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How many protected minke whales are sold in Japan and Korea? A census by microsatellite DNA profiling

Merel L. Dalebout[1], Gina M. Lento[1], Frank Cipriano[2], Naoko Funahashi[3] and C. Scott Baker[1]

[1]School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand
[2]Conservation Genetics Laboratory, Department of Biology, San Francisco State University, 1600 Holloway Avenue, San Francisco, CA 94132, USA
[3]International Fund for Animal Welfare, 1–2–10, Koyama, HigashiKurume-shi, Tokyo, Japan

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Abstract

Products from the protected East Sea/Sea of Japan ('J' stock) minke whales (*Balaenoptera acuturostrata*) are sold widely on the commercial markets of Japan and Korea despite the protection of this stock since 1986. To determine the minimum number of individual whales for sale, genotypes from six microsatellite loci were used to profile North Pacific (NP) minke whale products purchased on these markets between December 1997 and October 1999. Genotype differences showed that 99 NP minke whale products from the Japanese market represented 86 unique individuals. Of these, 33.7% were of likely J-stock origin based on mitochondrial (mt) DNA haplotypes. In Korea, genotyping showed that 23 NP minke products from March 1999 represented 18 individuals, and 19 products from October 1999 represented 16 individuals. No matches were found between the two sampling periods, giving a total of 34 unique individuals. A frequency-of-capture model suggests that 98 minke whales were present on the Korean market over the two brief sampling periods. No genotype matches were confirmed between the two countries, indicating that undocumented exploitation of this depleted stock must be additive, and greater than previously assumed.

INTRODUCTION

Minke whales (*Balaenoptera acuturostrata*) in the western North Pacific are thought to comprise at least two stocks; the 'O' stock, found in offshore Pacific waters, and the 'J' stock, which occupies the East Sea/Sea of Japan. These stocks are genetically distinct from one another, based on analyses of mitochondrial (mt) DNA control region restriction fragment length polymorphism (RFLP; Goto & Pastene, 1997) and sequence data (Baker *et al.*, 2000*b*). Preliminary studies using nuclear microsatellites also support the distinctiveness of these two stocks (e.g., Abe *et al.,* 1997; Abe, Goto & Pastene*,* 1998). While the 'O' stock is reported to be relatively abundant, the 'J' stock was exploited intensively by the Republic of (South) Korea, and to a lesser extent Japan, between 1962 and 1986, with a total of 13,734 animals taken during this period (Kim, 1999). In 1983, owing to a decrease in catch-per-unit effort, the Scientific Committee of the International Whaling Commission (IWC) concluded that the 'J' stock was depleted, and recommended it be granted protected stock status (International Whaling Commission, 1984). This classification was imposed in 1986, at the same time that the global moratorium on commercial whaling went into effect. In addition, the 'J' stock appears to be reduced in genetic diversity at both mitochondrial and nuclear loci in comparison to the 'O' stock (Abe *et al.*, 1997; Goto & Pastene, 1997; Baker *et al.*, 2000*b*).

The 'O' stock is the primary focus of the Japanese Whale Research Program under Special Permit for North Pacific Minke Whales (JARPN). This lethal research programme takes up to 100 animals annually from pelagic waters off the Pacific coast of Japan, with whalemeat products from this hunt sold on the Japanese domestic market. However, products from the scientific hunt appear to act as a cover for the sale of products from protected species and stocks (e.g., Baker *et al.*, 2000*a*,*b*). Analyses of mtDNA haplotypes from commercial products have indicated that J-type minke whales comprise 20–40% of the Japanese market for this species (Baker *et al.*, 2000*b*). One likely source of these products is fisheries bycatch or strandings along the East Sea/Sea of Japan coast. A government 'administrative order' allows the 'local use' of products from these sources (Mills *et al.*, 1997). Korea has no scientific whaling programme, and whalemeat products found for sale in the southeast coastal provinces of Korea are presumed

All correspondence to: C. Scott Baker. Tel.: 64–9–373–7599 ext.7280; Fax: 64–9–373–7417; E-mail: cs.baker@auckland.ac.nz.

to originate from stranded animals and local fisheries bycatch in the East Sea/Sea of Japan (Fig. 1). J-type minke whales are one of the most common products for sale on the Korean market (Baker, Cipriano & Palumbi, 1996; Lento, Dalebout & Baker, 2000), together with meat from dolphins and porpoises (Brownell *et al.*, 2000), as would be expected from coastal bycatch. A government requirement to document bycatch has been in effect since 1996 (Kim, 1999). The reported Korean annual bycatch of minke whales peaked in 1996 with a total of 129 animals (Kim, 1999). Reports of bycatch have declined since, with 56 minke whales killed in 1999 (Kim, 2001). Although products from minke whales can be distributed 'legally' in each country, products from whales killed incidentally or directly cannot be traded commercially between Japan and Korea. Under the Convention on International Trade in Endangered Species (CITES), international trade in products from

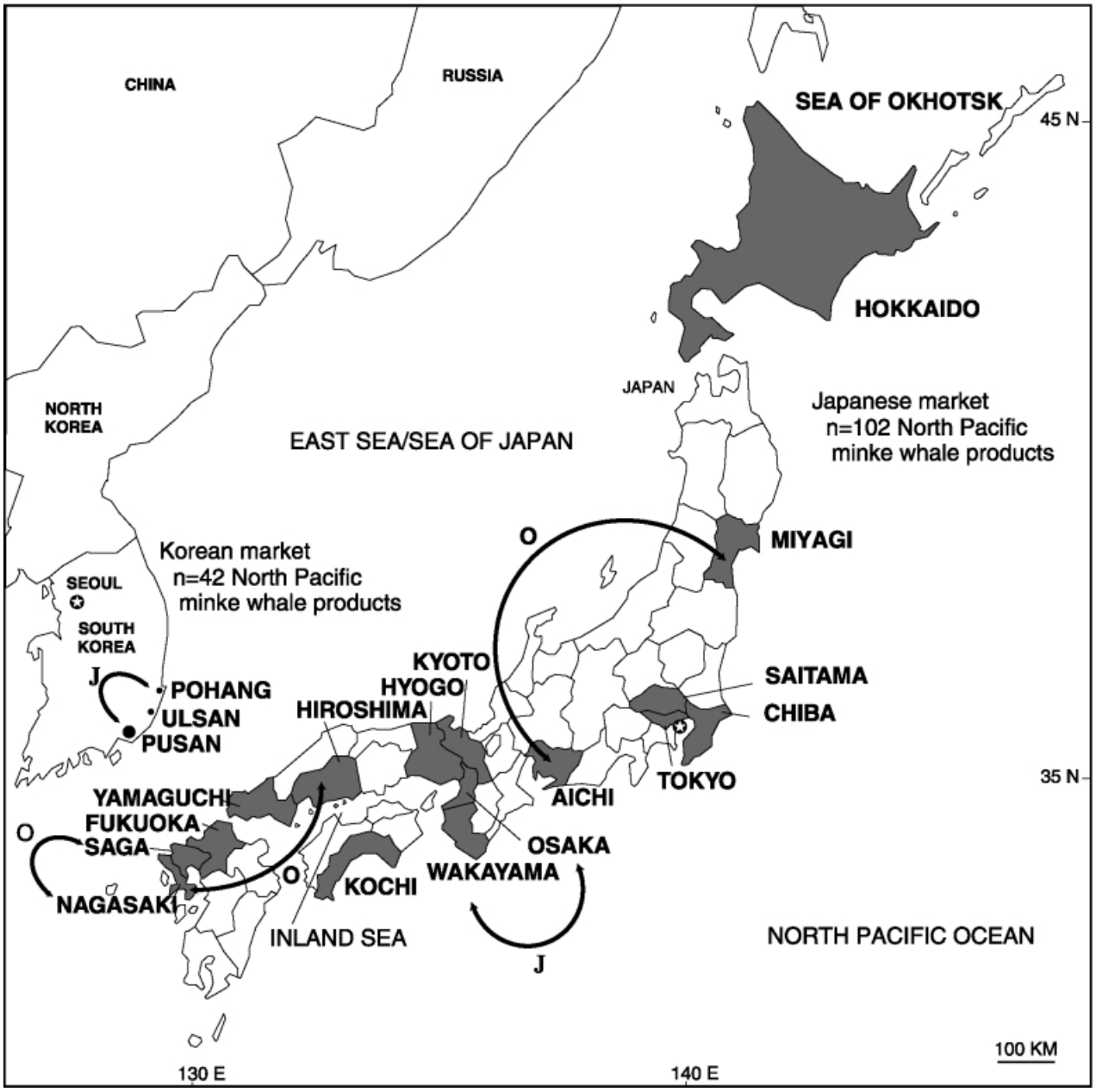

Fig. 1. The origins of North Pacific minke whale products purchased on the Japanese market between December 1997 and October 1999, and on the Korean market in March and October 1999. Prefectures in Japan where whalemeat products were purchased are highlighted in grey. North Pacific minke whales were not found among products purchased in Fukuoka and Yamaguchi prefectures. Cities on the southeastern coast of Korea where whalemeat products were purchased are indicated by black circles (sized to reflect comparative city size). Arrows indicate matches between prefectures (or cities in Korea) of replicate products from the same animal, as determined by microsatellite profiling. Letters adjacent to arrows indicate which minke whale stock these products represent. Matches within prefectures (or cities) are not shown, but see Table 4 and text for details. J, J stock (East Sea/Sea of Japan); O, O stock (offshore Pacific Ocean).

Appendix I species, such as minke and other baleen whales, is banned. We assume therefore that J-type minke whale products on Japanese and Korean markets should be derived solely from local bycatch and strandings, although we cannot rule out the possibility of smuggling and poaching.

Here we report a minimum census of the total number of North Pacific (NP) J-stock minke whales killed annually by Japan and Korea (i.e., total catch over time). We also examined the geographic distribution of J-stock products on the Japanese market to investigate patterns of trade in this protected stock. We first identified whalemeat products to species and stock through analyses of mtDNA sequences (e.g., Baker *et al.*, 2000*b*). We then used microsatellite profiles based on six loci to identify individually all products derived from NP minke whales. To allow comparison with other surveys of microsatellite diversity in NP minke whales, we used the same loci adopted for this purpose by the Institute for Cetacean Research of Japan (Abe *et al.*, 1997; Abe, Goto & Pastene, 1998). To increase further the sensitivity of our analyses, an additional locus, EV14 (Valsecchi & Amos, 1996), was also used. These loci (except EV14) are also a subset of those proposed as the basis for a 'DNA registry' of North Atlantic minke whales killed by Norway (Walløe & Grønvik, 2001). Norway continues whaling under an objection to the 1986 global moratorium on whaling. This system is intended to allow tracking of products originating from this hunt in the event of international trade in the future.

METHODS AND MATERIALS

Sample origins

Whalemeat products were purchased from shops and restaurants in 16 prefectures on the main islands of Japan between December 1997 and October 1999. Products were purchased over periods of several months each year. The overall sample period encompasses approximately 2 years of JARPN hunting (1997 and 1998). No products considered here were purchased after the release of products from the 1999 JARPN catch. In South Korea, whalemeat products were purchased in southeastern coastal cities in two short periods of 3 to 4 days each, in March 1999 and October 1999. In most cases, products were labelled, or described verbally, as 'kujira', the common name for whale in Japanese, or 'gorae', the common name for whale in Korean.

DNA extraction and general field protocols for PCR amplifications

As in previous surveys of commercial markets (e.g., Baker & Palumbi, 1994; Baker *et al.,* 1996, 2000*b*), we conducted DNA extractions from whale tissue and subsequent Polymerase Chain Reaction (PCR) amplifications on site in Japan and Korea. The field analyses in the current survey were performed using a Mobile Molecular Laboratory™ (MJ Research, Watertown, MA) specially designed for use in remote field locations. Tissue from each product was prepared for PCR amplification as described in Baker *et al.* (1996), using Chelex® resin (BioRad Laboratories) and following the protocol of Walsh, Metzger & Higuchi (1991). All amplified (synthetic) products were isolated from native template DNA (as required by CITES regulations; Bowen & Avise, 1994; Jones, 1994) by agarose gel electrophoresis and band excision, before transport to our home laboratory for direct DNA sequence analysis and microsatellite genotyping.

Species identification and amplification of microsatellites

Products were first identified to species based on phylogenetic analyses of mtDNA control region sequences as described by Baker *et al.* (1996). Products identified as NP minke whales were further identified to stock using the methods of Baker *et al.* (2000*b*): in a segment of the 5′ end of the mtDNA control region, starting 17 base pairs downstream from the end of the t-proline gene, an A at position 298 and a G at position 463 define the dominant haplotypes of the 'O' stock, while combinations of AA, GA or GG at these positions define the dominant haplotypes of the 'J' stock. The complete sequence of the mtDNA control region from position 17 to 463 was obtained for most samples but the variable sites defining unique haplotypes were not used in calculations of match probability.

Six microsatellite loci – three tetrameric repeats (GATA28, GATA417 and GATA98 (Palsbøll *et al.*, 1997)) and three dimeric repeats (EV14, EV37 (Valsecchi & Amos, 1996) and GT23 (Bérubé *et al.*, 2000)) – were amplified from all NP minke whale products. These loci were amplified individually to avoid competitive interactions among primers. Microsatellite primers were synthesized commercially, with a fluorescent label attached to one primer of each pair, for all loci (Table 1). Fluorescent labelling of each primer pair

Table 1. The six microsatellite loci used for profiling of North Pacific minke whales in this study

Locus	Allele sizes	Repeat sequence	Fluorescent label	Source
GATA417	198–226 bp	$(GATA)_n$	6–FAM (blue)	Palsbøll *et al*., 1997
GATA28	192–224 bp	$(GATA)_n$	TET (green)	Palsbøll *et al*., 1997
GATA98	98–118 bp	$(GATA)_n$	HEX (yellow)	Palsbøll *et al*., 1997
EV14	125–139 bp	$(GT)_n$	6–FAM (blue)	Valsecchi & Amos, 1996
EV37	176–206 bp	$(AC)_n$	HEX (yellow)	Valsecchi & Amos, 1996
GT23	~88–120 bp	$(GT)_n$	TET (green)	Bérubé *et al.*, 2000

was arranged such that for each individual analyzed, all six microsatellites could be electrophoresed in the same lane (i.e., 'multiplexed') on an automated DNA sequencer. As fluorescent labelling of primers can reduce their efficiency in PCR reactions, a set of unlabelled primers was used for initial amplifications in the field.

Microsatellite loci were reamplified from excised PCR products collected in the field for genotyping and analysis of alleles. Allele sizes for all loci were determined by electrophoresis on an ABI 377 Automated DNA Prism™ Sequencer, using a 4.75% polyacrylamide denaturing gel (Long Ranger™). For each sample, all six microsatellite products were electrophoresed at the same time in the same lane. An internal size standard (TAMRA 350 or TAMRA 500) was included in each lane for all runs. To allow standardization of allele sizes within and between gel runs, 'allelic ladders' were constructed for four of the loci by pooling amplifications from a number of reference samples. These allelic ladders were run in a separate lane on each gel. In addition, a subset of samples was repeated to check the internal consistency of allele sizing between gels.

Genetic analysis

Image analysis and fragment size determination was carried out using ABI Genescan®3.1.2 and ABI Genotyper®2.5 software programs. Fragment sizes were called automatically by Genotyper and further checked by eye. Peaks that could not be resolved unambiguously were excluded from the analyses. For each locus, peaks (alleles) were binned according to size, taking into account the repeat size of the locus (dimer or tetramer), and with reference to an allelic ladder where available.

Given the previously reported differences between stocks, products from the Japanese and Korean markets were analyzed separately, with allele frequencies and the probability of identity estimated for each locus using standard methods (Paetkau & Strobeck, 1994). The probability of identity (I) is the average probability that two animals (or two products) drawn at random from a population will have the same microsatellite genotype by chance. Probabilities for each locus were multiplied, assuming that these loci are unlinked, to obtain an overall probability of identity for samples in each market. We recognize that the inclusion of samples later identified as replicates from the same animal in the calculation of allele frequencies will create a conservative bias in the probability of identity. Finally, mtDNA haplotypes were checked for agreement with samples found to have matching microsatellite genotypes. Exact tests of population differentiation (with 10,000 Markov chain steps), as implemented in the program ARLEQUIN Ver. 2000 (Schneider, Roessli & Excoffier, 2000), were used to compare differences in allele frequencies between the Japanese and Korean markets. For each country's market sample, considered independently and for the pooled sample from both countries, observed and expected heterozygosity (± standard error) and departure from Hardy–Weinberg (HW) equilibrium was assessed using an exact test (with 100,000 Markov chain steps) as also implemented in this program.

Capture-recapture estimation of total catches in Korea

We considered it important to use information from the minimum census of individual whales sold on the Korean market to derive a more representative estimate of the total catches over time. At present, there is no accepted model of market dynamics that allows extrapolation or estimation of total catch from this type of survey (Dizon *et al.*, 2000). As a first attempt, however, we considered it reasonable to assume that market dynamics in Korea might conform to a simple Poisson process. Based on this assumption, we used a 'frequency-of-capture model' (Caughley, 1977; Baker & Herman, 1987) to estimate total bycatch based on information on replicate products in Korea. Frequency-of-capture analyses use information on the number of animals caught once, twice, three times, etc., during a sampling period. These captures (i.e., replicates in the case of market products) form a zero-truncated frequency distribution with the missing zero-class representing the unknown number of animals that were never caught. Under the assumption that market products from an individual whale were equally available for purchase (i.e., 'capture') during each of the two brief sampling periods, we used the Poisson distribution to estimate this zero-class. The size of the market 'population' was then calculated as the number of individual whales on the Korean market captured at least once plus the estimated number that were never captured. This model was considered appropriate for the Korean market where products were purchased in two brief survey periods. It was not considered valid for the Japanese market surveys where products were purchased across a wide range of dates. We were not able to derive a standard error or confidence limit for this estimate, given available development of this model (e.g., Caughley, 1977; Seber, 1982).

RESULTS

Species and stock identification

Of the total of 429 products purchased in Japan, 400 were successfully identified to species through phylogenetic analysis of a 500 base-pair fragment of the 5′ end of the mtDNA control region. The species identity of products purchased up to 1999 was published by Baker *et al.* (2000*b*). The species identity of products included in these analyses and purchased after this time were reported by Cipriano & Palumbi (1999) and Lento *et al.* (2000). Of these 400 products, 102 were identified as NP minke whales. Other products included protected sei, fin, Bryde's, gray, humpback and sperm whales (Baker *et al.*, 2000*a*,*b*). Products from NP minke whales were further categorized as either 'O' stock (offshore Pacific coast; n = 66) or 'J' stock (East Sea/Sea of Japan; n =

34) based on distinctive nucleotide substitutions at positions 298 and 463 of the mtDNA control region (Baker *et al*., 2000*b*). Two products could not be assigned to stock because of missing information from position 463. Of the 49 products purchased in South Korea, 45 were successfully identified to species. Of these, 42 were identified as NP minke whales. Only three of these showed nucleotide substitutions characteristic of the 'O' stock.

Genetic diversity

Microsatellite loci were amplified successfully from 99 of the 102 NP minke whale products from Japan and from all 42 NP minke whale products from Korea. For most samples, all six loci were reamplified successfully and genotyped. For a small number of samples, however, some loci failed to reamplify, or allele sizes could not be resolved unambiguously.

All six loci were found to be polymorphic for minke whale products from both markets. For five of the loci (GATA28, GATA417, GATA98, EV14 and EV37), allele sizes could, in general, be determined unambiguously. However, we were unable to determine allele sizes reliably for GT23 owing to multiple 'stutter' peaks. Instead, the Genotyper profiles of this locus (i.e., whether a homozygote or heterozygote, and the general size distribution of stutter peaks) were evaluated individually by eye when quantitative comparison of the other five loci suggested a match between samples. The bimodal pattern of allele sizes of EV37, together with irregularities in peak shape, also made this locus problematic for some individuals (see Table 2).

The expected heterozygosity for three of the five loci (GATA28, GATA417 and EV14), and the total number of alleles for all five loci, was lower for the Korean market than the Japanese market (Table 3). For GATA98 and EV37, expected heterozygosity was approximately the same for the two markets. For NP minke whales on the Japanese market the average expected heterozygosity was 0.677 with an average of eight alleles per locus, while for those on the Korean market the average expected heterozygosity was 0.607, with an average of five alleles per locus. For all five loci, exact tests of population differentiation indicated no significant difference in allele frequencies of NP minke whales on the two markets (Table 2).

Two of the five loci showed a significant deviation from HW equilibrium: GATA417 showed a heterozygote deficiency in the Japanese market sample and in the pooled sample from both countries; and GATA98 showed a heterozygote excess in both the Japanese and Korean market samples and in the pooled sample (Table 3).

Japanese market: individual identity

Comparison of microsatellite profiles from these 99 minke whale products indicated that they were derived from 86 unique individuals. The probability of identity for the Japanese market samples based on genotype profiles indicated that any given match based on chance alone would be approximately 1/14,500. On this basis,

Table 2. Summary of allele frequencies and allele sizes (in base pairs) for five microsatellite loci for North Pacific minke whales, by locus and market. Exact tests indicated no significant differences between the Japanese and Korean markets at any of the five microsatellite loci. *n*, number of chromosomes sampled for each locus

GTAA28		
Allele sizes	**Korean market** *n* = 78	**Japanese market** *n* = 158
192	–	0.032
196	0.103	0.082
200	0.205	0.171
204	0.115	0.152
208	0.154	0.266
212	0.372	0.228
216	0.051	0.057
220	–	–
224	–	0.013
P (Identity)	0.084	0.060
Exact test of population differentiation: 0.054		
GTA98		
Allele sizes	**Korean market** *n* = 80	**Japanese market** *n* = 174
98	–	0.011
102	0.088	0.092
106	0.488	0.540
110	0.375	0.259
114	0.038	0.092
118	0.013	0.006
P (Identity)	0.224	0.193
Exact test of population differentiation: 0.278		
EV14		
Allele sizes	**Korean market** *n* = 66	**Japanese market** *n* = 152
125	–	0.007
127	–	0.013
129	–	0.007
131	–	0.013
133	0.803	0.605
135	0.106	0.079
137	0.091	0.257
139	–	0.020
P (Identity)	0.467	0.247
Exact test of population differentiation: 0.227		
GATA417		
Allele sizes	**Korean market** *n* = 72	**Japanese market** *n* = 160
198	–	0.006
202	–	0.019
206	0.083	0.063
210	0.181	0.363
214	0.597	0.406
218	0.139	0.138
222	–	–
226	–	0.006
P (Identity)	0.217	0.160
Exact test of population differentiation: 0.132		
EV37		
Allele sizes	**Korean market** *n* = 70	**Japanese market** *n* = 140
176	0.014	0.007
178	0.357	0.423
180	–	0.021
182–90	–	–
192	–	0.070
194	0.043	0.007
196	–	0.014
198	0.414	0.359
200	–	0.021
202	0.014	0.014
204	0.157	0.042
206	–	0.007
P (Identity)	0.007	
Exact test of population differentiation: 0.081		

Table 3. Results of exact tests for Hardy–Weinberg equilibrium. Observed and expected heterozygosity (± standard error) are shown. Numbers in bold type indicate significant *P*-values ($P \leq 0.05$)

	Korean market			Japanese market			Pooled		
Locus	Observed	Expected	*P*-value	Observed	Expected	*P*-value	Observed	Expected	*P*-value
GATA28	0.74359	0.7796 ± 0.0271	0.14074	0.88608	0.8192 ± 0.0120	0.28998	0.83898	0.8110 ± 0.0099	0.45282
GATA417	0.55556	0.6092 ± 0.0523	0.35723	0.53750	0.6947 ± 0.0201	**0.00000**	0.54310	0.6804 ± 0.0189	**0.00000**
GATA98	0.80000	0.6203 ± 0.0320	**0.04564**	0.63218	0.6278 ± 0.0287	**0.04655**	0.68504	0.6272 ± 0.0217	**0.00306**
EV37	0.71429	0.6836 ± 0.0299	0.47215	0.70000	0.6801 ± 0.0254	0.18495	0.7046	0.6843 ± 0.0200	0.09353
EV14	0.30303	0.3408 ± 0.0692	0.34567	0.602526	0.5645 ± 0.0351	0.46504	0.51376	0.5093 ± 0.0344	0.29064

we made the assumption that samples with matching microsatellite profiles and mtDNA haplotypes came from the same individual. Of the 86 unique individuals found on the Japanese market, nine had been sampled twice, and two had been sampled three times (Table 4a). Comparison of full-length mtDNA sequences (from position 17–463) from the 99 minke whale products from the Japanese market distinguished 33 unique haplotypes, confirming a minimum of 33 unique individual whales (C. S. Baker, unpublished data). We found no disagreement between the mtDNA haplotypes and the genotype profiles (i.e., all products with matching genotype profiles also had matching mtDNA haplotypes and all products with different mtDNA haplotypes had different genotype profiles).

Prior classification of these products to stock based on mtDNA variation (Baker *et al.*, 2000*b*; Lento *et al.*, 2000) enabled further evaluation of replicate samples. The 34 J-type products represented 29 unique individuals (33.7%), three of which had been sampled twice, and one of which had been sampled three times. The 65 O-type products represented 57 unique individuals (66.3%), six of which had been sampled twice, and one of which had been sampled three times (Table 4a).

Japanese market: geographic distribution

The individual identification of these NP minke products provided some initial information on the distribution of whale products in Japan. NP minke whale products were found in 14 of the 16 prefectures where whalemeat products were purchased (exceptions, Fukuoka, Yamaguchi; Fig. 1). O-type minke whale products, assumed to be derived from the JARPN scientific hunt (and perhaps local bycatch), were found in all prefectures, while J-type products, assumed to be derived primarily from local bycatch (i.e., from the East Sea/Sea of Japan), were found in seven prefectures (Table 5). Although the proportion of J-stock products varied somewhat among prefectures, these differences were not significant given the samples sizes ($\chi^2_{\text{d.f.13}} = 16.525$, $P > 0.22$).

Of the 11 animals sampled more than once on the Japanese market (Table 4a), six were resampled within the same prefecture. The remaining five were resampled in two prefectures each. Replicate products from two J-type minke whales were found in both Wakayama and Osaka. Replicate products from O-type minke whales were found in (1) Saga and Nagasaki (one whale), (2) Hiroshima and Nagasaki (one whale), (3) Aichi and Miyagi (one whale).

Korean market: individual identity

Differences at one or more loci indicated that no Japanese product was derived from the same animal as a Korean product, with one exception (JW99–O13 and K9946). These two products matched at five microsatellite loci and shared the same mtDNA haplotype but showed an apparent mismatch at the GT23 locus. Further analyses are underway for these two samples.

Comparison of microsatellite profiles indicated that the 23 products purchased in March were derived from 18 unique individuals, and the 19 products purchased in October were derived from 16 unique individuals. The probability of identity for products from the Korean market based on genotype profiles was considerably lower than in Japan; approximately 1/3500. Based on this, we made the assumption that samples with matching microsatellite profiles and mtDNA haplotypes were likely to have come from the same individual but cannot discount with confidence the possibility of a match by chance in the multiple comparisons. We note that such a match by chance would result in a slight conservative bias in our within-country census. Comparison of full-length mtDNA sequences from the 23 products purchased in Korea in March and the 19 products purchased in Korea in October revealed eight unique haplotypes in each sample. As with the Japanese sample, we found no disagreement between the mtDNA haplotypes and the microsatellite genotypes.

Of the 18 unique individuals found in the March survey, three were sampled twice, and one was sampled three times. Of the 16 unique individuals found in the October survey, one was sampled twice, and one was sampled three times (Table 4b). Replicate products from a J-type animal were found in both Pusan and Pohang in March 1999. All other replicates were from the same location (city). Pohang lies approximately 100 km north of Pusan on the east coast of South Korea (Fig. 1). No replicate samples were found between the March and October collections.

Korea: estimate of bycatch

The 34 individual North Pacific minke whales identified among these 42 products provide a minimum 'census' of

Table 4. North Pacific (NP) minke whale products purchased on the commercial markets of (a) Japan, from December 1997 to October 1999, and (b) the Republic of (South) Korea, in March and October 1999, identified as replicate samples from the same animals through genotyping of six microsatellite loci. See text for discussion

	Sample code	Purchase location	Date of purchase
(a) Duplicate samples from the Japanese market			
J-type minke whale products			
(i)	J9896	Miyagi	11 Feb. 98
	J9897	Miyagi	11 Feb. 98
(ii)	J9941	Wakayama	3 Aug. 99
	J9949	Wakayama	1 Sept. 99
	J9966	Wakayama	10 Oct. 99
(iii)	J9935	Wakayama	31 Aug. 99
	J9979	Osaka	12 Oct. 99
(iv)	J9942	Wakayama	31 Aug. 99
	JW99–03	Osaka	15 Feb. 99
O-type minke whale products			
(i)	J98C21	Saga	1 Mar. 99
	J98D81	Nagasaki	25 Feb. 99
(ii)	J9938	Wakayama	31 Aug. 99
	J9962	Wakayama	10 Oct. 99
(iii)	J9959	Wakayama	10 Oct. 99
	J9963	Wakayama	10 Oct. 99
(iv)	JW99-A1	Miyagi	21 Feb. 99
	JW99-A7	Miyagi	21 Feb. 99
(v)	JW99-H8	Hiroshima	19 Feb. 99
	JW99-N1	Nagasaki	23 Feb. 99
(vi)	JW99-O2	Hyogo	15 Feb 99
	JW99-O4	Hyogo	15 Feb. 99
	JW99-O8	Hyogo	15 Feb. 99
(vii)	J9952	Aichi	1 Sept. 99
	JW99-A21	Miyagi	21 Feb. 99
(b) Duplicate samples from the Korean market			
J-type minke whale products			
(i)	K9902	Pusan	15 Mar. 99
	K9919	Pohang	16 Mar. 99
(ii)	K9904	Pusan	15 Mar. 99
	K9905	Pusan	15 Mar. 99
(iii)	K9907	Ulsan	16 Mar. 99
	K9909	Ulsan	16 Mar. 99
(iv)	K9911	Pohang	16 Mar. 99
	K9912	Pohang	16 Mar. 99
	K9918A	Pohang	16 Mar. 99
(v)	K9935	Pohang	26 Oct. 99
	K9936	Pohang	26 Oct. 99
	K9937	Pohang	26 Oct. 99
O-type minke whale products			
(i)	K9933	Pohang	26 Oct. 99
	K9934	Pohang	26 Oct. 99

Japanese market. Overall conclusion: Of 99 NP minke products genotyped, 34 were J-type and 65 were O-type. Of the J-type products, five were replicates, such that 34 products represented 29 animals. Of O-type products, eight were replicates, such that 65 products represented 57 animals. Overall, 99 NP minke products from the Japanese markets represented 86 unique individuals.

Korean market. Overall conclusion: Of the 42 NP minke products genotyped, 39 were J-type and three were O-type. Of the J-type products, seven were replicates, such that 39 products represented 32 animals. (March, 22 products minus five replicates = 17 animals; October, 17 products minus two replicates = 15 animals.) Of the O-type products, one was a replicate, such that three products represented two animals. (March, one product = one animal; October, two products minus one replicate = one animal.) Overall, 42 NP minke products from Korean markets represented 34 unique individuals. No replicates were found between the March 1999 and October 1999 surveys.

Table 5. Distribution of North Pacific (NP) minke whale products from the 'J' stock (East Sea/Sea of Japan) and 'O' stock (offshore Pacific) on the Japanese market by prefecture

Prefecture[a]	No. of O-type products	No. of J-type products	Total NP minke products	Coastal borders
Aichi	2	0	2	North Pacific Ocean
Chiba	3	3	6	North Pacific Ocean
Hiroshima	1	0	1	Inland Sea
Hokkaido	3	1	4	East Sea/Sea of Japan, North Pacific Ocean & Sea of Okhotsk
Hyogo	4	1	5	East Sea/Sea of Japan & Inland Sea
Kochi	4	0	4	North Pacific Ocean
Kyoto	1	0	1	East Sea/Sea of Japan
Miyagi	14	5	19	North Pacific Ocean
Nagasaki	2	0	2	North Pacific Ocean
Osaka	5	7	12	Inland Sea
Saga	3	4	7	East Sea/Sea of Japan
Saitama	1	0	1	No coasts
Tokyo	6	0	6	North Pacific Ocean
Wakayama	17	13	30	North Pacific Ocean
Total	**66**	**34**	**102**[b]	

[a]Whalemeat products purchased in Fukuoka and Yamaguchi prefectures did not include NP minke whales.
[b]Includes two NP minke whale products, one each from Tokyo and Wakayama, for which stock affinity could not be determined with certainty.

the 1999 bycatch of J-stock minke whales in Korean waters. Based on the number of replicate samples and the frequency-of-capture model, we estimated that 45 individual whales were available during the March sampling period and 54 during the October sampling period. In both sampling periods, the observed frequencies of replicate products in the purchases fit closely the expected Poisson distribution ($\chi^2 < 0.51$, $P > 0.5$). We note, however, that failure to reject a goodness-of-fit to a Poisson distribution does not represent strong evidence that this assumption is correct. Considering that no individuals were captured in both periods, we assume these estimates are independent and can be summed to provide a total estimate of about 98 minke whales for the Korean bycatch over a period of less than six months of 1999.

DISCUSSION AND CONCLUSIONS

Molecular genetic methods provide powerful new tools for the identification of the species origins of whale, dolphin and porpoise products in trade (Dizon *et al.*, 2000). Here we have confirmed the utility of microsatellite profiling for the individual identification of whalemeat products, and the censusing of the minimum number of unique animals present on the commercial market.

Standardization of alleles

Our attempts to compare the results of this study with previous surveys of microsatellite diversity in NP minke whales highlight the difficulties likely to be encountered if genotyping results are compared between laboratories.

Our allele size 'bins' differed consistently from those designated by Abe *et al.* (1997, 1998) even though products were electrophoresed on the same model of automated sequencer and analyzed using the same programs. Comparison of relative allele frequencies in the different populations suggested that our allele sizes differed by one (EV37) to two base pairs (GATA28, GATA 417, GATA98). With respect to the other microsatellite locus used in both studies (GT23), although the Abe *et al.* studies distinguished 15 alleles, we were not able to score allele sizes for this locus with confidence under the described conditions.

If DNA registries for the monitoring of whalemeat markets are to be utilized effectively (IWC Resolution 1999–8), these problems will need to be resolved. Standardization of microsatellite allele sizing and 'binning' between studies or institutions would be greatly facilitated by the exchange of reference samples and the construction of allelic ladders for each locus used. Aliquots of standardized allelic ladders could be distributed to all parties involved, to be run together with test samples on all gels. Sizing differences due to small variations in procedure and equipment between laboratories could then be easily resolved, allowing unambiguous comparison of results. Such extensive standardization procedures are considered mandatory in human forensic analyses (Ghosh *et al.*, 1997).

Other potential problems in the expanded application of genotyping to monitoring markets of whalemeat products should be considered. These include 'allelic dropout' (lack of amplification of an allele for a heterozygous locus creating a false homozygote) and 'false alleles' (e.g., the assignment of allelic identity to stutter peaks for a homozygous locus and creating a false heterozygote; Taberlet, Waits & Luikart, 1999). We did not find any evidence of systematic errors due to either allelic dropout or false alleles in our samples. For example, allelic dropout is more likely in poor-quality samples and should be evidenced by a deficiency of heterozygotes. Products sampled from the Korean market were of poorer quality than those from the Japanese market, but showed the least (no) evidence of heterozygote deficiency. Deviation from HW equilibrium (heterozygote deficiency) in the Japanese market sample and the pooled sample from both markets for the locus GATA417 is more probably due to the mixing of products from the genetically distinct J and O stocks (i.e., a Wahlund effect; Hartl & Clark, 1989). Subsets of samples from both markets were also subjected to repeated amplification and genotyping. No systematic loss of larger alleles (allelic dropout) was observed for these samples. Finally, we found no disagreement between matches based on genotype profiles and the mtDNA haplotypes of those samples. We did encounter problems with stutter peaks for some loci (e.g., GT23). To avoid a misclassification due to these peaks, we had to examine the genotype profiles of each potential match by eye. This would not be feasible logistically for a large-scale monitoring and observation programme based on matching of market products to a central register of legally hunted whales (Anonymous, 1998). The heterozygote excess observed at GATA98 is unlikely to be due to false alleles as tetrameric repeats show little or no stutter peaks.

Comparisons of markets and stocks

We found no significant differences in microsatellite allele frequencies at any of the five loci between NP minke whale products for sale on the Korean and Japanese markets. This is surprising given the differences between J-stock and O-stock minke whales reported by Abe *et al.* (1997, 1998). The discrepancy between our findings and those of Abe *et al.* is probably the result of two factors. First, the mixing of J- and O-stock products on the Japanese market (Baker *et al.*, 2000*b*), which would have the effect of 'diluting' any differences in microsatellite allele frequencies. Second, Abe *et al.* (1997, 1998) used a large number of samples from O-stock animals from the JARPN hunt, but only a small number of samples from J-stock animals. These 29 J-stock animals were killed during commercial hunting off the east coast of Korea in October 1982. This small sample collected from a small number of locations over a short period of time is unlikely to be fully representative of the J stock. Baker *et al.* (2000*b*) provide additional discussion of problems with sampling in the JARPN analyses.

Establishing individual identity of North Pacific minke whale products

The probability of identity (*I*) gives an indication of how useful these microsatellite loci are for excluding a match by chance for a given pair of samples. For individual identification of multiple samples, however, it is necessary to adjust this probability by the total number of pair-wise comparisons ($n(n–1)/2$). After making this adjustment, the possibility of a match by chance within each of the market samples increases substantially. Here we have emphasized exclusion to derive a minimum census. To establish a match with confidence, particularly between products from the two countries, will require several additional loci.

Bycatch for sale in Japan

Baker *et al.* (2000*b*) used a 'mixed-stock' analysis based on mtDNA control region haplotypes to estimate that J-stock minke whales comprised approximately 31% (95% confidence interval, 19 – 43%) of the total North Pacific minke whales on the Japanese market. By comparison, only 4.9% of the 368 animals reported from the Japanese scientific hunt between 1994 and 1998 could be classified as J stock (Goto & Pastene, 1999). Our finding that 33.7% of the unique individual NP minke whales on the Japanese market are J-type animals discounts the potential bias of replicate sampling in the mixed-stock analyses based on mtDNA haplotypes.

Our analysis of the geographic distribution of J- and

O-stock products in Japan further discounts a bias from regional sampling. The test of independence showed no evidence of a bias in J-stock products among the prefectures. Contrary to the government 'administrative order' that whales taken as bycatch should be 'used locally' (Mills *et al.*, 1997), four of the seven prefectures in which J-type products were found do not border the East Sea/Sea of Japan. This suggests that J-type minke whale products are distributed widely from their port of landing to markets around Japan, in a similar way to O-type products. The discovery of J-type minke whale products moving between two prefectures, neither of which borders the Sea of Japan, further supports the suggestion that local bycatch is distributed widely around commercial markets.

Alternatively, the J stock may have a more extensive coastal distribution than currently assumed from reports resulting from JARPN (Lento *et al.*, 2001). If the J stock is distributed along the Pacific coast of Japan during some times of the year, it could be inadvertently depleted as part of a hunt intended only for the O stock. A comprehensive genetic investigation of minke whales along the Pacific coast of Japan is required to exclude this possibility (e.g., along the coasts of the Miyagi, Chiba, Wakayama and Kochi prefectures).

Bycatch for sale in Korea

The absence of a match between the two sampling periods, March and October, argues against long-term storage of whalemeat products as a common practice in Korea. Instead, it seems that products from an individual whale are distributed in the markets for a period of less than 6 months. In this case, the census of individual whales from the two sampling periods is undoubtedly an underestimate of the true bycatch.

Although our efforts to apply a frequency-of-capture model to this market can only be considered preliminary, the estimated catch of 98 minke whales does not seem unreasonable given the minimum of 34 individuals found in the two brief (2–3 days) market surveys and the absence of replicates between surveys. Regardless of the precision of this estimate, the minimum census is difficult to reconcile with the total bycatch of 56 minke whales reported by the Fisheries Agency for 1999 and suggests that bycatch is increasing following an apparent 2-year decline (Kim, 2001). Simulations by Baker *et al.* (2000*b*) indicated that the 'protected' J stock would continue to decline under the official reported levels of bycatch. If, in fact, undocumented exploitation is higher than previously assumed on both sides of the East Sea/Sea of Japan, immediate action is required to prevent a more rapid decline of this depleted and genetically distinct population of whales.

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