The History of Whales Read from DNA

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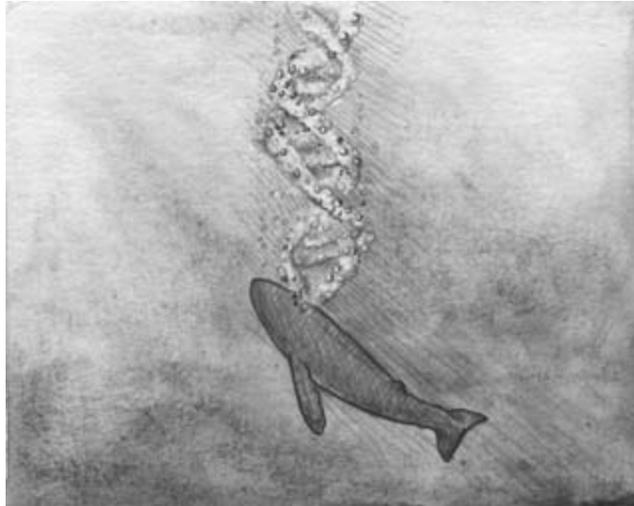
Of what value is knowledge about the history of a threatened species? Is it possible to chart the future of a species without information about its past? Or is knowledge about past population sizes and carrying capacities crucial to future management plans? These issues are particularly relevant to the management of populations of the great whales.

Populations of all the baleen whales were dramatically reduced by whaling, and unprecedented international cooperation has established a global moratorium on the hunting of whales for commercial purposes until stocks recover. But what does recovery entail? For gray whales that migrate along the western coastline of North America, removal from the United States Endangered Species list in 1994 (IUCN 1994) was heralded as the first time a whale population had recovered sufficiently so that it was no longer in danger of human-caused extinction. Removal from the list depended on population size increases that brought gray whales to about the same levels that were thought to have existed before whaling began (Scammon 1874). For this species, the estimated population sizes that existed before whaling have long served as a benchmark against which to evaluate recovery.

In addition, knowledge of the historical numbers of whales may be instrumental in determining how ocean ecosystems are constructed. Large numbers of whales may have been a key component of marine ecosystems before the oceans were disrupted by humans. Recent reports suggest many populations of large consumers, including whales, turtles, and sharks and other pelagic fish, have plummeted since the advent of global commercial fisheries (Jackson 1997; Baum et al. 2003; Myers and Worm 2003; Roman and Palumbi 2003). As a result, our current view of the oceans is missing a crucial set of organisms. How did marine ecosystems function before the near-extirpation of large consumers? The answer depends on values for historical population size.

In this chapter we describe a new method that employs the analysis of genetic variation to measure the numbers of whales before whaling. We present published accounts of prior methods of whale population estimation using catch data and compare results from both approaches. Genetic estimates of whale populations are far higher than those previously obtained from catch records. It is important to note that the fundamental data for both methods have uncertainty; for example, records may be missing in the whaling data, and the mutation rate may be higher than phylogenetic analyses suggest. This uncertainty may have a strong impact on the conclusions. We end with a discussion of potential ways to bring divergent estimates of whaling history into accord.

The Current View of the Past

For many species of whales, historical population values serve as a backdrop to management. The International Whaling

Commission (IWC) set targets for whale population recovery based on the idea that a population below about 54% of its prewhaling level should be protected to enable it to quickly increase. For populations above this level, careful management should regulate any allowable hunt, largely based on the trajectory of current populations.

Conventional estimates of current and past numbers, however, seem at odds with current protection schemes. For example, it has often been quoted that fin whales *(Balaenoptera physalus)* in the North Atlantic Ocean had population sizes of 30,000–50,000 individuals before whaling (Sergeant 1977). Yet current population size for this species in the North Atlantic is estimated at about 56,000 (Bérubé et al. 1998). Because fin whales were extensively hunted in the nineteenth and twentieth centuries, and because they were fully protected by the IWC only in 1984, it seems unlikely that current populations could exceed historical values. Are fin whales fully recovered in the North Atlantic, and thus open to exploitation? Have they breached their carrying capacity, or will populations continue to increase? Estimates for historical population size can help answer these questions.

Similar questions can be raised for one of the best-studied whale species in the North Atlantic—the humpback whale, *Megaptera novaeangliae*. Extensive survey work suggests that there are now about 12,000 humpback whales in the North Atlantic (Stevick et al. 2003). Using readily available published and unpublished works, including scientific papers, nineteenth-century annual reviews of the whale fishery, whale charts, and a sample of whaling logbooks, Mitchell and Reeves (1983) estimated a minimum number of humpback whales in the western North Atlantic of about 4,700 individuals. More recent work shows a record of kills totaling more than 29,000 humpbacks from the 1600s through the early 1900s (Smith and Reeves 2002). Although the accuracy of these historic reconstructions has increased greatly in the past decade, placing these data into a framework in which they can be related to historical numbers, and understanding the limits to precision of these estimates, has been challenging.

Genetic Approaches to Whale Populations

Recent theoretical work in population genetics has provided a new source of data for the measurement of historical population numbers. Patterns of genetic diversity within populations are controlled by many factors, including mutation, selection and migration. But one of the primary determinants of genetic diversity is the long-term average population size of a species. Mutation is always the primary source of genetic variation, and it tends to increase levels of DNA variation at a steady, slow rate. This variation is weeded out by natural selection against deleterious mutants or culled by *genetic drift*: the process by which alleles are lost randomly from one generation to the next, which tends to be more common when populations are small. In general, a population loses about $1/(2N_e)$ of its genetic diversity per generation

through drift (Hartl and Clark 1997), where N_e is the genetically effective population size—roughly the number of breeding adults. This simple relationship shows that small populations are subject to higher levels of inbreeding and a faster loss of genetic diversity.

For populations that remain at the same size for long periods of time, an equilibrium is reached between the addition of variation generated by mutation and the loss of variation deleted by genetic drift. Assuming that only mutation and drift are acting to control variation, the relationship between genetic diversity (measured by the parameter θ), mutation rate per generation (measured by μ), and effective population size is expected to be $\theta = 4N_e\mu$. If the population does not stay the same size for long periods, the equation can still apply, but the average genetic diversity depends on the long term average effective population size (Hartl and Clark 1997). This relationship applies to nuclear genes. For mitochondrial genes, the relationship between diversity and mutation and population size is similar, with two modifications. The population size is for females only, because only females transmit mitochondrial DNA (mtDNA) to offspring, and the factor of 4 in the formula drops to 2 because mtDNA is haploid, not diploid. As a result, for mitochondrial genes, $\theta = 2N_f\mu$, where N_f is the effective population size for females.

Armed with a measure of genetic diversity for a species and knowledge of the mutation rate, it should be possible to calculate values for effective population size. These values will not reflect the size of current whale populations, but they will tend to reflect the accumulation of genetic diversity over long periods of time. As a result, this diversity provides a molecular record of past population size that is independent of historical whaling records.

The level of mtDNA diversity in a population reflects accumulation of mutations over about N_f generations. Thus, estimates of historic numbers based on genetic diversity give a population size typical for the species over the past 10,000–100,000 years (for species with $N_f = 500$–5,000 and a generation time of 20 years), not just the past few centuries. If population size cycles over time, then the effective size can be smaller than the typical observed size. Populations that have grown steadily over time—such as humans—may have a genetic diversity that is far lower than expected (Takahata 1995). By contrast, populations that have experienced a bottleneck in the past may have a higher diversity than current populations would allow. Recent methods in DNA analysis can sometimes allow these circumstances to be distinguished based on branching patterns in gene genealogies (see, for example, Shapiro et al. 2004).

Just as for any type of historical data, historical levels of genetic diversity are subject to uncertainty. Mutation rate, unsampled populations, and variation between genetic loci can affect population estimates. Other factors besides population size and mutation rate are also known to affect levels of genetic diversity. The most important of these other factors are natural selection and population structure.

For moderate to large populations, selection generally weeds out mutations, and so decreases levels of genetic

variation, more quickly than drift does. So-called *genetic sweeps* may reduce genetic variation in a region of the genome to very low levels, while leaving levels of variation on other parts of the genome untouched. Because the mitochondrial genome is one long molecule, all genes in this genome are linked, and selection on one of them can reduce genetic variability in all of them. Selection can also *increase* genetic diversity when its direction varies over space or time, if it varies with the frequency of alleles in a population, or if individuals with heterozygous genotypes have an advantage.

Population structure can also increase overall genetic variation. When separate populations experience genetic drift independently, they may come to be dominated by different alleles. Diversity within any population may be low because of this domination, but genetic diversity in the species overall still remains high in these cases. For species with separate populations in separate ocean basins, such as many whales, it is critical to be able to correct measures of genetic diversity for potentially high levels of population substructure. If these separated populations are exchanging migrants at a low level, then genetic diversity in each local population may be enhanced by the immigration of novel genes from elsewhere. If this migration occurs often enough, then the diversity of a local population will be nearly as high as the diversity of the whole species. In such cases, local diversity would provide a measure of the population size of the entire species, not just the size of the local population. Because genetic studies of baleen whales, such as humpbacks and fins, have shown substantial divergence between oceans with occasional gene flow (Baker et al. 1998; Bérubé et al. 1998), this transfer of genetic variation could artificially elevate long-term population estimates in ocean basins such as the North Atlantic and must be taken into account.

In summary, genetic measures of past population sizes require (1) estimates of genetic diversity corrected for gene flow, (2) estimates of generation time, and (3) estimates of mutation rate. In the following sections we discuss the data currently available to estimate each of these values for several whale populations and show how these values are combined to provide estimates of historic population size.

Measuring Mutation Rate

Divergence Rate

Estimating the mutation rate for a genetic locus used to measure diversity requires data on the genetic difference at this locus between two species. It also requires information about their divergence time. Such information is often the bottleneck in establishing rates of molecular evolution, because divergence dates between species are often hard to estimate robustly. Instead, many calibrations of molecular rates rely on divergence of genera or families. In the case of cetaceans, well-established dates of species divergence are rare because species-level fossil data are scarce.

The divergence of odontocetes and mysticetes (see also Lindberg and Pyenson, Chapter 7 in this volume) is fairly well
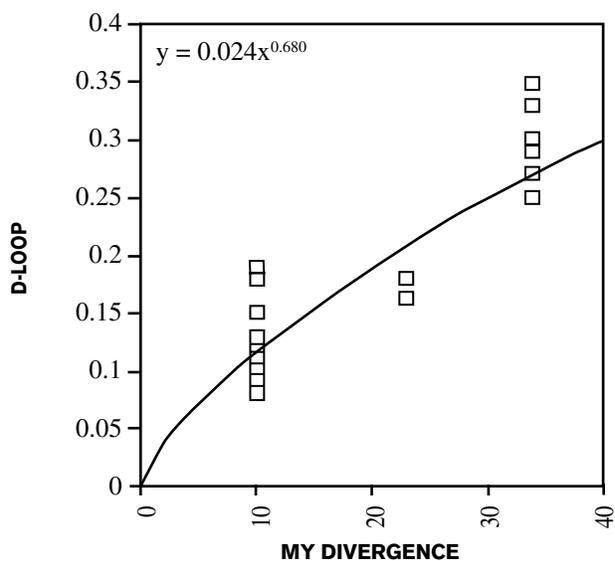
FIGURE 9.1. Genetic divergence in D-loop sequences among baleen whales. Genetic distances were calculated using a Tamura-Nei model with a gamma shape parameter of 0.2. Divergence dates are discussed in the text.

dated at about 35 million years ago, and the divergence of the mysticete families Balaenidae and Balaenopteridae is set at the base of the Miocene about 23 million years ago. Diversification of the species of the genus *Balaenoptera* is thought to have occurred by 6–20 million years ago, and the genera of Balaenids are thought to have diversified in the early Pliocene 3–5 million years ago (Rychel et al. 2004). Overall, these dates provide a few good temporal points on which to hang a measurement of substitution rates, but there are relatively few dates that are recent. The paucity of recent calibration points is important because rapidly evolving regions of mitochondrial DNA might have so many multiple substitutions that sequences will be *saturated* with changes—new changes will overwrite older ones making inferences about substitution rates difficult.

Fortunately, baleen whales tend to show some of the lowest rates of molecular evolution among mammals (Martin et al. 1990; Kimura and Ozawa 2002), reducing the problem of saturation. Yet it is crucial to understand the extent of multiple substitutions in whale DNA. One way to explore this issue is to examine patterns of molecular divergence at a series of timescales. If a measured rate of substitution is higher when the diverging taxa are more closely related and lower for older taxa, then saturation may be a problem. Typically, genetic divergence is plotted against time; if the curve asymptotes strongly, then saturation is suggested. In this situation, the estimated slope of the curve near the origin is the best estimate of short-term substitution rate.

For the mitochondrial control region, known as the *D-loop* in mammals, graphs of genetic difference versus temporal divergence in baleen whales show modest curvature, and the slope near the origin is about 1.5–2.0% change per million years (Figure 9.1). Because this value is the rate of divergence
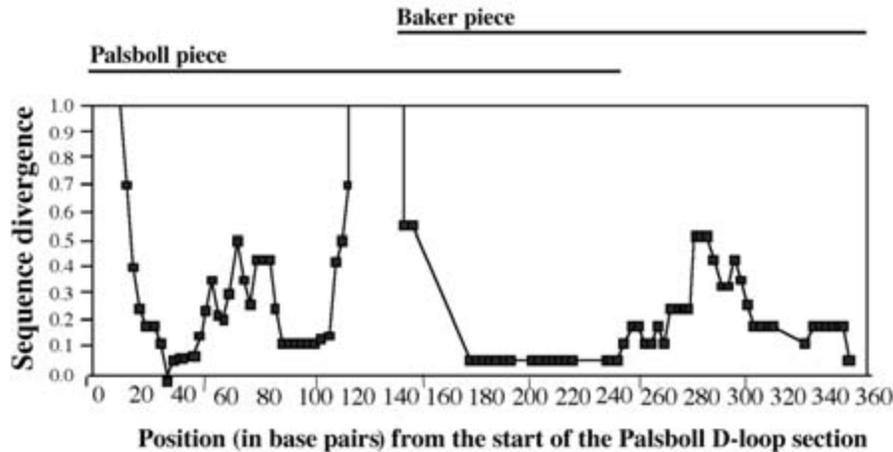
FIGURE 9.2. Sliding-window view of genetic differences between humpback and blue whales along the mitochondrial D-loop. Genetic differences (measured as corrected % sequence divergence) are calculated within 20 bp windows along the length of the D-loop region sequenced by Palsbøll et al. (1995) and Baker et al. (1993). Regions where divergence is greater than 1.0 represent areas where large numbers of insertions and deletions make comparisons across 20 base pairs difficult.

of two species evolving independently, the rate of evolution of each species is half this value, or about 0.75–1.0% per million years. The accumulated D-loop divergence within balaenopterids is about 10–15%, climbing to about 25–35% between odontocetes and baleen whales. This increase suggests that the D-loop values seen among balaenopterids are not as strongly saturated as they are in other mammalian lineages that have been separated for similar amounts of time.

Cautions about Rate Variation

Because different regions of the D-loop vary in their level of sequence conservation, this section of DNA can present special problems in molecular calibrations. Most models of DNA evolution assume that each base in a sequence has the same probability of change as every other base. If some bases have a higher chance of substitution (usually because selection is weaker at these positions), then a simple model of DNA evolution may underestimate the number of substitutions at these positions. One way to estimate the severity of this problem is to examine genetic distances between species in a sliding window that runs the length of the sequence. These graphic plots can reveal areas of particularly high substitution or show regions that are under such strong selection that no variation is allowed—in effect, they provide a topographic map of evolutionary rates along a stretch of DNA.

Such maps are important because the DNA sequences used to measure population structure in whales are from a small section of the control region. The D-loop has been shown to have alternating sections of conserved and highly variable regions (Franz et al. 1985; Andersen et al. 2003). If a population data set includes large portions of a highly conserved region, it may have lower genetic variation, reducing the estimated mutation rate. By contrast, if a data set includes

mostly variable regions, then both genetic diversity and mutation rate may increase. Because of this variation, it is critical that the rate calibration and the genetic diversity measurement be made from exactly the same section of DNA.

Population genetic comparisons have emphasized two partially overlapping pieces of the D-loop region. The first piece was a 264-bp region corresponding to positions 16043 to 16307 in the fin whale mtDNA sequence (Baker et al. 1993). Subsequent authors have used a section that is shifted about 130 bases upstream of this region (Palsbøll et al. 1995, Bérubé et al. 1998). Across the combined Palsbøll/Baker regions, there are some sections in which the amount of sequence change is so great that comparisons among species are difficult (Arnason et al. 1993). The most difficult section is in the middle of the Palsbøll piece, a region of the D-loop that is just before the beginning of the Baker piece. In this region (about position 120 of Figure 9.2), a comparison of humpback and blue whale sequences shows as many as 10 or 11 differences and 5 deleted bases within a given 20-bp window. Sequences with such a degree of variation are difficult to align, presenting challenges in the estimation of overall genetic divergence.

Within the region of DNA included in our analyses (approximately positions 130–360 in Figure 9.2), there are few insertions or deletions, and comparisons from one species to another are relatively straightforward. Most 20-bp windows show one to three substitutions. The largest divergence occurs at about position 240–340, where as many as 7 substitutions in 20 bases occur. Overall divergence of blue and humpback whales is between 10% and 16% across the entire region, corresponding to a divergence rate of about 1.5% per million years if humpback and blue whales diverged 10 million years ago. If we compare just the 100 bases from positions 240–340, however, then divergence increases to
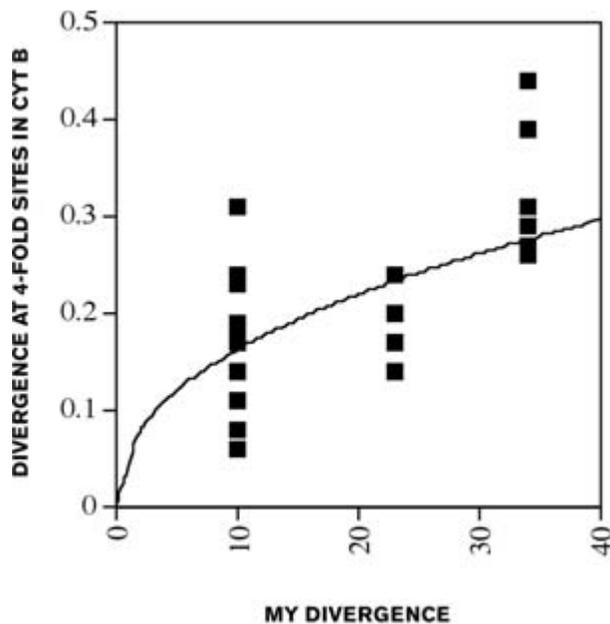
FIGURE 9.3. Genetic divergence at third positions of fourfold degenerate sites among baleen whales. Genetic distances were as the K4 statistic of Wu and Li (1985). Taxa and divergence dates are the same as in Figure 9.1.

13–25%, corresponding to a divergence rate of about 2% per million years.

If different sections of the D-loop show different levels of divergence, which section is best taken as a measure of neutral substitution rate? One way to address this is to examine a set of species for D-loop divergence and for divergence at *silent* positions of protein-coding genes—that is, positions at which a change does not affect the resulting amino acid sequence. Fourfold-degenerate codons are those for which any changes in the third position are silent. For these positions, the rate of change of bases is likely to be the closest measure of the neutral rate of substitution, although there are some cases in which codon bias may interfere (Li 1997). We measured the rate of change across species at third positions of fourfold codons in cytochrome b genes from the same species used in Figure 9.1 to estimate the rate of silent substitution in whale mitochondrial DNAs (Figure 9.3). The resulting estimate of slope near the origin is very close to the 1.5–2.0% measured from D-loop data, suggesting that divergence in silent positions across the mitochondrial genome occurs at about this rate.

Cautions about Hypermutation

Rate variation in the mitochondrial D-loop can occur on genetic spatial scales smaller than those seen in Figure 9.2. Some investigations of patterns of genetic divergence show that change occurs more commonly at some positions than at others. If the chance of substitution is the same among all bases in a DNA sequence, then in theory the number of times

a particular base shows a change should be approximately Poisson-distributed. In practice, different bases show different probabilities of substitution, and the number of times a particular position shows a substitution tends to follow a gamma distribution (Li 1997). The shape of the gamma distribution varies depending on the level of rate heterogeneity among nucleotide positions. For example, as the level of heterogeneity changes from strong to moderate to weak, the shape parameter *a* varies from below 0.5 to 1.0.

The shape parameter of the gamma distribution can be estimated from data on the number of polymorphisms in a collection of DNA sequences (Posada and Crandall 1998). Once estimated, this shape parameter can be included in estimates of genetic divergence, in essence correcting for rate heterogeneity in the sequence data. In the case of whale D-loop data, rate heterogeneity is strong ($a = 0.2$), and estimates of genetic divergence that take this heterogeneity into account are higher than those that do not. For example, divergence between humpback and blue whale D-loop sequences from the Baker segment range from 9% to 11% using a simple Kimura two-parameter model of DNA divergence that assumes no rate heterogeneity. This is 30–50% lower than the values we used, which are based on a model of DNA substitution that accounts for high levels of rate heterogeneity in the data. We conservatively used the higher rate estimate because it produces the lowest estimates of effective population size.

Extreme rate heterogeneity can occur if there are some nucleotide positions that accumulate changes very rapidly. If these positions exist, then comparisons among closely related sequences may show differences at these sites. More distant comparisons also show changes at these sites, but multiple changes overlay one another, and a later change can wipe out any evidence of the previous changes. Thus, a large number of hypermutable sites can increase rates of nucleotide diversity within species but decrease apparent rates of substitution between species. We estimated the number of these hypermutable sites by examining the number of times each base in our data set was estimated to change along the intraspecific phylogenetic tree (Roman and Palumbi 2003, supplement). For example, there were 14 nucleotide positions that were hypothesized to change more than three times in the phylogenetic history of humpback whales. One of these sites could have changed as many as eight times, although this number varies with the minimum-length phylogenetic tree used. Removing these 14 sites from the analysis reduced genetic diversity but also reduced the estimated mutation rate. This analysis suggests that D-loop hypermutation does not completely account for the high intraspecific variation we see in whale populations. However, the reliance on D-loop data for all analyses of whale genetic diversity is the single biggest problem with this approach and must be addressed by further data collection.

Currently, the best information we have suggests a whale D-loop substitution rate of about 0.75–1.0% per million years (note that substitution rate is half the divergence rates detailed above). In our analyses we conservatively doubled

this best estimate to account for hypermutation, providing a substitution rate range of 1.5–2% per million years.

The equations relating genetic diversity and mutation rate require that we calculate the mutation rate per generation, not per million years. To do this, we used an estimate of generation time derived from the average age of sexually mature females. These data derive from whaling studies of age at capture, and we also used a long-term photo-identification study of humpback whales to estimate generation time for this species (Table 9.1).

Measuring Diversity in North Atlantic Whales

We used the D-loop region of DNA to measure genetic diversity in North Atlantic populations of three whale species. We initially chose these species and these populations based on the abundance of existing data and the growing need to understand the population status of these populations.

Humpback Whales

Found in all the oceans of the world, the humpback whale is a highly migratory species, feeding in temperate waters and wintering in tropical calving grounds. A coastal species, with strong fidelity to natal feeding and calving grounds, humpbacks were often the first whales to be hunted in a newly discovered area (Clapham 2002). It was also one of the first whale species to be analyzed genetically (Baker et al. 1993) and has the best global representation, with sequences from the mitochondrial control region currently available from the North Pacific, Southern Hemisphere, and North Atlantic. Using two overlapping DNA fragments (Palsbøll et al. 1995; Baker et al. 1998; Bérubé et al. 1998), we assembled a data set of 312 individuals: 188 whales from the North Atlantic, 31 from the North Pacific, and 93 from the Southern Hemisphere. Geographic structure within the North Atlantic has been found between feeding populations in Iceland and western Atlantic populations (Palsbøll et al. 1995). Migration is high between these two regions, and analyses separating Iceland from the west Atlantic produced similar results to runs incorporating the entire ocean basin as a single population.

For humpbacks, the North Atlantic is populated by two independent genetic lineages: the IJ clade, which includes a large number of differentiated North Atlantic individuals, and the CD clade, which is dominated by Pacific and Southern animals (Baker et al. 1993). Phylogenetic analysis suggests that the IJ clade migrated into the North Atlantic from the Southern Oceans long ago and diversified there. Thus, the genetic patterns of the IJ clade may provide a view of North Atlantic humpback history that is independent of the view derived from the rest of the population.

Fin Whales

The fin whale, *Balaenoptera physalus*, is a cosmopolitan rorqual, found both along the continental shelf and in deep pelagic regions. Fin whales are fast-swimming whales; they cruise at 5–8 knots and are capable of bursts of up to 15 knots (Aguilar 2002). Since the development of bomb-lance technology in the mid-nineteenth century, they have been a primary target of whalers because of their large size. We analyzed data from 475 samples of Northern Hemisphere fin whales collected by Bérubé et al. (1998, 2002) from the eastern North Pacific Ocean (Sea of Cortez and coastal California), North Atlantic Ocean, and Mediterranean Sea. Sampling areas in the North Atlantic (*n* = 235) included the Gulf of Maine, Gulf of St. Lawrence, West Greenland, Iceland, and Spain. Fifty-six samples from the Gulf of California, 10 from California, and 69 samples from the Mediterranean were also available for comparison to the Atlantic.

To date, Southern Hemisphere control region samples are not available for fin whales. However, Wada and Numachi (1991) found a significant difference in gene frequencies for two of three polymorphic allozyme loci, suggesting low historical gene flow between the North Atlantic and the Southern Hemisphere.

Minke Whales

Minke whales are the smallest of the rorquals. There are two recognized species: *Balaenoptera acutorostrata,* found in the Northern Hemisphere, and *B. bonaerensis* in the south. The Northern Hemisphere minke, *B. acutorostrata,* is currently divided into three subspecies: the North Atlantic, North Pacific, and dwarf minke whale of the Southern Hemisphere. Although the capture of minke whales in Norway dates back to the Middle Ages, minkes were not heavily exploited until the twentieth century (Horwood 1990). In the North Atlantic, they have been hunted by both shore-based and pelagic whalers. It is presumed that most minke whale stocks are in better shape than those of other large whales (Perrin and Brownell 2002), and both aboriginal and commercial hunting of minke whales continues.

Because of the species and subspecies divisions between ocean basins, we analyzed minkes from the North Atlantic as a distinct, monophyletic population. North Atlantic minke whale data (*n* = 87) are derived from Bakke et al. (1996). A recent publication, including a 500-bp fragment for 306 individuals from the North Atlantic (Andersen et al. 2003), showed higher diversity values, suggesting that our values for minke whales may be conservative.

Estimating Migration and Genetic Diversity of Whale Populations

Given a data set showing the degree of variability in a region of DNA, we next need an estimate of the amount of intraspecific diversity within and between populations. In practice, comparisons between sequences within and between populations are used to estimate the level of population structure and genetic variability. Recently, several approaches to measuring genetic variation in populations with genetic structure have been devised.

TABLE 9.1
Historical Population Estimates Based on Genetic Diversity and Generation Time of
Baleen Whales in the North Atlantic Ocean

Species	n	θ		Generation time	$N_{e(f)}$ (thousands)		Genetic population estimates (thousands)		Current estimates (thousands)
		Mean	95% CI	(years)	Mean	95% CI	Mean	95% CI	
Humpback	188	0.0216	0.0179–0.0274	12–24	34	23–57	240	156–401	9.3–12.1
Fin	235	0.0430	0.0346–0.0526	25	51	38–65	360	249–481	56.0
Minke	87	0.0231	0.0161–0.0324	17	38	26–57	265	176–415	149.0
Total							865		214–217

Peter Beerli, Joe Felsenstein, Mary Kuhner, and colleagues have developed an approach using maximum-likelihood techniques to estimate effective population size and migration between populations using sequence or microsatellite data. The program MIGRATE (Beerli and Felsenstein 2001) employs a likelihood analysis to calculate migration events and long-term population size simultaneously. In this program, Markov chain Monte Carlo methods are used to estimate migration rates and θ, a measure of genetic diversity. Simulations have been used to test how well MIGRATE operates. Although the measurement of migration rate appears to be problematic with this approach, MIGRATE has been shown to provide fairly precise estimates of θ under a wide variety of population diversities and structures (Abdo et al. 2004). For example, when two populations connected by migration were simulated with $\theta = 0.025$, MIGRATE estimated $\theta = 0.0251$ to 0.0266 for a wide variety of different migration rates (Abdo et al. 2004, Table 9.1).

Migration

For all species, migration rates between oceanic populations were low. For humpback whales, results from MIGRATE indicate that the long-term average for transequatorial migration between the southern ocean and the North Atlantic is less than one female per generation. MIGRATE estimates of migration have high standard errors but are best under conditions that seem to pertain to whale populations: when θ is high and migration is low (Abdo et al. 2004). Indeed, similar migration values derive from different analyses. Baker et al. (1994) estimated the interoceanic divergence as $F_{ST} = 0.3$ and showed with phylogenetic analysis of gene flow that successful interoceanic migration was rare. For fin whales, the majority of genetic diversity is found in the North Atlantic, with low levels of gene flow between the North Pacific and North Atlantic. Based on MIGRATE, the average migration rate between these two basins was 0.19, or slightly less than one female per five generations. Bérubé et al. (1998) also

estimated high genetic divergence between ocean basins for fin whales ($F_{ST} = 0.42$–0.60), indicating low gene flow.

Migration rates between subpopulations within the North Atlantic are, unsurprisingly, much higher than transequatorial rates. For example, the long-term average of fin whale migration between the Mediterranean and the North Atlantic is approximately 7 females per generation. Migration rates between the West Atlantic and Iceland are also high for humpback whales, with approximately 11 females migrating per generation between these areas. These long-term averages, however, may be the result of recent colonization of Iceland by western Atlantic whales.

Genetic Diversity

Using sequence data from several populations, MIGRATE generates a likelihood profile for the parameter θ. This profile allows the calculation of 95% confidence intervals for each value of θ. Because the program uses a Monte Carlo simulator, it provides a slightly different result each time it analyzes a data set. To account for this variability from run to run, we used the program to generate 10 different values for θ and for the 95% confidence limits of θ. Independent runs gave very similar results, and we averaged the ten means and confidence limits to provide the most robust estimate of genetic diversity in each population.

These data sets provided estimates of genetic diversity for whale populations in the North Atlantic (Table 9.1). Values of θ were higher than would be expected if traditional historical values for fin and humpback whales were accurate. For North Atlantic humpback whales the mean value of θ is 0.022, with a 95% confidence interval (CI) of 0.018–0.027. For North Atlantic fin whales, the mean θ is 0.043, with 95% CI of 0.035–0.053. Estimates of θ for North Pacific fin whales were much lower than for those in the North Atlantic. (This difference may be the result of the relatively small sampling area for Pacific fins: Most whales were biopsied in the Sea of Cortez, with ten samples from coastal California.) The North Atlantic minke whale, which was not under heavy commercial

exploitation until the twentieth century, has estimates of θ that are roughly consistent with hunting records (mean value 0.023, with 95% CI of 0.016–0.032).

Genetic diversity estimates for the minke whale are restricted to the North Atlantic, because this is a recognizable separate subspecies that shares no mitochondrial lineages with the subspecies in the Pacific. For humpback whales the diversity value in the Atlantic is estimated in reference to diversity in four other population regions, including extensive sampling in the Southern Hemisphere. For fin whales, data from the Southern Hemisphere are the most limited, and diversity values are estimated only in reference to populations in the Mediterranean—which are genetically distinct—and the Pacific.

From Genetic Diversity to Effective Size to Population Estimates

Once mutation rate (μ) is established and we have reliable estimates of genetic diversity (θ), we can use the relationship $\theta = 2N_f\mu$ to calculate estimates of the genetic effective population size for females in each population (Table 9.1). For example, for humpback whales a mean genetic diversity value of 0.0216, generation time of 18 years, and mutation rate of 1.75% per million years translates into a genetically effective size for females of 34,000. This is the mean estimate, sitting inside a fairly wide 95% confidence limit resulting from uncertainty about the value of θ (Table 9.1). Because all estimated parameter values have uncertainty, we employed a Monte Carlo method of estimating population size and its 95% confidence limits (see below).

However, this number is not an estimate of the ancestral census size. To determine census size from effective size, we relied on three conversion factors. First, we converted N_f to total effective population size, N_e, by multiplying by two, because sex ratio of these whale species is 1:1 (Lockyer 1984). Second, N_e was converted to N_T, the total number of breeding adults. The N_T:N_e ratio approaches 2.0 in perfect populations with a constant population size (Nunney 1993). Numerous genetic studies suggest that this is a very conservative estimate. For example, Nunney (2000) has shown that for fluctuating populations the ratio would be closer to 10. Similarly, the average N_T:N_e ratio for 33 mammal species reviewed by Nei and Graur (1984) was 10, identical to the value Frankham (1995) found in his review of wildlife species. Mace and Lande (1991) have proposed an N_T:N_e ratio of 5–10 for a wide array of species; the IUCN (now the World Conservation Union) employs a ratio of 5. To ensure conservative estimates for baleen whales, we used Nunney's theoretical estimate, employing a multiplier of 2 to derive N_T from N_e. Third, to take into account the number of juveniles, we multiplied N_T by an estimate of (number of adults + juveniles)/(number of adults) derived from catch data. This ratio is 1.6 to 2.0 for humpbacks, assuming that whales in year classes 1 through 5 are equally abundant in the population (Chittleborough 1965). The ratio is 1.5 in gray whales

(Weller et al. 2002) and 2.5 to 3.0 in bowheads (Angliss et al. 1995). Considering these ranges, we used a multiplier of 1.5 to 2.0.

Thus, we estimate total population size as 6–8 times the number of breeding females ($N_C = 2 \times 2 \times 1.5N_{e(f)}$ or $2 \times 2 \times 2N_{e(f)}$). This highly conservative rate ignores fluctuation in population size, variation in female fecundity, and polygyny, all of which tend to reduce the ratio between effective population and census size (Avise et al. 1988; Nunney 2000). In particular, Nunney (1993) has noted that effective population size depends strongly on the mating system. Although rorqual whale mating systems are largely unknown (Lockyer 1984), Clapham (1996) has proposed that humpbacks form floating leks, a type of dominance polygyny, with male songs helping to establish dominance on the breeding grounds. Leks also increase values of N_T:N (Nunney 1993). If we had used a more likely estimate of 5–10, our suggested historical population sizes would be several times higher than presented here. Empirically estimating N_T:N_e for whale populations should be an important part of future research efforts.

Because several parameters are estimated as ranges, we employed a Monte Carlo resampling scheme to estimate historical population size. We randomly sampled μ and θ from within ranges estimated from the data analyses described in previous sections (assuming a uniform distribution) and used these chosen values to estimate N_f. We then randomly chose a multiplier from the range 6–8 to convert N_f to N_T and used 1,000 replicates to estimate mean values and 95% confidence limits on the number of breeding females and total census size (Roman and Palumbi 2003).

The final results suggest that North Atlantic whales had a population far in excess of those typically assumed (Table 9.1). For humpback whales, the genetic estimate of 240,000 animals (including juveniles and subadults) is about ten times higher than prior estimates of about 20,000. Confidence limits for these estimates are large, from 156,000 to 401,000, but even the lowest value is much higher than previous estimates. For fin whales, genetic diversity patterns suggest populations of about 360,000. This is also several times higher than previous estimates (Sergeant 1977). Minke whales show genetic estimates of population sizes that are closer to expectations, but in this case, commercial hunting began only in the twentieth century.

Reliability of Genetic Data and Analysis

Because nucleotide variation can be maintained through population bottlenecks of brief duration, genetic data may provide practical population estimates, independent of catch data and logbooks, for species that have been heavily exploited. This can be especially useful for species that are at historically low population levels. Baleen whales, which are long-lived and were exploited to levels that brought some species to the brink of extinction, are ideal organisms for exploring the practicality of this type of approach.

One important question is whether we are looking at populations from deep in the evolutionary history of North Atlantic rorquals or at more recent populations, when commercial hunting began. The data and their analysis suggest that we are looking at both. The large amount of genetic diversity of current whale populations has required more than a million years to accumulate; whale populations have probably been large over at least that time span. If population size fluctuates, genetic diversity is particularly vulnerable to periods of reduced numbers of reproductively active individuals, because such bottlenecks can rapidly winnow genetic diversity. As a result, fluctuation in population size has been suggested as a cause for widespread underestimates of population size of terrestrial and marine species from genetic data (Nei and Graur 1984; Avise 1994).

Could it be that whale populations have been typically large for long periods of time but fell to low levels *before* the start of industrial whaling? Although this has not been suggested by whale biologists previously, it is a formal possibility that could explain the data. If true, this decline in whale numbers would have had to be global in scale. Global levels of genetic diversity for humpback whales suggest world populations of about 1–1.5 million for this species (based on global diversity estimates of approximately 0.10), far higher than the 115,000 individuals estimated for this species at the start of whaling.

In addition, current analyses suggest that if a recent global bottleneck were the explanation, this depleted population size could not have extended for very long, because such an event would have reduced the diversity found in rorquals and would have changed the shape of the phylogenetic tree relating different mitochondrial haplotypes to one another. For whales, the shape of the tree (monitored using Tajima's [1989] *D* statistic) shows no signs of population decline. However, the power of this test to detect population trends is low, and further investigation using more sensitive techniques is warranted. Further evaluation of the hypothesis that whale population plummeted during human prehistory awaits evidence of natural declines in whale numbers over the last 10,000 years.

If such a crash occurred and was due to a climatological or ecological shift, this could signal dire consequences for current whale populations. We may be poised for a severe climate shift in coming decades. How will reduced populations of baleen whales respond? In fact, we have very little idea of how whale populations weathered the series of Ice Ages over the past two million years. The depth of phylogenetic trees in whales shows that many species stretch back this far in time, and research focused on the impact of climate change on whale populations would be very valuable.

A second concern is that analyses based on one genetic locus might incur uncertainty, resulting in an inaccurate population estimate. Analysis of nuclear data and other mitochondrial loci are a key part of future approaches to this problem (Roman and Palumbi 2003). Palsbøll et al. (2004) have found that genetic diversity of microsatellite data in humpbacks is largely concordant with the diversity signature in mtDNA. However, their data set did not address the effect of population subdivision on genetic diversity, and they could not yet answer the question of whether the nuclear genetic signature of large populations in North Atlantic humpbacks persisted until the start of industrial whaling. Thus the microsatellite data could not distinguish between a recent and an ancient population crash for humpbacks. Microsatellite data from all ocean basins will be needed to take the next step in this analysis.

A third concern is that absolute genetic measures of past population size require good estimates of sequence mutation rate. As detailed above, these rates are typically based on phylogenetic comparison of sequences from species with known fossil records. However, some studies suggest that rates estimated from known historical pedigrees may be higher than phylogenetic and fossil records suggest. A recent paper by Howell et al. (2003) suggests that the pedigree mutation rate is tenfold higher than rates obtained from phylogenetic analyses. If true, this discrepancy could reduce genetic estimates by an order of magnitude. However, there is considerable disagreement over whether pedigree rates are indeed higher than phylogenetic rates. In particular, use of the Howell rate to date the age of the diversification of humans and the spread of human lineages out of Africa gives a date of less than 10,000 years, whereas mtDNA and other loci agree that the date for these events is in excess of 150,000 years. Thus, the pedigree rate in this case is in conflict with rates derived from other loci and is not likely to provide a better view of the history of populations.

In our view, the most serious limitation of current data sets is that they are tied to a single piece of DNA whose patterns of molecular evolution are complex enough to engender caution. Providing data from additional loci is the key to overcoming this limitation, but it is challenging because the slow rate of mutation in whales produced few nuclear polymorphisms for analysis. Hare et al. (2003) analyzed intron polymorphism at four loci for delphinid dolphins and showed that intron lengths of 500–1,000 bp were needed in order to discover an adequate sampling of phylogenetically informative positions. Palumbi et al. (2001) and Palumbi and Baker (1994) showed a similarly low level of polymorphim in baleen whales. Nevertheless, such data sets for baleen whales will be required to provide data parallel to the mtDNA data discussed here.

As a consequence of the uncertainty of basing conclusions on a single locus, we have built a set of highly conservative assumptions into our analysis. Yet the final result remains that populations are typically high. For example, we have used a low value for the conversion of female effective size to total size (a multiplier of 6–8 instead of a multiplier of 15–30 or more, typical of other mammals). An empirically derived value for this multiplier—perhaps based on reproductive success in well-known populations—would be very valuable. It is possible that such empirical values will increase our estimate of population size.

Additionally, we would like to have a better understanding of the dynamics of whale population size in the past. Have

whale populations been higher in the past than in historical times? Have whale populations been stable during the serial Ice Ages of the past? Is there any evidence that whale populations plummeted before whaling began? Analysis of DNA patterns can reveal population trajectories in the past (Shapiro et al. 2004), but these approaches are more powerful when more than a single locus is applied.

Though these caveats are serious, we are left with a set of empirical conclusions that challenge current thinking about the history of whale populations. It seems to us that the genetic analysis of whale populations is inconsistent with a view that prewhaling population numbers were as low as previously suggested. These conclusions open a debate on the management of current whale populations that is timely and important.

Though initially surprising, our results are consistent with findings for other large marine vertebrates, such as green turtles (Jackson et al. 2001), pelagic sharks (Baum et al. 2003), and other fish (Myers and Worm 2003). The removal of turtles may have had an impact on the Caribbean ecosystem, and emerging research suggests a similar impact for the removal of pelagic predatory fish (R. A. Myers, personal communication). In parallel, it is likely that the removal of great whales has also had important ecological effects. Such changes may include prey shifting in predators (Springer et al. 2003) or perhaps alteration of the nitrogen cycle in coastal regions.

Management Implications of High Genetic Diversity

How might these findings affect the management of whales? In its original New Management Procedure, the IWC established that catches should not be allowed on stocks below 54% of the estimated carrying capacity. Under this criterion, humpbacks and fin whales in the North Atlantic might be considered exploitable even though they have been protected for only a generation or two. The Revised Management Procedure retains full protection for populations below 54% of carrying capacity, while recognizing the need for additional population models to set carefully monitored take limits above this level (Cooke 1999, Holt 2004).

In the North Atlantic, current populations of approximately 12,000 humpback and 56,000 fin whales are far lower than long-term population sizes based on genetic estimates. Instead of being close to exploitable, our new analyses suggest that these populations will require decades of continued full protection.

Only the minke whale, little exploited until the twentieth century, approaches the population size recommended by IWC models. However, genetic estimates of population size are usually several orders of magnitude too low—especially for fluctuating populations (Soulé 1976; Avise et al. 1988; Frankham 1996; Nunney 2000). If minkes are more vulnerable to such perturbations, genetic techniques could seriously underestimate population size. We also note that our diver-

sity estimates are from the eastern Atlantic stock of minkes only, and the IWC estimates of 120,000 to 182,000 for the North Atlantic do not include the Canadian East Coast. Clearly the population trends for this species—which is still hunted by Greenland, Iceland, and Norway—are in need of further analysis.

Regular hunting for minke whales in Greenland began in 1948, after fishing vessels were equipped with harpoon cannons (MacLean et al. 2002). Current catch limits are for 187 minke whales for both east and west Greenland (Reeves 2002). If greater genetic diversity were found in the western Atlantic (Andersen et al. 2003), pre-exploitation estimates would have to be corrected. A cautionary approach suggests that well above 150,000 minkes should be present in the North Atlantic before commercial exploitation is considered for other nations.

It is important to note that long-term reductions in some populations, such as right whales *(Eubalaena glacialis)*, have winnowed genetic diversity to the point where it is difficult to use contemporary DNA to make historical population estimates (Rosenbaum et al. 2000, Waldick et al. 2002). Unlike rorquals, which were heavily exploited only in the nineteenth and twentieth centuries, right whales have been commercially hunted for more than five centuries and are now completely extirpated from important breeding grounds. Low frequency microsatellite alleles are rare in *E. glacialis*, suggesting that genetic diversity has been reduced in this species (Waldick et al. 2002). Fortunately, humpbacks, fins, and minkes do not appear to have sustained such extended reductions in population size (Baker et al. 1993).

There is an important distinction between the regulatory apparatus of the IWC, which emphasizes carrying capacity, and population genetics, which produces long-term typical population size. Carrying capacity is an intrinsically ecological concept and can change if the state of an ecosystem changes. The current capacity of the oceans to support whales may be lower than before humans began removing 80 million metric tons of seafood a year from the sea. Yet, long-term population size may be a very good estimate of historical ocean carrying capacity, because long-term population size is a reflection of the numbers of whales that were supported by the oceans in the past. Current-day managers may find it necessary to limit whale populations to less than this value for economic reasons, but such debates have not been fully played out. The reason for lack of debate is that the *possibility* that oceans may have supported more whales than previously thought has not been thoughtfully advanced before. With new genetic results on the table, this debate should have greater scope.

Is a Synthesis of Two Historical Views Possible?

Estimates of prewhaling population sizes from summaries of whaling catch data suggest an order of magnitude fewer whales than do genetic estimates from humpback and fin whales. The discrepancy for minke whales in the North Atlantic is about threefold. Discrepancies for other species are
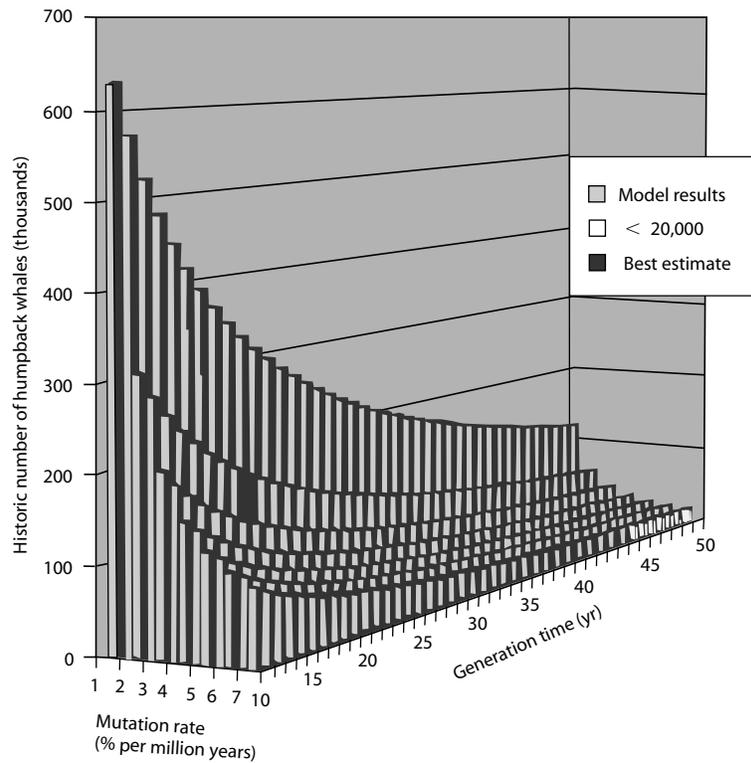
FIGURE 9.4. A sensitivity analysis of the impact of assumptions about mutation rate and generation time on conclusions about numbers of North Atlantic humpback whales before whaling. In this figure, we allowed mutation rate (μ) to vary between 1% per million years and 8% per million years and generation time to vary between 10 and 50 years. The product of these numbers is the amount of DNA sequence change per generation and is the value we need in the equation to calculate population size. The results of the analysis show that only extreme values of parameters will give population estimates consistent with previously published values for prewhaling population size (in white).

higher. Are these numbers reconcilable? Or do these severe differences signal a serious breach in one of these two approaches?

One way to approach this question is through *sensitivity analyses*—a process in which the same basic data are used in conjunction with a set of different assumptions to explore how the conclusions vary with the assumption sets used. For example, we estimated North Atlantic humpback genetic population sizes by measuring genetic diversity and mutation rate and applying a generation time estimate from the whaling literature. But what if either the mutation rate or the generation time were incorrect? To explore the impact of varying parameters on our conclusions, we recalculated population size for a variety of different mutation rates and generation times. The result (Figure 9.4) shows that an estimate of 10,000–20,000 humpback whales requires mutation rates of about 8% per million years and a generation time of 45 years. If generation time is held constant at 25 years, then the mutation rate must be about 16% per million years in order for our population estimate to be 10,000–20,000. These

values are outside reasonable ranges. For example, a mutation rate this high would imply that the *Balaenoptera* species, which have diverged by 10–20% in mtDNA at silent sites (Figures 9.1, 9.3) had diverged only 750,000–1.5 million years ago. For these reasons, we consider it unlikely that altering the assumptions of the genetic analysis will bring genetic and conventional numbers into full agreement.

Other analyses are not as clear-cut. For example, an estimate of 100,000 humpbacks in the North Atlantic requires mutation rates of 3% per million years and generation times of about 25 years. Furthermore, the best analysis—yielding an average of 240,000 humpback whales—has wide 95% confidence limits, ranging from 150,000 to 400,000. These explorations of the data analysis suggest that historical numbers of as few as 100,000 humpbacks probably could not be rejected by the mtDNA genetic analysis.

A minimum estimate of 100,000 animals from mtDNA or future nuclear analysis would remain far higher than suggested by current analyses of whaling records. Summaries of whale mortality from hunting by Smith and Reeves (2003)

show a total of about 29,000 killed from 1650 to 1910, and these data are thought to be consistent with a population of 40,000 (T. D. Smith, personal communication). Yet this analysis, like the one for genetic data, is based on a series of assumptions as well as a model relating the basic data (recorded whale kills) to conclusion about ancient populations. Whitehead (2000) has shown how a variety of different assumptions about population growth rates, generation times, and mortality rates can dramatically change estimates of past population sizes, but a rigorous sensitivity analysis has never been applied to the humpback whale data.

Although we cannot provide a rigorous sensitivity analysis here, we suggest there may be room for changes that would yield higher numbers of whales in the past. Two critical assumptions serve as examples. First, current analyses appear to assume that the loss rate during industrial hunting was only 2%; that is, the analysis assumes that 98% of all humpback whales struck by harpoons were killed and taken by whaling ships. Loss rates in earlier eras were far higher—at least 50%, according to Mitchell and Reeves (1983)—and it is not clear whether calves, injured to lure a mother toward a whale ship, were counted as individuals killed but not landed. If loss rates were higher than 2%, then the current views of mortality from whaling may be underestimates (see also IWC 2003).

Second, catch records are never complete, and a comprehensive analysis must take into account the whales that were killed but never recorded. This is a prodigious task for historians and remains a serious challenge. Estimates of the completeness of historical data are an important part of reconstruction of past views of many aspects of human industry and culture. Understanding the true history of whaling must include understanding the best ways to correct current data compilations for missing records.

The impact of both of these simple sources of error on analyses is strong. If it were discovered that whale loss rates from hunting were 50% instead of 2%, and that only half of the whales killed in the oceans during the three centuries of whaling were known to us, then we would need to adjust the total number of whales killed by whalers from about 30,000 to about 120,000. We do not suggest here that this fourfold correction is warranted at this time. We merely point out that correcting catch records upward is possible and could potentially help bridge the genetic gap.

The final assumption set needed to convert hunting mortality to standing population size is a set of models relating mortality to population trajectory over time (e.g., Whitehead 2002). Such models do not function well for humpback whales, suggesting that estimates of whaling mortality might be low (IWC 2003). In the absence of such quantitative models, it has been suggested that the minimum number of animals can be estimated if a pulse of hunting is followed by a dramatic reduction in catch. In such cases, Mitchell and Reeves (1983) argue that the sum of the animals killed during the hunting pulse is a good starting value for a historical population estimate.

A pulse of whaling from about 1870 to 1900 took 20,000 humpback whales from the North Atlantic (Smith and Reeves 2003). However, this number jumps to 80,000 if we change the loss rate and recording rate assumptions as described above. This number is similar to the lower bound of the estimate from genetics and could represent a value that might reconcile these approaches.

These changes in how catch data could be handled are heuristic devices only. Both whaling and genetic data are subject to uncertainty and rely on critical assumptions to make estimates about past population size (Clapham et al. 2004). Future work should concentrate on the validity of assumption sets for both types of data and analytical approaches (Clapham et al. 2004) in a framework that might reveal common ground.

Conclusions

DNA data add a new tool to our ability to research the past. Written into DNA sequence variation is a record of the population history of a species, and new theoretical tools are allowing us to read that variation in more and more powerful ways. Genetic data suggest that humpback and fin whales were much more abundant before whaling than conventional estimates suggest. For example, instead of a global total of 115,000 humpback whales, genetics estimates a world-wide abundance of about 1.5 million. Future analyses and additional data may refine these genetic estimates, but the major conclusion is that the number of whales that the oceans can support has been substantially underestimated.

For either DNA data or written history, it is a mistake to assume we have a perfect record of the past. The challenges for the future are to understand how different kinds of data jointly illuminate the past and to use as many perspectives as possible to try to reconstruct the history of whales before whaling.

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