Detecting genetic structure in migrating bowhead whales off the coast of Barrow, Alaska

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Abstract

We develop a general framework for analysing and testing genetic structure within a migratory assemblage that is based on measures of genetic differences between individuals. We demonstrate this method using microsatellite DNA data from the Bering-Chukchi-Beaufort stock of bowhead whales (Balaena mysticetus), sampled via Inuit hunting during the spring and autumn migration off Barrow, Alaska. This study includes a number of covariates such as whale ages and the time separation between captures. Applying the method to a sample of 117 bowhead whales, we use permutation methods to test for temporal trends in genetic differences that can be ascribed to age-related effects or to timing of catches during the seasons. The results reveal a pattern with elevated genetic differences among whales caught about a week apart, and are statistically significant for the autumn migration. In contrast, we find no effects of time of birth or age-difference on genetic differences. We discuss possible explanations for the results, including population substructuring, demographic consequences of historical overexploitation, and social structuring during migration.

Keywords: Balaena mysticetus, bowhead whale, genetic structure, microsatellites, statistical modelling, temporal pattern

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Introduction

Most previously developed methods for the assessment of genetic variability and the concomitant identification of mixed-stock assemblages (e.g. Smouse et al. 1990; Paetkau et al. 1995; Rannala & Mountain 1997) require genetic data from geographically separated breeding populations as reference material. However, in some cases data from breeding sites are not available because they are remotely located, unknown, or inaccessible. For example, genetics of freshwater eels (Anguilla) has been studied from their freshwater habitats in Europe and North America (Avise et al. 1986; Daemen et al. 2001; Wirth & Bernatchez 2001). But these species spawn at great depths in the Sargasso Sea from where samples are not available. Furthermore, genetic analyses of population structure in migratory marine animals typically require carefully planned temporal and spatial collections of individuals to be representative (e.g. Nesbo et al. 2000). For many organisms, including the cetaceans considered herein, such data are not available. Instead, samples are generally collected from animals killed near a few land-based villages during subsistence hunts or opportunistically during commercial hunts. For such organisms, few analytical tools are currently available, and those that are available (e.g. STRUCTURE and BAPS: Pritchard et al. 2000; Corander et al. 2003, respectively) typically do not utilize auxiliary information and may lack in statistical power in specific applications. The methods and analyses presented in this study directly address such problems.

The bowhead (Balaena mysticetus) is a large baleen whale inhabiting the Arctic oceans. Five stocks are presently...
recognized within the species' distribution range. These
to extensive commercial hunting in the 19th century, and the Bering-Chukchi-Beaufort Seas (BCB)
stock is the only one that is certain to be recovering success-
fully after commercial whaling ended in 1914 (Bockstoce
1986; Bockstoce & Burns 1993; Zeh et al. 1993; George et al.
2004). Whales of the BCB stock winter in the Bering Sea and
migrate northward to summering areas, predominantly in
the eastern Beaufort Sea (Moore & Reeves 1993). Based on
Russian observations and oceanographic data, there might
be two distinct patterns in feeding migration for whales
wintering in the Bering Sea. Melnikov et al. (2004) and
Bogoslovskaya (2003) describe a delay in spring migration of
bowheads out of the Gulf of Anadyr and north through the
Bering Strait as well as sightings on the Chukotka coast in
summer and early autumn. Some animals may reside in the
Bering or Chukchi seas during summer but the numbers
and whereabouts of these are poorly known. Mating likely
occurs from March to May, possibly in wintering areas or
during the spring migration (Koski et al. 1993).

In migration, whales are hunted for subsistence by
aboriginal peoples, primarily in Alaska. Of the 10 villages
that hunt in Alaska, Barrow is the only community that harvests
bowheads both during the spring and autumn migration,
and lands about 70% of the total catch. Examination of landed
whales has provided a great deal of information about the
biology of bowheads including some data on their unique
characteristics: they are very long-lived, perhaps up to
200 years or more, and attain sexual maturity at a rather
late age of approximately 20 years (George et al. 1999).

The International Whaling Commission (IWC) has man-
aged BCB bowheads as a single stock, and has developed
a management plan for Alaskan aboriginal subsistence
harvest based on the single-stock hypothesis. A general
management concern is the risk of depleting genetically
distinct population components when exploiting the stock
under the assumption of a single population. Different
populations may coexist temporally, but if they do so
during the subsistence hunt or commercial harvest, as is
typically the case, then stock management needs to take
this into account (Hoelzel 1998). Until recently, nearly all
available evidence suggested that a single-stock hypothesis
was the most reasonable one (Rugh et al. 2003). However,
the availability of new genetics data has motivated further
study of potential substructuring of BCB bowheads, uncover-
ing some reasons to question the single-stock hypothesis.
Aside from its management implications, the issue of potential
structure also has important implications for understand-
ing the biology and demography of this species.

Multilocus microsatellite DNA polymorphisms have
seen widespread use as a powerful tool for detecting popu-
lation structure in a wide range of species, including whales
and dolphins (Hoelzel 1994; references therein). Previous
genetic studies of the BCB stock of bowhead whales have
been restricted largely to testing for effects of putative popu-
lation bottlenecks caused by commercial whaling (Rooney
et al. 1999a, 2001). These studies have uncovered high levels
of genetic variability in BCB bowheads, but have yielded
limited information bearing on the issue of potential sub-
structuring of the stock.

In this study, we analyse microsatellite DNA poly-
morphisms in migrating bowhead whales off Barrow, Alaska,
with the objective of assessing to what extent there is a
structural substructure within the stock. As an aid in the analysis,
we develop a statistical framework that is based on genetic
differences within pairs of whales from the migratory
assemble (cf. Rousset 2000). The method is general and
may be applicable to other migratory species also, even
when no geographical reference samples are available.

Materials and methods
The data for this study are from Hunter (2005) which were
reported to the International Whaling Commission by
Bickham et al. (2004). All individuals were genotyped anew
relative to an earlier screening that contained a subset of the
present sample (Rooney et al. 1999a; Rugh et al. 2003). The
rescreening was done in order to ensure consistent allele
calling among older and new samples. The data refer to
samples of bowhead whales (n = 207) obtained from 1983
through 2003 and scored at up to 12 microsatellite loci: Ev1
and Ev104 (Valsecchi & Amos 1996); Gata28 (Palsbøll
et al. 1997); Tv7 (Rooney et al. 1999b); Tv11, Tv13, Tv14, Tv16,
Tv17, Tv18, Tv19, and Tv20 (Rooney et al. 1999a). For the
present analysis, we excluded two loci (Tv7 and Tv18) that
were found to be problematic during preliminary analysies,
displaying large deficiencies of heterozygotes and indicating
segregation of null alleles (Tv7) and short allele dominance
(Tv18), respectively. Tissue quality varied among samples
and not all individuals could be scored at all loci. We chose
to exclude all individuals that failed scoring at more than
one microsatellite after repeated attempts. The resulting
data set used in the present analyses included 10 microsatellite
loci scored in 134 bowhead whales, most of which were
captured at Barrow (n = 117), or from other Alaskan communities
(Point Hope, n = 3; Wainwright, n = 1; Nuiqsut, n = 1;
Kaktovik, n = 1), from St Lawrence Island (Gambell, n = 4;
Savoonga, n = 5), or from Russia (Chukotka, n = 2) (Fig. 1).
Individuals were caught during the spring (April–June)
and autumn (September–November) migration in approxi-
mately equal proportions (Fig. 2). The annual sample sizes
are not balanced (cf. Table 4), and we discuss potential
consequences for our analysis below.

Statistical model for genetic structure
The model that we propose is based on pairwise genetic
differences among individuals, following Rousset (2000).
Fig. 1 Map of the seasonal distribution (coloured areas) and migration routes (arrows) of BCB bowhead whales. Yellow dots denote whaling villages. Whales pass Barrow and are hunted there during both the spring and autumn migrations.

Fig. 2 Date of landing of individual whales in different years. Open circles: whales landed at Barrow; +: whales landed at other villages. Points are jittered for clarity.
The methods take advantage of the timing of individual catches as whales pass Barrow, the primary sample site, during their northward (spring) and southward (autumn) migrations (Fig. 1). We analyse data from spring and autumn separately to allow for possible differences in migratory behavior and structure in the two seasons. Calendar time of migration varies from year to year, and we consider only pairs of whales taken in the same year. Hence, data from spring and autumn are analysed separately, and there is stratification by sampling year. No temporal pattern should emerge if all individuals belong to the same biological population, assuming that the population is genetically well mixed. However, the population may not be well mixed for several reasons. For example, the sampling interval (20 years) is substantial and notable genetic change (e.g. random drift) may have occurred during the interval if the effective bowhead population size is small. Also, remnants of earlier (historical) drift may still exist in the population in the form of genetic differences among age classes (cf. Jorde & Ryman 1995; Ryman 1997), especially considering the exceptional longevity of this species. We used available length data to estimate age and test whether there is any effect of time of birth on the genetic differences among individual bowheads.

Patterns in pairwise genetic differences are detected using a statistical model with genetic differences between whales in pairs as the dependent variable and various covariates related to timing of sampling and birth as independent variables (equation 2). Genetic difference in a pair of whales was estimated from the microsatellite genotypes following the logic of Rousset (2000). Briefly, for each locus we estimated the probability that two individuals, i and j, carry the same allele (‘same’ in the meaning ‘alike in state’, not necessarily identical by descent). This was done by comparing the two gene copies carried within an individual with the two copies carried within the other individual in the pair and recording if they are the same (identity, \( I = 1 \)) or different (\( I = 0 \)). For each locus, \( I \), and pair of individuals, \( ij \), there are four such comparisons, with each of the two genes in individual \( i \) being compared to each of the two genes in individual \( j \). We calculated an average similarity score \( I_{ij} \), over the four comparisons in this pair and locus. This score was contrasted to the frequency of identity of genes within individuals, yielding an estimate of pairwise genetic difference between two individuals (cf. Rousset 2000):

\[
a_{ij} = \frac{1}{L_{ij}} \sum_{l} h_l - I_{ij} \frac{1}{1 - h_l}
\]  

(1)

Here, \( h_l \) is the average proportion of homozygous individuals at the \( l \)th locus (averaged over all sampled individuals from this season and not just the two individuals under consideration), and \( L_{ij} \) is the number of loci that were simultaneously scored in both individuals \( i \) and \( j \). The calculations were repeated for all pairs of individuals. We note that in the absence of any population substructuring, genetic similarity within individuals (\( a_{ij} \)) should on average equal that among individuals (\( I_{ij} \)) and the expected value of \( a_{ij} \) is zero. It remains zero when further conditioning on covariates not causing genetic segregation. With departure from Hardy–Weinberg equilibrium (e.g. inbreeding), the expected value might be different from zero, but should still not be affected by nonsegregating covariates.

Age of whales was estimated from their measured length by the von Bertalanffy growth function, using growth parameters from Rosa et al. (2004), separately for each sex. From ages thus estimated and from known years of sampling, we calculated the number of years \( y_{ij} \) between birth and the mean year of birth \( m_{ij} \) for each pair of individuals. With \( d_{ij} \) denoting the number of days between sampling in the pair, and focusing only on pairs caught in the same year and season and at the same locality (Barrow), we fitted the additive model:

\[
a_{ij} \sim s(d_{ij}) + s(y_{ij}) + s(m_{ij}).
\]  

(2)

Model (2) is denoted \( d + y + m \) for short, while \( d + y, d + m, \) and \( y + m \) denote submodels leaving out one of the covariates \( m, y, \) and \( d \), respectively. In equation 2, the genetic difference between individuals (\( a_{ij} \)) is the response, and \( s() \) are smoothing spline functions with degrees of freedom chosen by cross-validation.

The models were fitted using the S-plus function GAM (Venables & Ripley 1999) with weights proportional to the number of scored loci (\( L_{ij} \)) in each pairwise comparison. We did not separate between the sexes in the model as preliminary analyses indicated that there is no effect of sex on \( a \). Indeed, there are no detectable allele frequency differences between the sexes, and the average \( F_{ST} \) (Weir & Cockerham 1984) between males and females is negative (−0.003). Sex was therefore dropped from further consideration.

The statistical significance of genetic patterns is obtained by analysis of variance, with null distributions calculated from randomly permuted data. When testing for age effects (\( y \) and \( m \)) in models only including such effects, the variance of fitted values for observed pairs is compared to the distribution of variance of fitted values in replicated simulated data where the response is as observed and the birth years are randomly permuted within season and year. The same approach is used to test if days apart (\( d \)) has a significant effect on genetic difference in a model without age effects, by randomly permuting day of catch within season and year. To test whether there is a partial temporal effect on genetic difference, days of catch are randomly permuted while year of birth is kept as observed. The partial effect of days apart was tested also in the \( d + y \) model. The distribution of variance of fits to simulated data is a null distribution for the observed fits variance.

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The effects of factors $d$, $y$ and $m$ on $a$ are analysed as follows. First, we fit model (2) and all its six submodels (i.e. $d, y, m, d + y, d + m,$ and $y + m$) for each season separately. The fit of each model is measured by the $P$ value of $R^2 = \text{var}(\hat{a}) / \text{var}(a)$, where $\hat{a}$ is the fitted value for the model to be tested, and where the variance is taken over the paired individuals (within year). The null distribution of $R^2$ is estimated from 5000 replicate simulations. In each replicate, the vectors of day of catch and year of birth are randomly permuted over individuals within year. The model is then fitted and a simulated null value of $R^2$ is calculated from the variance of predicted genetic differences, $\text{var}(\hat{a})$, in the simulation. We concentrate on models that significantly explain some of the variation, as expressed by $R^2$, and which include the term $y$ since some genetic drift is expected in any finite population.

To test for partial effect of days apart, $d$, when controlling for age-related ($y$ and $m$) effects, a new set of 5000 simulations was performed. In this test, only day of catch is randomly permuted within year before the model is fitted and $R^2$ is calculated. In addition to the resulting $P$ value of $R^2$, a simultaneous $P$ value based on the confidence bands discussed below is also calculated. The analysis of variance test is more powerful since the confidence band method really consists of combining separate tests for each value of $d$ through a Bonferroni type construction.

The shape of the partial effect curve $s(d_j)$ in model (2), with or without the covariate $m$, might give a hint of a potential genetic segregation in the population. A graph of the estimated partial effect curve of days apart is obtained as the predicted genetic difference within pairs taken $0, 1, 2, \ldots$ days apart, assuming difference between birth years and mean birth years equal in all the hypothetical pairs, and equal to their medians in the sample. The following variation of the method of Beran (1988) is used to obtain simultaneous null-bands for the partial-effect curves. A 95% point-wise null-band is first calculated from simulated partial effect curves, using 5000 replicate permutations, with day of catch randomly permuted within season and year and with genetics and year of births as observed. At $d$ days apart, the point-wise 95% interval is $(\hat{a}_d - l\hat{d}_d, \hat{a}_d + h\hat{d}_d)$ where $l\hat{d}_d$ and $h\hat{d}_d$ are, respectively, the 2.5%, 50% and the 97.5% percentiles of the simulated curves at $d$ days apart. Simultaneous null-bands of confidence are obtained by replacing $l\hat{d}_d$ by $x^\ast l\hat{d}_d$ and $h\hat{d}_d$ by $x^\ast h\hat{d}_d$ where $x \geq 1$ is the multiplier required to contain the entire simulated curves in 95% of the simulations. Similar to the construction of these confidence bands we also adjusted the factor $x$ to obtain a null-band that just contained the observed partial effect curve at all points. The fraction of the null-simulated effect curves that were not entirely contained within this null-band was taken as the simultaneous $P$ value of the observed partial effect curve.

Cluster analysis of the total sample

The number of sampled whales at localities outside Barrow (1–5 whales from each locality; Fig. 1) is too small for statistically meaningful tests for geographical differentiation. Instead, we pooled all 134 individuals and tested for potential population structure in this combined sample using the software STRUCTURE (Pritchard et al. 2000) and baps (Corander et al. 2003). These programs implement algorithms to cluster individuals on the basis of their multilocus (microsatellite) genotype.

The algorithm in STRUCTURE attempts to minimize deviations from Hardy–Weinberg genotype proportions within loci and to minimize gametic phase disequilibrium among loci, while simultaneously maximizing differences among the specified (assumed) number of subpopulations, $K$ (Pritchard et al. 2000). We ran the software (version 2) with a burn-in period of 100 000 and with 100 million Markov chain Monte Carlo steps for different values of $K$, ranging from 1 to 5. In the analysis, we assumed correlated allele frequencies among putative subpopulations and allowed for population mixture (the default options). Inference on population structure was made by comparing the calculated log probability of the data given the assumed number of subpopulations ($\text{Ln}[\text{Pr}(X | K)]$) for different values of $K$.

baps (Bayesian analysis of population structure) is similar in scope to STRUCTURE in the sense that it attempts to uncover population structure from multilocus genotypes, assuming Hardy–Weinberg equilibrium and gametic phase equilibrium within the constituent subpopulations (Corander et al. 2003). The software treats allele frequencies and number of subpopulations as random variables and uses stochastic optimization to infer the posterior mode of the number of subpopulations. We used baps (version 3.2) to cluster individuals into an optimal number of subpopulations ($K$), and ran the software with a predefined maximum of $K = 5$. The software was supplied with a vector of 100 replicate maximum $K$ values, and we repeated the runs 10 times in order to check on the stability of the results. For each run, the software reports the probabilities for different numbers of clusters or subpopulations, $K \leq \text{maximum } K$, and we averaged probabilities over the 10 runs and calculated the standard errors of the means.

Results

The microsatellites reveal a fair amount of genetic variability in the BCB whales (Table 1). All 10 loci are highly polymorphic with observed number of alleles ranging from four (at Tv16) to 11 (Tv17) in the combined sample of 134 bowheads. The proportion of heterozygote individuals ranges from 0.4627 (at Tv16) to 0.8507 (Gata28), with an average of 0.6984. There is little or no evidence for deviations from Hardy–Weinberg expectations at any of these loci. Six loci display
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Table 1 Summary statistics of genetic variability in 10 microsatellite loci in 134 BCB bowhead whales. Alleles, observed number of alleles at each locus; \( H_0 \), proportion of heterozygote individuals; \( F_{IS} \), deviations from Hardy–Weinberg genotype proportions. \( P \) values refer to exact tests for Hardy–Weinberg disequilibrium using \texttt{geneq} (Raymond & Rousset 1995), with a joint \( P \) value calculated over loci using Fisher’s summation procedure.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Alleles</th>
<th>( H_0 )</th>
<th>( F_{IS} )</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tv11</td>
<td>5</td>
<td>0.5769</td>
<td>0.0576</td>
<td>0.654</td>
</tr>
<tr>
<td>Tv13</td>
<td>7</td>
<td>0.7090</td>
<td>-0.0209</td>
<td>0.563</td>
</tr>
<tr>
<td>Tv14</td>
<td>8</td>
<td>0.5821</td>
<td>0.0918</td>
<td>0.175</td>
</tr>
<tr>
<td>Tv16</td>
<td>4</td>
<td>0.4627</td>
<td>0.0425</td>
<td>0.571</td>
</tr>
<tr>
<td>Tv17</td>
<td>11</td>
<td>0.8175</td>
<td>-0.0184</td>
<td>0.598</td>
</tr>
<tr>
<td>Tv19</td>
<td>6</td>
<td>0.7438</td>
<td>0.0457</td>
<td>0.273</td>
</tr>
<tr>
<td>Tv20</td>
<td>6</td>
<td>0.6791</td>
<td>-0.0265</td>
<td>0.498</td>
</tr>
<tr>
<td>Gata28</td>
<td>10</td>
<td>0.8507</td>
<td>0.0177</td>
<td>0.996</td>
</tr>
<tr>
<td>Ev1</td>
<td>6</td>
<td>0.7559</td>
<td>-0.0047</td>
<td>0.842</td>
</tr>
<tr>
<td>Ev104</td>
<td>10</td>
<td>0.8062</td>
<td>0.0338</td>
<td>0.627</td>
</tr>
<tr>
<td>Average</td>
<td>7.3</td>
<td>0.6984</td>
<td>0.0204</td>
<td>0.881</td>
</tr>
</tbody>
</table>

positive \( F_{IS} \)-estimates (i.e. a deficiency of heterozygotes) and four are negative (excess heterozygotes), resulting in a small positive average of 0.0204. Neither the average nor any of the single-locus tests were significant, with \( P \) values ranging from 0.175 (at Tv 14) to 0.996 (at Gata28) and 0.881 for all loci considered jointly (Table 1).

In the pairwise analysis of whales caught at Barrow (\( n = 117 \) individuals), the average amount of genetic differentiation (equation 1) within all \( n(n-1)/2 = 6786 \) pairs is \( \bar{d} = 0.0240 \). Of these, pairs caught during the spring season in the same year (244 pairs) average 0.0505, and pairs caught during the autumn season in the same year (250 pairs) average 0.0254, respectively. These average genetic differences, being nominally larger than zero in both seasons, demonstrate that genes residing in different individuals are more often of different allelic types than are the two genes within individuals (cf. equation 1). This reflects the slight excess homozygosity in the sample.

Age-related effects
We find no evidence for any effect of difference in birth year \( y \) or mean birth year \( m \) on the genetic difference between whales, neither in the spring nor in the autumn. This holds true both when considered separately or jointly (Table 2: rows 2, 3, and 6). Including the age factors in models where catch days apart, \( d \), is present systematically increases the \( P \) value of tests involving \( d \).

Effects of time of capture
For whales caught during the spring migration, there is no significant effect of days apart (\( d \)) between landings of individuals on their genetic differences (Fig. 3a). The partial effect curve rises around \( d = 7 \) (cf. Fig. 4a), similar to that observed in the autumn (below), but the effect is weak and nonsignificant. The simultaneous \( P \) value for the partial effects curve in the spring is 0.392, and none of the covariates have significant effects in this season (\( P \) values range from 0.23 to 0.91: Table 2). During the autumn migration, on the other hand, there is a notable effect of days between capture (\( d \)) on genetic differences among whales. The effect takes the form of increasing genetic differences with days apart, from \( \bar{d} = 0.0254 \) for individuals caught on the same day to \( \bar{d} = 0.0848 \) for individuals caught 5–11 days apart, and a subsequent decline in genetic difference for individuals caught at still longer intervals (Fig. 3b). The effect on catch days apart is apparent from the partial effects curve (Fig. 4b), which has a simultaneous \( P \) value of 0.009. This effect also comes up as significant (\( P \) value = 0.016) in the GAM analysis (Table 2: row 1). The \( P \) value rises only slightly, to 0.021, when controlling for additive age effects, \( y + m \) (row 9). Hence, there is a significant temporal pattern in genetic differentiation during the autumn migration in these BCB bowhead whales.

Cluster analyses
Disregarding any information on timing of catches and year of birth, the analyses of potential substructure in the pooled material (\( n = 134 \)) with STRUCTURE and BAPS
yielded some, although conflicting, indications of population substructuring in BCB bowheads (cf. Table 3). Where BAPS found an optimal number of $K = 4$ genetically distinct groups or populations, STRUCTURE found either one or three groups with approximately the same likelihood. Convergence of the Markov chain algorithm in STRUCTURE was slow with these data and preliminary trial runs, based on 1 million iterations, resulted in variable log-likelihood values among runs for each $K$ value (with standard deviations of the same magnitude as the difference in the mean log-likelihood for different $K$ values). The results depicted in Table 3 for this software are based on 100 million iterations, which was the largest number feasible, but may still be unreliable.

Discussion

We have detected potential population substructure in BCB bowhead whales in the absence of adequate geographical sample coverage. However, explaining our finding biologically remains a challenge. Comparisons with alternative approaches demonstrate that our method of analysing genetic differentiation between individuals during migration represents a viable alternative when geographical data are unavailable or sparse, as for the bowhead whale. The software STRUCTURE obviously had problems with slow convergence when applied to the bowhead microsatellite data. Similar problems have been noted in computer simulations when genetic differentiation

Fig. 3 Genetic difference ($d_{ij}$ based on 10 microsatellite loci) among pairs of whales (points) caught different number of days apart ($d_{ij}$) at Barrow: (a) during spring; (b) during autumn. The solid lines are the estimated direct effects of days apart on pairwise genetic differences.
is weak (Waples & Gaggiotti 2006). Inference about $K$ is based on an approximation that Pritchard et al. (2000) characterize as ‘dubious at best’ (p. 949), and they strongly urge caution when interpreting such results. Noting this caveat, it appears that the STRUCTURE program preferred to cluster the whales into one or three groups.

When applying BAPS to estimate $K$, we ignored the little information that is available on geographical location of the samples (17 of 134 individuals were caught outside Barrow: Fig. 1). This was done because the whales were collected during migration and little, if any, information on population of origin is likely to be contained in the sample locations in this case. Nevertheless, BAPS apparently detected some genetic heterogeneity in the data and estimated the most likely number of subgroups to be four. It seems biologically doubtful, however, that three or four different populations of bowhead whales should exist in the Bering–Chukchi–Beaufort Seas and be included in the samples, which were largely from the single location of Barrow. Until the performances of BAPS and STRUCTURE are better understood, especially with regards to potentially false positives for BAPS, we choose to regard such results as doubtful.

While our analyses with STRUCTURE and BAPS are not exhaustive, the results are in line with computer simulations (e.g. Waples & Gaggiotti 2006) demonstrating the...
general difficulty, and failure of structure and baps in particular, on reliably uncovering population structure from genetic data alone when differentiation is not very strong.

Our method, in contrast, utilizes auxiliary information of potential relevance for population substructuring, including timing of catches during migration. This approach did indeed detect an effect-of-time of capture on the genetic differences among individuals. The effect is manifested as an elevated pairwise genetic difference among whales taken about a week apart (Fig. 4). This pattern is not associated directly with any particular date in the migration but rather with the length of the interval between when each whale in a pair passes Barrow. The effect-of-time separation in capture was significant only for whales caught during the autumn although a similar, but not significant, feature also appears in the spring. The time-of-capture effect was found to remain when correcting for birth year and age differences. Such a ‘bump’ in the distribution of genetic differentiation in whale pairs indicates a shifting pattern of genetic similarity among individuals during migration. If, for one of several reasons (below), genetically similar individuals tend to comigrate, individuals caught close in time should be more similar than those caught at longer intervals, as observed (the left part of the bump depicted in Fig. 4b). The subsequent decline observed at longer intervals (the right part of the bump) indicates that groups of genetically similar individuals reappear later, after about 2 weeks, in the migration. However, the number of individual whale pairs falls rapidly with increasing $d$ (cf. Table 4), and little weight can be given to the apparent very deep decline at large $d$s, as indicated by the expanding null-bands of confidence in this region (Fig. 4b). Possible biological explanations for the observed bump include genetic subdivision of geographically separated breeding populations that alternate during migration; demographic phenomena, like social structuring of the migratory population; or historical phenomena (commercial whaling) leaving a signal on the genetic composition of age classes. Both the spring and fall migrations are known to exhibit some age segregation; the extent of social structuring during migration is unknown.

In a single, randomly mating population, genotypes are expected to occur in Hardy–Weinberg proportions, and individuals should be equally similar genetically regardless of when (in the season) they were collected. Genetic differences among samples and deviations from Hardy–Weinberg proportions within them should be no greater than can be attributed to sampling variation. Such expectations for an ideal population must be modified for species, like the bowhead whale, in which age structure and overlapping generations cause deviations from strict panmixis. Age-structured populations consist of a series of cohorts that are born at different times and by different sets of parents. Cohorts will therefore differ genetically to an extent that depends on the amount of genetic drift (i.e. the effective size of the population) and the pattern of intermixing of genes among different cohorts during reproduction (Jorde & Ryman 1995). Both the effective size and the pattern

### Table 3

Results of cluster analyses of the total ($n = 134$) bowhead sample, using structure and baps. Values in boldface indicate the most likely number(s) of genetically different clusters of whales, as reported by each software. structure reports the log-likelihood of each $K$ value given the data, $\text{Ln}[\text{Pr}(X|K)]$, whereas baps reports the probabilities, $\text{Prob}$, of each $K$ value directly (SE, standard error, calculated over replicate runs).

| No. of clusters $K$ | structure $\text{Ln}[\text{Pr}(X|K)]$ | baps $\text{Prob}$ (SE) |
|--------------------|-----------------------------------|-----------------------------|
| 1                  | −3926.4                           | 0                           |
| 2                  | −3940.9                           | 0                           |
| 3                  | −3926.2                           | 0.047 (0.017)               |
| 4                  | −3951.9                           | 0.899 (0.036)               |
| 5                  | −3958.9                           | 0.054 (0.030)               |

### Table 4

Numbers of pairs and individuals in the GAM analysis of autumn whales.

<table>
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<td>15</td>
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<td></td>
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<tr>
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<td>6</td>
<td>4</td>
<td>3</td>
<td>17</td>
<td>50</td>
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</tr>
</tbody>
</table>

*excluding one pair caught 42 days apart in 1995.
†excluding four unpaired individuals (two in 1995 and one each in 1998 and 1999).
of intermixing may be drastically changed during periods of large demographic disturbances, such as seem likely to have occurred during commercial whaling up until about 1914. Moreover, there is strong evidence that 1914 is less than one bowhead lifetime ago.

We do not have sufficient demographic data on these BCB bowheads for a quantitative evaluation of the effects of age structure on temporal genetic shifts with or without demographic disturbances. However, there is substantial segregation by sex and age during migration (Angliss et al. 1995) and this segregation could lead to a temporal genetic pattern during the seasons if there are genetic differences between the sexes or between whales of different ages. We did not find any genetic differences among sexes nor did we find any age-related effects in our analyses, although ageing errors could have obscured such effects. Ageing is technically difficult in bowhead whales and the present analyses use age estimates that are obtained indirectly, from length information. These age estimates are subject to considerable uncertainties. The problem is greatest for the larger whales because there is little change in the length of a whale once it reaches physical maturity. Reliably estimating the age of whales that are older than age-at-physical-maturity from a growth curve is therefore difficult. Imprecise ageing may obscure detection of age effects on genetic differences and could explain why we find no such effects. On the other hand, our finding is also consistent with the notion (Rooney et al. 1999a, 2001) that commercial whaling did not have a persistent genetic impact on BCB bowheads.

Social structuring of the migrating population is another possible explanation for a temporal pattern in genetic differences during migration. If related individuals migrate in groups, genetic similarity is likely to be higher among whale pairs that were caught near in time as compared to those caught many days apart, as observed. Such a pattern has also been detected in humpback whales where calves accompany their mothers in their first year of life (Valsecchi et al. 2002). However, the most straightforward social-structuring hypothesis, with conmigrating family or kinship groups, is unlikely to explain the subsequent decline in genetic differences for pairs caught more than a week apart. More complicated temporal genetic patterns can be generated by interactions or associations between reproductive success and environmental factors, as observed in the humpback whale (Rosenbaum et al. 2002). Whether similar mechanisms occur in the BCB bowhead and might generate the observed temporal pattern is unclear and remains a subject for future studies.

Another possibility is that whales wintering in different areas in or around the Bering Sea are genetically differentiated. If members of these putative populations time their migration differently, or follow different migratory routes, genetically differentiated populations may pass Barrow in separate migratory groups or pulses. These pulses could create a temporal genetic pattern during the migration season that is similar to the observed. Such a pulsed migration is more effective at causing a genetic signal if the groups’ passages by Barrow are nearly nonoverlapping, if the degree of genetic difference is large, and/or if the groups are of approximately equal sizes. More refined hypotheses along these lines are possible, but remain speculative until further genetic data become available that strengthen or weaken the notion of genetically differentiated BCB bowhead populations.

One potential practical concern in application of our method is that the combinatorics of pairing individuals can exaggerate sampling imbalances. For example, in our autumn bowhead analysis, Table 4 shows the numbers of whales and whale pairs examined in each year. Since models are fit to points representing whale pairs, the analysis is dominated by years 1996 and 2002 to a greater extent than these years are actually represented in the samples. Because different sample years are not equally represented throughout the season, an apparent temporal pattern within seasons could result from this data patchiness if genetic differences among individuals systematically differed among years. Furthermore, the bowhead migration is highly labile and depends on ice and other poorly understood factors. Until more data become available, it is difficult to preclude the possibility that our findings are driven by something peculiar about 1996 and 2002.

In conclusion, a biological explanation for our finding remains uncertain. Future studies should aim at attesting the hypothesis of genetically differentiated BCB populations, by seeking to improve the geographical coverage of bowhead samples (particularly near St Lawrence Island and the Chukotka Peninsula), while also maintaining focus on possible population admixture, social structure, and better understanding of any genetic signals caused by historical overexploitation. The temporal pattern found in this study is a critical first step in this broad effort because our analysis has identified a genetic signal that needs explanation.

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References


The context of this work is the Scientific Committee of the International Whaling Commission (IWC), where four of the authors are members. IWC manages the aboriginal subsistence BCB bowhead whaling. The tissue samples were collected by North Slope Borough scientists and hunters of the Alaska Eskimo Whaling Commission, and genotyped in Texas at the laboratory of John Bickham. The data were made available, and the international cooperation and collaboration thus initiated, through the Data Availability Agreement procedure of the IWC (Journal of Cetacean Research & Management 2004, vol. 6 (Suppl.): 406–407). Statistical modelling and analyses were carried out in Oslo at the CEES, headed by Nils Chr. Stenseth (http://biologi.uio.no/cees/).