Estimation of the mixing proportion of the 'J' and 'O' stocks in sub-area 11 using uni- and bi-parental genetic markers

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ABSTRACT

The mixing proportion of the 'J' stock in sub-area 11, by month and sex, was estimated using three genetic markers: a) mtDNA control region RFLP haplotype frequencies, b) mtDNA control region sequencing haplotype frequencies and c) microsatellite allele frequencies. The three techniques are applied on the same sample set of 720 individual minke whales. Estimations obtained using RFLP and sequencing data were similar. In general mtDNA and microsatellite yielded the same results, both showing a significant mixing rate of 'J' stock female animals in April. The mixing rate obtained by the mtDNA analyses, however, was higher. On the other hand, a relatively high mixing rate of male 'J' stock individuals was found by the mtDNA analyses in August. Although the microsatellite analysis showed also a relatively high rate, the standard error of such estimation was high. Possible explanations for such differences are discussed e.g. differences in the level of differentiation at the markers between baseline stocks, limited number of loci used in the microsatellite analysis and the different nature of the markers examined, uni- and bi-parental, respectively.

INTRODUCTION

Studies based on genetics and on other biological parameters indicated that two biological stocks occur on either side of Japan: one distributed around Korean Peninsula and in the Sea of Japan and other distributed in the Sea of Okhotsk and Pacific side of Japan (see review by Pastene *et al.*, this meeting). The IWC refers to these stocks as 'J' and 'O' stocks, respectively.

Based on samples from past coastal whaling conducted in Korea and Japan, Wada (1991) showed a statistically significant allozyme heterogeneity in the April sample from the southern part of Okhotsk Sea (sub-area 11). Goto and Pastene (1997) reported also a significant mtDNA heterogeneity in the same sub-area and month using restriction fragment length polymorphism (RFLP) analysis of the control region. These authors interpreted such heterogeneity as the temporal mixing of 'J' and 'O' stocks in that locality in April.

The Japanese Whale Research Program under Special Permit in the North Pacific (JARPN) began with two feasibility studies in 1994 and 1995. The full research was conducted between 1996 and 1999 (see Fujise, this meeting). Under the JARPN, new samples from coastal and offshore areas in the western North Pacific became available.

The mixing proportion of 'J' and 'O' stocks in sub-area 11 was estimated using RFLP haplotype frequency data. Butterworth *et al.* (1996) estimated the mixing proportion of 'J' stock in 0.42 with associated 95% confidence interval (0.27, 0.57) in sub-area 11 in April. Subsequently Pastene *et al.* (1998) estimated the proportion of 'J' stock female animals in the April sample in sub-area 11 in 0.4075 (SE=0.0806) and that of male animals in August in 0.3147 (SE=0.1160).

Subsequent to these studies, mtDNA control region sequencing and nuclear DNA (microsatellite) data, which could be considered more powerful molecular techniques than RFLP analysis, became available. Then in addition to RFLP data, mixing proportion estimations can be made using these new set of genetic data.

In this study we estimate the proportion of the 'J' stock in sub-area 11, by month and sex, using three genetic markers: maternal inherited mtDNA RFLP and sequencing haplotype frequency data, and bi-parental inherited nuclear DNA (microsatellite) allele frequency data for five loci. We used these techniques on an identical sample set.

DATA AND METHODS

MtDNA analyses (RFLP and sequencing)

Samples

Samples available for this study were from past coastal whaling operation in Korea (sub-area 6, n=28), past coastal whaling operation in Japan in sub-area 7 (n=138) and 11 (n=147), JARPN surveys in sub-areas 7 (n=139), 9 (n=188) and 11 (n=80). A total of 720 samples were used. The data for 8 and 26 individuals sampled in sub-areas 7 and 11, respectively, have been omitted to ensure that the same data were used for two mtDNA analyses and that based on microsatellite analyses. The data from commercial operations and JARPN are pooled to increase the power to identify mixing proportions.

Mathematical model

We estimated the composition of the 'J' stock in the mixed assemblage of sub-area 11 by the maximum likelihood procedure. Variances of these estimates consist of the variance from the sampling from mixed stock (sub-area 11) and the sampling the baseline stocks ('O' and 'J' stocks) (Kishino *et al.*, 1994). This method had already been applied by Pastene *et al.* (1998) for estimation of mixing proportion in sub-area 11 using mtDNA RFLP haplotype frequency data.

Baseline stocks

We assumed minke whales from sub-area 6 (n=28), collected from a past Korean coastal whaling operations in September – October in 1982, as the representative sample of the 'J' stock (Table 1 and 2).

Whales taken in commercial operations in the past and by the JARPN surveys in sub-area 7 (n=277), were considered as the representative sample of the 'O' stock. In order to ensure that no whales from the 'J' stock are present in this sub-area, we conducted an estimation of mixing proportion in sub-area 7 having as baseline stock of the 'J' stock sub-area 6 (as above) and as baseline stock of the 'O' stock in sub-area 9 (samples from JARPN, n=188). The results of mixing proportion in sub-area 7 are shown in Table 3 for both RFLP and sequencing. In general results for RFLP and sequencing are similar. For both female and males, the estimations for most of the cases are zero. In some cases they are as large as 0.062 and 0.067, but these estimations have large standard errors, 0.079 and 0.120, respectively. These results indicates that no 'J' stock animals were found in sub-area 7 in each month This assumption is safe with 95% confidential intervals, when it is assumed that estimated mixing proportions are normally distributed. On the other hand, Abe *et al.* (this meeting) showed that no significant deviation from Hardy-Weinberg equilibrium was detected in sub-area 7 using microsatellite data. Therefore, for the analyses that follow, it is assumed that sub-area 7 contains only 'O' stock animals. The RFLP and sequencing haplotype frequency data for the representative sample of the 'O' stock (sub-area 7), are shown in Table 1 and 2, respectively.

Mixed stock

Tables 1 and 2 show the frequencies of RFLP and sequencing haplotypes in sub-area 11, by month and sex, respectively. The data for female in September and for males in April and September are ignored from this analysis due to small sample sizes (2, 2 and 4 animals respectively). In the case of RFLP haplotypes (Table 1), the main haplotype is haplotype '1', which is the main haplotype in 'O' stock samples. However, in April there is a relatively high number of individuals, especially females, with haplotypes '3' and '5', which are the main haplotypes in the 'J' stock samples. A similar situation is observed for males in the August sample. In the case of sequencing haplotype (Table 2), the predominant sequencing haplotype in April is haplotype '1', which is the main haplotype in the 'J' stock sample. A similar situation is observed for males in the August sample.

Microsatellite analysis

Samples used in the microsatellite analysis were the same as those used in the mtDNA analyses. Five microsatellite loci were used (GATA417, GT23, GATA98, GATA28 and EV37). See Abe *et al.* (this meeting) for references on these primers. The unpublished locus GT23 is from Berube *et al.* (in prep.). Sample sizes vary a little among loci as microsatellite regions of some animals were not amplified so that we could not get the allele data from some animals for each locus. The steps followed for the estimations were the same as mtDNA. Baseline stocks were sub-area 6 for the 'J' stock and sub-area 7 for the 'O' stock. Previous to the estimation of the mix proportions in sub-area 11, an estimation of mix proportion in sub-area 7 was conducted to ensure that no individuals 'J' stock were present in that sub-area.

The mathematical model for estimating the mixing proportion using microsatellite data was developed by Dr. A. E. Punt (CSIRO, Marine Research, Australia), who kindly made the calculations for this paper. The model, allele frequencies of baseline and mixed stocks and the results of the mixing proportions of the 'J' stock in sub-area 11 are shown in the Appendix, which was prepared by Dr. Punt.

RESULTS AND DISCUSSION

Mixing proportions in sub-area 11.

Table 4 shows the estimates (and their standard errors) of the mixing proportions for the 'J' stock in sub-area 11 by month and sex. These estimations are shown for both RFLP and sequencing.

Females

The mixing proportion of 'J' stock in sub-area 11 during April is 37.6% and 35.0% by RFLP and sequencing analyses, respectively. It should be noted that in April, most of the individuals sampled in this sub-area are females. In May, June and August the occurrence of 'J' stock animals can be disregarded. In July, however, the frequency of the 'J' stock is higher, 13% and 12.1% for RFLP and sequencing data, respectively.

Males

In May, June and July the occurrence of 'J' stock animals can be disregarded. However, in August the frequency is higher, 29.6% and 25.4% for RFLP and sequencing analyses, respectively.

For both male and female estimations results obtained by the RFLP and sequencing are similar. Estimations obtained for females in April are similar to those obtained by Butterworth *et al.* (1996) and Pastene *et al.* (1998), whose used RFLP data. The estimation for males in August is also similar to that obtained by the latter authors. The relatively high percentage of females in July has not been reported previously. This estimation was possible because samples became available for that month from the 1999 JARPN survey. It should be noted that the standard error for that estimation is large, in both RFLP and sequencing analyses so that this result should be seen with caution.

Comparison between mtDNA and microsatellites

The results of the microsatellite estimations are given in the Appendix. The main results are as follows: a) there are no significant differences in the results when the single and double alleles are included or not, b) for females there is a significant frequency of 'J' stock animals in the April sample. In July, the frequency is relatively high, however, like in the mtDNA analyses, the standard error of that estimation is high and c) for males the estimations in July and August are large but these estimations also have large standard errors.

Thus mtDNA and microsatellite in general yielded similar results. In particular it should be noted the significant mixing rate of females of the 'J' stock in April, although the estimations of the mtDNA analyses are higher than that of the microsatellite in that month, 37.6-35.0% and 25.4%, respectively. On the other hand mixing rate of males in August is relatively high in both methods, but the standard error of the microsatellite estimation was higher. Possible reasons for these differences could be the occurrence of stronger markers in the baseline stock in the case of the mtDNA. For example RFLP haplotype '5' is predominant in sub-area 6 with a frequency of 71.4%, while its frequency in sub-area 7 is only 3.2%. On the other hand, RFLP haplotype '1' is the predominant haplotype in sub-area 7 with a frequency of 91.3%, while there is no individual having this haplotype in sub-area 6. A similar situation occurs with sequencing haplotype '1'. In contrast, these striking frequency differences between baseline stocks are not observed in the microsatellite.

However, we can not get rid of the possibility that such a difference may simply result from the insufficient data on microsatellite analysis. Previous study (Abe *et al.*, 1997) showed that only a few microsatellite loci were enough to detect the genetic difference between two stocks. Nevertheless more microsatellite loci will be required under a situation of intermingling of two stocks.

Another explanation for this difference could be a sex-biased dispersal. The differential female and male dispersal patterns could be result in contrasting mitochondrial and nuclear genetic structure of stocks, where the former would be expected to show more differentiation owing to maternal inheritance, whereas male breeding dispersal may homogenize allele frequencies at the nuclear loci.

Future works

Pastene *et al.* (1998) already mentioned several future works related to the estimation of mixing rate. Some of them such as the use of mtDNA sequencing and microsatellite have been covered in this paper. Other pendent work is: investigate samples from sub-area 12 in different months to understand the spatial /temporal pattern of mixing of 'J' and 'O' stocks in summer and the increase of sample sizes for the 'J' stock baseline stock, including a larger geographical and period coverage.

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Table 3: Contribution of the J stock to the mixed assemblage of sub-area 7 from mtDNA RFLP and sequencing data, by month and sex when baseline stocks are sub-areas 6 and 9 (see text) (P = proportion; SE = standard error).

(a) Females

Month	RFLP		Sequence	
	P	SE	P	SE
April	0.000	(0.229)	0.000	(0.223)
May	0.000	(0.221)	0.000	(0.217)
June	0.062	(0.079)	0.052	(0.065)
July	0.067	(0.120)	0.000	(0.289)
September	0.000	(0.378)	0.000	(0.350)

(b) Males

Month	RFLP		Sequence	
	P	SE	P	SE
April	0.053	(0.066)	0.035	(0.049)
May	0.058	(0.037)	0.046	(0.030)
June	0.024	(0.031)	0.021	(0.026)
July	0.000	(0.267)	0.000	(0.267)
August	0.000	(0.229)	0.000	(0.217)
September	0.022	(0.041)	0.020	(0.032)

Table 4: Contribution of the J stock to the mixed assemblage of sub-area 11 from mtDNA RFLP and sequencing data, by month and sex when baseline stocks are sub-areas 6 and 7 (see text) (P = proportion; SE = standard error).

(a) Females

Month	RFLP		Sequence	
	Est	SE	Est	SE
April	0.376	(0.074)	0.350	(0.063)
May	0.000	(0.040)	0.035	(0.032)
June	0.017	(0.060)	0.015	(0.060)
July	0.130	(0.089)	0.121	(0.070)
August	0.018	(0.063)	0.013	(0.064)

(b) Males

Month	RFLP		Sequence	
	Est	SE	Est	SE
May	0.000	(0.333)	0.000	(0.314)
June	0.000	(0.354)	0.000	(0.354)
July	0.050	(0.065)	0.036	(0.035)
August	0.296	(0.110)	0.254	(0.094)

(Table 2 Continued)

Hap.	Sub-area6	Sub-area 11												Sub-area 7													Sub-area 9
		April		May		June		July		August		September		April		May		June		July		August		September		Total	
		F	M*	F	M	F	M	F	M	F	M	F*	M*	F	M	F	M	F	M	F	M	F*	M	F	M		
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	2	0
46	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
50	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	4
54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
60	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	3	2
61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
63	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64	0	5	0	1	0	2	0	4	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
65	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
74	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
75	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
78	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
80	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	28	55	2	41	9	17	8	23	[illegible]	16	22	2	4	19	20	16	66	[illegible]	55	12	14	3	19	7	31	277	188

Table 2: mtDNA sequencing haplotype frequency data by sub-area. The data in the columns marked by asterisks are omitted from the analyses due to small sample size.

Hap.	Sub-area6	Sub-area 11												Sub-area 7													Sub-area 9
		April		May		June		July		August		September		April		May		June		July		August		September		Total	
		F	M*	F	M	F	M	F	M	F	M	F*	M*	F	M	F	M	F	M	F	M	F*	M	F	M		
1	18	11	1	1	0	1	0	1	0	1	4	0	0	0	1	0	4	1	2	0	0	0	0	0	1	9	2
2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	6	5	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	1	2	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	1	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	1	1	1	0	1	0	0	0	2	0	4	0	4	1	0	0	0	0	1	12	9
7	0	1	0	5	1	2	1	4	1	0	1	0	0	1	0	4	3	1	7	0	3	0	3	0	0	22	15
8	0	1	0	1	0	2	0	0	1	1	1	0	0	0	0	0	1	1	2	1	1	0	1	0	2	9	3
9	0	5	0	6	2	1	1	0	3	1	4	0	0	8	7	2	5	0	6	4	0	0	5	2	1	40	30
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
11	0	1	0	2	0	0	0	0	3	0	0	0	0	0	0	0	1	0	4	1	0	0	1	0	1	8	10
12	0	0	0	2	1	1	0	0	0	1	0	0	0	0	1	0	2	1	2	1	1	0	1	0	1	10	7
13	0	1	0	2	0	1	2	0	1	3	0	0	1	0	1	1	4	2	5	0	1	0	2	0	3	19	4
14	0	0	0	0	0	1	1	1	4	1	0	0	1	2	1	0	2	2	1	0	2	0	1	0	3	14	11
15	0	1	0	2	2	0	0	0	2	0	2	0	0	1	1	0	4	0	1	0	1	0	1	1	1	11	6
16	0	3	0	4	0	0	0	0	2	0	1	1	0	1	1	1	4	2	2	1	0	1	1	1	2	17	11
17	0	1	0	2	1	0	1	0	0	1	1	0	0	2	1	0	5	0	3	0	0	1	1	0	1	14	16
18	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	2	3
19	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	1	1	4	1
20	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	3	0
21	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	1	3	3
22	0	4	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
23	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	3	4
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
27	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	3	1
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	1	0	0	4	0
29	0	0	0	3	0	0	0	1	0	0	0	0	1	0	0	2	2	1	1	0	0	0	0	0	0	6	5
30	0	1	0	3	0	1	0	0	0	2	0	0	0	1	0	1	4	0	4	0	2	1	0	1	0	14	9
31	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	3	1	0	1	0	0	0	0	0	6	2
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	2	0
34	0	0	0	0	0	1	1	1	0	1	0	0	0	1	0	0	2	0	1	0	0	0	0	0	0	4	3
35	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0	0	0	4	0
36	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	1	3	1	1	0	0	0	0	0	0	6	1
37	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	3	2
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	2	1
39	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	2	5
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 1: mtDNA RFLP haplotype frequency data by sub-area. The data in the columns marked by asterisks are omitted from the analyses due to small sample size.

Hap.	Sub-area6	Sub-area 11												Sub-area 7													Sub-area 9
		April		May		June		July		August		September		April		May		June		July		August		September		Total	
		F	M*	F	M	F	M	F	M	F	M	F*	M*	F	M	F	M	F	M	F	M	F*	M	F	M		
1	0	28	1	36	9	15	8	17	23	15	13	2	4	19	18	15	58	13	51	10	13	3	18	7	28	253	177
2	1	5	0	1	0	0	0	1	1	0	1	0	0	0	0	1	3	1	1	0	0	0	0	0	0	6	2
3	7	9	0	1	0	0	0	2	2	0	3	0	0	0	1	0	1	0	1	2	0	0	0	0	1	6	5
4	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	20	11	1	1	0	1	0	2	1	1	4	0	0	0	1	0	4	1	2	0	0	0	0	0	1	9	3
6	0	1	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	3	1
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	28	55	2	41	9	17	8	23	28	16	22	2	4	19	20	16	66	15	55	12	14	3	19	7	31	277	188

Appendix: Analysis of the (microsatellite) nuclear DNA data

The microsatellite DNA data can be used to estimate the rates of mixing of J and O stock animals in sub-areas 7 and 11. The model used to estimate these mixing rates is based on the assumption that two stocks (J and O) may be found in a sub-area. The model-estimate of the fraction of the alleles on locus l in sub-area S during month p that are allele a is given by:

$$\hat{P}_{a,p}^{l,S} = \alpha_a^S J_a^l + (1-\alpha_a^S) O_a^l \tag{1}$$

where J_a^l is the probability that a J stock animal has allele a on locus l,
O_a^l is the probability that a O stock animal has allele a on locus l, and
α_p^S is the fraction of animals in sub-area S during month p that are of stock J (assumed to be independent of time).

Note that J_a^l and O_a^l have to be interpreted in the sense of the entire stock (because a single animal can have two allele a's). The values for the mixing rates (the α_p^Ss) and probability of an animal from stock J / O having allele a on locus l, J_a^l / O_a^l, are estimated by assuming that the observed allele data for sub-area S are a multinomial sample from the population of alleles in sub-area S. The negative of the logarithm of the likelihood function (excluding constants that are independent of the model parameters) minimised to find the maximum likelihood estimates for the parameters is given by:

$$-\ln L = 0.5 \sum_p \sum_l \sum_s N_p^{l,S} \sum_a P_{a,p}^{l,S} \ln \hat{P}_{a,p}^{l,S} \tag{2}$$

where $P_{a,p}^{l,S}$ is the observed fraction of the alleles on locus l in sub-area S during month p that are allele a, and
$N_p^{l,S}$ is the sample size for locus l, sub-area S, and month p (the division by two arises because each animal has two alleles on each locus).

Now, it is assumed that all animals in sub-area 6 are J stock animals (i.e. $\alpha_p^6 = 1$) and that all animals in sub-area 9 are O stock animals (i.e. $\alpha_p^9 = 0$) while there may be mixing of J and O stock animals in sub-areas 7 and 11. The data for females in sub-area 7 during August and in sub-area 11 during September and for males in sub-area 11 during April and September are ignored due to low sample sizes (4, 2, 2 and 4 animals respectively). All of the analyses are based on data for five loci (GATA417, GT23, GATA98, GATA 28 and EV37). The data from commercial operations and JARPN are pooled to increase the power to identify mixing rates. The data for 12 individuals sampled in sub-area 7 have been omitted to ensure that the same data were used for these analyses and those based on RFLP and sequencing analyses of the mtDNA control region. Allele frequencies of each locus in sub-areas 6, 7, 11 and 9, are shown in Table 1.

Mixing rates in sub-area 7

Mixing rates (by month and sex) in sub-area 7 were determined by fitting model (1) to the data for the five loci. As noted above, the data for sub-area 6 were assumed to relate to the J stock only while those for sub-area 9 were assumed to relate to the O stock only. The fit of a model that assumed that no J stock animals were found in sub-area 7 was found not to be statistically inferior at the 5% level to a model that estimated mixing rates for each month and sex combination for which sufficient data were available ($\chi^2 = 1.20$; df=11). Therefore, for the analyses that follow, it is assumed that sub-area 7 contains only O stock animals.

Mixing rates in sub-area 11.

Table 2 lists estimates (and their asymptotic standard errors) of the mixing rates for the J stock in sub-area 11 by month and sex. These mixing rates were determined by assuming that the samples for sub-area 6 relate to the J stock while those for sub-area 7 relate to the O stock. The estimates of the mixing rates can be sensitive to including in the analysis data for alleles that appear only very infrequently. Therefore, in addition to presenting results for analyses based on the entire data set, results are also shown in Table 2 for analyses based on excluding alleles that only appear once or twice. However, the results insensitive to excluding these infrequent alleles. The mixing rate of J stock females in sub-area 11 during April is highly significant. In contrast, the estimated mixing rates for the remaining months, although on occasion large (e.g. for July), are barely significant at the 5% level, if at all.

Table 1 : The (microsatellite) DNA allele frequency data. The data in the columns marked by asterisks are omitted from the analyses due to small sample size

(a) GATA417

Allele	Sub-area 6	Sub-area 11												Sub-area 7												Sub-area 9
		April		May		June		July		August		September		April		May		June		July		August		September		
		F	M*	F	M	F	M	F	M	F	M	F*	M*	F	M	F	M	F	M	F	M	F*	M	F	M	
196	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
200	0	1	0	1	0	0	0	0	0	0	2	0	0	1	0	0	4	0	1	0	0	0	0	0	2	8
204	0	5	0	5	0	1	0	2	2	2	2	1	0	3	2	3	4	1	7	1	3	0	2	0	3	16
208	2	9	0	9	1	3	2	6	3	6	2	0	3	3	3	3	14	4	13	3	4	0	2	1	5	36
210	1	3	0	3	0	1	0	2	0	0	1	0	0	0	2	0	4	1	3	1	1	0	2	0	0	11
212	26	36	4	25	8	13	6	13	21	10	18	1	3	15	10	8	48	18	39	6	9	2	14	7	22	140
214	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	2	1	0	0	0	2	2
216	28	32	0	20	5	8	5	17	18	8	15	2	1	5	13	11	40	6	28	5	8	2	15	3	22	101
218	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
220	1	19	0	17	0	7	2	5	12	6	3	0	1	9	9	4	16	2	13	4	4	0	3	3	7	51
224	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	2
228	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Total	58	106	4	80	14	34	16	46	56	32	44	4	8	38	40	30	130	32	104	22	30	4	38	14	64	368

(Table 1 Continued)

(b) GT23

Allele	Sub-area 6	Sub-area 11												Sub-area 7												Sub-area 9
		April		May		June		July		August		September		April		May		June		July		August		September		
		F	M*	F	M	F	M	F	M	F	M	F*	M*	F	M	F	M	F	M	F	M	F*	M	F	M	
88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
94	6	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	3
96	5	2	0	2	0	0	0	2	1	1	2	0	0	0	1	0	0	0	0	0	0	0	0	1	0	2
98	0	5	0	8	0	3	2	2	2	3	3	0	0	4	2	3	14	4	12	1	5	0	3	2	6	32
100	1	8	1	3	0	1	0	1	5	2	2	0	0	0	2	0	4	1	2	3	0	0	0	0	1	7
102	0	2	0	0	1	2	0	1	0	1	1	0	1	1	3	1	9	0	5	0	0	0	0	0	2	11
104	4	13	1	9	0	3	3	8	8	5	9	3	0	4	6	2	18	7	15	1	4	1	6	2	8	50
106	9	8	1	3	2	3	3	3	2	1	4	0	2	5	2	3	11	4	8	1	1	1	5	3	4	37
108	2	5	0	9	2	2	1	5	4	3	2	0	0	1	4	3	5	2	6	1	2	0	3	1	4	30
110	3	9	1	4	2	2	1	10	3	5	4	0	1	5	3	5	18	4	15	4	4	1	5	3	9	50
112	22	25	0	9	0	3	1	7	11	4	7	0	1	8	8	4	21	2	21	9	5	2	7	1	15	75
114	0	8	0	7	0	6	3	5	11	2	3	0	3	6	1	5	12	4	13	2	1	2	4	1	5	48
116	0	6	0	4	0	1	2	2	5	5	7	1	0	3	4	6	15	2	11	0	7	1	5	0	7	28
118	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1
120	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Total	52	92	4	58	8	26	16	46	56	32	44	4	8	38	36	32	130	30	108	22	30	8	38	14	62	376

(Table 1 Continued)

(c) GATA98

Allele	Sub-area 6	Sub-area 11												Sub-area 7												Sub-area 9
		April		May		June		July		August		September		April		May		June		July		August		September		
		F	M*	F	M	F	M	F	M	F	M	F*	M*	F	M	F	M	F	M	F	M	F*	M	F	M	
100	0	0	0	1	0	1	0	2	0	1	1	0	0	0	0	0	2	0	1	0	0	0	0	0	0	3
104	3	7	0	4	1	1	2	3	5	3	4	0	0	2	2	1	11	2	9	1	1	1	5	0	6	34
108	41	59	4	47	10	17	9	22	31	15	19	4	5	19	21	21	74	18	55	16	14	4	20	11	44	188
112	12	32	0	21	2	10	4	15	16	7	13	0	2	10	9	6	27	7	24	2	6	3	7	1	10	104
116	0	9	0	8	3	5	0	4	4	6	7	0	0	3	5	4	14	3	16	4	6	0	5	2	4	45
120	0	3	0	1	0	0	1	0	0	0	0	0	1	0	1	0	2	0	1	1	1	0	1	0	0	2
Total	56	110	4	82	16	34	16	46	56	32	44	4	8	34	38	32	130	30	106	24	28	8	38	14	64	376

(Table 1 Continued)

(d) GATA28

Allele	Sub-area 6	Sub-area 11												Sub-area 7												Sub-area 9
		April		May		June		July		August		September		April		May		June		July		August		September		
		F	M*	F	M	F	M	F	M	F	M	F*	M*	F	M	F	M	F	M	F	M	F*	M	F	M	
148	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
193	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	2
194	0	0	0	5	0	0	1	0	1	0	1	0	1	0	0	0	2	0	0	0	1	0	0	1	0	11
198	0	10	1	7	2	2	0	3	3	3	2	0	0	1	5	3	4	0	11	0	2	0	2	0	3	1
201	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
202	15	20	2	14	2	7	6	2	7	5	8	1	1	6	5	6	19	2	21	6	6	2	7	2	10	54
205	0	0	0	2	1	0	0	0	0	1	0	0	0	2	1	0	1	1	0	0	0	0	0	0	1	5
206	0	12	0	12	2	6	2	7	9	4	5	0	1	4	4	6	24	3	17	5	3	1	3	0	9	52
209	0	1	0	1	1	0	0	1	3	0	1	0	0	0	1	0	3	1	5	1	1	1	1	0	2	12
210	16	24	0	13	3	9	5	11	9	3	6	1	0	5	7	4	17	7	9	5	3	1	5	3	9	63
213	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
214	25	32	0	13	4	7	2	17	16	5	15	2	3	12	11	8	36	11	29	5	10	2	14	3	21	102
218	0	3	1	5	3	2	0	3	5	4	5	0	1	4	2	2	6	1	6	0	2	0	3	2	5	46
222	0	2	0	2	0	1	0	1	2	4	1	0	0	0	3	1	7	1	7	1	2	0	1	2	1	17
226	0	0	0	1	0	0	0	0	0	1	0	0	1	0	1	0	2	2	2	1	0	1	0	0	1	7
230	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Total	56	104	4	78	18	34	16	46	56	30	44	4	8	34	40	30	121	30	108	24	30	8	38	14	62	372

(Table 1 Continued)

(e) EV37

Allele	Sub-area 6	Sub-area 11												Sub-area 7												Sub-area 9
		April		May		June		July		August		September		April		May		June		July		August		September		
		F	M*	F	M	F	M	F	M	F	M	F*	M*	F	M	F	M	F	M	F	M	F*	M	F	M	
179	17	43	0	33	4	13	7	25	18	10	12	2	5	10	14	10	47	14	38	2	11	3	8	7	17	121
181	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	2	0	0	0	0	0	0	5
193	0	1	0	7	0	2	2	1	0	1	3	0	0	1	4	2	12	1	4	5	1	0	4	0	1	23
195	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
197	0	0	0	1	2	0	0	1	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	8
199	18	44	2	31	4	13	7	15	22	10	20	1	2	18	13	14	47	9	37	10	10	2	14	5	26	147
201	2	6	0	3	4	2	0	0	1	3	1	1	0	1	0	0	7	0	12	4	2	1	3	0	7	18
203	0	1	0	0	0	1	0	0	1	0	2	0	0	0	2	1	1	1	0	1	0	0	0	0	1	4
205	17	11	1	8	2	1	0	2	10	4	6	0	1	3	3	2	15	2	13	1	4	2	4	1	7	30
207	0	1	0	0	1	0	0	0	1	0	0	0	0	1	1	0	1	1	1	0	0	0	0	0	0	1
209	0	0	0	1	1	0	0	1	1	0	0	0	0	1	1	1	0	0	0	1	0	0	1	0	0	5
Total	54	108	4	84	18	32	16	46	56	30	44	4	8	36	38	30	130	30	108	24	28	8	34	14	60	362

Table 2 : Estimates and asymptotic standard errors for the proportion of J stock animals in sub-area 11 by month and sex. Results are shown for analyses that (a) use all of the data (column "All data"), (b) exclude alleles that only appear once in the data set (column "Less single alleles"), and (c) exclude alleles that only appear twice in the data set (column "Less double alleles").

(a) Females

Month	All data		Less single alleles		Less double alleles	
	Est	SE	Est	SE	Est	SE
April	0.254	(0.097)	0.252	(0.097)	0.250	(0.098)
May	0.000	(0.000)	0.000	(0.000)	0.000	(0.000)
June	0.000	(0.000)	0.000	(0.000)	0.000	(0.000)
July	0.199	(0.115)	0.114	(0.131)	0.115	(0.131)
August	0.000	(0.000)	0.000	(0.000)	0.000	(0.000)

(b) Males

Month	All data		Less single alleles		Less double alleles	
	Est	SD	Est	SD	Est	SD
May	0.000	(0.000)	0.000	(0.000)	0.000	(0.000)
June	0.000	(0.001)	0.000	(0.000)	0.000	(0.001)
July	0.198	(0.098)	0.199	(0.097)	0.144	(0.108)
August	0.126	(0.118)	0.126	(0.118)	0.132	(0.119)