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THESIS APPROVAL

The abstract and thesis of Kristine Anne Gunter for the Master of Science in Biology were presented December 13, 1996, and accepted by the thesis committee and the department.

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ABSTRACT

An abstract of the thesis of Kristine Anne Gunter for the Master of Science in Biology presented December 13, 1996.

Title: Distribution of R-Banded Chromosome Variants in A
Bottlenose Dolphin (*Tursiops truncatus*)
Population

When combined with observational and behavioral data, chromosome variant analysis provides a means of assessing population structure in bottlenose dolphins, *Tursiops truncatus*. In this cytogenetic analysis of thirty-six bottlenose dolphins, a distribution of fluorescent R-band variants was catalogued and used as a way to characterize the genetic structure of a *Tursiops* population inhabiting the Matagorda Bay, Texas, area. Three different population genetic analytical techniques were evaluated for their usefulness in analyzing cytogenetic data. The chromosome variant data obtained from this study was then compared with cytogenetic data gathered from a resident dolphin population residing near Sarasota, Florida.

The purpose of this study was to measure the genetic differences that existed within the Matagorda Bay study population, as well as measure the differences which exist when comparing the Matagorda Bay animals to the Sarasota Bay population. The comparison of these two geographically separated populations gave a quantitative

baseline from which to evaluate the significance of the level of local differentiation and subgroup structure.

Each population genetic analytical method demonstrated that subgroup differentiation existed within the Matagorda Bay sample. The cytogenetic data, therefore, suggest that the *Tursiops* in Matagorda Bay most likely do not represent a single breeding unit or population. The Matagorda Bay area could instead be viewed as a region which is inclusive of the home ranges of multiple subgroups of *Tursiops*. These subgroups of animals presumably interact with one another, and the migration of animals into and out of subgroups serves as a method of genetic exchange among the animals in this region. The extent of migration of animals throughout the Gulf of Mexico is not known, but several chromosome variants previously identified in the Sarasota animals were not present in the Matagorda Bay sample and vice versa.

The cytogenetic data obtained through this study suggests that coastal, in-shore *Tursiops* populations in the Gulf of Mexico may be best characterized as a series of local, but inter-mixing populations. Chromosomal variants are transmitted from core area to core area via individual animals that do not maintain core area fidelity.

DISTRIBUTION OF R-BANDED CHROMOSOME VARIANTS IN A
BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*) POPULATION

by
KRISTINE ANNE GUNTER

A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE
in
BIOLOGY

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1997

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Introduction

Taxonomic Status of *Tursiops truncatus*

Bottlenose dolphins (*Tursiops truncatus*) reside worldwide in all tropical to temperate ocean locations (Darling et al., 1995), inhabiting in-shore areas as well as open ocean environments. Several issues regarding the taxonomic classification of in-shore and pelagic forms or regional distributions of *Tursiops* have not yet been resolved. For example, while differences in morphology, hematology and feeding habits have been found to distinguish coastal from off-shore populations (Duffield et al., 1983; Hersh and Duffield, 1990; Scott and Chivers, 1990), whether these differences warrant recognition as separate species has not been established.

Ross and Cockcraft (1990) reviewed the issue of speciation within the genus *Tursiops*, noting that some authors have suggested defining as many as four different species: *T. truncatus*, *T. gilli*, *T. aduncus* and *T. gephyreus*. Van Waerebeek et al., (1990), in a preliminary review of bottlenose dolphins residing along the Pacific coastline of South America, noted that while physical characteristics may distinguish off-shore from in-shore forms, the assumption should be that bottlenose dolphins consist of a single species, *T. truncatus*, distinguishable by a series of unique geographical forms.

Communities and Associations Formed Within Dolphin Populations

Site fidelity is thought to be a common feature of coastal populations of bottlenose dolphins (Irvine et al., 1981; Scott et al., 1990; Shane et al., 1986; Wells et al., 1980; Wells et al., 1987; Wells, 1991; Würsig and Harris, 1990). Studies in western Australia, central western Florida and Argentina have shown that *Tursiops* populations inhabit specific regions of coastline for extended periods of time. Würsig and Harris (1990) were able to resight ten bottlenose dolphins in the same region off the Argentina coastline some eight to twelve years after an initial study had been completed. In addition, affiliations between certain animals within the population appeared to be maintained over the course of the twelve year period. Studies in Sarasota, Florida, have shown affiliations lasting over three generations (Wells, 1991).

Extensive long-term observational studies on bottlenose dolphin populations have provided insight into the behavior and individual associations formed in local *Tursiops* communities (Scott et al., 1990; Shane et al., 1986; Wells, 1991). Observational studies have historically focused on coastal (in-shore) populations of *Tursiops* due to the ease with which these groups of

animals can be studied. Off-shore populations inhabiting pelagic oceanic zones, as well as populations residing around islands have been studied less frequently (Scott and Chivers,1990).

Long-term observational studies conducted on wild *Tursiops* populations residing near Sarasota, Florida, have provided insights into the group behavior of bottlenose dolphin communities (Scott et al.,1990; Irvine et al.,1981; Wells et al.,1980; Wells et al.,1987; Wells,1991; Würsig and Harris,1990). Four major observations about *Tursiops* population dynamics have been noted from on-going studies in the Sarasota area:

- 1) Most *Tursiops* individuals utilize specific 'core areas' within a larger home range.

- 2) Bands of adult females will form within a *Tursiops* population. Members of a given band share a common home range and display relatively high degrees of association with one another.

- 3) Adult males travel widely throughout the Sarasota Bay area. Most males do not associate exclusively with any particular female band. Instead, they have ranges which encompass the core areas of multiple female bands.

- 4) Males and females show substantial differences in home ranges; females occupy relatively limited home ranges while males will range over greater distances.

Adult males will travel from one female band to the next.

Such differences in male and female distribution and movement patterns create the potential for sampling from two separate subgroups within a single region. Males and females which reside in adjacent areas may be identified, through behavioral observations, as members of the same population when in fact, they are representatives of neighboring subgroups, but interacting socially with one another.

Genetic Studies of Cetacean Populations

Genetic analyses of cetacean populations have helped to clarify issues regarding speciation, to investigate the management of populations and to define how different cetacean groups utilize core areas (Baker et al., 1993; Bigg, 1982; Bigg et al., 1990; Daniëlsdóttir et al., 1991; Duffield, 1982; Duffield, 1986; Duffield and Wells, 1986; Hoelzel and Dover, 1991). By comparing genetic data both within and between populations, hypotheses can be generated regarding breeding behavior. Groups of animals can be studied to determine if they represent isolated breeding groups or subpopulations within a large, intermingling population.

Populations of humpback whales (*Megaptera novaeangliae*) were thought to be subdivided, based on

observational data, into discrete subpopulations (Baker et al.,1993). DNA fingerprint and mitochondrial DNA (mtDNA) studies performed on North Pacific humpback populations provided additional evidence to substantiate demographic and behavioral information which suggested that this population was subdivided into relatively discrete subgroups which were not obviously geographically separated (Baker et al.,1993).

Behavioral studies of killer whales (*Orcinus orca*) have shown the existence of numerous discrete pods that do not appear to intermingle, yet live within the same geographical location (Bigg,1982; Bigg et al.,1990). Genetic studies of Pacific Northwest killer whale populations, performed using genomic DNA fingerprinting and/or mtDNA analysis, revealed that the genetic differences existing within the Vancouver Island sympatric population are as great as the genetic differences which are observed when comparing geographically isolated populations (Hoelzel and Dover,1991; Stevens et al.,1989). Other differences, including color morphology, acoustic behavior and feeding strategies distinguish the sympatric Vancouver Island killer whale populations from each other. These differences, when considered along with the genetic data suggesting that sympatric populations of the same species

can be genetically isolated, led Hoelzel and Dover (1991) to suggest that the Vancouver Island killer whale population be managed as though it consists of separate stocks of animals.

Winans and Jones (1988) studied the electrophoretic variability present in a sample of 360 Dall's porpoises (*Phocoenoides dalli*) in order to help determine if the animals, taken from the North Pacific Ocean and from the Bering Sea, were representative of a single interbreeding population. Testing of the hypothesis involved comparing the protein electrophoretic variant frequencies among three selected subgroups within the population to those predicted by the Hardy-Weinberg equilibrium model. The subgroups were then pooled together and the variant frequencies for the population as a whole were calculated and compared to Hardy-Weinberg predictions. The authors reasoned that if the three subgroups sampled were indeed representative of a single breeding population, pooling of the data would not alter the Hardy-Weinberg results. When the analysis was performed, the Chi-square values in the "pooled" population exceeded those calculated for the individual subgroups at seven of nine variable loci tested, leading to the rejection of the null hypothesis that the samples were from a single interbreeding population.

Cytogenetic Analysis of Populations

Cytogenetic variant analysis has helped to clarify and resolve speciation issues in a number of mammalian species. Matayoshi et al., (1987), reported a characteristic heteromorphic chromosomal variant that appeared to be fixed within one subspecies population of *Cebus apella* (capuchin monkey). Taxonomic classification within this species has not been resolved with the number of subspecies and varieties reported as five, eleven and sixteen (Matayoshi et al., 1987). For one subspecies, *C.a. xanthosternos*, a characteristically different chromosome number eleven was identified with C-banding. All specimens of the subspecies were found to carry this distinguishable chromosome in a homozygous condition, suggesting that this is a fixed, not variable, chromosomal type. This finding, in conjunction with geographic distribution information and phenotypic identification helped to substantiate the existence of *C.a. xanthosternos* as a unique subspecies within the *Cebus* genus.

Cytogenetic variant analysis has been used in humans (*Homo sapiens*) as a method for paternity-testing, determination of uniparental disomy (UPD) and for assessing parental origin of extra chromosomes in trisomy

13 and trisomy 21 cases (Magenis and Toth-Fejel, 1992; Olson et al., 1983; Magenis et al., 1977). In 1975, an extensive study of a human newborn population was undertaken in order to determine the extent of polymorphic chromosome variation in humans. Of 376 newborns karyotyped using C- and Q-banding techniques, polymorphism rates were so extensive that no two infants in the study had identical variant patterns (Müller et al., 1975). Subsequent studies performed by Olson et al., (1986), found that within a human population the estimated probability of finding two persons with identical Q-band variants was 0.0003.

Cytogenetic Analysis of Cetaceans

Overall, cetaceans show a highly conserved chromosome complement with respect to chromosome number and general structure. Early investigations of cetacean chromosomes revealed that most species have a $2n=44$ chromosome count, with chromosomes in the majority of species having the same size and shape (Arnason, 1974; Arnason et al., 1977; Arnason, 1981; Duffield, 1977; Duffield-Kulu, 1972). Stability of chromosome number and morphology within the cetaceans are attributed to three factors (Arnason, 1972):

1) Cetaceans have a low reproductive rate, sexually maturing at a late age and producing only a single offspring each year.

2) Cetaceans show high degrees of mobility, often migrating thousands of miles each year.

3) Living in oceanic environments provides cetaceans with little opportunity to develop well defined niches.

All three factors promote a conservation of karyotype since a new rearrangement would have little chance of stabilizing in a mobile, inter-mixing population. Chromosome rearrangements are more likely to become fixed in small, isolated groups of individuals where inter-breeding is less likely to occur. Similarly, rare heteromorphic variants can be expected to become fixed only in small, closely related groups of individuals.

Despite the overall conservation of karyotype among the cetaceans, considerable variation in heterochromatic regions of chromosomes exists within this order (Arnason et al., 1980; Duffield, 1977; Duffield, 1986; Duffield et al., 1991; Duffield and Chamberlin-Lea, 1990; Stock, 1981). Cetacean chromosomes, therefore, may not provide an immediate means for identifying subspecies, but they can be used effectively as a method for comparing the variants between and among populations. These analyses

can show a flow of variants from one population to another or show that variants are specific to one population only.

Chromosome heteromorphism analysis has been used as a method to look at population substructures (Duffield,1986; Duffield and Chamberlin-Lea,1990; Duffield and Wells,1991; Lambertson and Duffield,1987), identify unique subspecies populations (Matayoshi et al.,1987), determine paternity (Duffield and Chamberlin-Lea,1990; Olson et al.,1986) and as a method for assessing the evolution of karyotypes among related species (Arnason et al.,1977; Arnason et al.,1980; Davis et al.,1986; Duffield,1977; Duffield et al.,1991; Stock,1981). Chromosome variants can be visualized through the use of different staining techniques, including fluorescent R-banding (reverse banding), Q-banding (quinacrine banding) and C-banding (centric banding) (Arnason et al.,1980; Duffield and Chamberlin-Lea,1990; Stock,1981). The variants which are visualized with each of these methods seem to have no phenotypic effect and are inherited in a Mendelian fashion (Olson et al.,1983; Duffield and Chamberlin-Lea,1990).

Fluorescent R-banding has previously been used as a method for identifying chromosome heteromorphisms in *Tursiops truncatus* (Duffield and Wells,1991; Duffield and

Chamberlin-Lea,1990). R-banding is the staining technique of choice for analysis of cetacean chromosomes because it allows for simultaneous visualization of heteromorphic variants, as well as provides a distinct banding pattern which allows for pairing of homologous chromosomes (Duffield and Chamberlin-Lea,1990; Duffield et al.,1991). Q-banding does not produce differential staining of cetacean chromosomal variants and C-banding, which preferentially stains centromeric regions of chromosomes, does not produce a banding pattern to allow for the unequivocal pairing of homologues.

Thesis Project: Matagorda Bay *Tursiops* Study

In my study, fluorescent R-banding techniques were used to evaluate the chromosomal variants of thirty-six bottlenose dolphins captured and released off the central Texas coast, near Matagorda Bay. Large-scale die-offs of *Tursiops* had been noted in the months prior to beginning this capture-and-release study. This study was initiated in order to try and more clearly understand how bottlenose dolphin populations utilize the Matagorda Bay area. Due to the availability of blood samples from the animals, it was suggested that an extensive genetic study of the sampled population be undertaken. It was hoped

that the combination of genetic and field observational data would allow for a more thorough understanding of the animals that make up the Matagorda Bay *Tursiops* population.

In this cytogenetic analysis of the 36 bottlenose dolphins, a distribution of fluorescent R-band variants was catalogued and used as a way to characterize the genetic structure of the Matagorda Bay *Tursiops* population. This distribution was also used to compare this group of animals with another *Tursiops* population residing in the Gulf of Mexico on the central west coast of Florida which had already been studied cytogenetically. Evaluations of maternity and paternity were made, as well as intrapopulation comparisons of variant frequencies among the animals sampled from Matagorda Bay. Three different methods of quantifying genetic differences between and within populations were assessed for their usefulness in evaluating potential genetic differences between groups of animals using chromosome heteromorphisms. Baseline levels for measuring cytogenetic differences between and among bottlenose dolphin populations were established for each test. It is hoped that this information will increase the understanding of how *Tursiops* utilize the Matagorda Bay area.

Materials and Methods

Matagorda Bay *Tursiops* Population

Thirty-six bottlenose dolphins, sixteen females and twenty males, were captured and released near Matagorda Bay, Texas, during a two-week period of July, 1992. All thirty-six animals were tagged with dorsal fin roto-tags and thirty-five of the animals were simultaneously freeze-branded with identification numbers. Radio transmitters were affixed to ten of the dolphins to aid in locating the animals after they were released (Würsig and Lynn, 1993). Monitoring of the subject animals continued via photographic surveys, and by periodically tracking the animals through the use of radio-tagging, through 1993.

At the time of capture, genders of the animals were ascertained and estimates of potential mother and calf pairs, were made based on proximal associations between animals. Estimates of the age of each animal were performed using tooth-aging methods (Hohn, 1980; Hohn et al., 1989). The sex, identification, capture location and estimated age of each animal are given in Table 1 and Figure 1.

One group of animals, which were captured within the Northeastern region of the study area, were not resighted

after the initial capture. These ten animals, nine of which were successfully cytogenetically analyzed, have been designated as the Northeast (NE) subgroup for the purposes of further genetic analysis. The remaining 26 animals, which comprise the Matagorda Bay sample population, are designated the Southwest (SW) subgroup. Members of the NE group are indicated on Table 1 by a double asterisk.

Chromosome Cultures

Whole blood samples were obtained from each of the thirty-six bottlenose dolphins involved in the study. The blood was refrigerated at 4 degrees Celsius and shipped, via next-day air service, to Portland State University where it was used for the initiation of chromosome cultures. Three separate chromosome cultures were established for each animal sampled.

Chromosome preparations were made using a leukocyte protocol for cetaceans (Duffield & Chamberlin-Lea, 1990). Approximately seven drops of whole blood (measured from a 1 ml disposable serological pipette) were added to 4 ml of RPMI 1640 media (Gibco) supplemented with 15% fetal calf serum, 1% penicillin-streptomycin and stimulated with various mitogenic agents. One culture from each individual (designated the PK culture) was supplemented

with 0.05 ml pokeweed mitogen. One sample was grown with 1% phytohemagglutinin as a mitotic stimulant (designated the PHA culture), and a third culture was supplemented with both 1% pokeweed mitogen and 1% phytohemagglutinin (designated the PHA + PK culture).

All cultures were incubated at 36.0 degrees C for 96 hours prior to the addition of 0.05 ml colcemid (Gibco). An additional sixteen hours of incubation at 36.0 degrees C followed the colcemid treatment after which time the cultures were harvested. Cultures were centrifuged at 1000 rpm for 10 minutes and then treated with 5.0 ml 0.075 M KCl hypotonic solution for ten minutes. Cells were fixed and stored in a 3:1 ethanol:glacial acetic acid solution.

Metaphase chromosome spreads were initially located on microscope slides by standard staining with Giemsa. Slides were then destained in fixative solution and banded using a fluorescent R-band technique employing both distamycin A and chromomycin A-3 (Duffield and Chamberlin-Lea, 1990; Sahar and Latt, 1978; Schweizer, 1980). Detailed protocols of blood culturing and harvesting techniques and R-banding methods are presented in Appendix 1. Metaphase cells were located and photographed using TechPan 2415 (Kodak) film on a Zeiss epifluorescence microscope equipped with an

ultraviolet light source. Film was developed using Diafine 2-step developer and cells were printed on Kodak and AGFA photographic paper.

Chromosome Variant Analysis

Metaphase chromosome spreads from each animal were banded, photographed, karyotyped and evaluated for heteromorphisms. When evaluating chromosomal variants, multiple cells were examined and compared to one another in order to distinguish between true variants and artifacts resulting from staining and photographic methods.

Variable regions were noted on eleven different chromosomes and each distinct variant was assigned a numerical value based on a scoring method previously used to evaluate *Tursiops* chromosome variants (Duffield and Chamberlin-Lea, 1990). Since heteromorphisms are not discrete entities but rather continuums of variation, the numerical assignments are used only to indicate major differences in the size or fluorescent intensity of the variable region.

Statistical Analysis

Three quantitative statistical methods were used to evaluate subpopulation structure, using cytogenetic variant analysis, within the Matagorda Bay population.

These same statistical analyses were applied to the Matagorda Bay and Sarasota *Tursiops* populations in order to quantify the cytogenetic differences which exist between these two populations.

1. Population Equilibrium Estimates

a. Hardy-Weinberg equilibrium analysis

Variable regions on the 11 chromosomes which exhibit cytogenetic differences were tested to see if the variants present were in Hardy-Weinberg equilibrium. A population distribution of heteromorphism scores for each variable region for both Matagorda Bay and Sarasota populations was tested for Hardy-Weinberg equilibrium using the log-likelihood ratio (G-test) analysis. G-statistics were applied to groups studied within the Matagorda Bay sample, as well as to test for differences between Matagorda Bay and a similar *Tursiops* population residing near Sarasota, Florida.

Calculation of G-statistics was performed using the following formula (from Zar, 1984):

$$G = 4.60517 [\sum f_i \log_{10} f_i - \sum f_i \log_{10} f_e]$$

f_i = observed values

f_e = expected values

For the purpose of evaluating significance of difference from predictions of the Hardy-Weinberg

equilibrium equation, three genotypic classes were used to group animals into sufficiently large data sets. These three classes were: 1) most common allele homozygotes; 2) most common allele heterozygotes; and 3) all other homozygotes and all other heterozygotes, grouped together.

b. Population Pooling

As a method to assess the presence or absence of subgroups from within the Matagorda Bay sample, a population pooling approach (Winans and Jones, 1988) was undertaken. The basis of this approach is comparing the G-statistic values between two or more possible subgroups with the G-statistic values which are obtained by pooling the subgroups together. If the pooled population has G-statistic values that are larger than those obtained in either or both subgroups, it may be indicative of genetic differentiation between the subgroups under investigation.

2. Pair-Wise Similarity Coefficients

Chromosomal variant sharing (S) was determined in a series of pair-wise comparisons as the number of variants which were shared between any two dolphins. This similarity value, S, between any two individuals in the

sample population was calculated (from Lehman et al., 1992) as:

$$S = 2n_{xy} / (n_x + n_y)$$

n_{xy} = number of variants shared between individuals x and y
 $n_x + n_y$ = total number of variants possessed by individuals x and y, respectively

Use of this formula allowed for comparisons to be made between males and females by adjusting for differences in the number of variants compared. Males were scored for 29 variants (28 autosomal regions as well as the 'Y' chromosome), while females were scored only for autosomal variants.

Z-scores were used to test how far the similarity values (S) for suspected mother and calf pairs, identified from within the Matagorda Bay population, deviated from the mean S-value calculated for the population as a whole. Z-scores (from McClave and Dietrich, 1988) were calculated by subtracting the mean S-value for the entire Matagorda Bay population from the S-value calculated for the potential mother and calf. This value is then divided by the standard deviation for the average S-value of the Matagorda Bay population.

3. Nei Index for Measuring Allelic Frequency Differences

A third method of evaluating intrapopulation and interpopulation genetic differences was used to quantify

the amount of difference in allelic frequencies between two groups of animals, using each chromosome variant as an allele. A similarity index (I), based on Nei's genetic distance (Nei and Tajima, 1983), was calculated to measure the probability that two alleles, drawn at random, one from each population, would be identical. If two populations share identical variant frequencies, the similarity index will be 1.0. The larger the difference in frequency of alleles between the groups, the lower the index, and hence the probability, will be. The calculation of the similarity index (I) is as follows (from Mettler et al., 1988):

$$I = j_{xy} / \sqrt{j_x j_y}$$

$j_{xy} = \sum x_i y_i$ where x_i is the frequency of an allele (the i th) in population x, and y_i is its frequency in population y

$j_x = \sum x_i^2$ = sum of squared allele frequencies in population x

$j_y = \sum y_i^2$ = sum of squared allele frequencies in population y

Results

Heteromorphism Analysis

Thirty-five of the thirty-six animals had a sufficiently high mitotic index to allow for variant analysis. One animal in the sample population (FB527) was deemed a culture failure after no metaphase cells

were found on seven slides examined. A total of 247 metaphase cells were karyotyped and analyzed in this study. The mean number of metaphase cells scored for variants, per animal, was 7.06. The minimum number of cells analyzed on an individual animal was three (FB517), while the maximum number of cells analyzed was thirteen (FB530 and RT412).

A total of 48 different variants (Figure 2), located on eleven different pairs of chromosomes, as well as a small marker chromosome, were identified in the Matagorda Bay population. Four chromosomes had variable regions in both the short (p) arm as well as in the long (q) arm.

Variant scores for each animal were assigned by examining multiple cells from an individual and comparing those variants present to a set of standards derived from variants in this population and variants reported for the Sarasota bottlenose dolphin population (Duffield and Chamberlin-Lea, 1991). A score was assigned to each homologue of a pair, creating a binomial value for each variable chromosome pair. Chromosomes 20 and 21 were also scored for the presence of satellites, indicated as an "S" after the numerical value. A complete listing of the variants identified in each animal is presented in Table 2. Appendix 2 presents a series of chromosome composite sheets. A representative cell from each of the

Matagorda Bay study animals was chosen for these composite sheets and only those chromosomes which have variable regions are shown in the composites. These sheets were compiled and used to analyze the variant regions for each animal sampled in Matagorda Bay. Appendix 2 also presents a representative complete karyotype from an individual member of the Matagorda Bay *Tursiops* sample. The polymorphic variation found in the Matagorda Bay *Tursiops* sample population was extensive enough that no two animals within the study had an identical set of variant scores.

Statistical Analysis

I. Population Equilibrium Estimates

a. Hardy-Weinberg Equilibrium Analysis

1. Matagorda Bay

Variant (allele) frequencies and genotype distributions for the Matagorda Bay population, listed by chromosome region, are presented in Tables 3 and 4. These observed genotype frequencies were compared to values predicted by the Hardy-Weinberg model and a G-statistic was calculated to see if statistically significant differences existed between the two values.

When the Matagorda Bay population was evaluated for goodness-of-fit to values predicted by Hardy-Weinberg

equilibrium, one variable region, 18p, deviated significantly ($p < 0.05$) from expected values (Table 5). A large number of heterozygous individuals was responsible for the skewing of the observed values. Further analysis of chromosome region 18p revealed that, when broken down into male and female subgroups (Table 6), the male population showed significant departure from expected Hardy-Weinberg values ($p < 0.05$) due to a large number of heterozygotes, while the female subgroup did not.

Because of the apparent discrepancy in genotype frequencies between males and females, identified in the evaluation of chromosome region 18p, a subsequent analysis of equilibrium estimates for all variable chromosome regions was performed for male and female members of the Matagorda Bay population.

2. Matagorda Bay Males and Females

Males and females were treated as separate populations and allelic frequencies for each variant were calculated (Table 7). Each group, males and females, were tested to see if the variant and genotype frequencies were in close agreement with values predicted by Hardy-Weinberg equilibrium. At one locus, chromosome region 2q, the Matagorda Bay females differed significantly ($p < 0.05$) in genotype frequency from the

values expected under Hardy-Weinberg rules. A large number of homozygotes led to this deviation. At a different locus, chromosome region 18p, the males showed a similar deviation from expected values, as mentioned above. In this case, a large number of heterozygotes led to the deviation of observed values from those which would be expected from an equilibrium population.

3. NE and SW Subgroups Within Matagorda Bay

Allelic frequencies, genotype frequencies and G-statistics were also calculated and compared for the nine animals (NE group) who were not resighted after capture, versus the values calculated for the twenty-six animals (SW group) from within Matagorda Bay (Table 8). It was found that the NE group also had a statistically significant deviation from expected values at chromosome region 18p ($p < 0.05$). As with the evaluation of the Matagorda Bay male subgroup, a large number of heterozygous individuals at this locus lead to the discrepancy between observed and expected results.

4. Sarasota Population

When variant distributions from the previously reported Sarasota population were tested for goodness-of-

fit to Hardy-Weinberg equilibrium expectations, statistically significant deviations from expected values were found at two variable regions (Table 5). For both of these regions, 1q and 2p, an increased number of homozygous individuals was responsible for the skewing of the observed frequencies from those expected in a population at equilibrium. A similar deviation, due to a large number of homozygotes, was detected in the Matagorda Bay female subgroup.

5. Matagorda Bay and Sarasota Pooled

When Matagorda Bay and Sarasota population samples were pooled and treated as a single breeding unit, three of fourteen variable regions showed statistically significant deviations ($p < 0.05$) from expected values (Table 5). Regions 1q, 2p and 4p all had an increased number of homozygous individuals. This number of discrepant regions is more than either the Matagorda Bay sample or the Sarasota sample showed when each was treated as a single population.

b. Population Pooling

G-statistics were also used to see if the genotype frequencies in a "pooled" population deviate farther from expected values than either or both of the individual

populations which were combined to create the pooled group (Winans and Jones, 1988).

When the Matagorda Bay females and males were treated as individual populations, and G-statistic values were compared to those calculated for Matagorda Bay as a whole, four of fourteen regions (28.6%) showed pooled values greater than either group treated alone (Table 7). Similarly, when the outgroup of nine individuals (NE group) from within Matagorda Bay were treated as their own population and compared to the frequencies calculated from the remaining twenty-six animals from the Matagorda Bay sample, 4 of 14 regions (28.6%) were also found to have larger G-statistic values in the pooled group than in either subgroup (Table 8).

When Matagorda Bay and Sarasota populations were treated separately and then as a pooled population, seven of fourteen (50%) variable regions had G-statistic values that were larger in the pooled population than in either individual population (Table 5).

II. Pair-Wise Similarity Coefficients (S)

A similarity value (S) was calculated to express the relative amount of variant sharing between any two individual animals. A mean S-value was calculated to assess the amount of variant sharing occurring within

groups of animals. The mean S-values were then used to compare average variant sharing between selected groups of animals.

The S-values for any two individuals from the Matagorda Bay sample (Table 9) ranged from 0.276 to 0.862. Of 595 pair-wise comparisons made, the mean S-value was 0.582. The standard deviation was 0.09. A graph of the S-value distribution for the entire Matagorda Bay population is shown in Figure 3.

Mean S-values were calculated for different groupings of animals selected from within the sample population. These calculations were used to see if any significant differences existed between subgroups. Mean similarity values were calculated to compare differences between all male animals and all female animals, as well as to compare average S-values between the nine animals which were not resighted after the initial capture (the NE group) with those calculated for the twenty-six animals which were seen again after the capturing and tagging period (the SW group). None of the mean S-values calculated for the groupings showed substantial deviations from one another, or from the mean S-value calculated for the population as a whole. The mean S-value for Matagorda Bay females was 0.577. The mean S-value for Matagorda Bay males was 0.535. The mean S-

value for the NE group was 0.603 while the mean S-value for the SW animals was 0.574. A graph showing the distribution of the pairwise similarity values for the NE group as compared to the SW group is presented in Figure 4.

III. Nei Index for Measuring Allelic Frequency Differences

Similarity index values were used to compare the allelic frequency differences between different groups of animals. Index values were calculated for the variants (used as alleles) for each individual chromosome region, as well as for the population as a whole, incorporating all 14 variable regions (Table 10).

When this method was applied to compare allelic frequencies from the Sarasota population with those in Matagorda Bay, similarity index values calculated for each variable region ranged from 0.73 to 1.00. The combined index was 0.88, which, when expressed as a probability, is interpreted as the chance of drawing identical alleles from the two different populations being only 0.88 as great as it would be if they were drawn from one population.

When Matagorda Bay was sub-divided into the nine animal (NE group) versus the twenty-six animal (SW group) subgroup, similarity index values ranged from 0.81 to

1.00 with an overall similarity index value between the two groups of 0.95. When the allelic frequencies among the Matagorda Bay males were compared to those found among the Matagorda Bay females, similarity index values range from 0.91 to 1.00 with an overall similarity index between these two subgroups of 0.97.

Evaluation of Maternity and Paternity

A. Maternity Analysis

Of the thirty-six animals involved in the study, five pairs of dolphins were thought to be mother and offspring pairs based on behavioral observations made during the capturing process. These potential mother and calf pairs, and the estimated ages of each, are shown in Figure 5.

Chromosome heteromorphisms in cetaceans have been shown to be inherited in a stable manner and can therefore serve as a method for exclusion of a female as mother of a particular calf (Duffield and Chamberlin-Lea, 1990). While the numerical values assigned to the individual variants of a potential mother and calf pair can be compared to one another to evaluate potential maternity (see Table 2), more frequently the evaluation is performed by simultaneously viewing multiple sets of cells from both the mother, as well as the suspected

offspring. This direct visual comparison allows for detection of subtle differences which are not revealed by the numerical value.

Three adult females (FB503, FB511 and FB521) suspected of being the mothers of calves were excluded as mothers using chromosome variant analysis. All three animals had variants at at least one locus which were not similar to the variants identified in the assigned calf, positively excluding the adult female as mother of the calf. An illustration of the variant differences between the discrepant pairs is shown in Figure 6.

One adult female (FB515) could not be excluded as the mother of calf FB517 on the basis of chromosomal variant inheritance due to the poor quality of cells analyzed in FB517. One pair, FB507 and FB509, had chromosome variants which were consistent with an inheritance pattern of mother-to-calf.

When the five sets of suspected mother-calf pairs were examined for similarity values (S), FB503 and FB508 had a similarity coefficient of 0.491, less than the population mean and also below the 0.500 level which would be the minimum similarity value expected when calculated for a parent and offspring pair. The four remaining pairs of animals (FB507/FB509; FB511/FB513; FB515/FB517; FB521/FB520) ranged from 0.536 to 0.714 (see

Table 11). An illustration showing the relationship between S-values for suspected mother-calf pairs, in relation to the distribution of S-values for the entire Matagorda Bay population is shown in Figure 7.

Z-scores were used to measure the distance of each mother-calf pair similarity coefficient from the average similarity value calculated for the entire Matagorda Bay population. Two of the potential parent-offspring pairs had negative Z-scores, reflecting that they fall below the mean similarity value for the population. Two pairs have Z-scores between zero and one, indicative of their values falling within one standard deviation of the mean. The fifth pair, FB507 and FB509 have a Z-score of 1.47, indicating that this pair has a similarity coefficient greater than one standard deviation above the population average. A list of the Z-scores for the five suspected mother-calf pairs is shown in Table 11.

B. Paternity Analysis

Previous observational and genetic studies of captive *Tursiops* populations have shown that males over the age of 20 years typically sire the majority of calves born into captive breeding groups (Duffield and Wells, 1991). In the Matagorda Bay population, three males (FB516, FB530 and FB532) were estimated to be approximately 20 years old at the time of their capture.

Of these, one (FB516) had an unusual combination of variants for three of the acrocentric chromosomes, being homozygous for a value of '1' at chromosome pairs 19, 20 and 21. Based on the frequencies of a '1' value for each of these acrocentric chromosomes in the sampled population, there is a 0.015% chance of an animal being homozygous for a '1' allele at all three loci. He was excluded as a potential sire of all five calves in the Matagorda Bay study with this variant combination.

The other two adult males, FB530 and FB532, were also excluded as sires of the five calves based on their combinations of variants. Both of these males were part of a group of ten individuals not resighted after the initial capturing period. Furthermore, they were captured in a part of the study area where none of the calves were captured or subsequently observed.

Marker Chromosome

A small extra chromosome was found to be present in a majority of the cells examined from one animal, FB534, in the Matagorda Bay study. A composite set of FB534's variable chromosomes, including the small marker chromosome (labeled 'mar'), is found in Appendix 2. Eight cells out of eleven photographed and examined from FB534 had the extra chromosome present.

No other animal in the Matagorda Bay sample was shown to have any supernumerary chromosomes present. FB534, the male with the marker chromosome, was estimated to be 4-6 years of age at the time of capture and therefore was too young to have sired any offspring.

Discussion

Behavioral Observations of Sample Population

In the Matagorda Bay study, ten individuals were radio-tagged and subsequently tracked for periods ranging from twelve to sixty days. Six of the ten radio-tagged animals stayed within an approximate twenty km radius area of where they were first caught. Four other tagged animals (FB501, FB502, FB504 and FB505) showed greater ranges. FB501, an adult female, showed the least amount of site fidelity, occasionally traveling 55 km over a twelve hour period to traverse her home range (Würsig and Lynn, 1993). Home ranges were found to overlap strongly for all animals in the study with the exception of the 10 animals (FB523-FB532) who were not resighted again after capture. In comparison, the entire Sarasota Bay *Tursiops* population inhabits a home-range area approximately 40 km long (Scott et al., 1990).

Animals in the Matagorda Bay study who were resighted after the initial capture were not observed

leaving the bay area to enter oceanic waters either for feeding or for other purposes. Similarly, at no time did any of the radio-tagged dolphins show evidence of leaving the confines of the bay system to swim in the open ocean. These observations allow for speculation that these animals are members of a resident, in-shore population, similar in structure and composition to the Sarasota Bay *Tursiops* population. The remaining ten animals that make up the Matagorda Bay sample were not resighted after the capturing process, so their use of ocean areas and neighboring in-shore areas is unknown.

Based on observational data collected during the study period, it was suspected that the Matagorda Bay area represented a major geographic area enclosing most of a breeding population. Some observations, such as noting that the animals did not appear to leave the bay system for any length of time, seemed to support this idea. Other observations, including the inability to resight one group of ten animals after the tagging procedure, seemed to suggest that the Matagorda Bay population was not necessarily closed to migration or that Matagorda Bay animals are members of a series of subpopulations with overlapping home regions. Genetic analysis of dolphins inhabiting this coastal region of

the Gulf of Mexico provides additional information about the population structuring of *Tursiops* within this Bay.

R-Banded Chromosome Variation as a Method for Comparing Populations

Previous cytogenetic analysis of a *Tursiops truncatus* population residing near Sarasota, Florida, provided an out-group for evaluating the cytogenetic variation which existed within the Matagorda Bay population. These comparisons together set baseline values for future comparisons of intra- and interpopulation structuring of *Tursiops* in Matagorda Bay and throughout the Gulf of Mexico.

By treating each chromosome variant as an individual allele, population genetic analyses used to evaluate polymorphisms in populations can be applied to the cytogenetic data. Three different quantitative analyses were used to examine the cytogenetic information compiled for the Matagorda Bay and Sarasota animals.

The first method applied the concept of Hardy-Weinberg equilibrium, where the distribution of variants at each chromosome region were evaluated for compliance to Hardy-Weinberg equilibrium expected values. Populations of animals were evaluated both as separate groups and then as pooled populations. The relative size of deviation of observed from expected values (as

measured by G-statistics) was used to estimate whether the pooled populations were similar to individual groups; pooled groups with larger G-values than individual groups, implies subgroup structure.

Secondly, a pair-wise similarity index was calculated to compare the relative amount of variants shared between any two animals. This method has most commonly been applied to DNA analyses as a method to compare band-sharing of DNA restriction fragments between animals in a population. Previous analysis of S-values applied to molecular studies have shown that large S-values are indicative of first generation relatives (Bishof and Duffield, 1994).

A third analytical method applied to the data gathered in this study involved the calculation of Nei Genetic Distance Similarity Index values to assess the "distance" of polymorphism differences between populations or subpopulations.

a. Hardy-Weinberg Equilibrium Estimates

Minkoff (1983) explains that if a population is divided into genetically separate subpopulations which mate randomly within themselves, each subpopulation can establish a Hardy-Weinberg equilibrated state. If the allelic frequencies from these subpopulations are pooled

and treated as a single population, an excess number of homozygotes would be observed. This effect, called Wahlund's principle, may explain the observance of excess homozygous individuals found within populations.

When the Matagorda Bay and Sarasota samples were pooled and treated as a single population, three loci had G-scores indicating significantly different observed values from expected values ($p < 0.05$), with each loci deviating due to an excess number of homozygous individuals. These two populations, known to have different allelic frequencies and to be fairly geographically isolated, show an increased number of homozygous individuals when pooled and treated as a single population. The Wahlund's effect suggests that, indeed, these two areas represent separate populations.

With regard to within region sub-structure, when genotypic and allelic frequencies for chromosomal variants found in the Matagorda Bay and Sarasota populations were treated individually and compared to Hardy-Weinberg equilibrium expectations, most variable regions had G-scores which revealed no significant deviations of observed values from expected values ($p > 0.05$). However, for the Sarasota population, two regions, 1q and 2p, had excessive numbers of homozygotes ($p < 0.05$). The Sarasota sample represents a population

known to consist of multiple interacting subgroups and also appears to follow Wahlund's principle.

A similar deviation from expected values, due to excessive numbers of homozygotes was described in the Matagorda Bay females for chromosome region 2q, and may suggest that the Matagorda Bay female subgroup also consists of female members from two or more different groups that are inhabiting the same or overlapping home ranges in this area.

When animals from Matagorda Bay were evaluated as a population as a whole, divided into male/female subgroups, or as NE/SW subgroups, statistically significant deviations from expected Hardy-Weinberg values in these three cases were attributable to an increased number of heterozygous individuals. Such deviations may be due to migration of animals into and out of the Matagorda Bay area, with reproductive exchange, suggesting that multiple subgroups of animals inhabit this overall region. In previous studies of *Tursiops* populations, males have been shown to exhibit the least amount of site fidelity and may be responsible for a significant degree of reproductive exchange between subgroups (Duffield and Wells, 1991).

b. Population Pooling

To further assess whether this sampling of animals from Matagorda Bay was representative of a single interbreeding group, or made up of two or more subgroups, the Winans and Jones (1988) technique of comparing Hardy-Weinberg equilibrium in individual groups versus pooled expectations was applied. The pooling of allelic and genotype frequencies between Matagorda Bay and Sarasota populations was used as a standard to see how two geographically separated populations would 'react' when pooled. It also provided an important calibration of the amount of deviation to be found between populations for a comparison of the degree of subgroup difference within the Matagorda Bay "population".

Calculations revealed that at seven of fourteen loci (50%), the pooled G-statistic values for Matagorda Bay and Sarasota exceed the individual G-statistic values for each population alone. Given the geographical separation of these two populations, this value may represent the maximum amount of dissimilarity which will be found between geographically separated populations inhabiting the Gulf of Mexico.

When a pooled population calculation was performed on Matagorda Bay males versus females compared to pooling the two groups, it was found that deviations from expected values, as measured by G-statistics, were larger

at four of fourteen loci (28.6%) for the pooled population over the individual male and female sets. Due to the nature of dolphin social interactions--i.e., the strong associations that are formed among female members and the male-only bands which are often present in populations--the male and female groups sampled from Matagorda Bay may indeed be representative of two or more neighboring subgroups.

Four of fourteen (28.6%) G-values from the pooled population were larger than both individual G-values calculated for the NE versus SW groups. This provides further evidence for potential subgroup separation existing within the Matagorda Bay region. The degree of deviation of pooled values (male versus female and NE group versus SW group) however, suggests that the differences between the local subgroups are considerably less than between the geographically separated populations. This supports the interpretation that genetic exchange between these local subgroups is greater, as would be expected by their sharing of a home range, than between geographically separate populations.

c. Pair-Wise Similarity Coefficients

Similarity coefficients were calculated for all pairs of animals from within the Matagorda Bay sample.

When average similarity values for males versus female comparisons were made, no substantial differences in values were noted. In addition, a mean S-value comparison made between the NE and SW subgroups also showed no substantial difference in values.

Similarity values were, however, useful for evaluating potential mother-calf pairs, as well as possible father-calf pairings. It has been suggested by previous studies, employing DNA techniques, that high similarity values may indicate first degree relatives. In the Matagorda Bay study, none of the mother-calf or father-calf pairs showed a substantially higher S-value than any other pairs of animals examined from within the population.

d. Nei Index for Measuring Allelic Frequency Differences

The similarity index value, calculated to compare allelic frequencies between Matagorda Bay and Sarasota, was 0.88. This value was smaller than either of the similarity index values calculated to compare the Matagorda Bay intrapopulation comparisons. The similarity index for males versus females was 0.97 and the index value for the NE versus SW subgroups was 0.95. These values suggest that the subgroup separation characterizing the Matagorda Bay sample is not as great

as the genetic separation found between Matagorda Bay and Sarasota. Such a finding is not unexpected, given the close proximity of the Matagorda Bay subgroups as compared to the large geographic distance which separates the Sarasota and Matagorda Bay resident groups, and it fits with the relative differences in pooled values found in the Hardy-Weinberg equilibrium population pooling approach. Furthermore, this finding suggests that the local subgroup differences are there, but at substantially reduced levels, as compared to values found between geographically separated populations.

e. Unique Variants

Identification of unique chromosomal variants also gives some idea of the amount of genetic differentiation separating any two groups of animals. An extreme example of the usefulness of this evaluation technique would be the discovery of a variant which is fixed in one subgroup (as in the capuchin monkey studied by Matayoshi et al., 1987) and not found at all in any other subgroups. Such an occurrence would be indicative that a subgroup of animals no longer interacts with neighboring groups.

Eight heteromorphisms identified in the study of chromosomal variants in *Tursiops* from Matagorda Bay were not present in the Sarasota sample. Similarly, six

variants which had previously been identified in the Sarasota *Tursiops* population samples were not found in the Matagorda Bay sample. Therefore, fourteen variants were found exclusively in one population or the other (Figure 8). None of these unique variants were found to be fixed in either population.

For the Matagorda Bay male versus female comparison, it was found that five variants were identified only in male members, while two other variants were seen only in females, yielding seven variants separating these two subgroups. The frequencies of each of these unique variants ranged from 0.025 to 0.10. However, it is possible that each variant would be identified in an animal of each sex if a larger sampling of animals from the region was obtained.

Seven of 48 (14.6%) of the chromosomal variants identified in Matagorda Bay were found only in the 26 animal (SW) subgroup. The frequencies for each of these variants were all relatively low, ranging from 0.02 to 0.08, leading to speculation that these particular variants might be identified in the NE subgroup if a larger number of animals were to be sampled and evaluated. No variants were found to be exclusive to the NE group.

The identification of unique variants in the Matagorda Bay subgroups lends further support to some degree of local subgroup population structure within the Matagorda Bay region. The number of unique variants identified within each Matagorda Bay subgroup is not as great as the number of variants which distinguish the Matagorda Bay animals from the Sarasota population. This is consistent with the idea that there is more genetic exchange occurring between local subgroups than between geographically separated populations.

Although it cannot be concluded, through cytogenetic variant analysis, that the NE group of nine animals not resighted after capture represents a separate population, it is likely that the animals sampled from the NE and SW regions of Matagorda Bay are, indeed, representative of separate subgroups. The analyses of mitochondrial DNA types from this same sample population have shown that these nine animals are, in fact, representative of a genetically separate group (Lenox, in preparation). If this group is genetically distinct from the remainder of the Matagorda Bay sample, the chromosomal variant frequencies between these two neighboring groups may be as similar as they are due to the migration of individuals and reproductive exchange between these subgroups. Further investigation of the degree of

distinction between these two groups will require the sampling of substantially more animals from both suspected groups.

An extra marker chromosome was identified in one male ($2n=45$) sampled from the Matagorda Bay region. A structurally similar marker chromosome was previously identified in the resident dolphin community in the Sarasota area. It was shown to be inherited in a stable manner, as it was transmitted from mother to calf (Duffield and Wells, 1991). Further banding studies of this chromosome revealed that it was a bi-satellited chromosome composed of two nuclear organizing regions (Duffield and Wells, 1991).

Ten animals from the Sarasota community were found to have the supernumerary chromosome. Mitochondrial DNA studies conducted on the Sarasota animals who had the extra marker chromosome revealed that these animals were of three different mtDNA types, suggesting that the marker had been passed by males to females of different maternal lineages (Duffield and Wells, 1991).

The discovery of a marker chromosome in an animal residing near Matagorda Bay supports the idea of possible transmission of chromosome variants from one population to another throughout the entire Gulf of Mexico region. The resolution of whether this is due to movement of

individual animals (probably males) between distant populations or the spread of variants from one local subgroup to another in a clinal fashion will require further field studies of dolphin populations in the Gulf of Mexico. FB534, the Matagorda Bay male with the marker chromosome, was a young male (4-6 years old) and was resighted only once after the initial capturing period.

f. Evaluation of Mother/Calf and Father/Calf Pairs

When five sets of suspected mother-calf pairs were evaluated using chromosomal variants, three pairs were directly excluded through identification of variants in the potential mother which were not found in the suspected offspring. Because chromosome variants are inherited in a Mendelian fashion, the non-identification of a variant in a calf precludes maternity.

In one pair of animals, the poor quality of cells analyzed in the calf did not allow for absolute exclusion of the potential mother, but the pair-wise S-value between this pair was low (0.536), suggestive of non-maternity.

For the fifth set of suspected mother/calf pairs, the variants present were consistent with that of a mother-calf pair. This pair, FB507 and FB509, also had the highest similarity coefficient ($S=0.714$) of the suspected mother-calf pairs. However, based on tooth-

aging methods, FB507 (the suspected mother) was estimated to be 4 to 6 years of age at the time of capture, and FB509 was estimated to be three years of age. Since bottlenose dolphin females are thought to reach sexual maturity (puberty) at five to seven years of age, and reproductive maturity (characterized by attainment of full adult size and maintenance of regular estrus cycles) at seven to ten years of age (Schroeder,1990), it would seem highly unlikely that FB507 and FB509 are in fact, a mother and calf pair. It is possible, given the high degree of variant similarity between these two individuals, that they are siblings or half-siblings. Other genetic methods, including DNA fingerprint analysis and protein electrophoresis analysis, might provide additional genetic information to further test this relationship.

The importance of using a variety of methods, genetic as well as observational, to study wild populations is emphasized by this mother-calf issue. Based on capture observations and association data collected during the study period, five pairs of animals were assumed to be mothers and calves (Würsig and Lynn,1993). In three of five cases, the chromosomal data, however, did not confirm this, suggesting that

other explanations of behavioral associations need to be formed.

When the three oldest males in the Matagorda Bay population were analyzed with respect to transmission of variants to the five suspected calves identified in the sample, all three males could be excluded from paternity of these calves on the basis of their variant combinations. Chromosome heteromorphism analysis is, therefore, also useful in investigating paternity within these social groups.

Analysis of Techniques To Assess Genetic Differences

This study has demonstrated that by calculating the number of chromosome variant regions which differ at a statistically significant level from values expected under the Hardy-Weinberg equilibrium law, and the magnitude of those discrepancies, two different population evaluations can be made. The first evaluation, identifying which variants are not within equilibrium expectations and interpreting why they are discrepant (i.e., too many heterozygous or homozygous individuals) can be used to assess general population structure. If multiple variable regions are found to be out of equilibrium status, due to an increased number of homozygous individuals, it may be indicative of a high

degree of in-breeding, or alternatively, an indication that two separate groups have erroneously been treated as a single group and Wahlund's effect is in operation. If an increased number of heterozygous individuals are found, it may be that the sample population is not closed to immigration or emigration, and animals are freely and randomly mating with animals from neighboring regions.

The second evaluation of population structure addresses whether a sample population is made up of two or more separate subgroups by a comparison of "pooled" equilibrium values.

When Winans and Jones (1988) used a population "pooling" method on populations of Dall's porpoise to assess whether they had sampled from one interbreeding but spatially separated group of animals or from two isolated non-interacting groups of animals, there was no established standard of how large the differences between pooled and individual values would be for known isolated populations of animals. This was because this study was initiated on a group of animals which were previously not studied, either behaviorally or genetically. Winans and Jones (1988) concluded that, because 7 of 9 variable loci (77.7%) studied had pooled values which exceeded those of each group when treated individually, the groups did not represent one interbreeding population. Because of the

ability to compare chromosome heteromorphisms in both the Sarasota and Matagorda Bay populations with variation within the Matagorda Bay study, a scale has been developed by which to measure relative differences between populations and subpopulations.

Pair-wise similarity coefficients provide useful gauges for the determination of relatedness among any two animals, but they are not precise enough to discriminate between subgroups residing in adjacent regions. Groups of animals occupying neighboring territories with a significant exchange of chromosome variants will presumably exhibit relatively high similarity values between animals.

When chromosomal variants are treated as alleles, polymorphic analyses such as the Nei Similarity Index can be used to quantify the differences in chromosomal variants from one group to another. This method appears to be a valuable technique for assessing the degree of genetic difference between intra- and interpopulation comparisons. When the technique was applied to compare the Matagorda Bay population chromosomal variants to those identified in Sarasota population, a value of 0.88 was calculated. This value is smaller than both intrapopulation comparisons made from within Matagorda Bay. Such a finding would be expected since the

Matagorda Bay/Sarasota comparison represents a comparison of two geographical isolated groups, while a subgroup comparison is made from within one localized region. Therefore, similarity index values based on chromosome heteromorphisms are useful for establishing a "scale" of the genetic differences between breeding groups and populations of bottlenose dolphins.

Using these three methods of analysis, it was found that the genetic differences existing between animals sampled from the Matagorda Bay region were less than differences that existed between Matagorda Bay and Sarasota, the two populations assumed to be the most genetically separate due to their large geographic isolation from each other. This result is not surprising since the Matagorda Bay subgroups, residing in close proximity to one another, are not obviously separated geographically from one another. These results underscore the fact that it is possible to detect genetically differing subgroups which occupy a single geographic location.

Thesis Conclusions

To date, no other cytogenetic studies have attempted to quantify the results of chromosome heteromorphism analysis in populations. Also, methods of quantifying

genetic data generally applied to studies involving DNA fingerprinting, protein electrophoresis variability and mtDNA analysis have not previously been evaluated for their applicability to chromosome variant analysis. When combined with observational and behavioral data, analysis of chromosomal variants provides a means of assessing the overall structure of wild populations. Each of the quantitative analyses used in this study indicates that there is potential subgroup separation present in the Matagorda Bay population area. It is, therefore, likely that because the Matagorda Bay captures occurred in several different areas encompassing a larger local geographic region, rather than occurring at a single, centralized location, sampling of multiple subgroups occurred.

Cytogenetic analysis of 36 bottlenose dolphins from the Matagorda Bay area revealed that:

- ♦ The Matagorda Bay *Tursiops* population most likely does not represent a single breeding unit or population, but instead consists of subgroups within a larger, geographically dispersed *Tursiops* population. This population may be inclusive of several neighboring bays, or it may encompass the entire Gulf of Mexico.

◆ Chromosomal variants not seen before in other *Tursiops* studies were found in Matagorda Bay. However, a number of variants were found in both Sarasota and Matagorda Bay populations, with no fixed variants found exclusively in either population. This may indicate that a series of local, but inter-mixing populations occurs with variants being transmitted from core area to core area via individual animals that do not maintain core site fidelity. It should be noted, however, that the sample populations from both Sarasota and Matagorda Bay most likely represent only in-shore forms of *Tursiops*. Due to the nature of the capturing process (i.e. having to locate and capture animals in water shallow enough for humans to stand in), the samples may not be indicative of more pelagic, possibly migratory, groups of dolphins that inhabit the deeper waters of the Gulf of Mexico. Therefore, the Matagorda Bay study area and the Sarasota Bay study area probably represent home-range situations of coastal *Tursiops* populations which are separated by numerous other *Tursiops* groups, each inhabiting their own stretch of Gulf of Mexico coastline. Adult male animals, the least likely members of a *Tursiops* population to maintain site

fidelity, may travel from home-range to home-range, helping to transmit variants from one area to another. Females, as exemplified by animal FB501 who traveled up to 55 km in a 24-hour period, may also be responsible for transmitting some genetic data from one region to another. Further analysis of mtDNA may clarify the extent of this transmission.

◆ The importance of combining observational data with cytogenetic information is emphasized by the evaluation of mother and calf pairs in this study. Cytogenetic analysis allowed for the exclusion of three suspected mothers in predicted mother-calf pairings. In one other pairing, a relatively high similarity value was described and the inheritance of variants was consistent with that expected of a mother-calf pair. However, tooth-aging analysis suggests that this pair of animals may be siblings or other first degree relatives.

◆ All three analytical methods used to evaluate cytogenetic differentiation showed consistent results when applied to inter- and intrapopulation comparisons. When analyses were used to evaluate the differences between two known geographically separated groups, the

results obtained consistently showed greater differences than when the same analyses were applied to groups taken from within the Matagorda Bay population. All three quantitative methods, therefore, proved valuable as methods for assessing cytogenetic data.

◆ These analyses suggest that future studies of *Tursiops* residing in the Matagorda Bay area should focus on sampling larger number of animals from locations flanking Matagorda Bay. By focusing on sampling animals from locations which are at least 55 km apart (a figure representing the farthest distance traveled by any single individual in this sample), the chances of sampling members of one subgroup or another exclusively, should increase.

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APPENDIX 1

Blood Culture Procedure

1. Three to five mls. sodium heparinized whole blood are obtained from each dolphin to be studied.
2. Seven drops from a pasteur pipette of whole blood are added to each of three tubes (labelled PHA, PK and PK+PHA) containing four mls. of RPMI 1640 culture media supplemented with 15% fetal calf serum, 1% penicillin-streptomycin and either 0.05 ml phytohemagglutinin (PHA culture), 0.05 ml pokeweed (PK culture) or 0.05 ml phytohemagglutinin and 0.05 ml pokeweed (PK+PHA culture).
3. The cultures are incubated 36.0 degrees C for 96 hours.
4. Sixteen hours prior to harvest, 0.05 mls colcemid (Gibco) are added to each culture.

References

This method adapted from Blood Culture Procedure protocols at Dr. Debbie Duffield's laboratory at Portland State University and the Oregon Health Sciences University Clinical Cytogenetics Laboratory.

Blood Harvest Procedure

1. Centrifuge each culture, after sixteen hour exposure to colcemid, for ten minutes at 1000 rpm; remove supernatent.
2. Add five mls. of 0.075 M KCl* to each tube and resuspend cell pellet; centrifuge immediately for ten minutes at 1000 rpm.
3. Remove all but 0.5 cc of supernatent and resuspend pellet in remaining supernatent with a pasteur pipette. Add two mls. of fixative (3:1 ethanol:glacial acetic acid) and gently but rapidly mix with the cell suspension. When this is accomplished, add another two mls. of fixative, mix well and centrifuge for ten minutes at 1000 rpm.
4. After this last centrifugation, dilute the cell pellet with fresh fixative and make slides.

References

This method adapted from Blood Harvest Procedure protocols at Dr. Debbie Duffield's laboratory at Portland State University and the Oregon Health Sciences University Clinical Cytogenetics Laboratory.

*0.075 M KCl=5.59 g/L

Fluorescent R-Banding

Dye Solutions:

Buffer: Dilute 50 mls. of McIlvane's buffer, pH 7.0, with 50 mls. of distilled water and add 0.102 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ for a final concentration of 5mM.

Chromomycin A₃ (CA₃): A 0.5 mg/ml solution of chromomycin A₃ is made up in the above buffer.

Distamycin A (DA): A 0.1 mg/ml solution is made up in the above buffer.

1. Place 5 drops of Chromomycin A₃ on slide and add coverslip. Keep slide in the dark for twenty minutes.
2. Rinse slide with McIlvane's buffer and shake off excess.
3. Place 5 drops of Distamycin A onto slide and add a coverslip. Keep in the dark for 5 minutes.
4. Again, rinse slide, blow dry with jet of air and mount a clean coverslip with glycerol.
5. Examine the slide on a fluorescent microscope using a 490 excitor, 510 reflector and 520nm barrier filters or their equivalents.

This method adapted from R-Banding Procedure protocols at Dr. Debbie Duffield's laboratory at Portland State University and the Oregon Health Sciences University Clinical Cytogenetics Laboratory.

R-Band Reagents:

Chromomycin A3:

Stock solution is 2.0 mg/ml

To arrive at 4.0 mls. of 0.5 mg/ml:

1.0 ml CA_3

3.0 mls. McIlvane's buffer (working solution with $MgCl_2$)

Distamycin A:

Dissolve 0.2 mg Distamycin A in 2-3 drops methanol.

Add 2.0 mls. McIlvane's buffer (with $MgCl_2$).

McIlvane's Buffer:

Solution A:

0.2 M dibasic sodium phosphate

Made by dissolving 28.38 g into 1.0 L deionized water

Solution B:

0.1 M citric acid

Made by dissolving 21.01 g into 1.0 L deionized water

Stock Solution-McIlvane's Buffer (pH 7.0):

Combine 164.7 mls. of solution A with 35.3 mls. solution B.

Working Solution-McIlvane's Buffer:

Combine 50.0 mls. stock solution with 50.0 mls. deionized water and add 0.102g $MgCl_2 \cdot 6H_2O$ for a final concentration of 5mM.

APPENDIX 2

Table 1: Summary information of Matagorda Bay Animals

Name	Sex	Age (years)	Capture location*	Date of Capture
FB501	Female	12-20	f	7/10/92
FB502	Male	10	j	7/9/92
FB503	Female	10-12	g	7/10/92
FB504	Male	10-12	j	7/9/92
FB505	Female	6-8	m	7/11/92
FB506	Male	8	k	7/9/92
FB507	Female	4-6	l	7/11/92
FB508	Male	2	g	7/10/92
FB509	Female	3	l	7/11/92
FB510	Male	2	l	7/11/92
FB511	Female	12-20	g	7/12/92
FB512	Male	4-6	l	7/11/92
FB513	Female	1	g	7/12/92
FB514	Male	12	g	7/14/92
FB515	Female	8-10	g	7/14/92
FB516	Male	20	i	7/14/92
FB517	Female	2	g	7/14/92
FB518	Male	8	h	7/15/92
FB519	Female	8-10	i	7/14/92
FB520	Male	2	h	7/15/92
FB521	Female	6-8	h	7/15/92
FB522	Male	5-7	e	7/17/92
FB523**	Female	20-40	b	7/17/92
FB524**	Male	5-7	d	7/17/92
FB525**	Female	3-4	b	7/17/92
FB526**	Male	6-8	d	7/17/92
FB527**	Female	6-8	b	7/17/92
FB528**	Male	3-5	d	7/17/92
FB529**	Female	4	c	7/18/92
FB530**	Male	20-40	a	7/18/92
FB531**	Female	3-4	c	7/18/92
FB532**	Male	20-40	a	7/18/92
FB534	Male	4-6	g	7/19/92
FB536	Male	4-6	h	7/19/92
FB538	Male	5-7	h	7/19/92
RT412	Male	2	g	7/10/92

Table 1, key:

* Refer to figure 1 for map of capture locations

** Members of a group of animals caught in the Northeastern portion of the study area. These animals were not resighted again after the capturing and tagging procedure.

Animal	Chromosome Region														Y
	1p	1q	2p	2q	3p	3q	4p	4q	5p	17q	18p	19p	20p	21p	
FB501	11	22	22	22	12	11	11	12	11	22	11	22	22	22	1
FB502	12	12	22	22	12	11	11	11	11	11	11	22	03	1s2	1
FB503	12	23	33	22	22	11	23	12	12	12	11	22	33	22	
FB504	12	12	12	22	11	12	12	11	11	11	12	12	12	2s2s	1
FB505	22	12	12	22	11	11	12	11	11	11	12	22	02	2s2	
FB506	22	12	22	22	22	12	12	22	22	12	01	22	1s3	1s1	1
FB507	11	22	12	13	12	12	01	11	11	12	12	13	23	2s2	
FB508	12	12	22	22	12	12	22	11	11	11	12	22	55	33	1
FB509	11	12	22	33	11	11	02	11	11	12	12	23	23	22	
FB510	11	11	12	23	11	11	11	11	11	22	12	13	02	23	1
FB511	11	12	22	22	12	11	22	11	12	12	22	22	33	22	
FB512	11	22	22	22	12	11	11	11	12	12	12	23	13	12	1
FB513	12	12	11	22	11	11	11	11	11	22	12	22	23	12	
FB514	12	12	33	12	12	12	01	11	11	22	12	11	1s1	2s1	1
FB515	12	22	33	22	12	11	22	12	22	22	12	22	3s3	22	
FB516	11	11	11	22	22	11	01	11	11	12	12	11	11	11	2
FB517	11	11	12	22	22	11	02	12	12	11	12	12	03	12	
FB518	11	23	22	23	22	12	12	12	22	22	12	22	55	24	1
FB519	12	11	22	11	12	11	12	11	11	11	12	22	12	1s1	
FB520	11	11	22	22	12	11	12	12	12	22	11	02	03	12	1
FB521	12	22	11	22	11	11	12	11	22	12	11	12	02	1s1	
FB522	11	12	22	23	12	11	02	11	12	12	12	12	22	22	0
FB523	11	12	22	22	12	11	12	12	22	22	12	12	13	23	
FB524	11	12	22	23	12	12	12	11	12	11	11	22	23	34	1
FB525	22	12	12	22	11	11	22	11	22	22	12	11	22	1s1	
FB526	11	22	12	22	12	12	02	11	12	11	12	13	22	12	1
FB528	12	12	22	22	22	12	11	11	11	11	12	12	01	22	1
FB529	12	11	22	22	12	11	11	11	12	12	12	12	11	11	
FB530	11	11	22	23	12	12	12	11	22	22	12	12	23	23	0
FB531	12	11	22	23	12	11	22	12	22	22	12	11	22	2s2	
FB532	12	12	23	12	22	12	11	12	12	12	12	12	23	23	2
FB534	11	22	12	22	12	11	12	11	12	12	12	22	12	12	1
FB536	12	22	12	22	12	22	11	11	12	12	22	12	12	23	1
FB538	12	22	22	12	12	11	12	11	12	11	12	23	23	12	1
RT412	12	23	23	23	12	12	12	22	22	22	12	12	33	22	2

Table 2: Summary of chromosomal variants for each of the 35 animals studied.
For each variable region, an animal is assigned two values, one for each homolog of a pair.
A score of "12" indicates a value of "1" was assigned to one homolog, and a "2" value was assigned to the other.

Chromosome Region						
1p	1q*	2p	2q	3p	3q	4p*
1-1 16	1-1 8	1-1 3	1-1 1	1-1 7	1-1 22	0-1 3
1-2 16	1-2 15	1-2 9	1-2 3	1-2 21	1-2 12	0-2 4
2-2 3	2-2 9	2-2 18	1-3 1	2-2 7	2-2 1	1-1 9
	2-3 3	2-3 2	2-2 22			1-2 13
		3-3 3	2-3 7			2-2 5
			3-3 1			2-3 1
4q	5p	17q	18p*	19p*	20p*	21p*
1-1 24	1-1 13	1-1 10	0-1 1	0-2 1	0-1 1	1-1 2
1-2 9	1-2 13	1-2 13	1-1 6	1-1 4	0-2 3	1-2 7
2-2 2	2-2 9	2-2 12	1-2 26	1-2 11	0-3 3	2-2 8
			2-2 2	1-3 3	1-1 2	2-3 5
				2-2 13	1-2 4	2-4 1
				2-3 3	1-3 2	3-3 1
					2-2 5	3-4 1
					2-3 7	1s-2 1
					3-3 3	2s-2s 1
					5-5 2	2s-2 3
					1s-1 1	1s-1 4
					1s-3 1	2s-1 1
					3s-3 1	

Table 3: Distribution of variant genotypes among Matagorda Bay sample population
 *Only those variant combinations which were present in the sample population are listed.

Chromosome Region					
1p	f(1)=0.69 f(2)=0.31	1q	2p	2q	3p
3q	f(1)=0.80 f(2)=0.20	4p	4q	5p	17p
18p	f(0)=0.014 f(1)=0.557 f(2)=0.429	19p	20p	21p	

Table 4: Frequencies of individual chromosomal variants for Matagorda Bay population

Chromosome Region		Location	Genotype						Allelic Frequencies				Hardy-Weinberg*								
			0/1	0/2	1/1	1/2	2/2	2/3	3/3	1/3	n	0	1	2	3	Individual G-score	Pooled G-score				
1p		Mat. Bay Sarasota			16 11	16 41	3 11								35 63	0.69 0.50	0.31 0.50			0.13 5.91	3.93
1q		Mat. Bay Sarasota			8 38	15 10	9 8	3 2							35 58	0.44 0.74	0.51 0.21	0.04 0.05		1.44 19.19	14.74
2p		Mat. Bay Sarasota			3 34	9 7	18 20	2 3							35 61	0.21 0.62	0.67 0.38	0.11		2.97 38.22	40.53
2q		Mat. Bay Sarasota			1 5	3 24	22 23	7 10	1 1						35 63	0.09 0.28	0.77 0.64	0.14 0.08		1.15 1.77	0.36
3p		Mat. Bay Sarasota			7 60	21 4	7								35 64	0.50 0.97	0.50 0.03		1.42 0.04	3.62	
3q		Mat. Bay Sarasota			22 64	12	1								35 64	0.80 1.00	0.20		0.20 0.00	0.55	
4p		Mat. Bay Sarasota	3	4	9 60	13 1	5	1							35 61	0.10 0.99	0.49 0.01	0.01	0.25 0.06	14.81	
4q		Mat. Bay Sarasota			24 56	9 6	2								35 62	0.81 0.95	0.19 0.05		0.71 0.18	1.22	
5p		Mat. Bay Sarasota			13 49	13 7	9 1								35 66	0.56 0.07	0.44 0.86	0.07	2.14 0.00	4.24	

Table 5: Results of Hardy-Weinberg tests--comparison of Sarasota population with Matagorda Bay. Genotypic and allelic frequencies are presented for variable chromosome regions for the two groups

* Hardy-Weinberg calculations based on three genotypic classes: 1) Most common allele homozygotes; 2) Most common allele heterozygotes; 3) All other homozygotes + all other heterozygotes. (d.f.=2).

Bold values for G-scores indicate statistically significant deviations from expected values (p<0.05).

Chromosome		Genotype*														Allelic Frequencies										Hardy-Weinberg**	
Region	Location	0/1	0/2	0/3	1/1	1/2	1/3	2/2	2/3	3/3	5/5	1/4	1/5	2/5	n	0	1	2	3	4	5	1s	2s	3s	Individual G-score	Pooled G-score	
17q	Mat. Bay				10	13		12							35		0.47	0.53							2.29		
	Sarasota				14	36	1	11							62	0.01	0.52	0.47							2.44	0.07	
18p	Mat. Bay	1			6	26		2							35	0.01	0.56	0.43							12.07		
	Sarasota	1	2		5	23		26	3						60	0.03	0.28	0.67	0.02						0.15	2.65	
19p	Mat. Bay		1		4	11	3	13	3						35	0.01	0.31	0.59	0.09						0.47		
	Sarasota				5	22	1	26	6	1					61		0.27	0.66	0.08						0.00	0.55	
20p	Mat. Bay	1	3	3	2	4	2	5	7	3	2		3		35	0.10	0.17	0.34	0.29	0.06	0.03		0.01	0.44			
	Sarasota				2	22	10	11	10	2			3		60		0.31	0.46	0.21			0.02	0.01	1.05	0.02		
21p	Mat. Bay				2	7		8	5	1		2	10		35	0.23	0.47	0.11	0.03			0.07	0.09	0.03			
	Sarasota					12	5	14	13	2			11	1	58	0.15	0.53	0.19		0.01	0.10	0.06	0.07	2.39	1.88		

Table 5: Results of Hardy-Weinberg tests--comparison of Sarasota with Matagorda Bay. Genotypic and allelic frequencies are presented for variable chromosome regions for the two groups

* Genotype classes are pooled: All "4" heterozygotes as well as all satellite-bearing homozygotes / heterozygotes are pooled together
 **Hardy-Weinberg calculations based on three genotypic classes: 1)Most common allele homozygotes; 2)Most common allele heterozygotes; 3)All other homozygotes + all other heterozygotes. (d.f.=2).
 Bold values indicate values which show significant deviations from expected values (p<0.05).

Sex	n	Genotype			Allelic Frequencies			Hardy-Weinberg* G-score**
		0/1	1/1	1/2	2/2	0	1	2
males	20	1	3	15	1	0.025	0.55	0.425
females	15		3	11	1		0.567	0.433
								8.32
								3.86

Table 6 : Comparison of variant and phenotypic frequencies for male and female populations sampled from Matagorda Bay for chromosome variable region 18p.

*Hardy-Weinberg calculations based on three genotypic classes: 1: Most common allele homozygotes; 2) Most common allele heterozygotes; 3) All other homozygotes + all other heterozygotes (d.f.=2)

**Bold values for G-scores indicate statistically significant deviations from expected values (p<0.05)

Chromosome Region		Sex	Genotype								Allelic Frequencies				Hardy-Weinberg*						
			0/1	0/2	1/1	1/2	2/2	2/3	3/3	1/3	n	0	1	2	3	Individual G-score	Pooled G-score				
1p		Male Female			10 6	9 7	1 2									0.73 0.63	0.27 0.37			0.27 0.01	0.13
1q		Male Female			4 4	9 6	5 4	2 1								.425 0.47	.525 0.50	0.05 0.03		0.19 1.31	1.44
2p		Male Female			1 2	5 4	11 7	2 2	1 2							.175 0.27	.725 0.6	0.1 0.13		0.20 3.03	2.97
2q		Male Female			1	3	11 11	6 1	1 1	1 1						.075 0.1	.775 0.77	0.15 0.13		2.53 9.22	1.15
3p		Male Female			2 5	13 8	5 2									.425 0.6	.575 0.4			2.01 0.21	1.42
3q		Male Female			8 14	11 1	1									.675 0.97	.325 0.03			1.47 0.28	0.20
4p		Male Female			2 1	6 3	9 4	1 4		1						0.1 0.1	.575 0.37	.325 0.50	0.03	0.25 0.06	0.25
4q		Male Female			15 9	3 6	2									.825 0.8	.175 0.2			4.22 1.55	0.71
5p		Male Female			7 6	9 4	4 5									.575 .533	.425 .467			0.46 3.36	2.14

Table 7: Results of Hardy-Weinberg tests--male and female comparison within Matagorda Bay. Genotypic and allelic frequencies are presented for variable chromosome regions for two groups within the Matagorda Bay sample population

* Hardy-Weinberg calculations based on three genotypic classes: 1) Most common allele homozygotes; 2) Most common allele heterozygotes;

3) All other homozygotes + all other heterozygotes. (d.f.=2).

Bold values for G-scores indicate statistically significant deviations from expected values (p<0.05).

Chromosome		Genotype*												Allelic Frequencies										Hardy-Weinberg**	
Region	Sex	0/1	0/2	0/3	1/1	1/2	1/3	2/2	2/3	3/3	5/5	4/4	1/4	1/5	2/5	3/5	4/5	5/5	1s	2s	3s	Individual G-score	Pooled G-score		
17q	Male				7	7		6						20	.525	.475						1.81			
	Female				3	6		6						15	0.4	0.6						0.428	2.29		
18p	Male	1			3	15		1						20	.025	0.55	.425					8.32			
	Female				3	11		1						15	0.57	0.43						3.86	12.07		
19p	Male		1		2	7	2	6	2					20	.025	.325	0.55	0.10				0.00			
	Female				2	4	1	7	1					15	0.30	.633	.067					1.19	0.47		
20p	Male	1	1	2	1	3	1	2	4	1	2			20	0.10	0.20	0.30	0.25	0.10	0.05		0.110			
	Female		2	1	1	1	1	3	3	2				15	0.10	0.13	0.40	0.33		.033		0.345	0.44		
21p	Male				1	5		3	4	1		2	4	20	.225	.425	.175	0.05	0.05	.075		0.327			
	Female				1	2		5	1			6		15	0.23	0.53	.03		0.10	0.10		0.470	0.03		

Table 7: Results of Hardy-Weinberg tests--Male and female comparison within Matagorda Bay. Genotypic and allelic frequencies are presented for variable chromosome regions for two groups within the Matagorda Bay sample population.

* Genotype classes are pooled: All "4" heterozygotes as well as all satellite-bearing homozygotes / heterozygotes are pooled together
 **Hardy-Weinberg calculations based on three genotypic classes: 1)Most common allele homozygotes; 2)Most common allele heterozygotes; 3)All other homozygotes + all other heterozygotes. (d.f. =2).
 Bold values indicate values which show significant deviations from expected values (p<0.05).

Chromosome Region	Locale	0/1	0/2	Genotype						Allelic Frequencies				Hardy-Weinberg*		
				1/1	1/2	2/2	2/3	3/3	1/3	n	0	1	2	3	Individual G-score	Pooled G-score
1p	NE			4	4	1						9	0.67	0.33		
	SW			12	12	2						26	0.69	0.31	0.19	0.13
1q	NE			3	5	1						9	0.61	0.39	0.26	
	SW			5	10	8	3					26	0.37	0.57	0.05	1.44
2p	NE				2	6	1					9	0.11	0.83	0.06	0.61
	SW			3	7	12	1	3				26	0.25	0.62	0.13	3.20
2q	NE				1	5	3					9	0.05	0.78	0.17	1.16
	SW			1	2	17	4	1	1			26	0.10	0.77	0.13	1.94
3p	NE			1	6	2						9	0.44	0.56	1.14	
	SW			6	15	5						26	0.52	0.48	0.64	1.42
3q	NE			4	5							9	0.72	0.28	1.98	
	SW			18	7	1						26	0.83	0.17	0.10	0.20
4p	NE		1	3	3	2						9	0.06	0.50	0.44	1.02
	SW	3	3	6	10	3	1					26	0.12	0.48	0.38	0.00
4q	NE			6	3							9	0.83	0.17	0.61	
	SW			18	6	2						26	0.81	0.19	1.01	0.71
5p	NE			1	4	4						9	0.33	0.67	0.00	
	SW			12	9	5						26	0.63	0.37	1.60	2.14

Table 8: Results of Hardy-Weinberg tests--Intrapopulation comparison within Matagorda Bay. Genotypic and allelic frequencies are presented for variable chromosome regions for two groups within the Matagorda Bay sample population

* Hardy-Weinberg calculations based on three genotypic classes: 1) Most common allele homozygotes; 2) Most common allele heterozygotes; 3) All other homozygotes + all other heterozygotes. (d.f.=2).

Chromosome		Genotype*																Allelic Frequencies										Hardy-Weinberg**	
Region	Locale	0/1	0/2	0/3	1/1	1/2	1/3	2/2	2/3	3/3	5/5	1/4	1/5	2/4	2/5	3/4	3/5	4	5	1s	2s	3s	Individual G-score	Pooled G-score					
17q	NE				3	2		4										0.44	0.56				2.87	2.29					
	SW				7	11		8										0.48	0.52				0.61						
18p	NE				1	8												0.56	0.44				7.36	12.07					
	SW	1			5	18		2										0.02	0.56	0.42			5.71						
19p	NE				2	5	1	1										0.56	0.39	0.05			1.14	0.47					
	SW		1		2	6	2	12	3									0.02	0.23	0.65	0.10		0.58						
20p	NE	1			1		1	3	3									0.06	0.22	0.5	0.22		1.02	0.44					
	SW		3	3	1	4	1	2	4	3	2		3					0.12	0.15	0.29	0.31	0.08	0.04		0.45				
21p	NE				1	1		1	3			1	2					0.22	0.39	0.22	0.06	0.06	0.05	0.26	0.03				
	SW				1	6		7	2	1		1	8					0.23	0.50	0.08	0.02	0.08	0.10	0.13					

Table 8: Results of hardy-Weinberg tests--Intrapopulation comparison within Matagorda Bay.
Genotypic and allelic frequencies are presented for variable chromosome regions for two groups
within the Matagorda Bay sample population.

* Genotype classes are pooled: All "4" heterozygotes as well as all satellite-bearing homozygotes / heterozygotes are pooled together
 **Hardy-Weinberg calculations based on three genotypic classes. 1)Most common allele homozygotes; 2)Most common allele heterozygotes;
 3)All other homozygotes + all other heterozygotes. (d.f.=2).
 Bold values indicate values which show significant deviations from expected values (p<0.05).

	FB501	FB502	FB503	FB504	FB505	FB506	FB507	FB508	FB509	FB510	FB511	FB512	FB513	FB514
FB501														
FB502	0.702													
FB503	0.607	0.596												
FB504	0.491	0.655	0.421											
FB505	0.571	0.737	0.5	0.807										
FB506	0.456	0.586	0.561	0.483	0.456									
FB507	0.571	0.526	0.393	0.632	0.571	0.351								
FB508	0.526	0.724	0.491	0.724	0.667	0.552	0.491							
FB509	0.607	0.561	0.5	0.596	0.643	0.386	0.714	0.561						
FB510	0.596	0.586	0.316	0.621	0.632	0.276	0.667	0.483	0.667					
FB511	0.643	0.667	0.679	0.526	0.607	0.526	0.536	0.632	0.714	0.491				
FB512	0.702	0.724	0.561	0.586	0.561	0.552	0.667	0.586	0.667	0.62	0.737			
FB513	0.679	0.702	0.536	0.667	0.75	0.456	0.571	0.561	0.643	0.702	0.607	0.667		
FB514	0.456	0.517	0.421	0.655	0.491	0.448	0.632	0.552	0.456	0.552	0.421	0.552	0.596	
FB515	0.643	0.526	0.75	0.421	0.5	0.526	0.429	0.526	0.5	0.386	0.643	0.596	0.607	0.491
FB516	0.456	0.483	0.421	0.552	0.491	0.379	0.561	0.448	0.491	0.586	0.491	0.586	0.596	0.62
FB517	0.5	0.596	0.607	0.561	0.607	0.526	0.536	0.526	0.571	0.561	0.643	0.632	0.536	0.421
FB518	0.596	0.483	0.561	0.488	0.421	0.621	0.491	0.586	0.491	0.483	0.561	0.586	0.456	0.448
Table 9: Similarity values for 595 pair-wise comparisons made among the Matagorda Bay population.														

	FB501	FB502	FB503	FB504	FB505	FB506	FB507	FB508	FB509	FB510	FB511	FB512	FB513	FB514
FB519	0.536	0.702	0.429	0.667	0.679	0.491	0.536	0.667	0.607	0.526	0.571	0.596	0.607	0.561
FB520	0.702	0.69	0.596	0.483	0.526	0.586	0.456	0.517	0.561	0.621	0.667	0.724	0.596	0.483
FB521	0.536	0.667	0.536	0.702	0.714	0.596	0.5	0.526	0.571	0.596	0.571	0.596	0.679	0.491
FB522	0.667	0.586	0.561	0.586	0.596	0.448	0.667	0.586	0.807	0.621	0.772	0.69	0.561	0.517
FB523	0.679	0.596	0.571	0.561	0.536	0.596	0.536	0.561	0.607	0.596	0.714	0.737	0.607	0.526
FB524	0.596	0.724	0.526	0.655	0.596	0.586	0.596	0.724	0.632	0.552	0.667	0.655	0.526	0.483
FB525	0.464	0.456	0.393	0.596	0.643	0.526	0.429	0.491	0.5	0.561	0.536	0.491	0.679	0.526
FB526	0.561	0.552	0.456	0.69	0.596	0.448	0.737	0.621	0.596	0.586	0.596	0.724	0.526	0.552
FB528	0.596	0.793	0.526	0.759	0.667	0.552	0.561	0.724	0.561	0.586	0.596	0.69	0.596	0.621
FB529	0.571	0.667	0.5	0.632	0.571	0.526	0.5	0.596	0.536	0.596	0.643	0.772	0.643	0.596
FB530	0.561	0.517	0.421	0.552	0.491	0.517	0.632	0.552	0.632	0.69	0.632	0.621	0.561	0.517
FB531	0.571	0.456	0.464	0.491	0.464	0.456	0.464	0.491	0.536	0.596	0.571	0.491	0.5	0.526
FB532	0.561	0.586	0.632	0.586	0.526	0.586	0.632	0.552	0.526	0.552	0.561	0.621	0.596	0.586
FB534	0.702	0.621	0.596	0.69	0.702	0.552	0.667	0.621	0.632	0.621	0.737	0.862	0.702	0.552
FB536	0.561	0.586	0.456	0.69	0.561	0.448	0.632	0.586	0.456	0.586	0.596	0.724	0.596	0.552
FB538	0.596	0.724	0.561	0.655	0.667	0.552	0.667	0.655	0.667	0.552	0.667	0.828	0.632	0.552
RT412	0.526	0.448	0.632	0.483	0.421	0.586	0.526	0.448	0.491	0.448	0.561	0.517	0.491	0.517

Table 9: Similarity values for 595 pair-wise comparisons made among the Matagorda Bay population.

	FB515	FB516	FB517	FB518	FB519	FB520	FB521	FB522	FB523	FB524	FB525	FB526	FB528	FB529
FB515														
FB516	0.351													
FB517	0.536	0.667												
FB518	0.596	0.379	0.491											
FB519	0.429	0.561	0.571	0.421										
FB520	0.596	0.517	0.737	0.586	0.561									
FB521	0.536	0.526	0.607	0.456	0.607	0.596								
FB522	0.561	0.552	0.667	0.586	0.596	0.552	0.632							
FB523	0.714	0.526	0.679	0.702	0.536	0.772	0.643	0.702						
FB524	0.456	0.414	0.561	0.655	0.632	0.655	0.596	0.69	0.632					
FB525	0.571	0.491	0.5	0.421	0.5	0.491	0.786	0.596	0.607	0.421				
FB526	0.491	0.552	0.702	0.517	0.526	0.552	0.596	0.724	0.561	0.655	0.596			
FB528	0.456	0.586	0.632	0.552	0.632	0.517	0.561	0.621	0.596	0.621	0.421	0.621		
FB529	0.5	0.737	0.643	0.456	0.714	0.667	0.679	0.667	0.679	0.561	0.571	0.561	0.702	
FB530	0.561	0.483	0.596	0.69	0.561	0.69	0.596	0.724	0.807	0.724	0.561	0.586	0.552	0.632
FB531	0.643	0.456	0.571	0.596	0.536	0.632	0.571	0.702	0.714	0.491	0.714	0.526	0.456	0.607
FB532	0.561	0.517	0.632	0.586	0.491	0.552	0.561	0.621	0.667	0.586	0.456	0.552	0.69	0.632
FB534	0.632	0.621	0.667	0.621	0.667	0.69	0.702	0.724	0.702	0.69	0.596	0.793	0.621	0.702
FB536	0.491	0.448	0.491	0.483	0.491	0.483	0.596	0.586	0.596	0.586	0.491	0.69	0.69	0.667
FB538	0.596	0.448	0.596	0.552	0.702	0.655	0.632	0.69	0.632	0.724	0.526	0.759	0.655	0.667
RT412	0.667	0.345	0.491	0.69	0.386	0.552	0.526	0.586	0.702	0.517	0.491	0.483	0.517	0.491

Table 9: Similarity values for 595 pair-wise comparisons made among the Matagorda Bay population

	FB530	FB531	FB532	FB534	FB536	FB538	RT412
FB530							
FB531	0.737						
FB532	0.655	0.561					
FB534	0.621	0.526	0.586				
FB536	0.586	0.456	0.69	0.759			
FB538	0.621	0.561	0.655	0.793	0.655		
RT412	0.69	0.632	0.69	0.517	0.517	0.552	

Table 9: Similarity values for 595 pair-wise comparisons made among the Matagorda Bay population

Chromosome Region	Similarity Index: Sarasota vs. Matagorda Bay	Similarity Index: Matagorda Bay Males vs Females	Similarity Index: NE vs. SW
1p	0.94	0.98	1.00
1q	0.84	1.00	0.91
2p	0.75	0.98	0.96
2q	0.96	1.00	1.00
3p	0.73	0.94	0.99
3q	0.97	0.91	0.99
4p	0.77	0.91	0.99
4q	0.98	1.00	1.00
5p	0.83	1.00	0.84
17q	0.99	0.97	1.00
18p	0.87	1.00	1.00
19p	1.00	0.99	0.81
20p	0.92	0.94	0.90
21p	0.97	0.95	0.94
Total Similarity Index	0.88	0.97	0.95

Table 10: Comparisons of similarity index values for three different pairs of population subgroups.

Suspected Mother	Suspected Calf	Similarity Value	Z-score
FB503	FB508	0.491	-1.01
FB507	FB509	0.714	1.47
FB511	FB513	0.607	0.277
FB515	FB517	0.536	-0.511
FB520	FB521	0.596	0.155

Table 11: Similarity values and Z-scores for the five potential mother and calf pairs from the Matagorda Bay sample.

Figure 1. Map of the Port O'Connor area of Matagorda Bay (After Top Spot Map F108, Pasadena Hot Spot, Inc., Pasadena, TX. and NOAA Charts 11317, 11319, NOAA, Washington, D. C.).

Letters a-m correspond to capture locations of dolphins in Matagorda Bay. See Table 1 for identification of animals captured in each locale.

Figure 2: Chromosome variants identified among the Matagorda Bay animals.

Figure 3: Distribution of pairwise similarity values for the Matagorda Bay sample.

Figure 4: Distribution of pairwise similarity values for NE group and SW group of animals from within Matagorda Bay sample

Estimated age,
in years, of
each animal

Figure 5: Ages and sex of suspected mother-calf pairs

Figure 6: Discrepant chromosome pairs in suspected mother-calf pairs

Figure 7: Similarity values for suspected mother-calf pairs in Matagorda Bay, in comparison to the similarity values calculated for the entire sample.

Figure 8: Distribution of unique variants between Matagorda Bay and Sarasota