaining assignment of Whale IDs could not be confirmed or refuted based on genetic data, as comparative samples were not available.

Conclusions:

The SNP data, although preliminary, have proven effective for genetic identification of samples, and for population structure analysis. Estimated power to detect population structure is estimated to be sufficient to detect F_{st} values ≥0.02 with the 19 loci reported in this paper, and is expected to increase substantially with the addition of 10 loci currently being genotyped. Estimated error rates were exceptionally low when modern samples were used, and an order of magnitude lower than is typical for microsatellite genotype data (Morin & McCarthy 2007).

The use of SNPs has allowed us to substantially increase our sample size for nuclear locus genotyping from SLI by making use of historical bone and baleen samples. We were able to successfully genotype 26 skull and baleen samples, adding 16 unique samples after matches were made between baleen and skull samples, or between historical and modern samples.

As reported in Morin and McCarthy (2007), the SNP genotyping process we have developed for historical and modern samples is efficient, cost effective, and produces high quality data. All of these factors, combined with the inherent advantages of SNPs over microsatellites (data portability, analytical models, ease of ascertainment) strongly support the use of SNPs as alternative nuclear markers that will be superior to microsatellite data in most, if not all, population genetic studies.

Acknowledgements:

We are grateful to George Noongwook and the residents of St Lawrence Island for collection of bone and baleen samples, to Lianne Postma for access to eastern Canadian bowhead samples, and to John Bickham and Ryan Huebinger at Texas A&M University for providing access to bowhead microsatellite library sequences for primer design. Janet Lowther provided technical assistance for SNP genotyping. Barb Taylor, Rick Leduc, and Karen Martien provided helpful advice on earlier drafts of this manuscript. Funding for the laboratory work was from the North Pacific Research Board and for Phillip Morin from Southwest Fisheries Science Center. All samples were collected by or transferred to the SWFSC under NMFS/MMPA and CITES permits.

References:

Aitken, N., Smith, S., Schwarz, C., and Morin, P. A. 2004. Single nucleotide polymorphism (SNP) discovery in mammals: a targeted-gene approach. Molecular Ecology **13**:1423-1431.

Asher, R. J., and Hofreiter, M. 2006. Tenrec phylogeny and the noninvasive extraction of nuclear DNA. Systematic Biology **55**:181-194.

B-Rao, C. 2001. Sample size considerations in genetic polymorphism studies. Hum Hered **52**:191-200.

Balloux, F., and Lugon-Moulin, N. 2002. The estimation of population differentiation with microsatellite markers. Mol Ecol **11**:155-165.

Bensasson, D., Zhang, D. X., Hartl, D. L., and Hewitt, G. M. 2001. Mitochondrial pseudogenes: evolution's misplaced witnesses. Trends in Ecology & Evolution **16**:314-321.

Broquet, T., and Petit, E. 2004. Quantifying genotyping errors in noninvasive population genetics. Mol Ecol **13**:3601-3608.

Brumfield, R. T., Beerli, P., Nickerson, D. A., and Edwards, S. V. 2003. The utility of single nucleotide polymorphisms in inferences of population history. Trends in Ecology & Evolution **18**:249-255.

Callen, D. F., Thompson, A. D., Shen, Y., Phillips, H. A., Richards, R. I., Mulley, J. C., and Sutherland, G. R. 1993. Incidence and origin of "null" alleles in the $(AC)_n$ microsatellite markers. American Journal of Human Genetics **52**:922-927.

Chakraborty, R., Stivers, D. N., Su, B., Zhong, Y., and Budowle, B. 1999. The utility of short tandem repeat loci beyond human identification: implications for development of new DNA typing systems. Electrophoresis **20**:1682-1696.

Elfstrom, C. M., Smith, C. T., and Seeb, J. E. 2006. Thirty-two single nucleotide polymorphism markers for high-throughput genotyping of sockeye salmon Molecular Ecology Notes **6**:1255-1259.

Excoffier, L., Laval, G., and Schneider, S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online **1**:47-50.

Excoffier, L., and Yang, Z. 1999. Substitution rate variation among sites in mitochondrial hypervariable region I of humans and chimpanzees. Mol Biol Evol **16**:1357-1368.

Gagneux, P., Boesch, C., and Woodruff, D. S. 1997. Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. Molecular Ecology **6**:861-868.

Givens, G. H., Huebinger, R. M., Bickham, J. W., George, J. C., and Suydam, R. 2007. Patterns of Genetic Differentiation in Bowhead Whales (Balaena mysticetus) from the Western Arctic. report No. SC/J07/AWMP 2.

Glaubitz, J. C., Rhodes, O. E., and Dewoody, J. A. 2003. Prospects for inferring pairwise relationships with single nucleotide polymorphisms. Molecular Ecology **12**:1039-1047.

Hare, M. P. 2001. Prospects for a nuclear gene phylogeography. Trends in Ecology & Evolution **16**:700-706.

Harpending, H. C., Batzer, M. A., Gurven, M., Jorde, L. B., Rogers, A. R., and Sherry, S. T. 1998. Genetic traces of ancient demography. Proceedings of the National Academy of Science U S A **95**:1961-1967.

Hedrick, P. W. 2005. A standardized genetic differentiation measure. International Journal of Organic Evolution **59**:1633-1638.

Hoffman, J. I., Matson, C. W., Amos, W., Loughlin, T. R., and Bickham, J. W. 2006. Deep genetic subdivision within a continuously distributed and highly vagile marine mammal, the Steller's sea lion (Eumetopias jubatus). Molecular Ecology **15**:2821-2832.

Kalinowski, S. T. 2002. How many alleles per locus should be used to estimate genetic distances? Heredity **88**:62-65.

Kohn, M. H., Murphy, W. J., Ostrander, E. A., and Wayne, R. K. 2006. Genomics and conservation genetics. Trends in Ecology and Evolution **21**:629-637.

Kuhner, M. K., Beerli, P., Yamato, J., and Felsenstein, J. 2000. Usefulness of single nucleotide polymorphism data for estimating population parameters. Genetics **156**:439-447.

LaHood, E. S., Moran, P., Olsen, J., Grant, W. S., and Park, L. K. 2002. Microsatellite allele ladders in two species of Pacific salmon: preparation and field-test results. Molecular Ecology Notes **2**:187-190.

LeDuc, R. G., Martien, K. K., Morin, P. A., Hedrick, N., Robertson, K., Taylor, B. L., George, J. C., Mugue, N., Borodin, R., and Zelennia, D. 2007. Mitochondrial genetic variation in bowhead whales in the western Arctic. report No. SC/59/BRG9.

LeDuc, R. G., Morin, P., George, Noongwook, G., Hancock, B., Robertson, K. M., and Taylor, B. L. 2006. Mitochondrial sequence variation in the Bering/Chukchi/Beaufort Seas bowhead whales. report No. SC/58/BRG9.

McKelvey, K. S., and Schwartz, M. K. 2004. Genetic errors associated with population estimation using non-invasive molecular tagging: problems and new solutions. Journal of Wildlife Management **68**:439-448.

Morin, P. A., Aitken, N. C., Rubio-Cisneros, N., Dizon, A. E., and Mesnick, S. 2006. Characterization of 18 SNP markers for sperm whale (*Physeter macrocephalus*). Molecular Ecology Notes **in press**.

Morin, P. A., Chambers, K. E., Boesch, C., and Vigilant, L. 2001. Quantitative polymerase chain reaction analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzees (*Pan troglodytes verus*). Mol Ecol **10**:1835-1844.

Morin, P. A., Luikart, G., Wayne, R. K., and SNP-Workshop_Grp. 2004. SNPs in ecology, evolution and conservation. Trends in Ecology & Evolution **19**:208-216.

Morin, P. A., and McCarthy, M. 2007. Highly accurate SNP genotyping from historical and low-quality samples. Molecular Ecology Notes **in review**.

Navidi, W., Arnheim, N., and Waterman, M. S. 1992. A multiple-tubes approach for accurate genotyping of very small DNA samples by using PCR: statistical considerations. Am J Hum Genet **50**:347-359.

Nielsen, R. 2000. Estimation of population parameters and recombination rates from single nucleotide polymorphisms. Genetics **154**:931-942.

Noonan, J. P., Hofreiter, M., Smith, D., Priest, J. R., Rohland, N., Rabeder, G., Krause, J., Detter, J. C., Paabo, S., and Rubin, E. M. 2005. Genomic sequencing of Pleistocene cave bears. Science **309**:597-599.

Paetkau, D. 2003. An empirical exploration of data quality in DNA-based population inventories. Molecular Ecology **12**:1375-1387.

Pastene, L. A., Goto, M., and Kitakado, T. 2007. Further mitochondrial DNA analysis on stock structure in the B-C-B stock of bowhead whale. report No. SC/J07/AWMP4.

Poinar, H. N., Schwarz, C., Qi, J., Shapiro, B., Macphee, R. D., Buigues, B., Tikhonov, A., Huson, D. H., Tomsho, L. P., Auch, A., Rampp, M., Miller, W., and Schuster, S. C. 2006. Metagenomics to paleogenomics: large-scale sequencing of mammoth DNA. Science **311**:392-394.

Primmer, C. R., Borge, T., Lindell, J., and Saetre, G. P. 2002. Single-nucleotide polymorphism characterization in species with limited available sequence information: high nucleotide diversity revealed in the avian genome. Molecular Ecology **11**:603-612.

Raymond, M., and Rousset, F. 1995. GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. The Journal of Heredity **86**:248-249.

Römpler, H., Rohland, N., Lalueza-Fox, C., Willerslev, E., Kuznetsova, T., Rabeder, G., Bertranpetit, J., Schoneberg, T., and Hofreiter, M. 2006. Nuclear gene indicates coat-color polymorphism in mammoths. Science **313**:62.

Rosenberg, N. A., Li, L. M., Ward, R., and Pritchard, J. K. 2003. Informativeness of genetic markers for inference of ancestry. American Journal of Human Genetics **73**:1402-1422.

Ryman, N. 2006. CHIFISH: a computer program testing for genetic heterogeneity at multiple loci using chi-square and Fisher's exact test. Molecular Ecology Notes **6**:285-287.

Ryman, N., and Palm, S. 2006. POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. Molecular Ecology Notes **6**:600-602.

Ryman, N., Palm, S., Andre, C., Carvalho, G. R., Dahlgren, T. G., Jorde, P. E., Laikre, L., Larsson, L. C., Palme, A., and Ruzzante, D. E. 2006. Power for detecting genetic divergence: differences between statistical methods and marker loci. Molecular Ecology **15**:2031-2045.

Seddon, J. M., Parker, H. G., Ostrander, E. A., and Ellegren, H. 2005. SNPs in ecological and conservation studies: a test in the Scandinavian wolf population. Molecular Ecology **14**:503-511.

Smith, S., Aitken, N., Schwarz, C., and Morin, P. A. 2004. Characterisation of 15 single nucleotide polymorphism markers for chimpanzees (*Pan troglodytes*). Molecular Ecology Notes **4**:348-351.

Surridge, A. K., Smith, A. C., Buchanan-Smith, H. M., and Mundy, N. I. 2002. Single-copy nuclear DNA sequences obtained from noninvasively collected primate feces. American Journal of Primatology **56**:185-190.

Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., Waits, L. P., and Bouvet, J. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Res **24**:3189-3194.

Wandeler, P., Morin, P. A., Smith, S., Pettifor, R. A., and Funk, S. M. 2003. Patterns of nuclear DNA degeneration over time - a case study in historic teeth samples. Molecular Ecology **12**:1087-1093.