MARINE MAMMAL SCIENCE, 4(1):1–12 (January 1988)
© 1988 by the Society for Marine Mammalogy

A METHOD TO COLLECT AND PROCESS SKIN BIOPSIES FOR CELL CULTURE FROM FREE-RANGING GRAY WHALES (*ESCHRICHTIUS ROBUSTUS*)

ELIZABETH A. MATHEWS
Applied Sciences 273, University of California, Santa Cruz, California 95060

STEVEN KELLER

DAVID B. WEINER[1]
Department of Biology, University of Cincinnati, Cincinnati, Ohio 45022

ABSTRACT

For researchers studying mysticete whales few methods for determining gender or for collecting biochemical and genetic information from unrestrained animals are available. The objective of this study was to develop a reliable method for collecting viable tissue samples for establishing continuous cell cultures from skin biopsies of free-ranging whales. A method to collect and process these samples is presented. Six of seven skin biopsies from gray whales were established in cell culture. Our results suggest that the viability of the samples is improved by (1) sterile processing in the field, (2) minimizing the time between collection and delivery to the cell culture facility, (3) reducing the concentration of antifungal agent, and (4) placing tissue explants under a coverslip. While the results reported in this paper are based on a small sample size, we believe that if the procedures are followed, they will increase the probability of successfully culturing cetacean tissue. Established cell lines can supply replenishable material from identified whales still living in the wild. These cultures can then be used for determination of sex from karyotypes, and for assessing genetic relationships of cetaceans from inherited protein, chromosomal and DNA polymorphisms. These much needed analytical tools can be used to determine familial and populational relationships, leading to a better understanding of mating systems, stock identification and effective population sizes of wild cetaceans.

Key words: cetaceans, gray whale (*Eschrichtius robustus*), determination of gender, determination of sex, skin biopsies, cell culture, karyotypes, genetics, DNA.

As field biologists and managers we need to understand phylogenetic as well as individual relationships of animals to one another. Chromosomes, DNA and proteins from cultured cells can be used in genetic and molecular studies to

[1] Present address: School of Medicine, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia 19104.

assess phylogenetic and familial relationships, to determine gender and in population studies of mammals (Selander *et al.* 1971, Ohno 1984, Modi 1986, O'brien *et al.* 1986). In humans, primates (Smith 1980, Stern and Smith 1984) and other mammals (McCracken and Bradbury 1977), cytogenetic, biochemical and molecular methods are used to detect genetic disorders (Davies *et al.* 1984), to construct family pedigrees (Craig-Holmes 1977, Bostein *et al.* 1980) and to resolve paternity exclusion cases (Keith 1981, Olsen *et al.* 1983, Chakraborty 1985). Recently, molecular DNA probes, particularly DNA 'fingerprinting', have been developed to detect polymorphisms for determining specific familial relationships in humans and birds (Jeffreys *et al.* 1985*a,* 1985*b,* 1985*c,* Quinn *et al.* 1987, Hill 1987). Some of these techniques may also be useful for sex determination (Wolfe *et al.* 1984).

For researchers studying mysticete whales few methods for determining gender or for collecting biochemical and genetic information from unrestrained animals are available. Cetacean tissues required for cell cultures and chromosome studies have typically come from captive, incidentally killed or beachcast animals. Karyotypes and molecular data derived from these cultures have been used to evaluate evolutionary relationships (Makino 1948, Walen and Madin 1965, Arnason 1972, 1974, Duffield 1977, Arnason *et al.* 1978, Arnason and Widegren 1984*a,* 1984*b*). Skin biopsies from one blue whale (*Balaenoptera musculus*) (Arnason 1985) and several from humpback whales (*Megaptera novaeangliae*) (Duffield *et al.* 1985*b*) have been used to determine the sex of free-ranging animals. Even though the marriage of cell and molecular genetics with field studies of living cetaceans is of obvious benefit (Duffield *et al.* 1985*a,* 1985*b*), attempts to combine these disciplines remain rare. This is primarily because samples from free-ranging whales and dolphins are difficult and expensive to obtain. Once obtained, establishing healthy cell cultures from these samples is not a trivial task.

To collect skin samples from wild cetaceans Winn *et al.* (1973) developed a biopsy dart which could be fired from a harpoon gun or a crossbow. Preserved, non-living skin samples collected from two free-ranging sperm whales (*Physeter catodon*), two humpback whales (*M. novaeangliae*), and one bridled dolphin (*Stenella frontalis*) were analyzed for sex chromatin (Barr and Bertram 1949) in an attempt to determine the gender of whales at sea. Later Hoelzel and coworkers (1983) used a modified version of the dart and crossbow system to collect skin biopsies from free-ranging minke whales. Although the collection methods used were successful, tests for sex chromatin from minke whale skin revealed considerable differences both between individuals and within different tissue layers. Because the individual differences were not related to gender Hoelzel *et al.* (1983) concluded that sex chromatin in minke (*Balaenoptera acutorostrata*), fin (*B. physalis*), and sei (*B. edeni*) whales was not a reliable indicator of gender. An advantage to gender determination using sex chromatin analysis is that living cells, difficult to maintain in the field, are not required. However, live cell cultures of tissue from free-ranging whales and dolphins provide a continuous supply of cells, allowing the use of several different techniques to assess genetic variation as well as gender determination.

The objective of our study was to develop a reliable method for collecting viable tissue samples for establishing continuous cell cultures from skin biopsies of free-ranging whales. We report a method for collecting and sterile processing of skin samples from free-ranging gray whales (*Eschrichtius robustus*) and their use in establishing cell cultures of fibroblasts. In addition to cell culture, each biopsy was utilized in (1) an assay of natural or introduced microorganisms (Mathews 1986); (2) sex chromatin analysis of fixed tissue (Mathews 1986); (3) karyotypic analysis, DNA isolation, restriction enzyme digests, DNA gel electrophoresis, protein gel electrophoresis and preparation of frozen, viable cultures for future studies.

Materials and Methods

Tissue collection—We collected skin samples from seven whales off the west coast of Vancouver Island, British Columbia, Canada, in the Barkley Sound area between 3 August and 1 October 1984. Whales were approached for sampling in a 6-m Boston Whaler. At least two, and preferably three, people were required to help with sample collection, behavioral observations, photography and boat operation.

Skin samples were obtained with a compound bow (Darton 40) and aluminum arrows with specially designed biopsy tips provided by Dr. Jon Lien (Memorial University, Newfoundland, Canada). The cylindrical stainless steel tips are 6 mm in diameter and 10 mm long (Fig. 1). A flared, cushioned backing at the base of the tip prevented penetration beyond 10 mm, and insured that the arrows were freed immediately by the resilient compression of the underlying blubber, or by the force of the water as the whale swam forward and down. Before each sampling attempt, the biopsy tip was submersed in 95% ethanol. Arrows were launched from 10 to 25 m with only 11.3 kg of thrust to reduce the possibility of deeper penetration. To avoid the risk of entanglement we did not attach a retrieval line to the arrows. Instead, as suggested by Ellie Dorsey (personal communication), a 5.1-cm collar of bouyant, closed-cell foam was added just behind the base of the tip (Fig. 1). The floating arrow was retrieved from the water as soon as possible (within a few minutes) to minimize tissue damage from prolonged exposure to sea water. Samples were taken from just below the dorsal ridge or from the peduncle.

All of the sampled whales were identified by photographing the unique markings on the left, right or both sides of the dorsal ridge (Hatler and Darling 1974, Darling 1984). We used Tri-X (Kodak) film pushed to an ASA of 1600 and exposed at 1/1000 sec. These I.D. photographs will be used to reidentify individual whales and in comparisons with identification photographs taken in previous or subsequent years or from other study sites. The skin biopsies were collected and shipped in accordance with all required permits.

Biopsy processing—In most field situations researchers do not have ready access to a cell culture facility or to a laminar flow hood for sterile media preparation and tissue manipulations. A portable unit equipped with an ultraviolet (U.V.) sterilizing system was designed to meet this need (Fig. 2). Two portals allow

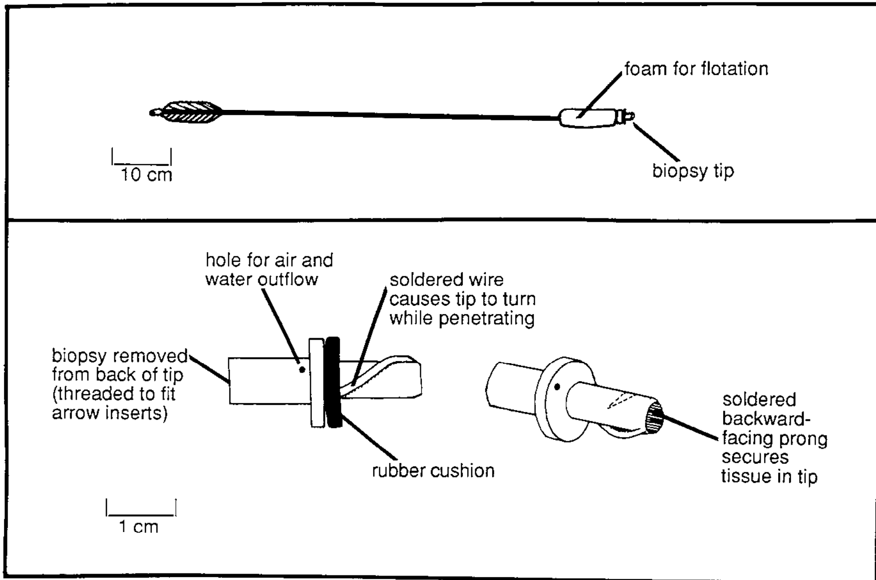

Figure 1. Top: Aluminum arrow with biopsy tip and collar of closed-cell foam for buoyancy. Used with a compound bow to collect skin biopsies from free-ranging gray whales (*Eschrichtius robustus*). *Bottom:* Stainless steel biopsy tip used to collect whale skin samples.

insertion of the hands for aseptic manipulations and minimize the entrance of air-borne contaminants.

All inner surfaces of the container, and materials used while working with the samples, were sterilized by wiping with 70% ethanol or by autoclaving. Sterilized disposable surgical gloves were worn during sterile procedures. The U.V. light and fan were activated for at least 1 h prior to tissue handling on land. During the initial processing on the boat the U.V. system was not used since an electrical source was not available. An alcohol lamp, secured to the portable sterile box, was used to flame the mouths of media bottles and culture tubes.

Immediately after the arrow was retrieved from the water the biopsy tip was removed from the arrow and placed in the portable sterile hood. The biopsy was then taken out of the back of the stainless steel tip with forceps. The tissue sample removed by the biopsy tip included the outer epidermal/dermal layer plus a portion of the underlying blubber. A layer of the outermost epidermis about 0.5 mm thick was cut off with a razor and used to culture and identify 'normal' or introduced microorganisms on the surface of the whale's skin (Mathews 1986). The remainder of the sample was placed in a petri dish, and rinsed three times in phosphate-buffered saline (0.9%) followed by a rinse and incubation in McCoy's 5A medium with L-glutamine (GIBCO, Grand Island, N.Y.) with 300 to 500 μg/ml of gentamycin sulfate (obtained as Garamycin, an antibiotic with broad spectrum antimicrobial activity, from the Schering Corporation, Kenilworth, N.J.) and up to 100 units/ml of an antifungal agent,

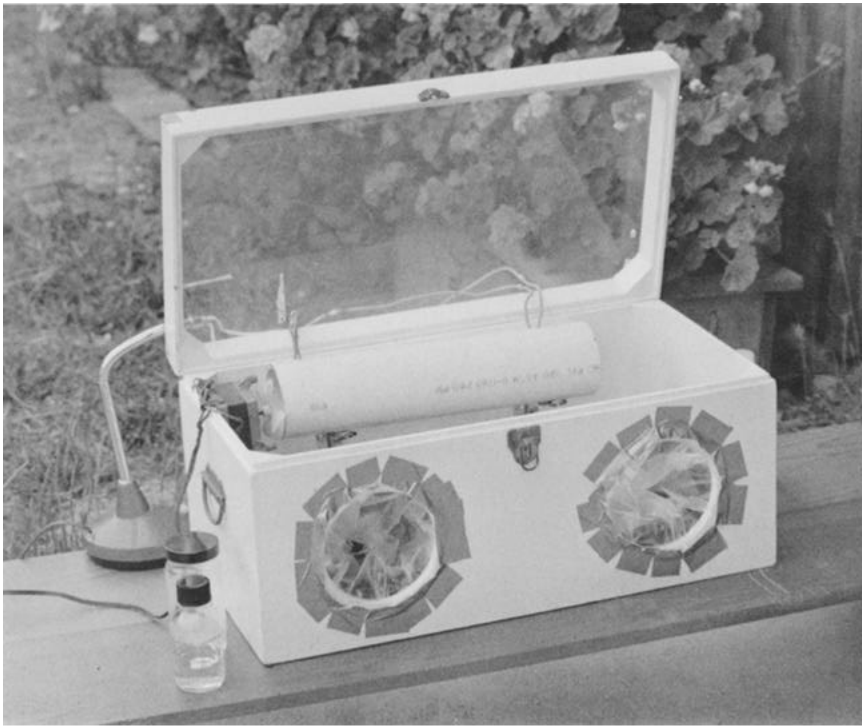

Figure 2. Portable container for sterile processing of skin biopsies from free-ranging whales. A small electrical fan and a sterilizing ultraviolet light (suspended in 10.2 cm PVC tubing) were activated to eliminate air-borne contaminants.

mycostatin (Nystatin, GIBCO). After 4 to 12 h in media with antibiotics each specimen was transferred into a Corning 15-ml disposable centrifuge tube containing McCoy's 5A medium with reduced concentrations of Garamycin and Nystatin (Table 1).

Samples in the 15-ml centrifuge tubes were packaged for shipping in styrofoam containers (~ 25.4 × 25.4 × 30.5 cm) with a cold pack to maintain the temperature between 10°C and ambient. Direct contact between the cold pack and the sample was prevented. Processed biopsies were shipped via express mail to Dave Weiner at Dr. S. Keller's cell culture laboratory at the University of Cincinnati, in Ohio. Samples typically arrived within 4 d by which time the cold pack had thawed. The length of time each sample remained in the different concentrations of antibiotics, the duration of shipping and the length of time before growth varied (Table 1).

Cell culture—Upon arrival at the tissue culture facility, each sample was washed with fresh RPMI 1640 media (Flow Laboratories, McLean, Va.) and transferred to 100-mm glass petri dishes for cell separation. All specimens were handled using sterile technique.

In order to evaluate different plating methods for growth and survival of the biopsies, dermal tissue from samples 3, 4, 5 and 6 was divided into three parts

Table 1. Length of time each skin biopsy spent in culture medium, concentrations of antibiotic and antifungal agents, duration of shipping, and days between collection and when growth was first observed.

	First incubation			Second incubation			
Sample	Garamycin μg/ml[a]	Nystatin[b] units/ml	H	Garamycin μg/ml[a]	Nystatin units/ml[b]	Days[c]	Days before fibroblast growth
gw1	310	0	4	160	0	7	no growth
gw2	310	100	8	160	100	5	54
gw3	310	100	11	160	100	3	39
gw4	310	100	9	160	100	3	37
gw5	500	80	10	200	80	3	30
gw6	500	80	8	200	80	3	30
gw7	440	10	12	100	10	3	15

[a] Schering (Kenilworth, N.J.): 50–100 μg/ml culture medium recommended for growth.
[b] GIBCO (Grand Island, N.Y.): 2 mg/ml culture medium recommended for growth.
[c] Days between collection and delivery to the tissue culture facility including first incubation in media with antibiotics.

which were minced to a fine pulp by scalpel, curved surgical scissors or grinding between glass slides. The cell suspension was then diluted slightly with media until a thick slurry was formed. Suspensions were seeded onto 100-mm tissue culture plates in both the presence or absence of 18-mm glass coverslips. These cultures were then tested for their ability to form at least three growing colonies within 40 d in culture. Samples 2 and 7 were treated in a similar manner, but were not scored in this study.

Cultures were covered with medium (RPMI 1640 + 15% fetal bovine serum + 1X penicillin/streptomycin) and incubated at 37°C in a 0.2% CO_2 in-air incubator. Media were changed by one-half replacement with fresh RPMI 1640 every 8 d until growth was noted; thereafter samples were fed every 3 d. Cultures were considered positive for growth when at least three independent colonies of more than five cells per colony were observed. Cell viability was tested during passage by staining with either 0.01% acridine orange/ethidium bromide (Parks *et al.* 1979), or by Trypan blue exclusion (Kruse and Patterson 1973).

Results

Six of the seven skin samples collected from free-ranging gray whales were successfully cultured. Only the first sample, which was detained by custom's agents, did not grow. This was probably due to heavy fungal contamination and prolonged exposure to ambient temperatures. The remaining six samples grew continuously for over 4 mon. Viability during passages was always greater than 90%. However, the length of time before the first dividing fibroblast cells were observed for each of the six samples varied widely (Table 1).

After 4 mon the culturing incubator was inadvertently contaminated with a

fungus from an adjacent laboratory and all but two of the cell lines were destroyed. Up to this time all of the fibroblast cultures were growing well, showed no signs of senescence and multiple transfers into fresh media had been made without decreased viability. Once the cultures were established, cell counts of trypsinized cells using a hemocytometer demonstrated that the average doubling time was 12 d (±3 d). Whales 4 and 6 were later determined to be a female and a male, respectively. The cytogenetically determined gender of both animals was confirmed by direct observation of the genitalia (Whale 6 observed by Don Croll, personal communication). Cell cultures from the two surviving specimens were still growing after 20 mon.

When comparing the different plating methods, samples which were set up under coverslips established colonies by 40 d in culture. In contrast only one plate from any of the cultures without coverslips showed growth by this time. Furthermore, after 2 mon in continuous culture, 21 out of the 24 samples set up under coverslips yielded usable colonies, while only 2 of 24 samples which were plated without coverslips exhibited growth. The method used to dissociate the cells did not affect cell viability.

Discussion

Reported success rates for culturing cetacean tissues have ranged from 7% to 33% (1 out of 14 primary cultures from *P. catodon*; 1 out of 3 cultures for *B. borealis,* Arnason 1970) up to 50% (Duffield, personal communication *in* Medway 1983), and Arnason (1985) successfully cultured fibroblasts from one biopsy of a free-ranging blue whale. Worthen (1981) reported no growth from 61 skin samples collected from dolphins captured incidentally in the tuna fishery, whereas 25% to 30% of the lung and embryo tissues grew from these same animals.

Six of the seven skin biopsies collected for our study yielded healthy fibroblast cultures. Only the first sample, which was in transit for 7 d, could not be used in cell culture. All samples delivered to the tissue culture laboratory within 5 d grew. Among the six viable samples there was variation in the time before fibroblasts grew (Table 1). Samples which grew relatively soon probably contained more viable cells to begin with. That is, fewer cells from these samples died during shipment. Variability in the condition of the biopsies when they reached the cell culture facility may be a result of several factors.

First, shortening the length of time between collection and delivery to the culture facility may increase the likelihood of earlier detectable growth. For example, sample 2 was delivered in 5 d while sample 7 took only 3 d to reach the cell culture laboratory. Growth from sample 2 was first observed after 54 d, whereas sample 7 showed growth in only 15 d. The average time for growth was 33 d. Thus, reducing the shipping time may be a crucial factor in enhancing the probability for cell growth.

Second, the concentration of antifungal agent (Nystatin) may affect cell viability. The major difference in the shipping conditions for samples 5, 6 and

7 (Table 1) was in the concentrations of Garamycin and Nystatin. Mycostatins (*e.g.*, Nystatin) are known to have cytotoxic effects (Freshney 1983), so we reduced the concentration from 80 units/ml to 10 units/ml for the last sample (Whale 7). Although the dose of Garamycin was reduced from 200 μg/ml to 100 μg/ml for sample 7, this is less important than the reduction in Nystatin, since Garamycin is not reportedly cytotoxic at 200 μg/ml (Schering 1979). Thus, the 8-fold reduction of Nystatin was more likely a critical factor in improving the viability of the cells from sample 7; however, the reduction in Garamycin may also be a factor in the observed differences in growth. Some antifungal agent is essential, since fungal contamination was observed in sample 1 which did not contain any antifungal agent. Furthermore, *Aspergillus* spp. and *Penicillium* spp. were isolated from untreated portions of these biopsies (Mathews 1986).

The temperature during shipping is another important factor which can affect the viability of living cells. Warmer temperatures will accelerate bacterial proliferation as well as increase the metabolic activity of the mammalian cells. These factors contribute to depletion of the nutrient medium and the accumulation of waste products which may induce autolysis. Consequently samples should be kept cool (between approximately 10° and 30°C), but not frozen.

Contamination by microorganisms is also a major cause of limited cell viability or slow cell growth (Freshney 1983). Rapid proliferation of bacteria produces an unfavorable environment for the more slowly growing mammalian cells by depletion of the available nutrients. In addition to this depletion, changes in the pH may occur and this may have a detrimental effect on culture viability.

Bacteria, yeasts and fungal spores may be introduced into a culture from several routes including: 1) culture media or other contaminated solutions; 2) specimens taken from non-sterile environments; and 3) exposure to air or work surfaces during handling (Coriell 1973, Freshney 1983).

Because our samples were collected directly from whales in the wild, they were unavoidably contaminated by microorganisms normally associated with the integument, or subsequently introduced by exposure to the biopsy tip, sea water or during handling. Thus, our objectives were to eliminate these contaminants and to maintain a barrier between the tissue and the non-sterile environment.

Since the highest concentration of microorganisms is located on the outer surface of the skin, this layer was removed and used in a separate epxeriment in which the growth of contaminants was encouraged for diagnostic purposes (Mathews 1986). The rinses in sterile saline followed by a rinse and incubation in culture medium with Garamycin were established to dilute and eliminate bacteria while Nystatin inhibited fungal growth. Garamycin was chosen for the field study over penicillin/streptomycin because of its stability and because it does not require constant cold storage. Shipping in media with antimicrobial agents was a final precaution against contamination during transit from British Columbia, Canada to Cincinnati, Ohio.

To our knowledge previous attempts to culture fibroblasts from whales have not achieved the high degree of success as those reported in this paper. We believe that several factors increased the viability of our samples:

1) stringent adherence to aseptic technique,
2) use of a portable hood for sterile media preparation and tissue handling in the field,
3) rapid delivery of samples to the culture facility,
4) reduced concentration of Nystatin during shipping of our samples,
5) use of a cold pack and insulated packaging during shipping, and
6) placing tissue explants under a coverslip during cell culture initiation.

The genetic and biochemical information obtainable from living skin biopsies will provide valuable information to long-term field studies of free-ranging whales. Aside from temporary restraint, impractical with large cetaceans, genetic and biochemical data can only be obtained from free-ranging whales by remote sampling. Although the results reported in this paper are based on a small sample size, we believe that if the procedures are followed, they will increase the probability of successfully culturing cetacean fibroblasts from dermal tissue. Once a cell culture is established, it will supply replenishable material from identified whales still living in the wild. These cultures can then be used for gender determination from karyotypes, and for detecting inherited protein, chromosomal and DNA polymorphisms. Using the reported method for processing tissue samples from free-ranging whales, information on the genetic relationships of carefully selected key animals can be gathered to assess more accurately the social dynamics of cetaceans. Accelerated advances in cytogenetic and molecular techniques employed routinely in humans will provide much needed analytical tools for evaluating familial and populational relationships in wild cetaceans, leading to improved understanding of mating systems, stock identification and effective population size.

Acknowledgments

This study was conducted as part of the first author's Master's thesis research at the University of California at Santa Cruz. I am especially grateful to Dr. Kenneth S. Norris, my major professor and a constant source of inspiration. Dr. James Darling's assistance vastly improved our ability to collect samples with a minimum of disturbance to the whales. Ellie Dorsey, Dr. Deborah Duffield, Judith Perkins, Susan Shane and Dr. Peter Tyack provided valuable guidance during various aspects of the study. I gratefully acknowledge assistance from Virginia Barrister, Don Croll, Dr. Mike Dalbey, Jo Guerero, Dr. Jon Lien, Helen Lawse, Dave Meyers, the cytogenetic technicians at the Kaiser Permanente Clinic (San Jose, CA) and especially James and Bettie Mathews. Ted Goodspeed, Nathan Comfort, Enid Cummings, Tina Gemeinhardt, Karen Richardson and Mitchell Swartz provided indispensible field assistance. This research was supported in part by scholarships from the Center for Marine Studies, UCSC; the Stockton Sportsman's Club; the American Cetacean Society, Monterey Chapter; Sigma Xi Grant-in-Aid of Research; and the Bamfield Marine Station. We thank two anonymous reviewers for helpful comments on the manuscript.

Literature Cited

Arnason, U. 1970. Karyotype of a male sperm whale (*Physeter catodon,* L.) and a female sei whale (*Balaenoptera borealis,* Less.). Brief report in Hereditas 64:291–293.

ARNASON, U. 1972. The role of chromosomal rearrangement in mammalian speciation with special reference to Cetacea and Pinnipedia. Hereditas 70:113–118.

ARNASON, U. 1974. Comparative chromosome studies in Cetacea. Hereditas 77:1–36.

ARNASON, U. 1985. Conventionally stained and C-banded karyotypes of a female blue whale. Hereditas 102:251–253.

ARNASON, U., I. F. PURDOM AND K. W. JONES. 1978. Conservation and chromosomal localization of DNA satellites in balaeanopterid whales. Chromosoma (Berl) 66: 141–159.

ARNASON, U., AND F. WIDEGREN. 1984*a*. Studies on ribosomal DNA in cetaceans (whales). Hereditas 101:149–154.

ARNASON, U., AND F. WIDEGREN. 1984*b*. Different rates of divergence in highly repetitive DNA of cetaceans. Hereditas 101:171–177.

BARR, M. L., AND E. G. BERTRAM. 1949. A morphological distinction between neurones of the male and female, and the behavior of the nucleolar satellite during accelerated nucleoprotein synthesis. Nature 163:676.

BOSTEIN, D., R. L. WHITE, M. SCOLNICK AND R. W. DAVIS. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics 32:314–331.

CHAKRABORTY, R. 1985. Paternity testing with genetic markers: are Y-linked genes more efficient than autosomal ones? American Journal of Medical Genetics 21:297–305.

CORIELL, L. L. 1973. Methods of prevention of bacterial, fungal, and other contaminations. Pages 29–49 *in* J. Fogh, ed. Contamination in tissue culture. Academic Press, New York, NY.

CRAIG-HOLMES, A. P. 1977. C-band polymorphism in human populations. Pages 161–178 *in* E. B. Hook and I. H. Porter, eds. Population cytogenetics: studies in human populations. Academic Press, Inc., New York, NY.

DARLING, J. D. 1984. Gray whales off Vancouver Island, British Columbia. Pages 267–287 *in* M. L. Jones, S. L. Swartz and S. Leatherwood, eds. The gray whale, *Eschrichtius robustus*. Academic Press, Inc. New York, NY.

DAVIES, K. E., K. HARPER, D. BONTHRON, R. KRUMLAUF, A. POLKEY, M. E. PEMBREY AND R. WILLIAMSON. 1984. Use of a chromosome 21 cloned DNA probe for the analysis of non-disjunction in Down's syndrome. Human Genetics 66:54–56.

DUFFIELD, D. A. 1977. Fluorescent karyotype of the California gray whale. Marine Fisheries Review 36:25–28.

DUFFIELD, D. A., J. CHAMBERLIN-LEA, W. GILMARTIN, I. KANG AND R. LAMBERTSEN. 1985*a*. Chromosomes of the Pacific and Atlantic humpback whale, *Megaptera novaeangliae*. *In* Abstracts of the Sixth Biennial Conference on the Biology of Marine Mammals. Vancouver, B.C.

DUFFIELD, D. A., J. CHAMBERLIN-LEA, R. S. WELLS AND M. D. SCOTT. 1985*b*. Inheritance of an extra chromosome in a resident female band of bottlenose dolphins in Sarasota, Florida. *In* Abstracts of the Sixth Biennial Conference on the Biology of Marine Mammals. Vancouver, B.C.

FRESHNEY, R. I. 1983. Culture of animal cells. Alan R. Liss, Inc. New York.

HATLER, D. F., AND J. D. DARLING. 1974. Recent observations of the gray whale in British Columbia. Canadian Field Naturalist 88:449–459.

HILL, W. G. 1987. DNA fingerprints applied to animal and bird populations. Nature 327:98–99.

HOELZEL, A. R., E. M. DORSEY, D. A. DUFFIELD AND R. H. LAMBERTSEN. 1983. The absence of distinguishable sex chromatin in the integument of three species of mysticetes. *In* Abstracts of the Fifth Biennial Conference on the Biology of Marine Mammals. Boston, MA.

JEFFREYS, A. J., V. WILSON AND S. L THEIN. 1985*a*. Hypervariable 'minisatellite' regions in human DNA. Nature 314:67–73.

Help Volumes Main Menu

JEFFREYS, A. J., V. WILSON AND S. L THEIN. 1985*b*. Individual-specific 'fingerprints' of human DNA. Nature 316:76–79.

JEFFREYS, A. J., J. F. Y. BROOKFIELD AND R. SEMEONOFF. 1985*c*. Positive identification of an immigration test-case using human DNA fingerprints. Nature 317:818–819.

KEITH, R. E. 1981. Resolution of paternity disputes by analysis of the blood. Family Law Reports 8:4001–4008.

KRUSE, P. F., JR., AND M. K. PATTERSON, JR., EDS. 1973. Tissue culture: methods and applications. Academic Press, New York.

MAKINO, S. 1948. The chromosomes of Dall's porpoise, *Phocoenoides dallii* (True), with remarks on the phylogenetic relations of the Cetacea. Chromosoma (Berl) 3: 220–231.

MATHEWS, E. A. 1986. Multiple use of skin biopsies collected from free-ranging gray whales, *Eschrichtius robustus*: sex chromatin analysis, collection and processing for cell culture, microbiological analysis of associated microorgansisms, behavioral responses of whales to biopsying, and future prospects for using biopsies in genetic and biochemical studies. Master's thesis, University of California, Santa Cruz, CA. 118 pp.

MCCRACKEN, G. F., AND J. W. BRADBURY. 1977. Paternity and genetic heterogeneity in the polygynous bat, *Phyllostomus hastatus*. Science 198:303–306.

MEDWAY, W. 1983. Evaluation of the safety and usefulness of techniques and equipment used to obtain biopsies from free-swimming cetaceans. Final Report to the Marine Mammal Commission. P.O.#MM2324809-8. (Unpublished). 14 pp.

MODI, W. S. 1986. Karyotypic differentiation among two sibling species pairs of New World microtine rodents. Journal of Mammalogy 67:159–165.

O'BRIEN, S. J., W. G. NASH, D. E. WILDT, M. E. BUSH AND R. E. BENEVENISTE. 1986. A molecular solution to the riddle of the giant panda's phylogeny. Nature 317: 140–144.

OHNO, S. 1984. Conservation of linkage relationships between genes as the underlying theme of karyological evolution in mammals. Pages 1–10 *in* A. K. Sharma and A. Sharma, eds. Chromosomes in evolution of eukaryotic groups. CRC Press, Inc., Boca Raton, Florida.

OLSEN, S. B., R. E. MAGENIS, S. I. ROWE AND E. W. LOVREIN. 1983. Chromosome heteromorphism analysis in cases of disputed paternity. American Journal of Medical Genetics 15:47–55.

PARKS, D. R., V. M. BRYAN, V. M. OI, V. T. OI, AND L. A. HERZENBERG. 1979. Antigen specific identification and cloning of hybridomas with a fluorescence activated cell sorter (FACS). Proceedings of the National Academy of Science 76:1962–1966.

QUINN, T. W., J. S. QUINN, F. COOKE AND B. N. WHITE. 1987. DNA marker analysis detects multiple maternity and paternity in single broods of the lesser snow goose. Nature 326:392–394.

SCHERING CORPORATION. 1979. GRS®, Garamycin®. Product Information. Kenilworth, N.J. 07033.

SELANDER, R. K., M. H. SMITH, S. Y. YANNG, W. E. JOHNSON AND J. B. GENTRY. 1971. IV. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Studies in genetics VI. University of Texas Publication 7103:49–90.

SMITH, D. G. 1980. Paternity exclusion in six captive groups of rhesus monkeys (*Macaca mulatta*). American Journal of Physical Anthropology 53:243–249.

STERN, B. R., AND D. G. SMITH. 1984. Sexual behavior and paternity in three captive groups of rhesus monkeys (*Macaca mulatta*). Animal Behavior 32:23–32.

WALEN, K. H., AND S. H. MADIN. 1965. Comparative chromosome analysis of the bottlenosed dolphin (*Tursiops truncatus*) and the pilot whale (*Globicephale scammonii*). The American Naturalist 99:349–354.

WINN, H. E., W.L. BISHOFF AND A. G. TARUSKI. 1973. Cytological sexing of Cetacea. Marine Biology 23:343–346.

WOLFE, J., R. P. ERIKSON, R. P. RIGBY, P. W. J. RIGBY AND P. N. GOODFELLOW. 1984. Cosmid clones derived from both euchromatic and heterochromatic regions of the human Y chromosome. The EMBO Journal 3:1997–2003.

WORTHEN, G. L. 1981. Preliminary analysis of the potential for stock assessment of Pacific Ocean delphinids by G- and C-chromosome banding. Final report to NMFS, NOAA 79-ABC-00205. Southwest Fisheries Center, La Jolla, CA.

Received: October 6, 1986
Accepted: July 7, 1987