Phylogeny of Whales: Dependence of the Inference on Species Sampling

Jun Adachi* and Masami Hasegawa*†
*Department of Statistical Science, Graduate University for Advanced Studies and †Institute of Statistical Mathematics

From phylogenetic analyses of the 12S and 16S mitochondrial ribosomal DNA and of myoglobin amino acid sequences, Milinkovitch et al. (1993) proposed the hypothesis that one group of toothed whales (Odontoceti), the sperm whales (Physeteridae), is more closely related to the baleen whales (Mysticeti) than to other alleged odontocetes such as dolphins. This hypothesis is in conflict with the traditional view (Barnes et al. 1985; Novacek 1993) that the odontocetes form a monophyletic clade. From a maximum parsimony analysis of the cytochrome b gene, Arnason and Gullberg (1994) recently challenged Milinkovitch et al.'s (1993) hypothesis as well as the traditional tree, claiming that the mysticetes are closer to the dolphins rather than to the sperm whales. They used the cow as the only outgroup and the giant sperm whale as the only representative of Physeteridae, but the estimated tree may depend on the sampled species (Lecointre et al. 1993; Cao et al. 1994a). It is now highly likely that artiodactyles are the closest relatives of whales (Novacek 1992; Milinkovitch et al. 1993; Janke et al. 1994; Cao et al. 1994b), and by including many alternative artiodactyl outgroups (Irwin et al. 1991) in their cytochrome b dataset, we show that Arnason and Gullberg's conclusion is shaky and that an argument based on a single species as an outgroup is unstable.

Although the maximum parsimony method is used widely in molecular phylogenetics (Stewart 1993), it sometimes gives biased estimate of the phylogeny, particularly when the evolutionary rate differs among lineages (Felsenstein 1978; Hasegawa et al. 1992; Cao et al. 1994a), while the maximum likelihood method that does not assume the rate constancy is robust against the violation of the constancy (Hasegawa and Fujiwara 1993; Kuhner and Felsenstein 1994). For this reason, we used the PROTM program in our package MOLPHY (Adachi and Hasegawa 1994). This program is for the maximum likelihood inference of protein phylogeny (Kishino et al. 1990), and it was based originally on the amino acid substitution matrix of Dayhoff et al. (1978).

Recently, Jones et al. (1992) updated the amino acid substitution matrix by using about 40 times more abundant substitution data than those of Dayhoff et al. The new version of PROTM (version 2.1.2) allows us to use this model (called the JTT model) as well as the Dayhoff, Proportional, and Poisson models (Hasegawa et al. 1992), and it turned out that the JTT model best approximates the evolution of diverse proteins among these alternative models (Cao et al. 1994a).

Figure 1 illustrates the three competing hypotheses on the relationships among mysticetes, sperm whales, and dolphins; that is, (1) Milinkovitch's tree in which sperm whale is closer to mysticetes rather than to dolphins, (2) the traditional tree of Odontoceti monophyly, and (3) Arnason's tree in which dolphins are closer to mysticetes than to sperm whales. Figure 2 shows that the inferred tree is highly sensitive to the choice of the outgroup species, and although Arnason's tree is favored when only the cow is used as an outgroup (with 70% bootstrap probability), this does not necessarily hold when other species are used. For example, when goat is used as an outgroup, Arnason's trce is least supported among the alternative trees only with 11% bootstrap probability. It is preferable to cut long branches on the tree (hence, allowing for a better polarization of characters) by using at least two divergent outgroup species. When two species are used as outgroups, Arnason's tree is favored only in three of 24 cases, while Milinkovitch's tree is favored in 14 cases and the traditional tree is favored in seven cases. Furthermore, in 18 of 24 cases the bootstrap probability of Arnason's tree is the lowest of the three alternatives. Consequently, Arnason and Gullberg's (1994) hypothesis is the least likely among the alternatives as far as their cytochrome b data are concerned.

It must be noted that, although Milinkovitch et al.'s (1993) analysis included only two rorquals as baleen-whales representatives, Milinkovitch et al. did not suggest paraphyly of mysticetes as Arnason and Gullberg (1994) misinterpret. Indeed, Milinkovitch et al.'s main hypothesis is the suggested sister relationship between sperm whales and all mysticetes. In addition, on the basis of the assumption that the molecular clock of the mitochondrial genes is similar in whales and ungulates, Milinkovitch et al. suggested the additional, but distinct hypothesis that sperm whales and baleen whales separated 10 to 15 Mya. However, the fossil record (Barnes...
1984) strongly suggests that the sperm-whale and baleen-whale lineages are at least 20 Myr old. Consequently, it is likely that the dating of the separation of sperm whales and baleen whales is older than 20 Myr and that the molecular clock is slower in whales than in ungulates (as far as the mitochondrial ribosomal genes are concerned). Årnason and Gullberg erroneously confused these considerations about molecular clock calibration with those on the branching pattern of the tree. It is likely that the Milinkovitch et al.'s 10–15 Myr estimate is too low, but it does not affect the robustness of their main hypothesis that concerns the topological relationships of the major groups of cetaceans.

Although more data are obviously needed to determine which of the phylogenetic hypotheses is the truth, the cytochrome b data marginally favor Milinkovitch's tree consistently with the 12S, 16S, and myoglobin data. Many species included in Årnason and Gullberg's dataset are very closely related baleen whales; hence they are poorly informative for testing Milinkovitch et al.'s hypothesis. This study demonstrates that an argument based on a small dataset with respect to the number of relevant species may be unstable (Lecointre et al. 1993; Cao et al. 1994a).

**Fig. 2.—Analysis of the cytochrome b amino acid sequences (Irwin et al. 1991; Årnason and Gullberg 1994) by the maximum likelihood program, PROTMML (Adachi and Hasegawa 1994), based on the JTT model. Bootstrap probabilities estimated by the RELL method (Kishino et al. 1990; Hasegawa and Kishino 1994) with $10^4$ replications shown. The "win" indicates the tree that wins. The same dataset from whales as in Årnason and Gullberg (1994) was used. Within the Mysticeti (b bowhead, right), pygmy right, [Antarctic minke, N. Atlantic minke], gray, humpback, blue, fin, sei, Bryde's whale) was assumed according to the local PROTMML analysis, and this is compatible with the Mysticeti part of fig. 1 in Årnason and Gullberg. First, one species was chosen as an outgroup from cow, sheep, goat, black-tailed deer (b-deer), fallow deer (f-deer), giraffe, pronghorn, chevrotain, camel, peccary, and pig, and second, two divergent species, one from cow, sheep, goat, b-deer, f-deer, giraffe, pronghorn, and chevrotain, and the other from camel, peccary, and pig, were chosen.

**Fig. 1.—Three competing phylogenies of whales

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