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Genetic heterogeneity in the B-C-B stock of bowhead whale as revealed by mitochondrial DNA and microsatellite analyses

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ABSTRACT

Genetic analyses based on mtDNA control region sequences and microsatellites were conducted on samples of the bowhead whale collected from different villages engaged in aboriginal whaling. The main objective of this study was to evaluate the single B-C-B stock hypothesis adopted for the Scientific Committee. Laboratory work on mitochondrial DNA sequencing and microsatellite profiling was carried out by US scientists and access to these data was possible under the Committee's rules for data availability. The number of samples available for mtDNA and microsatellite was 221 and 201, respectively. An 86.4% and 85.1% of these samples, respectively, come from a single locality (Point Barrow). Therefore the analysis on genetic heterogeneity was focused on this particular locality. An additional 25 samples from the Okhotsk Sea stock was used in the mtDNA analysis for comparison. Significant mtDNA heterogeneity (based on Fst) was found when the samples from Barrow were divided into spring and fall migrants. Furthermore a significant deviation from Hardy-Weinberg equilibrium was observed in that locality for both spring and fall migrants as well for all samples combined. Based on these results the possibility of additional stock structure in the B-C-B stock can not be discarded. The scarcity of genetic and biological data from other localities and seasons preclude a comprehensive evaluation of stock identity in bowhead whale. A comprehensive evaluation on stock structure will require the following sampling/analyses: surveys to investigate the number and distribution of breeding grounds in the Bering Sea and collection of genetic samples from these grounds; genetic sampling in the Bering Sea and Chukotka Peninsula in summer and comparison with whales passing Barrow and summering in the Beaufort Sea; genetic analysis of whales from the B-C-B stock summering in Canada waters and a more detailed comparison among B-C-B, Hudson and Davis Strait stocks; collection of non-genetic biological materials from other localities so that genetic results can be interpreted in the context of other biological results. Given these gaps in the study of stock identity in the bowhead whale and our preliminary results that indicate some degree of genetic heterogeneity, the adoption of the B-C-B single stock, as the sole scenario for management purpose, is risky from the conservation point of view.

INTRODUCTION

For management purposes the IWC has recognized five stocks of the bowhead whale (*Balaena mysticetus*) (Fig. 1). All these stocks, but the Bering-Chukchi-Beaufort (B-C-B) stock, are in the category of heavily depleted (in some cases perhaps extinguished e.g. the Spitsbergen stock). The B-C-B stock is the target of aboriginal whaling and the IWC manages this stock in the context of the Aboriginal Whaling Management Procedure (AWMP). During the 2002 Committee Meeting an estimation of 9,860 animals was given for this stock (IWC, 2003). The estimation come from sighting data obtained during the spring migration at Point Barrow.

The B-C-B stock winters in central and western Bering Sea. From April to June whales migrate north and east until they pass Point Barrow where they travel east toward the south-eastern Beaufort Sea. Whales spend most of the summer through the Beaufort Sea. During the fall whales migrate west out the Beaufort Sea. From mid-September to mid October bowhead whales are seen in the northeast Chukchi Sea. Whales

migrate from Point Barrow into the Chukchi Sea heading toward Wrangel Island. When they reach the Siberian coast, they follow it southeast to the Bering Sea. Fig. 2 is a modified version of Fig. 9.7 in Moore and Reeves (1993). This figure shows the generalized seasonal migration of the B-C-B stock and the locations of the villages where whales are hunted for aboriginal purpose.

The Committee has addressed the issue of stock identity of the B-C-B stocks in several opportunities. However, it should be recognized that those discussions have been based on a very limited number of data and almost no comparative analyses on stock identity within the B-C-B (genetic and non-genetics) have been presented (see review by Rugh *et al*., 2003). The available genetic samples come mainly from a single locality (Point Barrow at the northern coast of Alaska). Despite the limited availability of data and comparative analyses within the distribution area of the B-C-B stock, the Committee recommended again in 2002 the single B-C-B stock scenario for management (IWC, 2003). This is the only stock structure scenario being considered by the AWMP.

In contribution for the in-depth assessment of the B-C-B stock of bowhead whale to be conducted by the Scientific Committee in 2004, genetic analyses based on both mtDNA and microsatellite were conducted to test for the single stock hypothesis. Information on the number and distribution of breeding grounds in the Bering Sea is very limited. Without such information it is difficult to infer the actual number of stocks and their pattern of migration toward the feeding grounds. A question on stock identity relevant to management, which we wanted to address, is: are all the whales passing Point Barrow (migrating toward and from their summering ground in the Beaufort Sea) from a single stock? Therefore our analysis was concentrated mainly on this locality from where a relatively large number of genetic data are available. Substantial geographical/temporal genetic heterogeneity on that locality could suggest a scenario of multiple stocks, e.g. different stocks passing Point Barrow at different periods or more than one stock mixing to each other while they pass Point Barrow. This can be investigated by a combined analysis of mtDNA and microsatellites.

MATERIALS AND METHOD

Available data

Following the new Procedure of Data Availability adopted by the Committee during the 2003 meeting (IWC, 2004), genetic data of the B-C-B stock was requested to and provided from US scientists. These included mtDNA control region sequences (397bp) as well as genotype profiles of 12 microsatellite loci (TV7, TV11, TV13, TV14, TV16, TV17, TV18, TV19, TV20, GATA28, EV1, EV104). A part of these data (some of the loci and samples) was used in previous studies (LeDuc *et al*. 1998; Rooney *et al*. 1999; 2001). All laboratory work was made by US scientists and details can be found in these papers. It should be noted that in a message from the US scientific delegation dated 26 April 2004 it was informed about some 'problems' with the bowhead genetic data base (mainly microsatellite). A response to that message was given in a letter dated on 7 May. By the reasons given in that response the present analysis only deals with the problem related to duplicate samples (two cases).

The total number of samples available for the mtDNA analysis is 221 and the distribution by locality is: Commander (4), Gambell (4), Savoonga (7), Chukotka (3) Point Hope (3), Wainwright (2), Barrow (191), Nuiqsut (1), and Kaktovik (6). For comparative purposes a sample of the Okhotsk Sea stock was used (25). These samples were obtained between 1983 and 2003. Only one sample of the duplicate cases, (one from Barrow and one from Chukotka as informed through the message from the US scientific delegation dated 26 April) was used in the analysis. The number of samples used in the mtDNA analysis grouped by locality, month and sex is shown in Table 1.

The number of samples available for the microsatellite analysis is 201 and the distribution by locality is Commander (4), Gambell (4), Savoonga (7), Chukotka (3) Point Hope (3), Wainwright (2), Barrow (171), Nuiqsut (1), and Kaktovik (6). A sample of the Okhotsk Sea stock (25) was available for comparative analysis. These samples were obtained between 1983 and 2003. Only one sample of the duplicate cases (one from Barrow and one from Chukotka as informed through the message from the US scientific delegation dated 26 April) was used in the analysis. The number of samples used in the microsatellite analysis grouped by locality, month and sex is shown in Table 2.

mtDNA analysis
The evolutionary distance between two nucleotide sequences was calculated according to Kimura's two parameters method (Kimura, 1980). The degree of genetic diversity within each locality was estimated using the nucleotide diversity (Nei, 1987). Phylogenetic reconstruction of unique sequences (haplotypes) was made using the neighbor-joining method (Saitou and Nei, 1987). To evaluate the confidence intervals, the bootstrap method was used (Felsenstein, 1993).

As shown in Table 1 most of the available samples come from Point Barrow. The hypothesis testing analysis in this locality followed a stepwise fashion. First we tested for differences between sexes in each month. If no significant differences were found then we combined female and male samples in each month. The next step was to test for differences among 'spring' months (April, May and June) and fall months (August, September and October). Finally we tested for differences between spring and fall seasons.

Following LeDuc *et al*. (1998) the Fst in AMOVA (Excoffier *et al*., 1992) was used to investigate the differentiation of mtDNA variation. In addition we used the randomized chi-square Test of Independence (Roff and Bentzen, 1989) to test genetic differences, as recommended by the Committee. A nested AMOVA was conducted, which included several localities and a temporal division in Barrow. In each test a total of 10,000 randomizations of the original data set were performed. A 'P' value below 5% was used as criteria for rejecting the null hypothesis of panmixia.

Microsatellite analysis
The samples used for the microsatellite analysis are shown in Table 2. The level of variation at nuclear loci was estimated as the number of alleles per locus and the expected heterozygosity as implemented in GENEPOP program (ver. 1.31) PC software package (Raymond and Rousset, 1995). Deviation from the expected Hardy-Weinberg (HW) genotype frequencies for all loci and grouping were examined using the chi-square test as implemented in the GENEPOP program. We employed the homogeneity test implemented in GENEPOP. For each locus, an unbiased estimate of the P value was obtained after 10,000 permutations. The P values from the 12 loci were combined into a single P value as described by Sokal and Rohlf (1995, p.795). The grouping of samples in Barrow was similar to that followed in the mtDNA analysis.

RESULTS

mtDNA
Variability of mtDNA control region sequences
A segment of 397bp of the mtDNA control region was determined in 246 animals. A total of 42 polymorphic sites defined 58 unique sequences (haplotypes) (Fig. 3). Nucleotide diversity by locality is shown in Table 3. As reported by LeDuc *et al*. (1998) the diversity in the Okhotsk Sea samples is lower than in the B-C-B samples.

Phylogenetic analysis
Fig. 4 shows the neighbor-joining tree of mtDNA haplotypes. Closed circles indicate those nodes for which bootstrap values were above 50% in 1,000 simulations. Some nodes are supported by relatively high bootstrap values; however haplotypes represented in these nodes were distributed in more than one locality of the B-C-B stock. In general the tree was not informative on the stock structure.

Geographical and temporal distribution of haplotypes
Table 4 shows the distribution of haplotypes among localities. Samples in Barrow were grouped into 'spring' and 'fall' groups. The sample of the Okhotsk Sea is included for comparison. The main haplotype in the B-C-B localities was haplotype '2' (23.2%) followed by haplotype '8' (6.8%). The main haplotype in the B-C-B localities was also the main haplotype in the Okhotsk stock (52.0%). The four haplotypes in this stock were represented in the B-C-B localities. The Commander Island, which is located between Okhotsk

Sea and Bering Sea, showed three haplotypes (n=4). All of them were represented in the B-C-B localities. One of them (n=2) was represented in the Okhotsk Sea samples.

Differences in the frequency of main haplotypes '2' and '8' between spring and fall samples, are observed in the Barrow locality. The frequencies of these haplotypes in the spring samples are 28.6% and 4.1% while in the fall samples the frequencies are 18.1% and 10.6%, respectively.

Test of sex and temporal differences at Barrow
By using both Fst and chi-square no significant differences were found between sexes in each month. Furthermore no significant differences were found among spring months (April-May-June). Also no significant differences were found among fall months (August-September-October). Male and female samples were compared also within season (e.g. within spring and fall). Again no significant differences were found between sexes.

Hierarchical analysis by AMOVA
This analysis involved the four localities with larger sample sizes: Barrow, Savoonga+Gambell, Kaktovik and Barrow. Further the Barrow locality involved two temporal components: spring and fall. The nested analysis of molecular variance using Fst (Table 5) revealed that 3.57% of the total molecular variance is due to among locality partitions, 0.77% accounted for the temporal division within the Barrow locality and 95.7% accounted for diversity within localities. Significant P values were obtained for the within groups as well between temporal groups in Barrow.

The chi-square test showed no significant differences among localities although a near-to-significant difference was found in the comparison between spring and fall samples in Barrow (P=0.053).

Microsatellites
Level of polymorphism
The total number of alleles per microsatellite locus in the sample from Barrow ranged from 4 (TV16) to 14 (TV7) with an average of 7.8. The mean expected heterozygosity in that locality varied from 0.686 to 0.702 (Table 6).

Heterogeneity test
Table 7 shows the results of the heterogeneity test at Barrow. Tests were conducted by each locus and for all loci combined. No significant differences were found when the samples were grouped by temporal group (spring and fall) as well by sexes.

Test of H-W genotypic proportion
Table 8 shows the results of the test for H-W equilibrium for samples grouped by sex and temporal groups (spring, fall). Results are shown for each locus as well for all loci combined. For three particular loci (TV7, TV18 and TV 14) a significant departure from H-W equilibrium is observed for several combinations of the data set as well for the total samples. A similar result is obtained for all loci combined. Significant departure from H-W equilibrium is due to homozygote excess.

DISCUSSION

The most effective way to address questions on stock identity is to consider results from several techniques, genetics and non-genetics (Donovan, 1991; Pastene *et al*., 2000; Perrin, 2001; Rugh *et al*., 2003). A good example of this was the comprehensive results on stock identity on North Pacific minke whale presented during the JARPN review meeting (IWC, 2001). An attempt was made to investigate stock structure in the bowhead whale B-C-B localities by considering different approaches; genetics (this paper) as well examination of biological parameters (SC/56/BRG33, this meeting).

Unfortunately data from bowhead whale are scarce and fragmented and this prevented to conduct a comprehensive evaluation on stock structure. This is in contrast to North Pacific minke whale where the data and analyses accumulated are far more comprehensive than those available for bowhead whales. Notwithstanding the Committee has repeatedly cited the lack of data on stock structure, and created

complicated and unrealistic stock scenarios in the case of the minke whale. As shown in Tables 1 and 2 and in Bando *et al*. (SC/56/BRG33), data available for stock structure studies in the case of the bowhead whale is limited. However in the case of bowhead whale the Committee has not considered alternative hypotheses on stock structure to cover for the lack of data. The difficulty for biological sampling during aboriginal whaling is recognized. Notwithstanding the number of samples available for genetic analysis (genetic sampling does not require a large effort) is still being limited and fragmented with regard the number of whales taken. The number of samples available for genetic analysis in Table 1 and 2 should be considered in the context of total landing for the period 1973-1999: Gambell (41); Savoonga (40); Wales (7); Kivalina (15); Pt. Hope (83); Wainwright (68); Barrow (354) and Nuiqsut (25) (Demaster *et al*., 2000). More effort to obtain sample tissues could have been done.

Previous genetic studies in the bowhead whale were focused to compare the genetic composition among IWC stocks. For example LeDuc *et al* (1998) used mtDNA and microsatellite to compare genetic composition of whales from the Okhotsk and B-C-B stocks and they found significant differences by using the Fst statistics. We confirmed this result by using a larger number of samples for B-C-B stock than that used by LeDuc *et al*. (1998). However, we did not find significant differences in this comparison using the chi-square test.

Maiers *et al*. (2001) used the same genetics markers to compare the genetic composition of whales from the B-C-B, Hudson and Davis Strait Stocks. They concluded that Hudson Bay stock is more similar genetically to the B-C-B stock than it is to the Davis Strait stock. Some localities from the Davis Strait stock showed significant differences to the B-C-B samples, others not. These results suggest that, if B-C-B, Davis Strait and Hudson stocks are biological differentiated populations, the effect size among them is small and that large number of samples and markers is necessary to detect significant differences.

There are limited comparative genetic analyses within the B-C-B localities. Rooney *et al*. (1999) and Rooney *et al*. (2001) examined mtDNA and microsatellite in whales from the B-C-B stocks but these works were focused to investigate possible genetic bottleneck in the population. The scarcity in the number of genetic samples from most of the B-C-B localities (Tables 1 and 2) prevent a more detailed comparative geographical analysis. The only locality for which a relatively large number of samples are available is Point Barrow. Our comparative genetic analysis in this locality found significant level of heterogeneity in mtDNA when the samples were grouped into spring and fall migrants. Genetic results are consistent with analysis of biological parameters, which also found some degree of heterogeneity (SC/56/BRG33, this meeting).

With regard our microsatellite analysis it should be noted that a significant departure from H-W equilibrium was observed in the Barrow samples but that the heterogeneity test produced no significant results for several combination of the samples. Notwithstanding the results of heterogeneity found in the mtDNA analysis and the significant departure from H-W equilibrium are somewhat surprising given the previous information on seasonal movement of this stock (Fig. 2) that whales passing Point Barrow in spring belong to the same stock of whales passing (returning) at that locality in fall. Under such scenario we would have expected a high degree of homogeneity in all samples from Barrow. Below are some possible explanations and interpretations for the heterogeneity found in our study:

a) Heterogeneity found reflect sampling/data bias

The deviation from H-W equilibrium found in our analysis of the Barrow samples is due to excess of homozygosity. Therefore it could be the results of additional stock structure or that some of the loci demonstrating disequilibrium may contain null alleles (alleles actually present but apparently undetected by the probe). The latter issues can be further investigated by re-designing the primers (i.e. moving them to elsewhere in the flanking region and re-analyzing all homozygote individuals). As we had access to data and no the samples, we were unable to check for the possibility of null alleles.

The other issue is quality of samples. The letter from the US scientific delegation referred to the issue of genetic data quality. They argued that bias can be introduced for low quality samples by over-estimating the number of homozygote because of allelic drop-out. To avoid such bias they used a sample sub-set

including only individuals where at least 10 of 12 alleles amplified successfully. The analysis conducted by US scientists based on this sub-set and an arbitrary bowhead whale sample taken in fall was still suggesting a significant departure from H-W equilibrium. Therefore such heterogeneity is not the result of including 'low quality' samples.

Low quality and degraded DNA, which yield unreliable genotypes, is a problem that people typically have with DNA from fecal samples and it is surprising for the case of DNA extracted from tissue samples. In the case of the fecal samples each sample is genotyped multiple times at each locus to make sure that there are not spurious alleles or allelic drop-out.

Furthermore the data quality issue mentioned by the US scientific delegation refers only to microsatellite. The analysis of mtDNA, which is not affected by the issue of data quality they suggested, also revealed significant degree of heterogeneity in the Barrow samples.

It is likely that the heterogeneity found in the Barrow samples is better explained by some biological event.

b) Heterogeneity found reflect additional stock structure

The number and distribution of bowhead whale breeding grounds in the Bering Sea is still being poorly understood. Furthermore no genetic samples are available from whales in their breeding grounds. Therefore the possibility of more than one stocks moving from breeding areas to feeding areas can not be discarded, as suggested by the results of our genetic analyses.

The possibility of additional stock structure has been mentioned previously. For example Bogoslovskaya *et al*. (1982) suggested that some whales migrate west along the north coast of Chukotka in late spring resulting in two migration routes, one in the western Chukchi Sea, the second in the eastern Chukchi Sea. Melninov *et al*. (1998) also suggested the whales winter in leads and polynyas on the leeward shore and capes and points of the Asian coastline of the Bering Sea. In every spring some of the whales remain in the Gulf of Anadyr and the whales' northward migration along the eastern coast of the Chukotka Peninsula take place much later than along the shore of northwestern Alaska. This information could suggest the occurrence of two stocks within the B-C-B moving from winter breeding areas in the Bering Sea to northern feeding areas in spring. The first could moves mainly along the Chuckchi Peninsula (western Chuckchi Sea) but also along northern Alaska (eastern Chuckchi Sea) toward the feeding ground in the Beaufort Sea. The second move only along the coast of northern Alaska toward the feeding ground in the Beaufort Sea. Then two stocks mix to each other, under an unknown pattern of mixing, as they move toward the feeding ground in the Beaufort Sea. This is consistent with the results of significant heterogeneity at mtDNA and significant departure from H-W equilibrium of nuclear markers at Point Barrow.

The scenario of two B-C-B stocks can not be rejected without genetic analysis of samples summering along the Russian coast and Islands in the Bering Sea to whales summering in the Beaufort Sea (passing through Pt. Barrow). Such analyses have not been made because the collection of samples in such localities is difficult to achieve. DeMaster *et al*. (2000) reported that whales from northern coast of Alaska (93) were not statistically different in mtDNA or microsatellite from St. Lawrence Islands (6) but no conclusion was obtained from this comparison due to the small sample size from St. Lawrence Island. A similar result was found in the present analysis using a larger sample size for Barrow. However the samples in St. Lawrence Island were obtained in April and May, not in summer.

The possibility of more than one stock which mix together as they pass through the area close to Point Barrow has been rejected by the Committee saying it is known that young whales generally migrate past Barrow before older whales and such fact is difficult to reconcile with a multiple stock hypothesis (IWC, 2001 pp. 418). However the Committee has rejected such argument for supporting the single stock scenario in the case of the North Pacific minke whale where a large proportion of immature animals migrate close to the coast while mature animals migrate in more offshore areas. Therefore the Committee has not been consistent in the use of this argument.

The other possibility is that the heterogeneity found reflects intrusion of adjacent stocks into the area of distribution of the B-C-B stock. The movement of the J stock in areas where O stock could be harvested has been a subject of deep discussion within the Committee and the *ISTs* consider detailed information on stocks mixing between O and J stocks in the case of North Pacific minke whale. For scientific consistency, the possibility of intrusion of endangered stocks into the area of distribution of the B-C-B stocks should be also considered and discussed. There is some evidence of possible interchange between B-C-B stock and adjacent stocks. The evidence was based on two whaling irons taken from whales in the Chukchi Sea that apparently came from ships than only cruised in arctic waters of the western North Atlantic sector (Bockstoce and Burn, 1993). These authors noted that it is highly unlikely that these irons would have been carried to the Chukchi Sea aboard a ship. Therefore it is possible that some whales from adjacent stocks move into the area of distribution of the B-C-B stock, which is also consistent with our results.

Maiers *et al*. (2001) found that the B-C-B stock, represented by samples from Mackenzie Delta, was closely related to whales from the Hudson Bay stock than to whales from the Davis Strait stock. Further one of the loci used showed significant departure from the H-W equilibrium in the B-C-B stock sample. An alternative explanation for the heterogeneity found in our analysis in Barrow is that the samples examined include an unknown proportion of the adjacent Hudson stock. That possibility could be further investigated by incorporating into our genetic analysis data available for both Hudson and Davis Strait stocks (Maiers *et al*., 2001).

A more comprehensive analysis of stock structure in the bowhead whale is required for elucidating the different explanations given above. This comprehensive analysis will require the following sampling and analyses:

- Surveys to investigate the number and distribution of breeding grounds in the Bering Sea and collection of genetic sampling from the breeding grounds. This information can assist the interpretation of genetic variability in migratory corridors and feeding areas. In the case of North Pacific minke whale the Committee has recommended on several occasions the analysis of genetic data from putative breeding areas and even some scientists have argued that without such information it will not be possible to clarify the stock structure of minke whale.
- Genetic sampling and analyses of samples summering along the Russian coast and Islands in the Bering Sea to whales summering in the Beaufort Sea (passing through Pt. Barrow).
- A more detailed genetic examination of samples of the B-C-B stock summering in Canadian waters and a more detailed comparison among B-C-B, Hudson and Davis Strait stocks.
- Collection of additional data and further analysis on biological parameters to expand the study presented in SC/56/BRG33 (this meeting). These data are necessary for a comprehensive examination on stock structure.
- A more rigorous laboratory work to address possible effect of sample quality on microsatellite profiling.

Furthermore it is necessary a standardization of the criteria used by the Scientific Committee for examining stock identification in different species subjected to assessment (e.g. Pastene, 2003).

Given these gaps in the study of stock identity in the bowhead whale and our preliminary results that indicate some degree of genetic heterogeneity, the adoption of the B-C-B single stock, as the sole scenario for management purpose, is risky from the conservation point of view.

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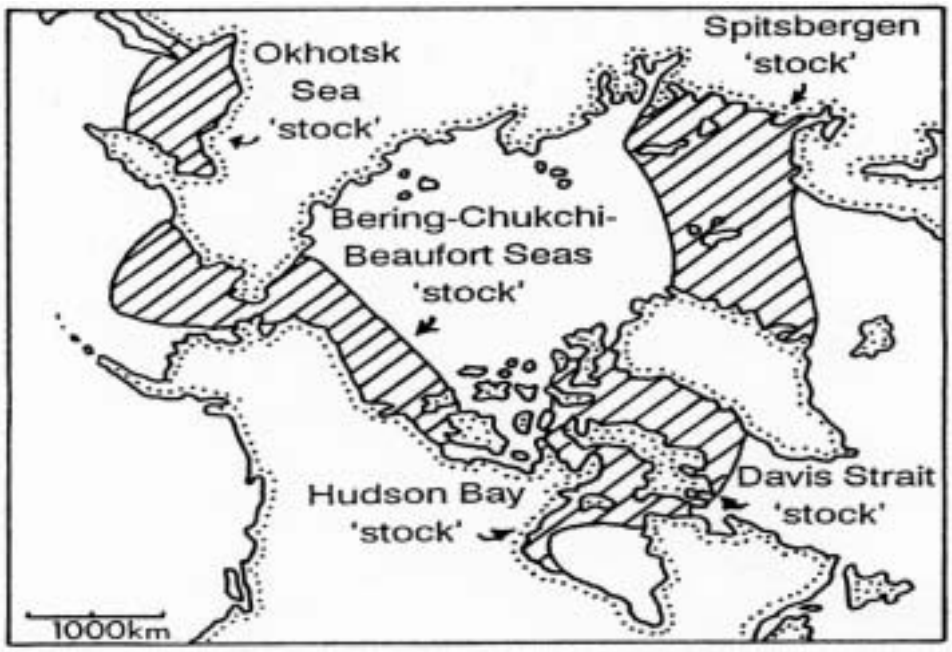

Fig. 1: Putative stocks of the bowhead whale (modified from IWC, 1992).

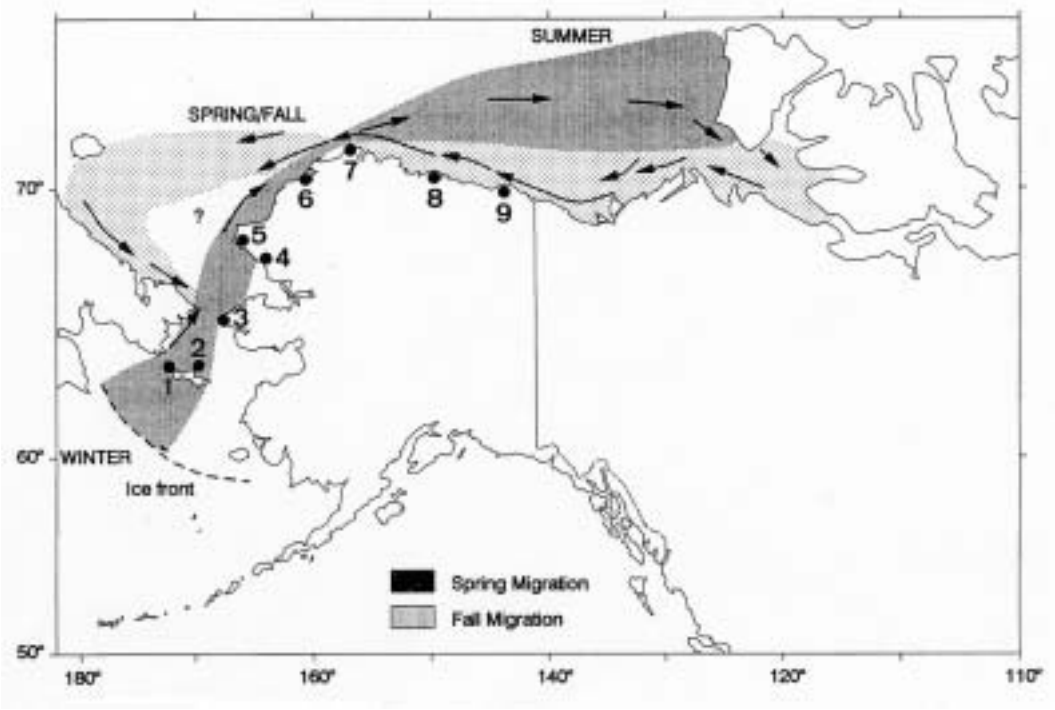

Fig. 2: Generalized seasonal occurrence and migration corridor for the B-C-B bowhead whale stock depicting spring and fall pathways. The figure also shows the villages where whales are taken by aboriginal whaling: 1= Gambell (Lawrence Island, Bering Sea), 2=Savoonga (Lawrence Island, Bering Sea), 3= Wales (Bering Sea), 4=Kivalina (Chuckchi Sea), 5= Pt. Hope (Chuckchi Sea), 6= Wainwright (Chuckchi Sea), 7= Barrow (Chuckchi Sea), 8= Nuiqsut (Beaufort Sea), 9= Kaktovik (Beaufort Sea) (modified from Moore and Reeves, 1993).

	10	20	30	40
	11	1111111112	2222222222	2222233333 33
	1578889900	0111124440	2245556677	7788912578 88
Hap	6774574812	9013741395	1693474501	4734090245 89
1	ACTCTGTCCA	GGCGCCGAGG	ACGGGTTCCA	TACTCGGCAC TC
2	....	....	....	.........T ..
3	..C.......	....	......C...	..T......T ..
4	....	......A...	...AT.....	C...T....T ..
5	....	......AG..	...A.C.T..	...C...... .T
6	....C.....	......A...	....	....TA...T C.
7	....	....	....	.G.......T ..
8	....	......A...	....	.........T ..
9	....C.....	......A...	....	.........T ..
10	....C.....	......A...	....	....T....T ..
11	....	....	....	.....A.T.T ..
12	.........G	......AG..	...A.C.T..	...C...... ..
13	....	......A...	........T.	.........T ..
14	....	A.........	......C.T.	.........T ..
15	....C....T	......A...	....	.........T ..
16	....	......A...	...AT.....	....T....T ..
17	....	....	......C...	.........T ..
18	....	......A...	.T.AT.....	....T....T ..
19	....	......AG..	...A.C.T..	...C...... ..
20	....	....T.A...	.T........	.........T ..
21	....	....	....	.......T.T ..
22	....	.....TA...	...A.C.T..	....TA.... .T
23	...TC.....	.....TA..T	...A.C.T..	....TA...T ..
24	....	....	....	....T....T ..
25	.A........	......A...	.T.A.C.T..	...C...... .T
26	....	......A...	.T.AT.....	.G..T....T ..
27	G.........	....	....	.........T ..
28	.......T..	....	....	....
29	....	..TAT.AG..	...A.C.T..	.... .T
30	.....A..TG	......A...	....	....T....T ..
31	....	....	.........G	.........T ..
32	....	......A...	..AAT.....	....T....T ..
33	....	.........A.	....	.......T.T ..
34	....	....	....	....T..T.T ..
35	....	......AG..	.....C.T..	...C...... ..
36	....	......A...	.T........	.........T ..
37	....	......A...	GT........	....T....T ..
38	....	....	......C...	......A..T ..
39	....C.....	......A...	....	.........T C.
40	....	...A......	.........G	...C.....T ..
41	....	......A...	...AT.....	.........T ..
42	........T.	..TAT.AG..	...A.C.T..	.... .T
43	....	......A...	......C...	.........T ..
44	....	......AG..	...A.C.T..	....
45	......C...	......AG..	...A.C.T..	...C...... .T
46	....	......A...	...AT.....	....T...CT ..
47	....	...A..A...	....	.........T ..
48	....	.A........	....	.........T ..
49	....	.....TA...	...A.C.T..	....TA.... ..
50	.........G	....	....	.........T ..
51	....	......AG..	.T.A.C.T..	...C...... ..
52	...T......	....	....	.........T ..
53	........G.	....	....	.......T.. ..
54	.......T..	....	....	.........T ..
55	....	....	.T........	....T..T.T ..
56	....	......A...	....	....T....T ..
57	...TC.....	......A...	.........G	.........T ..
58	........T.	..TAT.AG..	.T.A.C.T..	.... .T
	2222222233	2222222222	2222222222	2222222222 22

Figures 3: Variable sites defining 58 mtDNA haplotypes in the bowhead whale. The numbers above list the nucleotide position of the polymorphic sites starting from the 5' end of the mtDNA control region. Haplotypes '2' through '58' are listed with reference to haplotype '1'. A dot indicates an identical nucleotide at the position relative to haplotype '1'

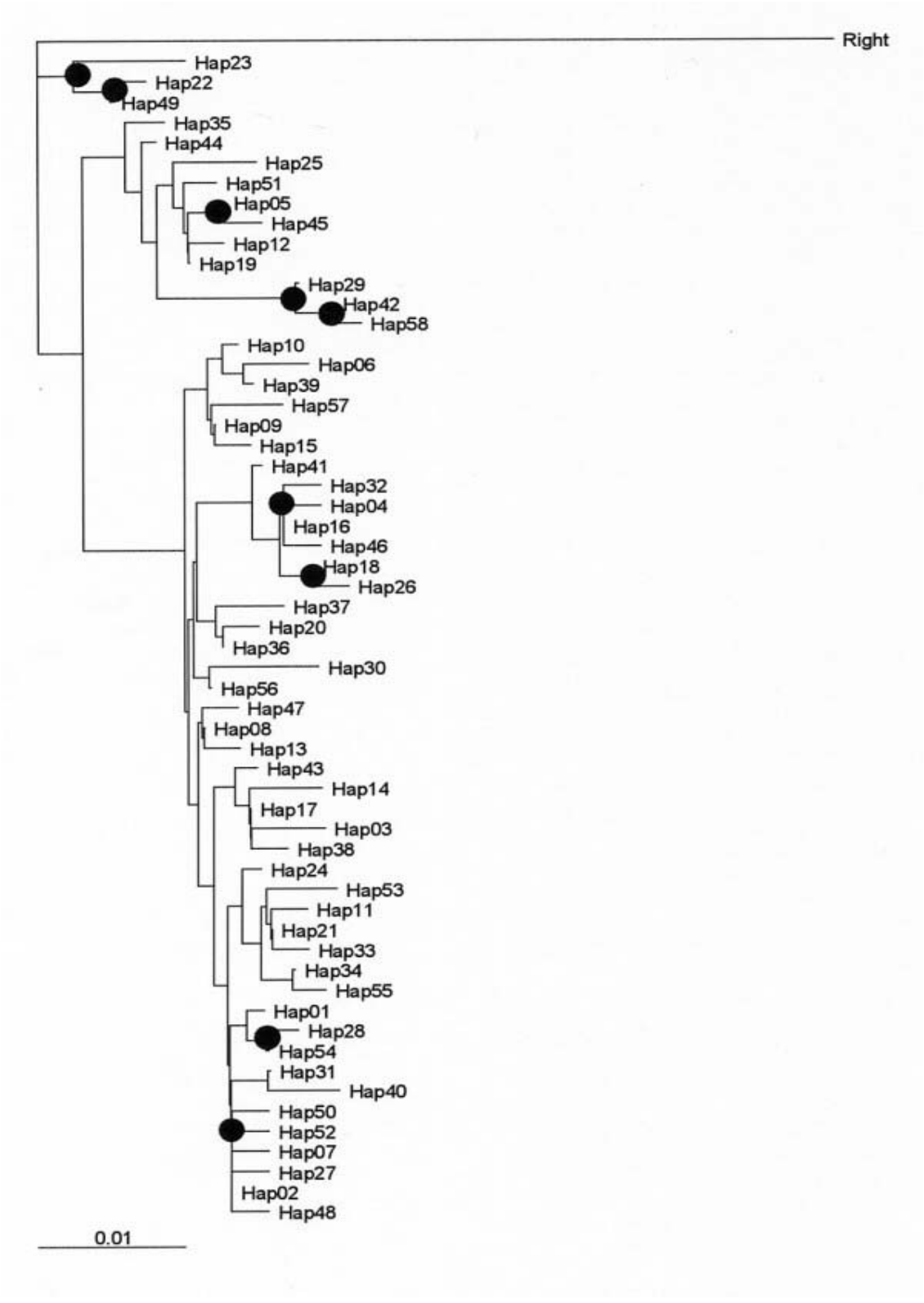

Fig. 4: Phylogenetic reconstruction of mtDNA haplotypes in bowhead whale based on neighbor-joining method. Closed circles show nodes with bootstrap values higher than 50% in 1,000 simulations.

Table 1: Number of samples of bowhead whale used in the mtDNA analysis, by locality, month and sex.

	April		May		June		July		August		September		October		November		December		Total
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	
Kaktovik	0	0	0	0	0	0	0	0	0	0	3	3	0	0	0	0	0	0	6
Barrow	9	4	46	36	3	0	0	0	2	0	19	28	20	23	0	0	0	0	191
Wainwright	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Point Hope	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Chukotka	0	0	1		0	0	0	0	0	0	1		0	0	1		0	0	3
Savoonga	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	7
Gambell	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	4
Nuiqsut	0	0	0	0	0	0	0	0	0	0	1		0	0	0	0	0	0	1
Commander	0	0	0	0	4		0	0	0	0	0	0	0	0	0	0	0	0	4
Okhotsk Sea Stock	0	0	0	0	0	0	0	0	25		0	0	0	0	0	0	0	0	25

Table 2: Number of samples of bowhead whale used in the microsatellite analysis, by locality, month and sex.

	Apr.		May		Jun.		Jul.		Aug.		Sep.		Oct.		Nov.		Dec.		Total
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	
Kaktovik	0	0	0	0	0	0	0	0	0	0	3	3	0	0	0	0	0	0	6
Barrow	9	4	41	32	3	0	0	0	2	0	16	25	19	20	0	0	0	0	171
Wainwright	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Point Hope	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Chukotka	0	0	0	0	0	0	0	0	0	0	1		0	0	2		0	0	3
Savoonga	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	7
Nuiqsut	0	0	0		0	0	0	0	0	0	1		0	0	0	0	0	0	1
Gambell	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	4
Commander	0	0	0	0	4		0	0	0	0	0	0	0	0	0	0	0	0	4
Okhotsk Sea	0	0	0	0	0	0	0	0	25		0	0	0	0	0	0	0	0	25

Table 3: Nucleotide diversity in bowhead whale from different localities (in parenthesis is the standard error).

	Savoonga+ Gambell	Barrow		Kaktovik	Okhotsk Sea
		Spring	Fall		
Sample size	11	98	93	6	25
Nucleotide diversity	0.0152 (0.0023)	0.0108 (0.0011)	0.0126 (0.0010)	0.0123 (0.0037)	0.0080 (0.0025)

Table 4: Distribution of bowhead mtDNA haplotypes among different localities.

	Barrow		Kaktovik	Wainwright	Point Hope	Chukotka	Savoonga	Gambell	Nuiqsut	Commander Is	OS
Hap	Spring	Fall									
1	2	0	0	0	0	0	0	0	0	0	0
2	28	17	1	0	0	0	2	1	0	2	13
3	2	1	0	0	0	0	0	0	0	0	0
4	3	1	0	0	0	0	0	0	0	0	0
5	2	1	0	0	0	0	1	0	0	0	0
6	0	0	0	1	0	0	0	0	0	0	0
7	4	2	1	0	0	1	0	0	0	0	0
8	4	10	0	0	1	0	0	0	0	0	0
9	8	4	0	0	0	0	0	0	0	1	0
10	2	0	1	0	0	0	0	0	0	0	0
11	0	0	1	0	0	0	0	0	0	0	0
12	0	2	0	0	0	0	0	0	0	0	0
13	1	0	0	0	0	0	0	0	0	0	0
14	1	0	0	0	0	0	0	0	0	0	0
15	1	1	0	0	0	0	0	0	0	0	0
16	1	4	0	0	0	0	0	0	0	0	0
17	1	2	1	0	0	0	0	0	0	0	0
18	2	0	0	0	0	0	0	0	0	0	0
19	3	2	0	0	1	0	0	1	0	0	0
20	0	2	0	0	0	0	0	0	0	0	0
21	3	7	0	0	0	0	1	0	0	0	8
22	0	3	0	0	0	0	0	1	0	0	0
23	2	2	0	0	0	0	1	0	0	0	3
24	0	1	0	0	0	0	0	0	0	0	0
25	0	4	0	0	0	0	0	0	0	0	0
26	1	1	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	1	0	0
28	6	1	0	0	0	0	0	0	0	1	0
29	2	0	0	0	0	0	0	0	0	0	0
30	2	0	0	0	1	1	0	0	0	0	0
31	0	2	0	0	0	0	0	0	0	0	0
32	1	1	0	0	0	0	0	0	0	0	0
33	1	0	0	0	0	0	0	0	0	0	0
34	1	1	0	0	0	0	0	0	0	0	0
35	0	2	0	0	0	0	0	0	0	0	0
36	2	4	0	0	0	0	0	0	0	0	0
37	0	1	0	0	0	0	0	0	0	0	0
38	0	1	0	0	0	0	0	0	0	0	0
39	2	2	0	0	0	0	0	0	0	0	0
40	1	0	0	0	0	0	0	0	0	0	0
41	1	0	0	0	0	0	0	0	0	0	0
42	1	2	0	1	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	1	0	0	0
44	0	0	0	0	0	0	1	0	0	0	0
45	1	1	0	0	0	0	0	0	0	0	0
46	0	1	0	0	0	0	0	0	0	0	0
47	1	2	0	0	0	0	0	0	0	0	0
48	1	0	0	0	0	0	0	0	0	0	0
49	0	2	0	0	0	0	0	0	0	0	1
50	0	0	0	0	0	1	0	0	0	0	0
51	0	0	1	0	0	0	0	0	0	0	0
52	0	1	0	0	0	0	0	0	0	0	0
53	0	1	0	0	0	0	1	0	0	0	0
54	1	0	0	0	0	0	0	0	0	0	0
55	0	1	0	0	0	0	0	0	0	0	0
56	1	0	0	0	0	0	0	0	0	0	0
57	1	0	0	0	0	0	0	0	0	0	0
58	1	0	0	0	0	0	0	0	0	0	0
	98	93	6	2	3	3	7	4	1	4	25

Table 5: Results of the nested analysis of molecular variance of bowhead whale mtDNA control region haplotypes (using Fst).

Partitions	df	% total variance	PHI	P
Among localities	3	3.57	0.036	0.083
Among temporal groups	1	0.77	0.008	0.038
In localities				
Within groups	228	95.66	0.043	<0.0001

Table 6: Number of samples analyzed (N), number of alleles detected (A) and expected heterozygosity (H) at each of 12 microsatellite loci in the samples of bowhead whale from Barrow. 'Spring' includes samples from April, May and June; 'Fall' includes samples from August, September and October.

	Female Spring			Female Fall			Male Spring			Male Fall			Total alleles
Locus	N	A	H	N	A	H	N	A	H	N	A	H	detected
TV 7	47	10	0.638	35	9	0.663	31	9	0.698	42	11	0.679	14
TV11	49	6	0.641	29	5	0.576	31	5	0.586	36	5	0.574	6
TV13	48	6	0.685	33	6	0.699	32	5	0.664	41	7	0.708	7
TV14	49	7	0.660	33	5	0.615	32	5	0.632	43	7	0.630	8
TV16	52	4	0.505	36	4	0.470	33	4	0.390	43	4	0.441	4
TV17	39	10	0.829	27	7	0.758	27	10	0.810	35	8	0.770	10
TV18	43	4	0.584	28	5	0.646	31	6	0.650	37	6	0.674	7
TV19	39	6	0.792	27	6	0.770	27	6	0.737	33	5	0.761	6
TV20	47	5	0.672	32	5	0.676	34	6	0.654	41	5	0.578	6
GATA28	49	9	0.867	31	10	0.861	31	9	0.839	41	10	0.859	10
EV1	44	6	0.726	26	5	0.734	23	6	0.763	38	6	0.760	6
EV104	45	9	0.831	31	7	0.817	32	8	0.805	41	9	0.833	10
Average	46	6.8	0.702	31	6.2	0.690	30	6.6	0.686	39	6.9	0.689	7.8

Table 7: Results of the heterogeneity tests in the bowhead whales from Barrow.

By Season	Female x Male		All
Locus	Spring	Fall	Spring x Fall
TV 7	0.7338	0.3347	0.2658
TV11	0.9176	0.6562	0.8622
TV13	0.3679	0.6830	0.4420
TV14	0.4879	0.2232	0.1748
TV16	0.3080	0.4002	0.2834
TV17	0.9293	0.5081	0.3537
TV18	0.2829	0.9478	0.0925
TV19	0.0116	0.4590	0.2851
TV20	0.4503	0.1429	0.9399
GATA28	0.3512	0.9716	0.2418
EV1	0.7907	0.7992	0.2201
EV104	0.2930	0.6943	0.2291
Total	0.4181	0.8582	0.2248

By Sexes	Spring x Fall		All
Locus	Female	Male	Female x Male
TV 7	0.9151	0.1571	0.3260
TV11	0.6025	0.9691	0.8637
TV13	0.5698	0.2613	0.5952
TV14	0.4038	0.2343	0.2375
TV16	0.1873	0.3936	0.5240
TV17	0.3924	0.5740	0.9206
TV18	0.2462	0.1819	0.5829
TV19	0.2931	0.0044	0.6789
TV20	0.5363	0.4836	0.2849
GATA28	0.7773	0.2009	0.7841
EV1	0.0745	0.9741	0.9597
EV104	0.4240	0.1433	0.7077
Total	0.5040	0.0660	0.9565

Table 8: Results of tests for deviation from Hardy-Weinberg genotypic proportion in the samples of bowhead whales from Barrow. Asterisk in Fis column indicates homozygote excess. F= female, M= male, Sp= spring and Fa= fall.

	F-Sp		F-Fa		All F		M-Sp		M-Fa		All M		All Sp		All Fa			
Locus	P-value	Fis	P-value	Fis	P-value	Fis	P-value	Fis	P-value	Fis	P-value	Fis	P-value	Fis	P-value	Fis	All in one	Fis
TV 7	0.0039	*	0.0136	*	0.0000	*	0.0554		0.0557		0.0102	*	0.0000	*	0.0368	*	0.0000	*
TV11	0.2877		0.0380	*	0.0314	*	0.2638		0.6995		0.7165		0.1954		0.2262		0.1281	
TV13	0.5688		0.2610		0.3361		0.2554		0.8717		0.3401		0.2519		0.6878		0.2740	
TV14	0.6433		0.0561		0.1158		0.2234		0.0927		0.0408	*	0.7987		0.0044	*	0.0164	*
TV16	0.3902		0.9465		0.8966		0.2415		0.6402		0.3234		0.1787		0.6881		0.6633	
TV17	0.5845		0.6004		0.1814		0.1398		0.1169		0.5247		0.6780		0.0576		0.3362	
TV18	0.0000	*	0.0082	*	0.0000	*	0.0014	*	0.0002	*	0.0000	*	0.0000	*	0.0000	*	0.0000	*
TV19	0.0703		0.1114		0.0077		0.6261		0.4106		0.3348		0.3268		0.1854		0.4009	
TV20	0.9969		0.1647		0.7820		0.2679		0.7775		0.2574		0.6526		0.3867		0.3464	
GATA28	0.3719		0.7719		0.7534		0.5716		0.2553		0.6450		0.7561		0.1528		0.9296	
EV1	0.9254		0.2558		0.7355		0.0967		0.7852		0.9187		0.9857		0.8029		0.9390	
EV104	0.4716		0.1141		0.2529		0.8800		0.2930		0.6843		0.8037		0.3357		0.4571	
Total	0.0003		0.0015		high.sign		0.0083		0.0161		high.sign		high.sign		high.sign		high.sign	