

# Bayes and Empirical Bayes approaches to addressing stock structure questions using mtDNA data, with an illustrative application to North Pacific minke whales

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## ABSTRACT

Bayesian methods using mtDNA data are developed to compare single- and multiple-stock hypotheses. The likelihood of the data is assumed to be multinomial and the multivariate prior for the probability of an individual having a particular haplotype is assumed to be of the Dirichlet- $\beta$  form. The values for the parameters of this prior are either determined using an Empirical Bayes approach or assumed to be distributed according to a log-normal hyper-prior (the 'Full Bayes' approach). The Empirical and Full Bayes methods are examined using simulation. The performance of the Empirical Bayes method is found to be much worse than that of the Full Bayes method. Illustrative comparisons for North Pacific minke whales based on the latter method confirm previous results that sub-areas 6 and 7 contain different stocks. Results of the application of this method to the mtDNA data for the sub-areas to the east of Japan, although generally uninformative, are nevertheless consistent with analyses based on hypothesis testing using allozymes and mtDNA. The results from this method should, however, be used for management purposes with some caution. This is because, although some testing of the Full Bayes method has been completed and suggests that when applied to data for two stocks that differ substantially in haplotype frequency, or when sample sizes are large and there is only one stock, performance is adequate, in common with most other methods for analysing genetics data, its performance has yet to be fully evaluated.

KEYWORDS: BAYES; GENETICS; STOCK IDENTITY; NORTHERN HEMISPHERE; NORTH PACIFIC OCEAN; MINKE WHALE

## INTRODUCTION

One of the key uncertainties identified during the development of the Revised Management Procedure (RMP) for baleen whales was that of uncertainty regarding stock structure (IWC, 1992; Hall and Donovan, 2001). Fig. 1 illustrates the problem generically. Areas A and B are areas covered during abundance surveys while all of the historical catch is taken from Area A. The future intention of the fishery is to operate in Area A (which may, for example, be the closest to port). The catch limit for Area A can be based either on (1) the survey and catch data for Area A only, or (2) on the survey and catch data for both Areas combined.

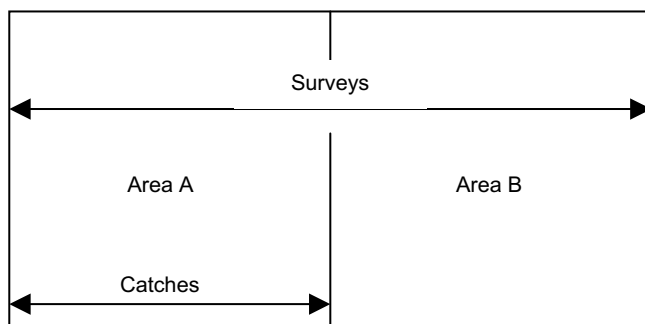


Fig. 1. The generic one-stock / two-stock problem. The historical (and future) catches are taken from Area A and surveys cover both Areas A and B. It is uncertain whether Areas A and B contain one or two stocks (and in the latter case in a manner that their relative proportions in Areas A and B differ appreciably).

Option (1) is appropriate if separate stocks<sup>1</sup> are found in Areas A and B, whilst option (2) is appropriate if there is only a single stock. If the catch limit is based on data for Area A only and there is in fact a single stock, the resource will be underutilised. Conversely, if it is based on the data for both Areas when Area A contains a separate stock from Area B, overexploitation will occur in Area A. The RMP can overcome some of the problems associated with uncertainty about stock structure through its catch capping and catch cascading options (IWC, 1994), but will yield improved performance (better catches for the same perceived risk) if some of this uncertainty can be resolved.

One of the most common recent approaches to attempt to resolve stock structure questions is collection and analysis of genetics data (e.g. see IWC, 1991; Dizon *et al.*, 1997). Traditionally, this has been examined using classical (frequentist) statistical methods based on the null hypothesis of panmixia. This classical approach is based on well-established statistical techniques (e.g. Excoffier *et al.*, 1992; Hudson *et al.*, 1992). Statistics related both to haplotypes (based on the haplotype frequencies only) and to sequencing (based on haplotype frequencies and genetic distances among haplotypes) are used. These techniques provide clear guidance regarding the most appropriate stock

<sup>1</sup> For simplicity, this paper discusses stock differentiation issues in the context of the existence of a stock boundary which exactly and completely separates stocks. In reality, of course, there will be a region of overlap, and any boundary line specified would constitute a trade-off choice which attempts to minimise the proportions of each stock likely to be present on each's 'other' side of that line.

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structure hypothesis if a statistically significant result is obtained. However, the implications of a non-significant result are unclear. This is because a non-significant result can arise either because there is only a single stock in the area from which the data were collected, or because there is stock structure in the area but the sample size is too small to detect this. Furthermore, a non-significant result could also arise if there are really two stocks but the boundary between the strata chosen for data analysis does not correspond to that between the stocks.

In principle, the use of Bayesian techniques can overcome these problems. The outcome of a Bayesian comparison of single- and two-stock hypotheses (models) is the posterior odds ratio (Jeffreys, 1961; Kass and Raftery, 1995). The posterior odds ratio is the ratio of the relative probability of the one- to the two-stock hypothesis. Thus, a very large value will indicate preference for the one-stock hypothesis, a value close to zero preference for the two-stock hypothesis, and a value close to unity preference for neither hypothesis. The posterior odds ratio is the product of the prior odds ratio and the Bayes Factor. For the illustrative calculations of this paper, the prior odds ratio is assumed to be unity (i.e. the one- and two-stock hypotheses are equally likely *a priori*) so the posterior odds ratio is equal to the Bayes Factor.

Use of Bayesian methods is perhaps preferable to the use of classical statistical methods in any case. This is because they provide the (relative) probability of alternative hypotheses rather than simply the ability to reject one of the two models at some pre-specified level of type I error. Determining the relative probability of alternative hypotheses is preferable because it avoids the need for the specification of a somewhat arbitrary level of type I error, and because risk in fisheries management is related not only to the probability of an event but also to the severity of possible outcomes given that event. Thus, a stock structure hypothesis that has major management implications may warrant consideration by the decision makers even if it has relatively low probability.

Bayesian methods are being used increasingly to analyse genetics data (e.g. Lulhart and England, 1999; Shoemaker *et al.*, 1999; Kitada *et al.*, 2000; Pella and Masuda, 2001) and hence to determine the relative probabilities of alternative stock structure hypotheses. Punt *et al.* (2000) developed an approach for determining the relative probability of alternative stock structure hypotheses using allozyme data. Although allozyme data have been widely used in studies of stock structure (e.g. Butterworth *et al.*, 1996; Gardner and Ward, 1998), allozymes mutate at a slower rate than mtDNA and microsatellites so they have lower power to detect genetic differences (Bossart and Pashley Powell, 1998). This paper therefore develops a Bayesian framework within which single- and multiple-stock hypotheses can be compared using mtDNA data. The approach is evaluated using simulation and then, for illustrative purposes, applied to data for North Pacific minke whales (see Fig. 2 for the management sub-areas defined for North Pacific minke whales).

## METHODS

### Basic formulation

The region to be sampled (and for which stock structure hypotheses are postulated) is assumed to be divided into  $n$  sub-areas. For each sub-area  $i$ , a number,  $N^i$ , of animals are sampled. This leads to a total dataset  $\{x_j^i: j = 1, 2, \dots, k; i = 1, 2, \dots, n\}$  where  $x_j^i$  is the number of animals sampled in sub-area  $i$  that have haplotype  $j$ , and  $k$  is the total number of haplotypes in the whole dataset (by definition  $\sum_{j=1}^k x_j^i = N^i$ ).

Given a random sampling scheme (as is the case for the mtDNA data for North Pacific minke whales; e.g. Fujise (2000)), the dataset for sub-area  $i$  can be considered to be a multinomial sample from the population in sub-area  $i$ . If  $p_j^i$  is the proportion of animals in sub-area  $i$  with haplotype  $j$ , then  $\underline{x}^i \sim MN(\underline{p}^i, N^i)$  and the likelihood for the dataset for

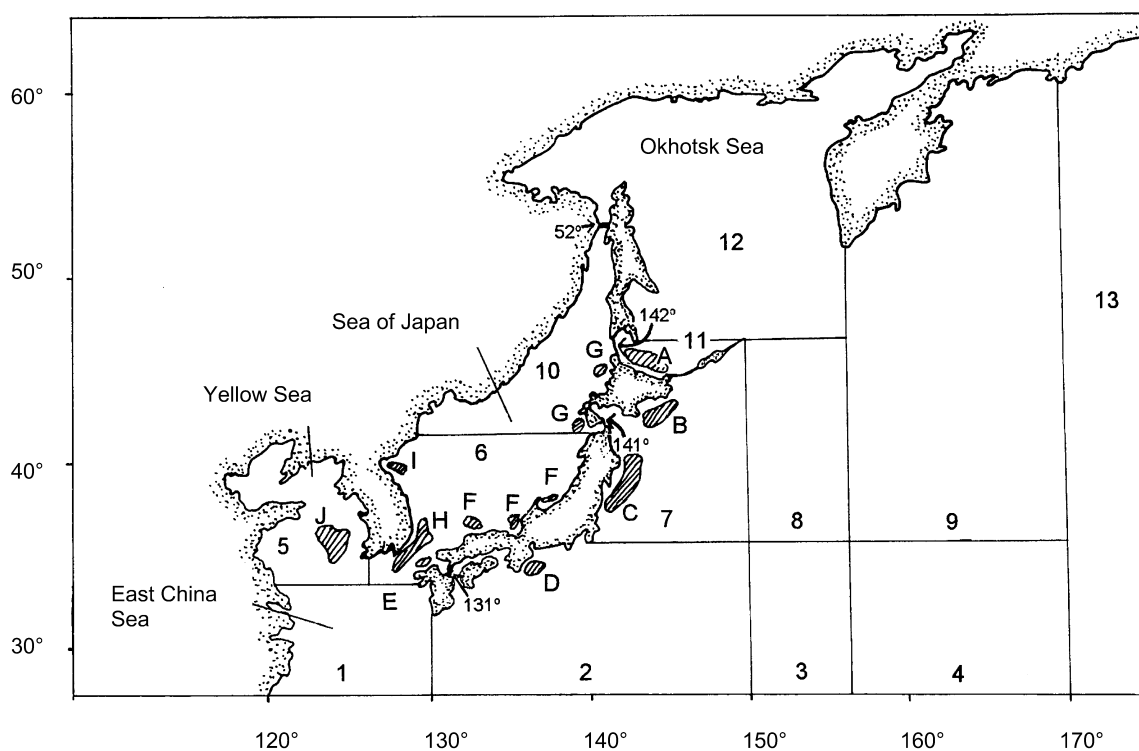


Fig. 2. Sub-areas of the western North Pacific as defined for minke whale management purposes (from IWC, 2000).

sub-area  $i$  is given by:

$$L(D^i | \underline{p}^i) = \frac{N^i!}{\prod_{j=1}^k x_j^i!} \prod_{j=1}^k (p_j^i)^{x_j^i} \quad (1)$$

where  $D^i$  is the dataset for sub-area  $i$ .

It is necessary to specify prior distributions for the parameters of model (1) to apply a Bayesian estimation approach and hence to compute Bayes Factors. The prior chosen for the parameters is the Dirichlet- $\beta$  distribution, i.e.:

$$P(\underline{p}^i) = \frac{\Gamma\left(\sum_{j=1}^k \alpha_j\right)}{\prod_{j=1}^k \Gamma(\alpha_j)} \prod_{j=1}^k (p_j^i)^{\alpha_j - 1} \quad (2)$$

where  $\alpha$  are the parameters of the prior distribution.

The Dirichet- $\beta$  distribution was chosen as the prior distribution because it is the conjugate prior to the multinomial distribution (Johnson and Kotz, 1970; Gelman *et al.*, 1995). It can be shown (Johnson and Kotz, 1970) that the marginal posterior distribution for sub-area  $i$  is given by:

$$\int_{\underline{p}^i} L(D^i | \underline{p}^i) P(\underline{p}^i | \underline{\alpha}) d\underline{p}^i = \frac{N^i! \Gamma\left(\sum_{j=1}^k \alpha_j\right) \prod_{j=1}^k \Gamma(x_j^i + \alpha_j)}{\prod_{j=1}^k x_j^i! \prod_{j=1}^k \Gamma(\alpha_j) \Gamma\left(\sum_{j=1}^k \alpha_j + N^i\right)} \quad (3)$$

The marginal posterior across all sub-areas is therefore the product over sub-areas of Equation (3), i.e.:

$$P^m(\mathbf{D} | \underline{\alpha}) = \prod_i \left( \frac{N^i! \Gamma\left(\sum_{j=1}^k \alpha_j\right) \prod_{j=1}^k \Gamma(x_j^i + \alpha_j)}{\prod_{j=1}^k x_j^i! \prod_{j=1}^k \Gamma(\alpha_j) \Gamma\left(\sum_{j=1}^k \alpha_j + N^i\right)} \right) \quad (4)$$

Fig. 3 explores the impact of different choices for the values for the parameters of the prior for the simple case in which there is only one sub-area and two haplotypes. For this example, the posterior can be summarised by the probability of getting one of the two haplotypes. The potential for the

prior to ‘bias’ the posterior away from the probability implied by the data alone depends on (1) the sum of  $\alpha$  over all haplotypes relative to the total sample size and (2) the relative difference between the ratio of the number of animals observed with each haplotype and the ratio of the  $\alpha$ s. Furthermore, Equation (3) can be interpreted by noting that including the prior is equivalent to ‘adding’ a sample where the number of individuals with haplotype  $j$  is equal to  $\alpha_j - 1$  to the actual data.

Now Equations (1)-(4) are based on the assumption that the proportion of animals in sub-area  $i$  with haplotype  $j$ ,  $p_j^i$ , depends on sub-area, i.e. this is a multi-stock assumption. To develop the marginal posterior across all sub-areas for a single-stock model, the likelihood for the dataset for sub-area  $i$  and the prior are given by Equations (1) and (2) where the dependence of  $p$  on sub-area is dropped. The marginal posterior across all sub-areas for the single stock model is given by:

$$P^s(\mathbf{D} | \underline{\alpha}) = \int \prod_i L(D^i | \underline{p}) P(\underline{p} | \underline{\alpha}) d\underline{p} \quad (5)$$

which can be shown to be:

$$\frac{\prod_i N^i!}{\prod_i \prod_{j=1}^k x_j^i!} \frac{\Gamma\left(\sum_{j=1}^k \alpha_j\right)}{\prod_{j=1}^k \Gamma(\alpha_j)} \frac{\prod_{j=1}^k \Gamma\left(\sum_i x_j^i + \alpha_j\right)}{\Gamma\left(\sum_{j=1}^k \alpha_j + \sum_i N^i\right)} \quad (6)$$

**Dealing with the parameters of the prior**

The specification of the values for the parameters of the Dirichlet- $\beta$  prior (the  $\alpha$ s) can be achieved using Empirical Bayes or Full Bayes approaches. The Empirical Bayes approach involves pre-specifying the values for the hyper-parameters (the  $\alpha$ s) based on the actual data (i.e. the  $x$ s), while the Full Bayes approach involves placing a (hyper)prior<sup>2</sup> on the  $\alpha$ s. One immediate difference between the Empirical and Full Bayes approaches is therefore that only the latter deals with the uncertainty associated with the  $\alpha$ s. Pella and Masuda (2001) apply two methods (‘maximum prior predictive distribution’ and ‘minimum squared-error risk’) to determine Empirical Bayes estimates for the  $\alpha$ s. In common with Pella and Masuda (2001), it was found here that the former method often leads to unrealistically large (i.e. very informative – see Fig. 3) values for the  $\alpha$ s.

<sup>2</sup> A hyper-prior is the prior distribution for the parameters of the prior distribution.

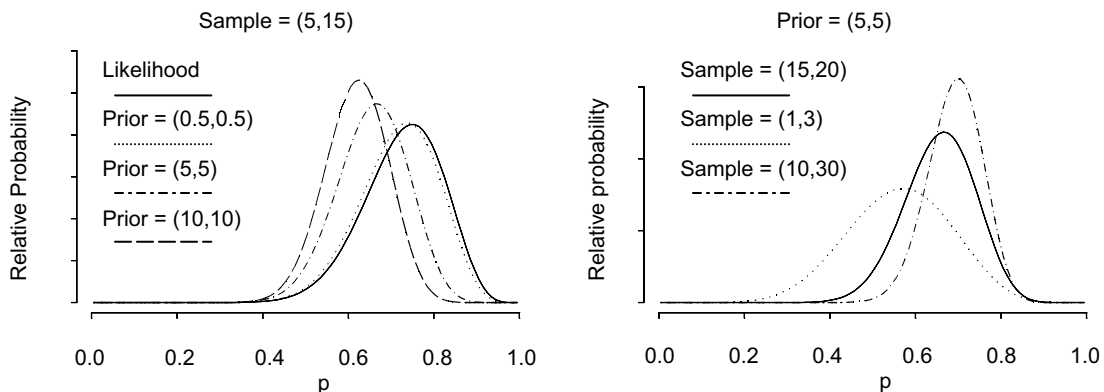


Fig. 3. Sensitivity of the posterior distribution for the probability of selecting an animal with a particular haplotype from a population where there are only two haplotypes. The likelihood is assumed to be binomial and the prior a beta distribution. The base-case specifications are that 15 animals out of a sample of 20 had the particular haplotype and the parameters of the beta distribution prior = (5,5).

Therefore, the Empirical Bayes results reported in this paper are based on the ‘minimum squared-error risk’ method (see Appendix 1 and Bishop *et al.* (1975) for details).

The hyper-prior for the Full Bayes approach needs to satisfy the constraint  $\alpha_j > 0 \forall j$ . One widely-used distribution that automatically imposes this constraint is the log-normal distribution, i.e.:

$$P(\alpha_j) = \frac{1}{\sqrt{2\pi} \sigma_\alpha \alpha_j} e^{-\frac{1}{2\sigma_\alpha^2} (\ln \alpha_j - \ln \bar{\alpha})^2} d\alpha \quad (7)$$

where:

$\bar{\alpha}$  is the median of the hyper-prior for  $\alpha$ ; and

$\sigma_\alpha$  is the standard deviation of the hyper-prior for  $\ln \alpha$ .

The values for  $\bar{\alpha}$  and  $\sigma_\alpha$  are taken to be the mean and standard deviation respectively of the Empirical Bayes estimates for the  $\ln \alpha$ . Therefore, the Full Bayes approach has been designed to be roughly comparable with the Empirical Bayes approach<sup>3</sup>.

### Computational aspects

Computing the Bayes Factor is straightforward if an Empirical Bayes approach is adopted because the Bayes Factor is simply the ratio of the marginal posteriors (Equations 4 and 6) given the values calculated for the  $\alpha$ s. In contrast, the Full Bayes approach involves integrating over the hyper-prior for the  $\alpha$ s; i.e., in this case, the Bayes Factor is defined as:

$$BF = \frac{\int_{\alpha} P^s(\mathbf{D} | \alpha) P(\alpha) d\alpha}{\int_{\alpha} P^m(\mathbf{D} | \alpha) P(\alpha) d\alpha} \quad (8)$$

Evaluation of the numerator and the denominator of Equation (8) cannot be achieved analytically, and consequently a numerical integration approach needs to be applied. Three alternative approaches to computing the integrals were considered; two of these (Equations 9a and 9b) are based on samples from the posterior distribution for

the  $\alpha$ s (i.e. the distribution defined by  $\frac{P^{s/m}(\mathbf{D} | \alpha) P(\alpha)}{\int P^{s/m}(\mathbf{D} | \alpha) P(\alpha) d\alpha}$ )

while the third (Equation 9c) relies on samples from an approximation to that posterior distribution:

$$\hat{I} = \left\{ \frac{1}{w} \sum_{l=1}^w \frac{1}{P^{s/m}(\mathbf{D} | \underline{\alpha}_l)} \right\}^{-1} \quad (9a)$$

$$\hat{I} = \left\{ \frac{1}{w} \sum_{l=1}^w \frac{f(\underline{\alpha}_l)}{P^{s/m}(\mathbf{D} | \underline{\alpha}_l) P(\underline{\alpha}_l)} \right\}^{-1} \quad (9b)$$

$$\hat{I} = \frac{\sum_{l=1}^q P^{s/m}(\mathbf{D} | \underline{\alpha}_l) P(\underline{\alpha}_l) / g(\underline{\alpha}_l)}{\sum_{l=1}^q P(\underline{\alpha}_l) / g(\underline{\alpha}_l)} \quad (9c)$$

where:

$\hat{I}$  is the integral needed to compute the Bayes factor;

$w$  is the number of parameter vectors generated from the (numerical representation of the) posterior distribution;

$q$  is the number of parameter vectors generated from  $g()$ , the approximation to the posterior distribution; and

$P^{s/m}(\mathbf{D} | \underline{\alpha}_l)$  is the value of the marginal posterior (see Equations 4 and 6 for the multiple and single stock marginal posteriors) for the  $l$ th parameter vector.

Equations (9a) and (9b), due to Newton and Raftery (1994) and Gelfand and Dey (1994) respectively, utilise a sample of  $w$  independent parameter vectors from the posterior distribution for the  $\alpha$ s. Equation (9c), on the other hand, is based on a sample of  $q$  points from an approximation to this posterior distribution (the importance function). The function  $f()$  in Equation (9b) can be any proper density function. For the purposes of this study,  $f()$  and  $g()$  have been taken to be the multivariate normal distribution with multivariate mean given by the vector  $\underline{\alpha}_{\max}$  at which the marginal posterior attains its maximum and the variance-covariance matrix obtained by inverting the Hessian matrix about  $\underline{\alpha}_{\max}$ . The samples needed to apply Equations (9a)<sup>4</sup> and (9b) can be obtained using the Markov Chain Monte Carlo (MCMC) (Hastings, 1970; Gelman *et al.*, 1995) or Sample-Importance-Resample (SIR) (Rubin, 1987) algorithms, while those needed to apply Equation (9c) can be obtained using the SIR algorithm if the function  $g()$  is taken to be the importance function.

A major problem associated with the application of Bayesian methods to complex problems is how to assess whether the algorithm used for numerical integration has converged to the posterior distribution (Gelman *et al.*, 1995). Assessing convergence can be divided into two parts in the context of this study: (a) whether the MCMC and SIR algorithms have been run for long enough that the resultant samples represent the posterior distributions; and (b) whether Equations (9a)-(9c) are numerically stable. The convergence of the SIR algorithm has been evaluated in this paper by the proportion of replicate parameter vectors in the sample from the posterior (typically no more than 0.5%), while the convergence of the MCMC algorithm has been evaluated using the magnitude of the correlation between ‘adjacent’ parameter sets (both visually and by means of correlation coefficients) and by using the statistics contained in the ‘Bayesian Output Analysis’ set of routines for assessing convergence of MCMC chains<sup>5</sup>. Based on these considerations, it was concluded that an adequate representation of the posterior could be obtained by conducting 5,570,000 cycles of the MCMC algorithm, ignoring the first 15% as a ‘burn in’ period, and then selecting every 2,000<sup>th</sup> parameter vector in the remaining chain. This resulted in a sample of 2,500 parameter vectors from the posterior distribution on which the integrals could be based. The SIR results are based on 5,000,000 draws from the importance function and 2,500 resamples from these draws.

The results in this paper are based on Equations 9b and 9c. Equation 9a was not used because it was found to be numerically unstable. The lack of stability of Equation (9a) is not surprising because a parameter vector with small likelihood can have a large impact on the value for  $\hat{I}$  (Kass

<sup>3</sup> Basing the values for  $\bar{\alpha}$  and  $\sigma_\alpha$  on the Empirical Bayes estimates implies some use of the haplotype frequency data in developing the hyper-prior and, as such, the ‘Fully Bayes’ approach therefore has a slightly Empirical Bayes flavour.

<sup>4</sup> Equation (9a) follows from Equation (9c) taking as the sample from the importance function a sample from the posterior, i.e.  $g(\underline{\alpha}) = P^{s/m}(\mathbf{D} | \underline{\alpha}) P(\underline{\alpha}) / \hat{I}$ .

<sup>5</sup> <http://www.pmech.uiowa.edu/boa/>.

and Raftery, 1995; Carlin and Louis, 2000). Table 1 illustrates this potential sensitivity for the simple case in which the prior is  $U[-5,5]$  and the likelihood is  $N(0; 1^2)$ . For this case, it is possible to generate samples directly from the posterior distribution and to compute the integral over the product of the likelihood and prior analytically. While illustrative, the results in Table 1 indicate that improved performance arises from larger samples from the posterior distribution and that Equation 9a gives results which are much more variable than Equations 9b and 9c. It would appear that Equation 9a is only first order correct while Equations 9b and 9c are second order correct.

**Example application**

The *Implementation Simulation Trials* for the North Pacific minke whales (IWC, 2000; 2001a) include two hypotheses regarding stock structure in the western North Pacific: a two stock- ('J' and 'O') model and a three stock- ('J', 'O' and 'W') model<sup>6</sup>. There is support for at least two stocks in the Western North Pacific from analyses of allele frequency (Wada, 1984; 1991; Punt *et al.*, 1995; Butterworth *et al.*, 1996), conception date (Best and Kato, 1992), mtDNA (Goto and Pastene, 1997; 1998) and morphological (Kato *et al.*, 1992) information. The evidence for a 'W' stock is low *p*-values found in comparisons involving sub-area 9 (IWC, 2001b).

The mtDNA control region sequencing data used in this study were from minke whales taken during Korean and Japanese coastal small-type whaling operations (1982-1987; Goto and Pastene, unpublished data) and during the Japanese Whale Research Programme under Special Permit in the western North Pacific (JARPN) (1994-1999; Goto and Pastene, 2000). Some of the analyses conducted excluded samples from the western part of sub-area 9 (west of 162°E) in 1995. The reason for doing this is that the results of previous hypothesis testing based on mtDNA data showed some heterogeneity in this particular group of animals (Goto *et al.*, 2000). Given these previous results, it was of interest to examine the sensitivity of the results from the Bayesian approach to including and excluding the data from the

<sup>6</sup> More recent versions of these trials (IWC, 2001a) include the hypothesis that there is limited interchange between the 'O' and 'W' stocks but this hypothesis is not considered in this paper.

western part of sub-area 9 in 1995. The results of Goto *et al.* (2000) suggest the possibility of some temporal component to the distribution of stocks in the western North Pacific.

**RESULTS AND DISCUSSION**

**Simulation evaluation**

The objective of the method developed in this paper is that the resultant Bayes Factor should be very large if the one-stock hypothesis is correct, 0 if the two-stock hypothesis is correct and 1 if the data are unable to identify which stock structure hypothesis is correct. The ability of the method to achieve this objective can be evaluated by means of simulation (e.g. Martien and Taylor, 2000; Taylor *et al.*, 2000). Detailed simulations are, however, beyond the scope of the current paper. Nevertheless, some simulations have been conducted to evaluate the performance of the method given different sample sizes and true stock structure hypotheses.

Fig. 4 plots the logarithms of the ratio of the probability of the one-stock hypothesis to the sum of the probabilities of the one- and two-stock hypotheses (essentially the relative weight that should be assigned to the one-stock hypothesis) against the logarithms of the *p*-values from likelihood ratio tests comparing the one- and two-stock hypotheses<sup>7</sup>. The results in Fig. 4 are based on applying the Full Bayes version of the method to 20 datasets, each of which includes two areas. The haplotype frequency data for sub-areas 6 and 7 were used as the basis to generate the simulated data for the two areas; i.e. the two-stock hypothesis is correct for these simulations. As expected from previous studies, the one-stock hypothesis is rejected by both the Bayesian and frequentist methods even for low (25 per area) samples sizes. This result suggests that the method of this paper performs adequately when there are major differences in haplotype frequencies among areas.

Fig. 5 plots the relative weights that should be assigned to the one-stock hypothesis against the *p*-values from a likelihood ratio test comparing the one- and two-stock hypotheses for the case in which both datasets are generated from the haplotype frequency data for sub-area 7. This is a

<sup>7</sup> More powerful versions of the likelihood ratio test based on randomisation techniques exist, but the qualitative features of Figs 4–7 should be insensitive to using such tests.

Table 1

Sampled-based estimates of the integral of the product of a  $U[-5,5]$  prior and a  $N(0;1^2)$  likelihood. Results are shown for three alternative approximating equations, three alternative posterior sample sizes, and 10 individual samples from the posterior distribution for each sample size. The row 'SD' lists the standard deviation (multiplied by 100) of the 10 values for each combination of approximating equation and sample size.

Simulation number	Sample size/approximating equation								
	1,000,000			100,000			10,000		
	Eqn 9a	Eqn 9b	Eqn 9c	Eqn 9a	Eqn 9b	Eqn 9c	Eqn 9a	Eqn 9b	Eqn 9c
1	0.099	0.100	0.100	0.111	0.100	0.100	0.110	0.101	0.101
2	0.094	0.100	0.100	0.094	0.100	0.100	0.135	0.100	0.101
3	0.103	0.100	0.100	0.107	0.100	0.100	0.119	0.098	0.100
4	0.098	0.100	0.100	0.114	0.099	0.100	0.103	0.099	0.099
5	0.108	0.100	0.100	0.105	0.100	0.100	0.118	0.100	0.101
6	0.093	0.100	0.100	0.105	0.100	0.100	0.105	0.099	0.100
7	0.108	0.100	0.100	0.118	0.100	0.100	0.122	0.099	0.101
8	0.102	0.100	0.100	0.100	0.100	0.100	0.083	0.097	0.100
9	0.099	0.100	0.100	0.106	0.100	0.100	0.118	0.099	0.100
10	0.100	0.100	0.100	0.121	0.100	0.100	0.110	0.099	0.100
SD	0.523	0.021	0.015	0.820	0.033	0.021	1.378	0.105	0.072

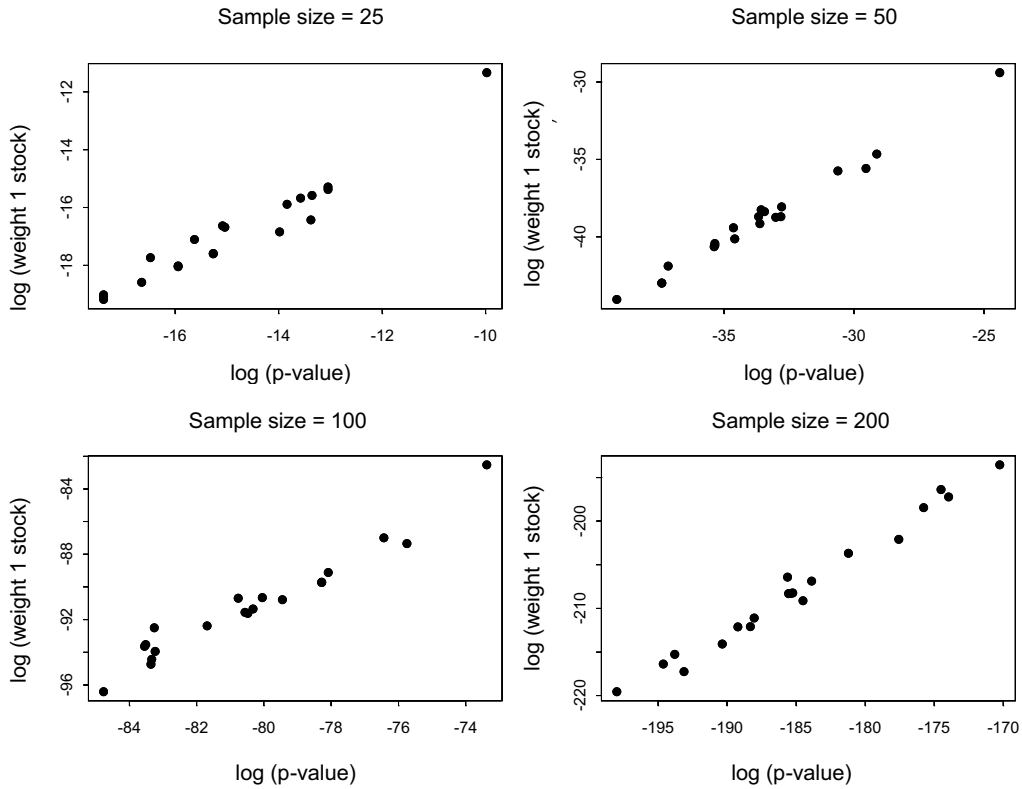


Fig. 4. Logarithms of the relative weights assigned to the one-stock hypothesis based on the Full Bayes method versus logarithms of likelihood ratio test  $p$ -values. The results in this Figure are based on 20 simulations in which the two-stock hypothesis is correct.

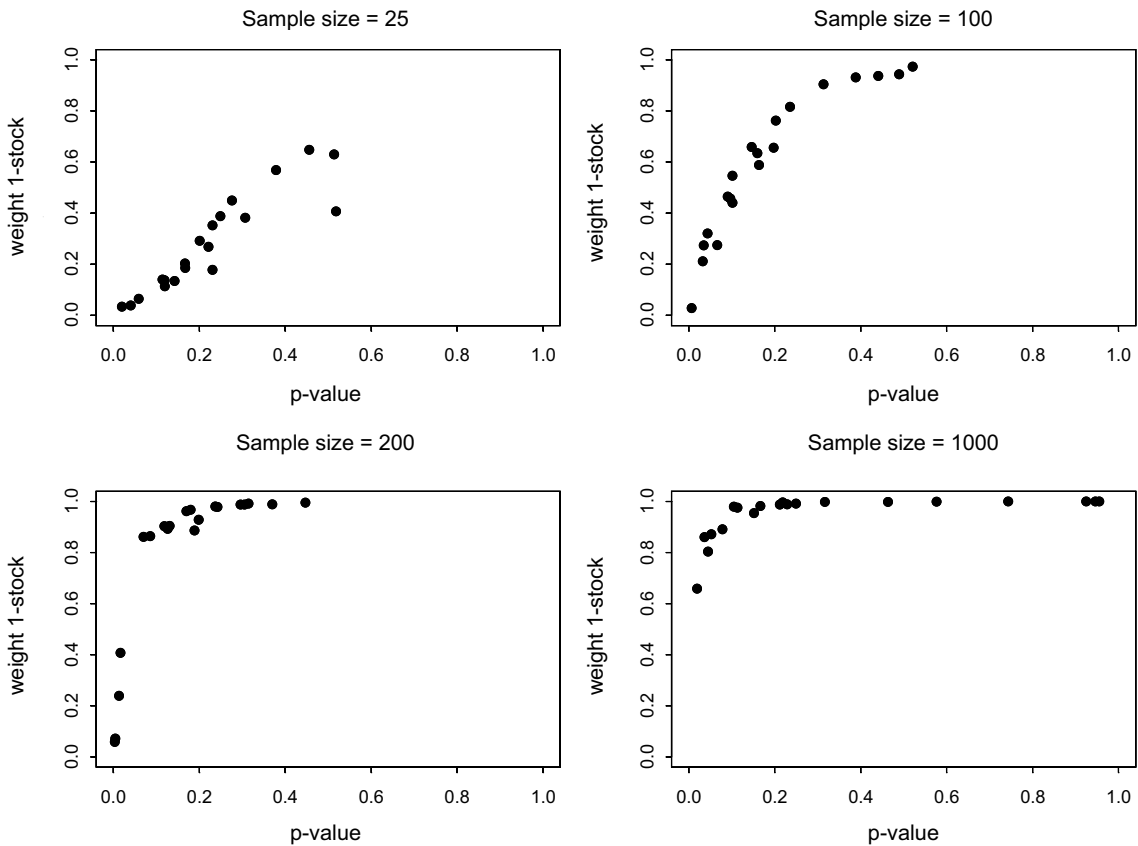


Fig. 5. Relative weights assigned to the one-stock hypothesis based on the Full Bayes method versus likelihood ratio test  $p$ -values. The results in this Figure are based on 20 simulations in which the one-stock hypothesis is correct.

case in which the one-stock hypothesis is correct. The results of the Full Bayes approach for the smallest sample size (25 per area) inappropriately indicate support for the two-stock hypothesis (17 of the 20 relative weights are smaller than

0.5) while three of the 20 likelihood ratio test  $p$ -values were smaller than the nominal level of 0.05. However, increasing the sample size from 25 to 100, to 200 and then to 1,000, results in much better performance, with increases in the

proportions of weights larger than 0.5 to 0.6, 0.8, and 1 respectively. In contrast, it is noteworthy that even for a sample size of 1000, three of the 20 simulations led to likelihood ratio test *p*-values less than the nominal level of 0.05. The reasons for the poor performance of the Full Bayes approach for a sample size of 25 are unclear but are probably related to the nature of the prior distribution, the effect of which is minimised given large sample sizes.

Although performance is adequate for large sample sizes, the results in Figs 4 and 5 suggest that the Full Bayes method may not provide reliable results for very small sample sizes if the one-stock hypothesis is correct. The simulations conducted to date are, however, relatively limited and additional work in this area is needed. Additional simulation work should be conducted in which the pseudo datasets are generated using either the types of operating models considered by Martien and Taylor (2000) or those based on coalescence simulations (e.g. Hudson, 1991). Both of these approaches to generating pseudo datasets allow these to be generated such that they are bounded by the limitations imposed by evolution and gene flow, although coalescence simulations are likely to be more efficient computationally.

Figs 6 and 7 show results analogous to those in Figs 4 and 5, except that results are shown for the Empirical Bayes approach (based on the ‘minimum squared-error risk’ method) and a variant of this approach in which the  $\alpha$ s are set to ‘uninformative’ values (i.e.  $\alpha = 1/k$  – Pella and Masuda (2001)). The performance of the Empirical Bayes approach (open symbols in Figs 6 and 7) is very poor for the case in which the one-stock hypothesis is correct (Fig. 6); even with very large sample sizes, the Empirical Bayes approach indicates a preference for the two-stock hypothesis. The performance of the ‘uninformative’ approach is markedly better than the Empirical and Full Bayes approaches when the one-stock hypothesis is correct (Figs 5 and 6). However, its performance is less than ideal for the case in which the

two-stock hypothesis is correct (Fig. 7). Although the ‘uninformative’ approach places greatest weight on the two-stock hypothesis when it is correct, the weight assigned to the one-stock hypothesis is much greater than the *p*-value from the likelihood ratio test – this suggests that the ‘uninformative’ approach tends to ‘favour’ the one-stock hypothesis. The extent to which this is actually a concern is not entirely clear because even the ‘uninformative’ approach indicates that the two-stock hypothesis is far more likely than the one-stock hypothesis. A further problem with the ‘uninformative’ approach is that not all authors agree on the values to assign to the  $\alpha$ s in order to obtain an ‘uninformative’ conjugate prior for a multinomial likelihood (Gelman *et al.*, 1995).

The only potential impediment to evaluating Bayes Factor approaches to addressing stock structure questions using simulation is their computational demands. This is not a problem with the Empirical Bayes or uninformative approaches as they do not involve any numerical integration. In contrast, this is certainly potentially a major problem for the Full Bayes approach. However, the software on which the calculations of this paper are based has been optimised so that roughly 10-20 Bayes Factors can be calculated in a twelve-hour period. This suggests that a full evaluation of even the Full Bayes approach should be feasible in short- to medium-term.

**Illustrative application of the data for North Pacific minke whales**

Table 2 lists Bayes Factors for a variety of comparisons among sub-areas for minke whales in the western North Pacific. Sub-area 7 comparisons are shown based on commercial samples, JARPN samples, and commercial and JARPN samples combined. The values for the Bayes Factor can be interpreted in terms of the support for (or against) the one-stock hypothesis (more positive values indicating

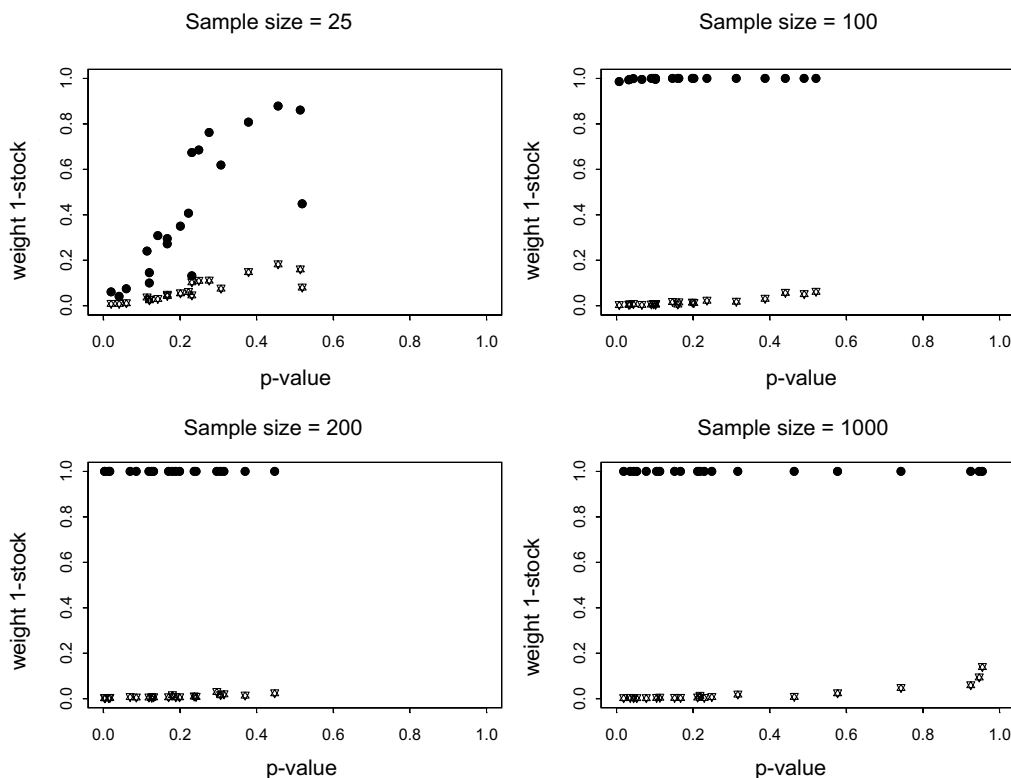


Fig. 6. Relative weights assigned to the one-stock hypothesis (solid dots – uninformative prior; open symbols – Empirical Bayes) versus likelihood ratio test *p*-values. The results in this Figure are based on 20 simulations in which the one-stock hypothesis is correct.

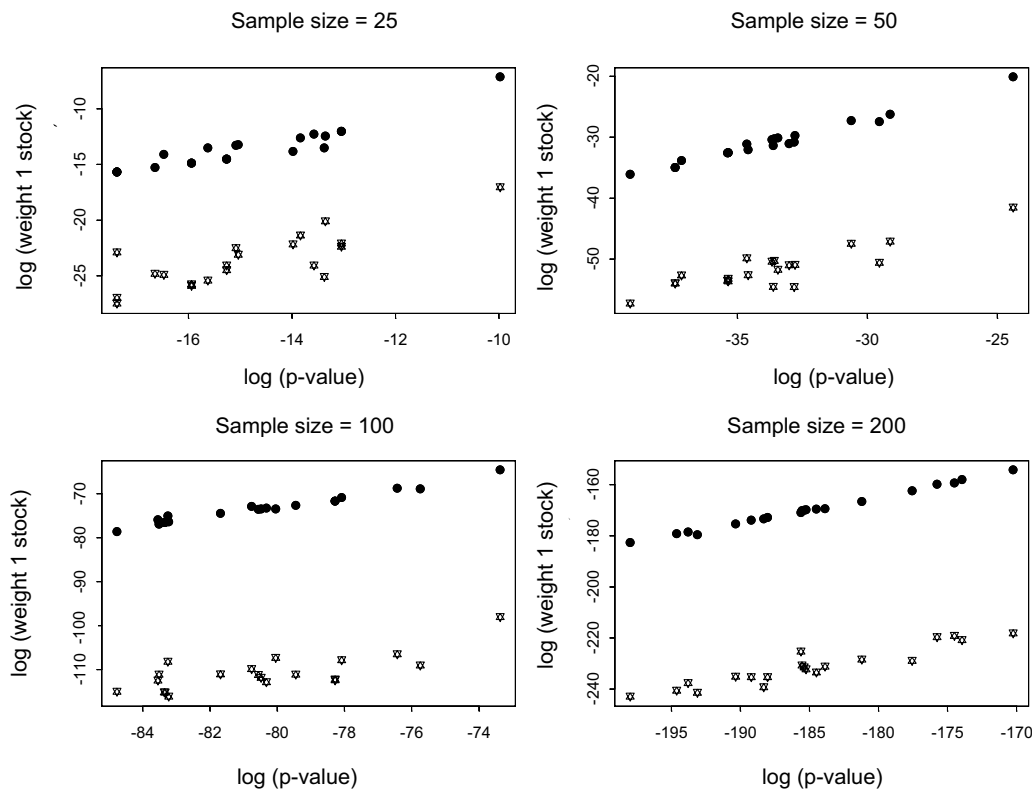


Fig. 7. Logarithms of the relative weights assigned to the one-stock hypothesis (solid dots —uninformative prior; open symbols —Empirical Bayes) versus logarithms of likelihood ratio test  $p$ -values. The results in this Figure are based on 20 simulations in which the two-stock hypothesis is correct.

greater support). Table 2 indicates the qualitative strength of evidence for the one-stock hypothesis using the scheme developed by Kass and Raftery (1995). Results are shown in Table 2 for the Full Bayes method (based on Equation 9c), and the ‘uninformative’ approach. Results are not shown for the Empirical Bayes approach given its poor performance in Fig. 6.

Account needs to be taken of the potential numerical uncertainty associated with the calculation of Bayes Factors using the Full Bayes approach when interpreting the results

in Table 2. Fig. 8 shows the distribution for the Bayes Factor for a comparison of sub-areas 7 (JARPN and commercial samples combined) and 9 (less west 1995) that results from changing the random number sequence used when applying the MCMC and SIR algorithms. It is clear that the value of the Bayes factor can be sensitive to the random number sequence when Equation (9b) is used (it would be even more sensitive had the Bayes Factor been based on Equation 9a) while the results for Equation 9c are relatively insensitive to the random number sequence.

Table 2

Results for comparisons of some one- and two-stock hypotheses. The column ‘Total haplotypes’ lists the total number of haplotypes included in the analysis. The Bayes Factor is defined as the ratio of the probability of the one-stock hypothesis to that of the two-stock hypothesis. C = Commercial data; J = JARPN data; \* = Positive; \*\* = Strong; and \*\*\* = Very strong.

Area 1	Area 2	Sample sizes		Total haplotypes	$\ell_n$ (Bayes Factor)	
					Uninformative.	Full Bayes
6	7 (C+J)	28	285	49	-31.89***	-46.02***
9 (less west 95)	7 (C+J) + 8	110	376	60	26.89***	5.18***
9 (less west 95)	7 (C+J)	110	285	52	20.28***	2.24*
9 (less west 95)	7 J	110	139	44	13.62***	1.21*
9 (less west 95)	7 C	110	146	45	10.87***	-0.35
8+9 (less west 95)	7(C+J)	201	285	60	41.50***	0.97
9	7 (C+J)+8	188	376	64	48.12***	1.28*
9	7 (C+J)	188	285	56	24.29***	-1.13*
9	7 J	188	139	50	27.01***	-0.63
9	7 C	188	146	50	20.44***	-1.92*
8+9	7 (C+J)	279	285	64	51.68***	-0.40
8	7 (C+J)	91	285	56	13.78***	-1.56*
7C	7J	146	139	46	13.08***	0.85



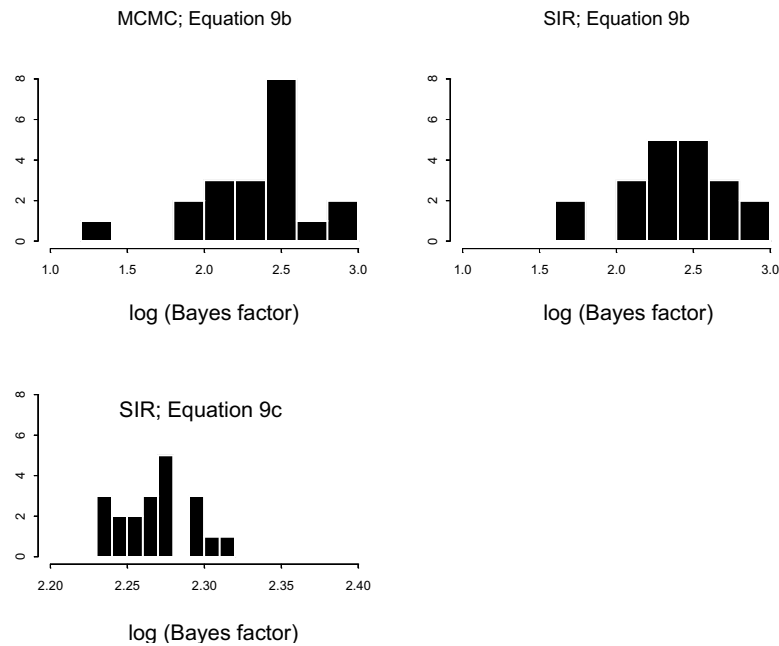


Fig. 8. Sensitivity of the logarithm of the Bayes Factor (probability one-stock hypothesis divided by probability two-stock hypothesis) to the choice of the random number sequence used when conducting the Monte Carlo integrals and the equation used to approximate the probability of the data. The results in this Figure relate to a comparison of sub-areas 7 (JARPN and commercial samples combined) and 9 (less west 1995).

The results, except for the comparison of sub-areas 6 and 7, are very sensitive to how the  $\alpha$ s are specified. For example, there is very strong evidence for the one-stock hypothesis if an ‘uninformative’ prior is assumed for the  $\alpha$ s. This may, however, be a consequence of the ‘uninformative’ approach tending to prefer the one-stock hypothesis. Nevertheless, it should be borne in mind that it is hard to extrapolate from the results in Fig. 7 (where the two-stock hypothesis is clearly correct) to the situation to the east of Japan.

The results for the Full Bayes approach suggest that the data generally provide little information regarding comparison among sub-areas 7, 8 and 9. The only marked exception to this is the comparison between sub-area 9 (less west 95) and the data for sub-areas 7 and 8 pooled. This result is consistent with those from a Bayesian analysis of the allele frequency data (Punt *et al.*, 2000) as well as with those from previous analyses based on hypothesis testing using allozymes (Wada, 1984) and mtDNA RFLP data (Goto and Pastene, 1997). For all combinations of factors, the probability that sub-area 9 contains a separate stock from sub-area 7 decreases if the data for the west of sub-area 9 in 1995 are omitted. This result is consistent with previous analyses based on hypothesis testing (Goto *et al.*, 2000).

## DISCUSSION

The use of Bayesian methods to analyse genetics data, while preferable theoretically, is a relatively recent development. A prime reason for this is that the computational requirements of the calculations can be prohibitive. However, the opportunities for using these methods should increase with the advent of faster personal computers. Some of the assumptions made in this paper (for example that the prior for the proportion of animals in a sub-area with a given haplotype is of the Dirichlet- $\beta$  form) were made largely for computational convenience (so that the marginal posterior could be evaluated analytically) rather than for good theoretical reasons. Although examining different choices for this prior is beyond the scope of the current study,

development of more powerful computers should enable this to be carried out in the future.

The use of the log-normal hyper-prior for the  $\alpha$ s is relatively arbitrary. It would seem prudent to examine the sensitivity of any results to be used for management purposes to other probability distribution functions that have similar properties to the log-normal (e.g. the gamma distribution). In addition, basing the mean and coefficient of variation of the hyper-prior on the values for the  $\alpha$ s used in the Empirical Bayes calculations is also relatively arbitrary. However, no more objective way to define these parameters is immediately obvious. It should also be noted that the illustrative simulations only considered as the case when the two-stock hypothesis is correct, an example where there are clear differences in haplotype frequencies. Future simulations should consider scenarios in which the differences are less clear.

An advantage of Bayesian over frequentist approaches is that the former can be used to assign probabilities to alternative hypotheses. This cannot be achieved using frequentist techniques *inter alia* because the ‘effect size’ is unknown. This problem is not removed through the Bayesian approach. In fact, the ‘effect size’ is implicit in the priors. Fig. 9 illustrates the ‘effect size’ in terms of the prior distribution implied for the *Fst* statistic under the one- and two-stock models (solid and dotted lines respectively). Results are shown in Fig. 9 for two different choices for  $\sigma_\alpha$  (0.5 and 1.5) and two choices for the sample sizes from the two sub-areas. As expected, there is considerable overlap between these distributions, particularly for the lower sample sizes and the high value for  $\sigma_\alpha$ . High values for  $\sigma_\alpha$  imply a more skewed haplotype frequency distribution (a few very common haplotypes and many rare haplotypes) due to the prior assigning higher probability to occasional large values for  $\alpha$ . The distributions in Fig. 9 raise the intriguing question of whether a Bayesian analysis could be based on the implied prior distribution for a quantity such as *Fst* rather than having to be based on the Full Bayesian analysis.

At the present stage of development, the results from Full Bayes method should only be used for management purposes

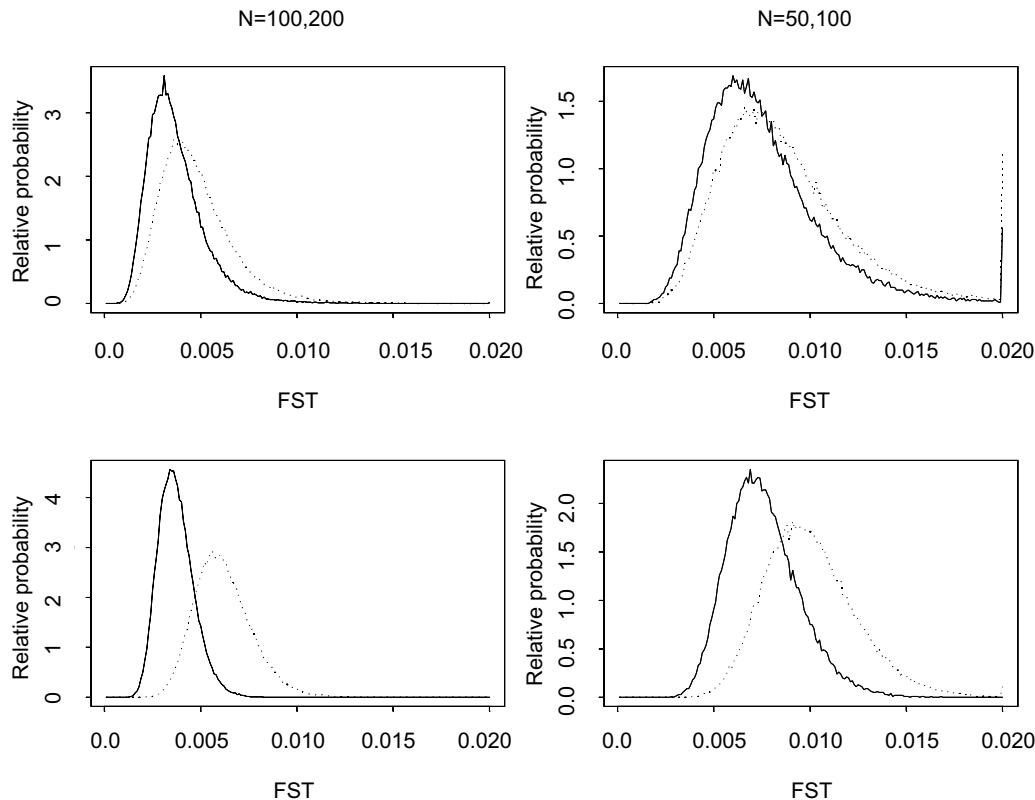


Fig. 9. Distributions for  $F_{st}$  implied by the prior distributions (solid lines —one stock; dotted lines —two stocks). The upper and lower panels correspond to  $\sigma_{\alpha} = 1.5$  and  $\sigma_{\alpha} = 0.5$  respectively. The left and right panels correspond to different sample sizes for the two areas: (100, 200) and (50, 100) respectively.

with caution. Some testing of the method has been completed and this suggests that, when applied to data for two stocks that differ substantially in haplotype frequency, or when sample sizes are large and the one-stock hypothesis is correct, performance is adequate. However, in common with most other methods for analysing genetics data, performance of this method has yet to be fully evaluated, particularly for cases in which there are two stocks but their haplotype frequencies differ only slightly.

Although the use of Bayesian methods for resolving stock structure questions is still in its infancy, we believe that these methods show considerable promise. For example, Pella and Masuda (2001) developed an approach based on similar assumptions regarding the likelihood function and the prior for the proportion of animals with a particular haplotype to estimate probability distributions for stock mixture rates. Further development of the technique outlined in this paper should provide a firmer basis for the development of *Implementation Simulation Trials*<sup>8</sup> is, in some cases, already based on a Bayesian assessment (e.g. Punt and Smith, 1999; IWC, 2002). In fact, there is no reason (barring computational constraints) therefore that genetics data could not be included in the conditioning process so that the probability of alternative stock structure hypotheses is one outcome of this process.

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<sup>8</sup> Conditioning involves selecting the values for the parameters of the operating model that represents the 'true situation' for the trials so that this model adequately mimics the observed data.

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### Appendix 1

#### The minimum squared-error risk approach to selecting values for the $\alpha$ s

The values for the  $\alpha$ s are defined according to the formula:

$$\alpha_j = \alpha^T \bar{x}_j \quad (1.1)$$

where:

$\alpha^T$  is a value that minimises the expected squared-error between the posterior means for the relative frequencies of each haplotype and the observed relative frequency of each haplotype; and

$\bar{x}_j$  is the arithmetic average of the relative frequency of haplotype  $j$  across samples:

$$\bar{x}_j = \frac{1}{n} \sum_{i=1}^n \frac{x_j^i}{N^i} \quad (1.2)$$

Now, it can be shown (Bishop *et al.*, 1975) that the value of  $\alpha^T$  satisfies the equation:

$$\alpha^T \sum_{i=1}^n \left\{ \frac{(N^i)^2}{(N^i + \alpha^T)^3} \sum_{j=1}^k (x_j^i - \bar{x}_j)^2 \right\} = \sum_{i=1}^n \left\{ \frac{(N^i)^2}{(N^i + \alpha^T)^3} \left[ 1 - \sum_{j=1}^k (x_j^i)^2 \right] \right\} \quad (1.3)$$

Solving Equation (1.3) for  $\alpha^T$  and applying Equation (1.1) provides the 'minimum squared-error risk' values for the  $\alpha$ s.