FINAL REPORT

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GENETIC ANALYSIS TO DETERMINE MIXING PROPORTIONS BY SEASON OF WESTERN ATLANTIC AND GULF OF MEXICO STOCKS OF KING MACKEREL

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SUMMARY

A total 1,006 king mackerel representing 20 discrete samples collected over a three-year period (from 1996 to 1998) along the east (Atlantic) and west (Gulf) coasts of Florida and the Florida Keys were assayed for allelic variation at seven, nuclear-encoded microsatellites. The sampling area extended from offshore of Jacksonville on the Atlantic coast to Panama City on the Gulf coast. The overall goal of the project was to examine rigorously by means of genetic markers (microsatellites) the spatial-temporal limits of the two (presumed) stocks (migratory units) of king mackerel along peninsular Florida. Subsumed within this goal was a test of the two stock hypothesis. A second goal was to estimate the proportions of both stocks (assuming separate stocks exist) in a mixing zone that was presumed to occur around southern Florida. Genotype proportions at the seven microsatellites in each sample were tested for conformance to expectations of the Hardy-Weinberg equilibrium and for independence. Following Bonferroni corrections, no significant deviations from Hardy-Weinberg equilibrium expectations were found among the 20 samples at six of the microsatellites. Genotype proportions at one microsatellite, *Sca* 23, differed from those expected under Hardy-Weinberg equilibrium at four samples. The four significant tests may be anomalous. Genotypes at all seven microsatellites were independent in pairwise comparisons. Allele distributions at the seven microsatellites also were tested for independence with sex and age, and no significant departures following Bonferroni corrections were found. Results of homogeneity tests of the spatial distributions of alleles at the seven microsatellites were consistent with the hypothesis that there are two, *very* weakly differentiated 'genetic' stocks (subpopulations) of king mackerel within the area sampled: one in the Atlantic that extends as far south as offshore of West Palm Beach on the east coast of Florida, and one in the Gulf that extends as far south as Marco Island on the west coast of Florida. These 'stock' boundaries appear not to vary temporally, in that significant (genetic) heterogeneity was found only when samples were pooled along geographic lines independent of season. Tests of homogeneity of allele distributions when samples were pooled along seasonal (temporal) boundaries, consistent with the temporal boundaries used currently for stock assessment and allocation of the king mackerel resource, were non-significant. However, the degree of genetic divergence between the two 'genetic' stocks is very small. On average, only 0.19% of the total genetic variance across all samples assayed occurs between the two regions; whereas, on average, 99.74% of the total genetic variance occurs within samples. Cluster analyses and assignment tests, respectively, were used to determine whether samples could be placed into clustered temporal or geographic units and to estimate the proportion of Atlantic or Gulf fish within individual samples. Neither approach generated patterns consistent with either geographic or spatial-temporal boundaries: no stable or geographically cohesive sample relationships were revealed by cluster analysis, and assignment proportions indicated a more-or-less even mixture of Atlantic and Gulf fish among most samples. There also was no indication in cluster analyses that samples from the Florida Keys represented any more of a mixture of Atlantic and Gulf fish than did other samples. This is of note because samples from the Florida Keys were obtained during January and March, when current temporal-spatial boundaries place the fish in the Gulf Migratory Unit. Assignment tests (a form of mixed stock analysis) did suggest larger differences in proportions of Atlantic and Gulf fish in samples from the Florida Keys than elsewhere. However, these differences were inconsistent among different samples from the Florida Keys. Moreover, assignment proportions for two of the samples from the Florida Keys were opposite to what would be expected given the temporal boundaries currently in effect for stock assessment: one sample was obtained in March and would be considered Gulf stock, yet was estimated to contain 58.6% Atlantic fish; whereas a second sample was obtained in January (also considered to be Gulf stock), yet was estimated to contain 64% Atlantic fish. A third sample from the Florida Keys also was obtained in March and was estimated to contain only 37.2% Atlantic fish. Spatial autocorrelation analysis essentially revealed no relationship between genetic divergence and geographic distance among samples. Correlations of allele frequencies were essentially the same between geographically proximal and geographically distant localities, indicating that fish

taken in the same or different year or season from the same or different locality were equally similar or different from one another. Results of spatial autocorrelation analysis were consistent with results of assignment tests, where the proportion of Atlantic and Gulf fish within most samples was approximately 50:50. The genetic data obtained in this study are compatible with the hypothesis that two, *very* weakly differentiated 'genetic' subpopulations of king mackerel exist in peninsular Florida and that considerable, perhaps extensive, mixing occurs between them. King mackerel sampled from the Florida Keys cannot easily be assigned unequivocally to either 'genetic' stock, and like most other samples, contain mixtures with approximately the same proportion of fish from both 'genetic' stocks. These results are not consistent with the current spatial-temporal boundaries employed in stock assessment and allocation of the king mackerel resource. The results are consistent with the hypothesis that considerable gene flow occurs among all of the localities sampled, and that differences in gene flow likely do not arise as a function of geographic distance. Similar findings were obtained previously in a study of variation in king mackerel mitochondrial DNA. The very slight genetic differences between king mackerel in the Atlantic versus those in the Gulf most likely stem from slightly reduced gene flow (migration) between the Atlantic and Gulf relative to gene flow (migration) along the Atlantic and Gulf coasts of peninsular Florida. This is consistent with findings in other marine fishes that the southern Florida peninsula serves (or has served) as a biogeographic boundary.

INTRODUCTION

The king mackerel (*Scomberomorus cavalla*) is a widely distributed, coastal pelagic fish of considerable economic importance. The species occurs in the western Atlantic Ocean from Massachusetts to Rio de Janeiro in Brazil and throughout both the Gulf of Mexico and the Caribbean Sea (Rivas 1951; Collette and Nauen 1983); management of the king mackerel resource in U.S. waters is under the jurisdiction of two regional fishery management councils. Both recreational and commercial catches of king mackerel in U.S. waters are substantial, and recreational landings are generally greater than commercial catches (Manooch 1979; U.S. Dept. Commerce 1985-1987). King mackerel are critical to the southeastern Atlantic (hereafter Atlantic) and northern Gulf of Mexico (hereafter Gulf) charter-boat industries, and the total value of the U.S. king mackerel fishery exceeds $100 million. Mexican commercial catches also are substantial (Fortune 1987; Collins and Trent 1990).

The present management regime for king mackerel is based on a two migratory unit (stock) hypothesis: one stock (Atlantic Migratory Unit) occurs in the U.S. southeastern Atlantic, whereas the other (Gulf of Mexico Migratory Unit) occurs in the Gulf. Separation of the two migratory units (hereafter stocks) is based primarily on mark-and-recapture studies carried out prior to 1984 and on growth rate differences (Williams and Godcharles 1984; MSAP 1994). The two stocks appear to mix extensively during the winter months along the southeast coast of Florida, and for purposes of stock assessment and resource allocation, the boundary between the two stocks is specified as the Volusia/Flagler county line (east coast of Florida) in winter (1 November - 31 March) and the Monroe/Collier county line (west coast of Florida) in summer (1 April - 31 October). Pragmatically, this means that king mackerel caught south of the Volusia/Flagler county line (including the Florida Keys) between 1 November and 31 March are considered as belonging to the Gulf stock. Between 1 April and 31 October, king mackerel caught south of the Monroe/Collier county line are considered as belonging to the Atlantic stock. These boundaries also were based primarily on the pre-1984 mark-and-recapture studies of Williams and Godcharles (1984), where it was estimated that in winter roughly 60% of fish along the east coast of Florida were from the Gulf stock.

Data from additional mark-capture (Fable 1988; Fable et al., 1987; Sutter et al. 1991; Schaefer and Fable 1994), growth rate (DeVries and Grimes 1997), and temporal/geographic sampling studies (Collins and Stender 1987; Trent et al. 1983; Grimes et al. 1990), are consistent with the hypothesis that king mackerel in U.S. waters are comprised of distinct stocks (populations) in the Atlantic and Gulf. However, based on allozyme evidence (Johnson et al. 1993) and studies of early life history (Grimes et al. 1988, 1990), it has been suggested (DeVries and Grimes 1997) that there may be two stocks or populations of king mackerel in the northern Gulf. The allozyme evidence (Johnson et al. 1993) was in the form of electrophoretic patterns of a nuclear-encoded, polymorphic dipeptidase locus (*PEPA-2*). Briefly, two common alleles at *PEPA-2* were found to vary in frequency within the U.S. king mackerel fishery: one (*PEPA-2a*) appeared to be in high frequency among king mackerel sampled from the western and northwestern Gulf; whereas the other allele (*PEPA-2b*) appeared to be in high frequency among king mackerel sampled from the Atlantic and the northeastern Gulf. Johnson et al. (1993) hypothesized the occurrence of a western Gulf stock, with wintering grounds in Mexican waters, that migrated northward during the spring and summer; and an eastern Gulf stock, with wintering grounds in waters off of southern Florida, that migrated northward along both coasts of Florida during the spring. They also hypothesized that both Gulf stocks mixed to varying degrees in the northern Gulf. The studies of early life history (Grimes et al. 1988, 1990) suggested the presence of at least two spawning locations in the Gulf: one located in the northcentral Gulf (from Texas to northwest Florida), with peaks in spawning activity occurring in June and September; and one located in Mexican waters, with spawning possibly occurring during the late winter through summer months. Of particular interest were bimodal spawning peaks occurring in the northcentral Gulf, which Grimes et al. (1988, 1990; Grimes, pers. comm.) interpreted as early and late spawning waves

of larger and smaller spawning adults. Such a pattern also could be explained by the occurrence of two discrete, breeding stocks or populations. Considering all the data acquired to date, DeVries and Grimes (1997) suggested there may be three migratory units (stocks) of king mackerel in U.S. waters: one in the Atlantic, one in the eastern Gulf, and one in the western Gulf.

The electrophoretic data of Johnson et al. (1993) did not distinguish king mackerel in the eastern Gulf from those in the Atlantic, and to that extent, argued against the hypothesis that king mackerel in the Atlantic and Gulf represented two distinct stocks. However, Gold et al. (1997) assayed variation in restriction sites of mitochondrial (mt)DNA among king mackerel collected from 13 localities along the U.S. Atlantic coast and northern Gulf and found significant (but weak) heterogeneity only in comparisons of pooled mtDNA haplotypes from Atlantic localities versus pooled haplotypes from Gulf localities. The mtDNA data thus did not support the hypothesis that two genetically identifiable stocks of king mackerel occur in the northern Gulf, but rather were consistent with the hypothesis that separate stocks of king mackerel exist in the Atlantic and in the Gulf. Estimates of F_{ST}, a measure of population subdivision, between king mackerel in the Atlantic and Gulf were small, indicating that mixing between Atlantic and Gulf king mackerel occurs. Gold et al. (1997) also examined spatial variation in frequencies of the two alleles at *PEPA-2*. Results were essentially the same as those reported by Johnson et al. (1993): one allele (*PEPA-2a*) was in higher frequency among king mackerel from the western Gulf, whereas the other allele (*PEPA-2b*) was in higher frequency among king mackerel from the eastern Gulf and the Atlantic. Tests of independence of *PEPA-2* genotypes with age and sex of individual fish, however, revealed significant non-random associations among Gulf fish of *PEPA-2a* homozygous genotypes with males and of *PEPA-2b* homozygous genotypes with females. Moreover, among fish sampled from the Atlantic, there was a highly significant decrease in the frequency of *PEPA-2b* alleles with increasing fish age. The same trend was found among fish sampled from the Gulf, but to a lesser extent. Tests of independence of sex versus age, and of mtDNA variation versus sex or age, were non-significant. These findings strongly indicate that the use of *PEPA-2* alleles to distinguish stocks of king mackerel is compromised, and that the hypothesis of eastern and western stocks of king mackerel in the Gulf needs to be re-evaluated. Finally, Broughton et al. (2000) recently surveyed allelic variation at five nuclear-encoded microsatellites among a subset of the samples of king mackerel studied by Gold et al. (1997). Tests of homogeneity in allele distribution at the five microsatellites indicated that samples from Port Aransas, Texas (western Gulf) and Gulfport, Mississippi (central Gulf) differed from each other and from the remaining samples (including two samples from the Atlantic, one from the Florida Keys, one from the eastern Gulf, one from the western Gulf, and one from Veracruz, Mexico). No significant differences at any microsatellite was found between samples representing geographic extremes, and no significant geographic patterns were found when samples were combined into regional groupings reflecting current hypotheses of king mackerel stock structure in U.S. waters.

Because the mtDNA study of Gold et al. (1997) utilized much larger sample sizes (in pooled comparisons) than did the study of Broughton et al. (2000), the genetic data taken together are consistent with the notion that two stocks of king mackerel exist along peninsular Florida (one along the Atlantic coast and one along the Gulf coast) but that considerable mixing occurs between the two. Of concern is the degree of mixing between the two stocks. Analysis of mark-and-recapture data collected during 1985 to 1993 (SEFC 1994; MSAP 1994) indicated that roughly 3.0% of fish tagged in the Atlantic were recaptured in the Gulf, whereas 6.4% tagged in the Gulf were recovered in the Atlantic. These results suggest more limited movement of king mackerel between the Atlantic and Gulf than indicated by the genetic data. Alternatively, more liberal estimates (SEFC 1994) of recaptures (generated when utilizing the summer and winter seasons in the "mixing" zone) suggested that 2.6 - 30.9% of recaptures tagged in the Atlantic were returned as Gulf fish and 1.5 - 13.6% of recaptures tagged in the Gulf were returned as Atlantic fish. Jones et al. (1994), however, seriously questioned the mixing rates of Atlantic and Gulf king mackerel based on mark-and-recapture experiments. They pointed out that virtual population analysis (VPA) based estimates

of fishing mortality for the directed king mackerel fisheries in the Gulf and Atlantic corresponded to annual exploitation rates of 0.30 and 0.11, respectively. Exploitation rates calculated from the 1985-1993 (uncorrected) tag returns, however, ranged from 0.027 to 0.033 (Gulf) and 0.036 to 0.045 (Atlantic). The difference between the two estimates of exploitation rates implies either that the true exploitation rate was overestimated by VPA or underestimated by uncorrected tag-return data. Because of unaccounted sources of error in the mark-and-recapture data, and the observation that the unaccounted sources of error were not consistent between the two regions, Jones et al. (1994) concluded that little to no confidence should be placed in reported mixing rates based on the mark-and-recapture data. Assuming that VPA estimates are essentially correct, a further implication of the panel's finding is that mixing rates between the Atlantic and Gulf (and vice-versa) could be higher than the values of 3% and 6%, respectively, estimated from mark-and-recapture data. The issue of the mixing zone, its boundaries, and the seasonal proportion of fish from either stock in the zone is of importance relative to assessment and allocation, particularly during the winter season, of the king mackerel resource. Mark-recapture data cited in MSAP (1994), for example, indicate that 22% of fish tagged in the mixing zone in southeastern Florida moved into the Gulf. One impact of this (i.e., only ~20% of winter catches from the east coast of Florida are Gulf stock, as opposed to 100% under the current management plan) is that the Allowable Biological Catch (ABC) for the Gulf stock would decrease significantly (MSAP 1994). Because the Gulf stock of king mackerel is currently considered overfished (MSAP 2000), reductions in ABC of the Gulf stock could have significant economic impact. This underscores the need to know the seasonal and spatial boundaries of the two stocks of king mackerel.

In this project, extensive sampling along peninsular Florida and genetic assays employing nuclear-encoded microsatellites were carried out to assess rigorously the seasonal and spatial borders of the two migratory units (stocks). In the process, the stock structure of king mackerel in waters off peninsular Florida, from Jacksonville on the east coast to Panama City on the west coast also was assessed. The goals of the project were to define more rigorously the spatial/temporal limits of the two stocks (if, in fact, separate stocks exist), and, if possible, to estimate the proportions of both stocks in the mixing zone. The choice of microsatellites for the project was obvious. Briefly, microsatellites are rapidly evolving, short stretches of DNA composed of di-, tri-, and tetranucleotide arrays that are abundant, highly polymorphic, and inherited in a codominant fashion (Weber 1990; Wright 1993; Wright and Bentzen, 1994). Because allele frequencies at microsatellites are generally consistent with equilibrium expectations of diploid, Mendelian loci; and because identification of each microsatellite is via amplification using specific polymerase-chain-reaction (PCR) primers, removing problems associated with homology of alleles (Weber 1990; Wright and Bentzen 1994; Edwards et al. 1992), microsatellites have proven to be useful genetic markers of population structure in numerous taxa, including fishes (Jarne and Lagoda 1996; Ruzzante et al. 1996; Estoup and Angers 1998). In addition, new alleles at microsatellite loci appear to arise rapidly (Schug et al. 1998), meaning that microsatellites may reveal both short-term gene flow (O'Connell and Slatkin 1993) and population structure (Ruzzante et al. 1996) at small spatial and temporal scales.

Results of this study indicate that there are two, *very* weakly differentiated 'genetic' stocks of king mackerel, one in the Atlantic that extends as far south as offshore of West Palm Beach on the east coast of Florida, and one in the Gulf that extends as far south as Marco Island on the west coast of Florida. On this basis, one might infer that a mixing zone that includes the Florida Keys may exist at the very southern tip of the Florida peninsula. However, assignment tests (a form of mixed stock analysis) indicated that most of the samples of king mackerel assayed in the study contain roughly equivalent proportions of both Atlantic and Gulf fish, and that proportions of Atlantic or Gulf fish within a sample was inconsistent with the spatial-temporal boundaries currently employed in stock assessment and resource allocation.

METHODS AND MATERIALS

A total of 20 samples of king mackerel were procured between 1996 and 1998 from 11 different offshore localities (Table 1; Figure 1). The sample from Panama City was obtained from charter boat catches, and the samples from Sarasota and Jacksonville were obtained from tournaments. The remaining samples were obtained from commercial catches. Tissue samples (heart and muscle) were removed from each fish, frozen in liquid nitrogen, transported to College Station, and stored at -80° C. Sex of individuals was recorded for all samples except for the March 1997 sample from the Florida Keys. Approximate ages of individuals from all samples except for the July 1998 sample from Jacksonville, the March 1997 sample from the Florida Keys, and the April 1997 sample from Sarasota, were determined by otolith-increment analysis. The latter was carried out by D. DeVries of the Panama City Laboratory of the Southeast Fisheries Science Center (National Marine Fisheries Service).

Initially, we planned to deploy the five microsatellites developed in our prior study (Broughton et al. 2000) of king mackerel. Two of these (*Sca* 8 and *Sca* 47), however, had proven difficult to amplify consistently in the prior study, and similar difficulties with these two microsatellites were encountered in the early stages of this project (leading to the decision not to include them in this study). We also excluded a third microsatellite, *Sca* 30, developed by Broughton et al. (2000), in part because of difficulties with consistent amplification, and in part because the allele distribution at *Sca* 30 was highly leptokurtic (Broughton et al. 2000). A total of five new microsatellites were then developed from the microsatellite-enriched genomic library generated by Broughton et al. (2000). Briefly, we sequenced a number of candidate microsatellites from either or both ends by using standard M13 sequencing primers and an Applied Biosystems (Perkin Elmer) 377 automated DNA sequencer. Identification of primers from regions flanking microsatellites employed the OLIGO software package. Primers were designed according to preset criteria that included product length, internal stability, proportion GC content, and primer Tm difference. PCR amplifications were performed under a variety of experimental conditions to optimize procedures that produced high yields of target sequence and minimized additional fragments ("stutter" bands). Experimental tractability (reproducibility, consistency, range in allele size, frequency of "stutter" bands [if present], and microsatellite polymorphism) of PCR-amplified microsatellites were evaluated by screening a panel of king mackerel samples available in the laboratory. PCR primer sequences, the length (in base pairs) of the cloned allele, and the annealing temperature in PCR amplification for the seven microsatellites utilized in the project are given in Appendix Table A1. Two of these, *Sca* 37 and *Sca* 44, were developed previously by Broughton et al. (2000).

For assay of individual fish, genomic DNA was isolated from frozen tissues as described in Gold and Richardson (1991). Genotypes at the seven microsatellites were determined by PCR amplification and gel electrophoresis. Prior to amplification, one of the primers was kinase-labeled with γ^{32}P-ATP by T4 polynucleotide kinase (30 min, 37°C). PCR reactions contained approximately 5 ng of genomic DNA, 0.1 units of *Taq* DNA polymerase, 0.5 μM of each primer, 800 μM dNTPs, 1-2 mM $MgCl_2$, 1X *Taq* buffer at pH 9.0 (Promega, Inc.), and sterile deionized water in a total volume of 10 μL. Thermal cycling was carried out in 96-well plates as follows: denaturation (45 sec, 95°C), annealing (30 sec, temperature as per Table 1), and polymerization (30 sec, 72°C) for 30 cycles. Aliquots (3 μL) of each PCR reaction were electrophoresed in 6% denaturing polyacrylamide ("sequencing") gels. Gels were dried and exposed to X-ray film. Alleles at individual microsatellites were scored as number of repeats by comparison to the cloned (and sequenced) allele. Genotypes at each microsatellite for each individual were scored and entered into a database.

Initial statistical analysis involved generation of allele frequencies and (direct-count) heterozygosity values, and significance testing of genotypic proportions relative to those expected under conditions of

Hardy Weinberg equilibrium. Significance testing of Hardy-Weinberg equilibrium proportions involved exact tests performed using Markov-chain randomization (Guo and Thompson 1992); probability (P) values for tests at each microsatellite within each sample were estimated via permutation (bootstrapping) with 1000 resamplings (Manly 1991). Significance levels for simultaneous tests were adjusted with the sequential Bonferroni approach (Rice 1989). Tests of genotypic equilibrium at pairs of microsatellites were carried out as a surrogate to assess whether any microsatellites were genetically linked. Probability values for (exact) tests of genotypic equilibrium were generated by 1000 resamplings, and significance levels for simultaneous tests were adjusted with the sequential Bonferroni approach. Allele frequencies and heterozygosity values were obtained using BIOSYS-1.7 (Swofford and Selander 1981), and tests of Hardy-Weinberg and genotypic equilibria employed the package GENEPOP (Raymond and Rousset 1995). Exact tests also were used to test independence of the distribution of genotypes at each microsatellite with the sex and age of individuals. Initial tests involved each of the 20 samples separately. We then pooled individuals sampled at Atlantic localities (nine samples), in the Florida Keys (three samples), at Gulf localities (eight samples), and over all localities (20 samples) in order to increase cell sizes in individual tests. Probability (P) values for these tests of independence were estimated via permutation (1000 resamplings) and significance levels for simultaneous tests were adjusted with the sequential Bonferroni approach.

Tests of genetic homogeneity among samples followed the approach employed by Ruzzante et al. (1998). The underlying null hypothesis in each test (comparison) was that allele distributions are homogeneous among localities. Tests (analyses) included exact tests, as implemented in GENEPOP, the Monte Carlo procedure of Roff and Bentzen (1989), as implemented in the Restriction Enzyme Analysis Package of McElroy et al. (1992), and the molecular analysis of variance (AMOVA) of Excoffier et al. (1992). Significance of tests of genetic homogeneity employed permutation with 1,000 resamplings per individual comparison, and significance levels for simultaneous tests were adjusted by using the sequential Bonferroni approach. Tests of genetic homogeneity were carried out separately for each of the seven microsatellites. Individual tests were carried out (i) among all 20 samples, (ii) among samples (nine) from Atlantic localities, (iii) among samples (three) from the Florida Keys, and (iv) among samples (eight) from Gulf localities. Molecular analysis of variance (AMOVA) was employed to generate estimates of (genetic) variance components and Φ statistics. The latter are a set of hierarchical F-statistic analogs that consider evolutionary distance among alleles. Significance of Φ statistics employed permutation (1000 resamplings). Sample localities were nested into regional groupings for input into AMOVA. For one analysis, we grouped all samples from the Atlantic into one group and all samples from the Gulf into a second group. This analysis excluded the samples from the Florida Keys. For a second analysis, we used the two groups from the first analysis and a grouping that included the samples from the Florida Keys. In this way, we tested genetic homogeneity (i) between samples from the Atlantic versus those from the Gulf, (ii) among samples from the Atlantic, the Florida Keys, and the Gulf, and (iii) among samples within the three regional groupings. These tests were designed to examine spatial genetic homogeneity among king mackerel in accordance with a geographic boundary defined by peninsular Florida.

A second series of homogeneity tests were carried out that were designed to examine the temporal stock boundaries currently used in management planning for the king mackerel resource. Briefly, each of the 20 samples of king mackerel were designated as either Atlantic or Gulf stock based on the time during a year in which they were sampled. Six of the samples thus were designated Atlantic stock and 14 of the samples were designated Gulf stock (Table 1). We then carried out three separate tests of genetic homogeneity: the first compared Atlantic versus Gulf stock; the second compared Atlantic stock versus those samples from the Atlantic but which are considered Gulf stock because of the time of year when they were sampled; the third compared Atlantic stock versus those samples from the Gulf which are

considered Gulf stock because of the time of year when they were sampled. Homogeneity tests included exact tests and the Monte Carlo procedure of Roff and Bentzen.

In addition to homogeneity testing of microsatellite allele distributions, we also employed clustering of genetic distances, spatial autocorrelation analysis, and assignment tests as a means to assess temporal and spatial variation of microsatellites. Cluster analysis of genetic distances between pairs of samples was used to generate an estimate of the degree of genetic divergence/similarity among samples. We employed both Rogers (1972) 'modified' genetic distance, as implemented in BIOSYS-1.7 (Swofford and Selander 1981), and Cavalli-Sforza's chord distance (Cavalli-Sforza and Edwards 1967) between pairs of samples, as implemented in the GENEDIST program in Version 3.4 of the Phylogenetic Inference Package (PHYLIP) of Felsenstein (1992). Pearson's product-moment correlation was used to estimate the co-phenetic correlation between the two genetic distance matrices. Both genetic distance matrices were clustered by neighbor joining (Saitou and Nei 1987), using the NEIGHBOR program in PHYLIP. A consensus of 500 neighbor-joining topologies based on the chord distance matrix was constructed using the CONSENSE program in PHYLIP. Spatial autocorrelation analysis was carried out to determine whether allele frequencies at each microsatellite at any given sample locality were independent of those in adjacent localities. Correlograms that plot autocorrelation coefficients (Moran's I values) as a function of geographic distance between pairs of sample localities were used to summarize patterns of geographic variation of allele frequencies at each microsatellite. Positive autocorrelations between adjacent localities, with decreasing autocorrelation as geographic distance between localities increases, are generally interpreted as an isolation-by-distance effect (Sokal and Oden 1978a). We employed the Spatial Autocorrelation Analysis Program (SAAP) of Wartenberg (1989) and followed procedures outlined in Sokal and Oden (1978a,b). 'Noise' was minimized by including only alleles that occurred in frequencies of 20 or greater over all individuals assayed. The first of two SAAP runs employed equal geographic distances between each of five distance classes; the second employed equal numbers of pairwise comparisons in each distance class. Finally, assignment tests (Paetkau et al. 1995, 1997) were used to 'assign' individuals within each of the 20 samples to one of three regional groupings (Atlantic, Florida Keys, and Gulf). The three groups were employed largely as a result of homogeneity tests of allele distributions, where the existence of the three groups was weakly supported. Assignment tests have a number of uses (Waser and Strobeck 1998): in this case we were interested in the proportion of individuals within a sample that could be assigned to each regional group, relative to the locality of the sample and the season in which it was procured. Assignments tests were carried out employing the 'Assignment Calculator' software available at http:\\www.biology.ualberta.ca/jbruzusto/Doh.html.

RESULTS AND DISCUSSION

Summary statistics for the seven microsatellites used in the study are given in Table 2. The summary statistics include for each microsatellite (i) the repeat sequence of the cloned allele, (ii) the number of alleles detected, (iii) the average (direct count) heterozygosity (± SE) observed among samples, and (iv) results of tests of conformance of observed genotype proportions to expectations of Hardy-Weinberg equilibrium. [Allele frequencies at the seven microsatellites in each of the 20 samples are given in Appendix Tables B1 and B2. Number of individuals assayed, heterozygosity (direct count) values, and probability of conformance to expected Hardy-Weinberg proportions per microsatellite per individual sample are given in Appendix Tables C1 and C2.] Cloned alleles at the seven microsatellites included simple and complex dinucleotide repeats (*Sca* 49 and *Sca* 65 vs *Sca* 14, *Sca* 23, *Sca* 37, and *Sca* 61) and one complex tetranucleotide repeat (*Sca* 44). All of the dinucleotide repeats included CA (or complementary TG) dinucleotide repeats, with number of alleles per microsatellite ranging from five (*Sca* 14) to twenty-four (*Sca* 23 and *Sca* 65). Direct count heterozygosity, averaged over the twenty samples, ranged from 0.311 ± 0.018 (*Sca* 61) to 0.803 ± 0.105 (*Sca* 23). These results indicate that the

seven microsatellites assayed in king mackerel are typical of microsatellites found in other vertebrate organisms, including fishes (e.g., Turner et al. 1998; Gold et al. 2000). Following (sequential) Bonferroni correction (Rice 1989), genotype proportions at six of the microsatellites in all twenty samples did not deviate significantly from proportions expected under Hardy Weinberg equilibrium. Genotype proportions at *Sca* 23 among three of the samples (SEB^2, KEY^1, and SAR^4) differed significantly ($P = 0.000$) from Hardy-Weinberg equilibrium expectations, and at a fourth sample (SAR^1), the probability value of 0.006 was very close to the Bonferroni adjusted alpha of 0.003 (Appendix tables C1 and C2). F_{IS} values (after Weir and Cockerham, 1984) for these four samples were all positive, indicating a deficit of heterozygotes and the possible presence of a null allele. However, probability values for tests of Hardy-Weinberg equilibrium at *Sca* 23 among the remaining 16 samples were non-significant and averaged (± S.E.) 0.385 ± 0.076. This may suggest that the four significant tests were anomalous, although the possibility of a null allele at *Sca* 23 cannot be discounted. Finally, tests of genotypic equilibrium between pairs of microsatellites (samples pooled) yielded only one significant value (*Sca* 44 x *Sca* 61) following Bonferroni correction (Table 3). Probability values of tests involving these two microsatellites carried out within each of the 20 samples were all non-significant and averaged (± S.E.) 0.338 ± 0.067. Of the remaining (pairwise) tests carried out within samples (420 tests in all), only four significant probability values were obtained: *Sca* 37 and *Sca* 44 (CCN), *Sca* 23 x *Sca* 44 (PCY), *Sca* 37 x *Sca* 49 (BCG), and *Sca* 23 x *Sca* 44 (SEB^2). These results strongly indicate that none of the seven microsatellites are genetically linked in king mackerel.

Tests for independence of allele distributions at each of the seven microsatellites versus both the sex and age of individuals were carried out (i) within each of the 20 samples, and (ii) among individuals (pooled) sampled from the Atlantic, the Florida Keys, the Gulf, and overall. It was necessary to carry out these tests of independence before executing tests of the spatial distribution of microsatellite alleles, as Gold et al. (1997) previously found that allelic variation at the protein-coding gene *PEPA-2* was not independent of either sex of age in king mackerel. Non-independence of allelic variation at any microsatellite would mean that tests of the spatial distribution of alleles at that microsatellite (as a means of inferring population structure) would be compromised. In tests for independence with sex, eight significant probability values ($P < 0.05$) were found prior to Bonferroni correction for simultaneous tests (Table 4). None of these, however, were significant following Bonferroni correction. In addition, one would expect that eight of 162 tests would be significant by chance alone at $\alpha = 0.05$. Finally, only one of the significant probability values occurred in a pooled comparison (*Sca* 49 in the test of individuals from the Atlantic), where larger sample sizes should increase robustness of tests of independence. In tests for independence of allele distributions with the age (year class) of individuals, eight significant probability values ($P < 0.05$) were found prior to Bonferroni correction for simultaneous tests (Table 5), two of which (*Sca* 23 in SAR^4 and *Sca* 61 in SAR^4) were significant following Bonferroni correction (at least when using eight simultaneous tests per microsatellite to estimate adjusted α levels). In both instances, the non-independence appeared to stem from a elevated incidence of specific alleles: *Sca* 23-19 occurred at a frequency of 36% in the 1989 year class, as opposed to other year classes where its frequency ranged from 0 – 15%; and similarly, *Sca* 61-12 occurred at a frequency of 25-30% in the 1986 and 1989 year classes, as opposed to a frequency of 0 – 10% in the other year classes. We suspect these are anomalous instances that do not reflect an age-related effect of allele distribution, in part because allele distributions at *Sca* 23 and *Sca* 61 were independent of year class in all other samples, and in part because allele distributions at all microsatellites were independent of year class in pooled comparisons, where larger sample sizes should increase robustness of tests of independence. We conclude based on the above that allelic variation at the seven microsatellites essentially is independent of variation in both sex and age (year class).

Spatial homogeneity in allele distributions at each microsatellite initially were tested (i) over all 20 samples, (ii) among samples from the Atlantic, (iii) among samples from the Florida Keys, and (iv) among samples from the Gulf. We chose this design *a priori*, in part because it was geographically logical, and in part because the southern Florida peninsula apparently serves (or has served) as a biogeographic boundary for a number of marine species (Avise 1992; Gold and Richardson 1998). Tests included both exact tests and the Monte Carlo procedure of Roff and Bentzen. Only three significant probability values were found among the four sets of homogeneity tests prior to Bonferroni correction: the exact test at *Sca* 23 in the comparison over all 20 samples, and both the exact test and the Roff-Bentzen procedure at *Sca* 37 in the comparison among samples from the Florida Keys (Table 6a). None of these probability values were significant following Bonferroni correction. We then tested spatial homogeneity in allele distributions (i) between (pooled) samples from the Atlantic versus (pooled) samples from the Gulf, and (ii) among (pooled) samples from the Atlantic, Florida Keys, and Gulf. For these tests, we employed exact tests, the Monte Carlo procedure of Roff and Bentzen, and the molecular analysis of variance (AMOVA). The last generates a series of F-statistic analogs called Φ statistics that can be employed to assess homogeneity between or among regions and among samples within regions. Results of these tests of homogeneity are presented in Table 6b. Significant heterogeneity for the comparison (pooled) Atlantic versus (pooled) Gulf was found prior to Bonferroni correction at *Sca* 14 (all three statistical approaches), *Sca* 23 (exact test and the Roff-Bentzen procedure), and *Sca* 44 (for the probability that $\Phi_{CT} > 0$). Probability values for *Sca* 14 and *Sca* 23 were marginal relative to the (initial) Bonferroni adjusted α of 0.007, whereas the probability that $\Phi_{CT} >$ zero at *Sca* 44 was non-significant after Bonferroni correction (Table 6b). For the comparison Atlantic versus Florida Keys versus Gulf, significant heterogeneity was found at *Sca* 14 (all three statistical approaches) and *Sca*23 (exact test only) before but not after Bonferroni correction; heterogeneity at *Sca* 37 and *Sca* 44 in the same comparison was significant both before and after Bonferroni correction in at least one of the three statistical approaches (Table 6b). In general, results of the three approaches to homogeneity testing were fairly consistent, with one notable exception. At *Sca* 37, probability values from the exact test and the Roff-Bentzen procedure were 0.009 and 0.001 in the comparison Atlantic versus Florida Keys versus Gulf, respectively, while the probability that Φ_{CT} differed from zero was 0.794 (Table 6b). We examined this discrepancy further by carrying out "V" tests of homogeneity (DeSalle et al. 1987) for each allele at *Sca* 37. Significant heterogeneity ($P < 0.05$) was found only at *Sca* 37-12: this allele was found only in the March 1997 sample from the Florida Keys, where it occurred at a frequency of 6.9% (Appendix Tables B1 and B2). Because there were far fewer alleles at *Sca* 37 sampled from the Florida Keys (258) than from either the Atlantic (884) or Gulf (872), the disproportionate frequency of this allele within the Florida Keys likely skewed the exact test and the Roff-Bentzen procedure.

Collectively, results of pooled homogeneity testing (after Bonferroni correction) indicate that all three regional groupings differ genetically, albeit weakly, from one another. Samples from the Atlantic appear to differ from those from the Gulf at *Sca* 14 and *Sca* 23; whereas samples from the Florida Keys appear differ from the other two at *Sca* 44. Frequency differences at *Sca* 14, *Sca* 23, and *Sca* 44 among the three regional groupings are shown in Table 7, and indicate that small differences in frequency of several alleles at each microsatellite appear to account for observed heterogeneity among the (pooled) sample comparisons. A final point to note is that although the foregoing indicates that allele-frequency differences exist among the three regions, the differences are very small and account for only a fraction of the overall genetic variance. Results of AMOVA for the comparison Atlantic versus Gulf (Table 8a) demonstrate that on average 99.74% of the total genetic variance at the seven microsatellites occurs within samples, whereas on average only 0.19% of the total genetic variance occurs between regions. For the comparison Atlantic versus Florida Keys versus Gulf (Table 8b), 99.78% of the genetic variance occurs on average within samples and only 0.11% of the genetic variance occurs on average among regions. [For both comparisons, the proportion of the variation among samples within regions accounts

for the remainder of the genetic variance, and for both comparisons, this proportion is small and statistically non-significant (Tables 8a and 8b)].

The next set of homogeneity tests involved comparisons designed to examine the temporal stock boundaries currently used in management planning for the king mackerel resource. Briefly, these boundaries are the Volusia/Flagler county line (east coast of Florida) southward around the Florida Keys and into the Gulf between 1 November and 31 March (Gulf stock), and the Monroe/Collier county line (west coast of Florida) southward around the Florida Keys and into the Atlantic between 1 April and 31 October (Atlantic stock. King mackerel north of the Volusia/Flagler county line are Atlantic stock year-around, and king mackerel north of the Monroe/Collier county line are Gulf stock year-around. We classified each of the 20 samples as either Atlantic or Gulf stock based on these boundaries (Table 1), then carried out both exact tests and the Roff-Bentzen procedure on each microsatellite for the following groupings: (i) between Atlantic stock and Gulf stock. (ii) between Atlantic stock and Gulf stock sampled from the Atlantic; and (iii) between Atlantic stock and Gulf stock sampled from the Gulf. No significant heterogeneity at any of the seven microsatellites (before of after Bonferroni correction) was found for any of the three sets of homogeneity tests (Table 9). These results provide no genetically based evidence for the existence of the currently used temporal boundaries dividing Atlantic and Gulf migratory units (stocks).

Neighbor-joining clustering of genetic distance between pairs of samples was employed to display genetic similarities among the 20 samples. Pairwise genetic-distance matrices using Rogers (1972) 'modified' genetic distance and Cavalli-Sforza's (Cavalli-Sforza and Edwards 1967) chord distance are shown in Tables 10 and 11, respectively. Topologies (Figures 2 and 3) generated from the two distance matrices shared relatively few similarities, as might be expected based on the estimated co-phenetic correlation ($r_{xy} = 0.56$) between the two matrices. Examples of the dissimilarities between the two topologies include clustering of SEB^2 - KEY^3, CCN - SAR^1, and SAR^5 - JCK^1 in the topology derived from Roger's distances (Figure 2) versus clustering of SAR^1 - JCK^1 and CCN - KEY^3 in the topology derived from Cavalli-Sforza's chord distances (Figure 3). Differences between the two topologies may stem in part from differences in the approaches used to estimate genetic distance. However, based on the low reproducibility (bootstrap proportions) of nodes in the topology derived form chord distance (Figure 3), the differences likely are due primarily to the low (probably non-significant) genetic divergence among the samples.

Spatial autocorrelation analysis was employed to determine the relationships (patterns) of correlations of allele frequencies over geographic distance. Initially, the analysis employed both equal geographic distances between each of five distance classes and equal numbers of pairwise comparisons in each distance class. Analysis involving equal geographic distances between distance classes generated a very uneven number of pairwise comparisons among distance classes, i.e., 18, 14, 15, 5, and 3 pairwise comparisons in distance classes 1 - 5, respectively, resulting in a high variance in Moran's I values among alleles in distance classes 4 and 5. Accordingly, we restricted the analysis to equal numbers of pairwise comparisons in each distance class, where the number of pairwise comparisons was 11 in all five distance classes. A total of 50 alleles (five at *Sca* 14, sixteen at *Sca* 23, four at *Sca* 37, six at *Sca* 44, five at *Sca* 49, three at *Sca* 61), and eleven at *Sca* 65) was tested in each distance class, resulting in 250 Moran's I values. Only 10 significant ($P < 0.05$) Moran's I values generated: one at *Sca* 14 (positive in the third distance class); seven at *Sca* 23 (two positive in the second distance class, four positive in the third distance class, and one negative in the fifth distance class); two at *Sca* 37 (one negative in the fourth distance class and one positive in the fifth distance class), and one at *Sca* 65 (negative in the fifth distance class). No significant Moran's I values were found at *Sca* 44, *Sca* 49, and *Sca* 61. Only one of the 'significant' Moran's I values (a positive value for *Sca* 23-22 in the third distance class) remained significant after Bonferroni correction. Collectively, these results indicate that there is no significant relationship of allele frequencies with geographic distance between pairs of samples. This is reflected in

the correlogram (Figure 4) where average (± SE) Moran's I values for each microsatellite is plotted by distance class. Most importantly, results of spatial autocorrelation do not indicate an isolation-by-distance effect, or that gene flow in king mackerel occurs in a stepping-stone fashion. Rather, the absence of spatial autocorrelation indicates that gene flow in king mackerel is consistent with expectations of an island model (*sensu* Wright 1943) of population structure, meaning that there is roughly an equal probability of gene flow between any of the 20 sample localities.

The last analysis carried out with the microsatellite data involved 'assignment tests' (Paetkau et al. 1995, 1997), where the expected frequency of an individual's genotype at all seven microsatellites was calculated and the individual 'assigned' to a population (stock) where the expected genotype frequency of the individual was the highest. We employed two populations (stocks), Atlantic and Gulf, based on the pooled tests of allele distribution homogeneity where samples from the Atlantic were found to be weakly (but significantly) divergent from samples from the Gulf. We then computed the proportion of individuals from each of the 20 samples that were 'assigned' to the Atlantic or Gulf groups. As shown in Table 12, samples from each of the 20 samples include high proportions of both Atlantic and Gulf king mackerel. On average, samples from the Atlantic contain more 'Atlantic' fish (54%), whereas samples from the Gulf contain more 'Gulf' fish (51.2%). The three samples from the Florida Keys, on average, contained more 'Atlantic' fish (53.3%), but this is misleading as the proportion of 'Atlantic' in the three samples from the Florida Keys ranged from 37.2% to 64.0 % (Table 12). In addition, the estimated proportions of Atlantic versus Gulf fish in samples from the Florida Keys were not consistent with what might be predicted based on the time of sampling and the spatial-temporal boundaries used currently in king mackerel stock assessment. The KEY^1 and KEY^2 samples were obtained in March, a time when both would be considered Gulf stock, yet close to the temporal boundary (1 April) when they would be considered Atlantic stock. The estimated proportion of Gulf fish in these two samples was 62.8% (KEY^1) and 41.4% (KEY^2). Alternatively, the KEY^3 sample was obtained in January, firmly within the time king mackerel in the Florida Keys are considered Gulf stock. The estimated proportion of Gulf fish in the KEY^3 sample was 36.0%. In general, the assignment tests were concordant with all of the other analyses of king mackerel microsatellites: fish from the Atlantic are very weakly divergent genetically from fish in the Gulf. The assignment tests also are consistent with the results of spatial autocorrelation analysis, in that there is no strong geographic pattern evident in the proportions of fish assigned to either the Atlantic or Gulf group.

CONCLUSIONS

The overall goal of the project was to examine rigorously by means of genetic markers (microsatellites) the spatial-temporal limits of the two (presumed) stocks (migratory units) of king mackerel along peninsular Florida. Subsumed within this goal was a test of the two stock hypothesis. If separate stocks existed, a second goal was to estimate the proportions of both stocks in a mixing zone that was presumed to occur around southern Florida. This mixing zone is thought to occur primarily during the winter months, and for purposes of stock assessment and resource allocation, the boundary between the two stocks is specified as the Volusia/Flagler county line (east coast of Florida) in winter (1 November - 31 March) and the Monroe/Collier county line (west coast of Florida) in summer (1 April - 31 October. Extensive sampling (1,006 fish total) of king mackerel was carried out between 1996 and 1998, from Jacksonville on the northeast coast of Florida to Panama City on the northwest coast of Florida. Each fish was assayed for genotype at seven, independent, nuclear-encoded microsatellites.

Results of homogeneity testing were consistent with the hypothesis that there are two, *very* weakly differentiated 'genetic' stocks (subpopulations) of king mackerel within the area sampled: one in the Atlantic that extends as far south as offshore of West Palm Beach on the east coast of Florida, and one in

the Gulf that extends as far south as Marco Island on the west coast of Florida. These 'stock' boundaries appear not to vary temporally, in that significant (genetic) heterogeneity was found only when samples were pooled along geographic lines independent of season. Tests of genetic homogeneity when samples were pooled along seasonal boundaries, consistent with temporal boundaries used currently for stock assessment and resource allocation, were non-significant. It is to be emphasized that the degree of genetic divergence between the two 'genetic' stocks is very small. On average, only 0.19% of the total genetic variance among the seven microsatellites assayed occurs between the two regions; whereas, on average, 99.74% of the total genetic variance occurs within samples.

Cluster analyses and assignment tests (the latter is a form of mixed stock analysis) were employed, respectively, to determine whether samples could be placed into clustered temporal or geographic units and to estimate the proportion of Atlantic or Gulf fish within individual samples. These approaches represented a different technique to determine whether genetic data were consistent with geographic or spatial-temporal boundaries. Of particular interest in these analyses were the samples from the Florida Keys, an area situated geographically between the two regions. Neither approach generated patterns consistent with either geographic or spatial-temporal boundaries: no stable or geographically cohesive sample relationships were revealed by cluster analysis, and assignment proportions indicated a more-or-less even mixture of Atlantic and Gulf fish among most samples. There also was no indication in cluster analyses that the samples from the Florida Keys represented any more of a mixture of Atlantic and Gulf fish than did other samples. This is particularly noteworthy, as the samples from the Florida Keys were obtained during January and March, when current temporal-spatial boundaries would place fish in the Gulf Migratory Unit. Assignment tests did suggest larger differences in proportions of Atlantic and Gulf fish in samples from the Florida Keys than elsewhere. The observed differences, however, were inconsistent, and for two samples, opposite to what would be expected given the temporal boundaries currently in effect for stock assessment. The KEY^2 sample was obtained in March and would be considered Gulf stock, yet was estimated to contain 58.6% Atlantic fish; whereas the KEY^3 sample was obtained in January (also Gulf stock), yet was estimated to contain 64% Atlantic fish. The KEY^1 sample also was obtained in March (making it Gulf stock) and was estimated to contain only 37.2% Atlantic fish. While seemingly consistent with present temporal stock boundaries, the percentage of Atlantic fish in the KEY^1 sample was not concordant with the percentage of Atlantic fish in the KEY^2 sample, nor was it consistent with the notion that March is very close to the temporal change in boundaries when one might expect the proportion of Atlantic fish in the Florida Keys to be increasing.

Spatial autocorrelation analysis essentially revealed no relationship between genetic divergence and geographic distance among samples. Correlations of allele frequencies were essentially the same between geographically proximal and geographically distant localities. This indicates that samples taken in the same or different year or season from the same or different locality are equally similar or different from one another. This interpretation is consistent with results of assignment tests, where the proportion of Atlantic and Gulf fish within most samples was approximately 50:50.

Taken together, the genetic data obtained in this study are compatible with the hypothesis that two, *very* weakly differentiated 'genetic' subpopulations of king mackerel exist in peninsular Florida and that considerable, perhaps extensive, mixing occurs between them. King mackerel sampled from the Florida Keys cannot easily be assigned unequivocally to either 'genetic' stock, and like most other samples, contain mixtures with approximately the same proportion of fish from both 'genetic' stocks. These results are not consistent with the current spatial-temporal boundaries employed in stock assessment and allocation of the king mackerel resource. The results are consistent with the hypothesis that considerable gene flow occurs among all of the localities sampled, and that differences in gene flow likely do not arise as a function of geographic distance. Similar findings were obtained by Gold et al. (1997) in their study of variation in king mackerel mitochondrial DNA. The very slight genetic differences between king

mackerel in the Atlantic versus those in the Gulf most likely stem from slightly reduced gene flow (migration) between the Atlantic and Gulf relative to gene flow (migration) along the Atlantic and Gulf coasts of peninsular Florida. This is consistent with the notion based on studies in other marine fishes (Avise et al. 1992; Gold and Richardson 1998) that the southern Florida peninsula serves (or has served) as a biogeographic boundary.

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Table 1. Localities, acronyms, dates of collection, and number of individuals (by sex) of king mackerel (*Scomberomorus cavalla*) sampled from the east and west coasts of Florida and the Florida Keys.

Sample locality	Acronym	Date of capture	Number of individuals ♀♀	♂♂	Total	Migratory group*
Atlantic Ocean (east coast):						
Jacksonville, FL	JCK[1]	July 1996	48	0	48	Atlantic
Jacksonville, FL	JCK[2]	July 1998	28	3	31	Atlantic
New Smryna Beach, FL	NSB	July 1996	41	9	50	Atlantic
Cape Canaveral, FL	CCN	December 1998	24	26	50	Gulf
Sebastian, FL	SEB[1]	March 1997	29	21	50	Gulf
Sebastian, FL	SEB[2]	March 1998	24	26	50	Gulf
Sebastian, FL	SEB[3]	December 1998	35	15	50	Gulf
Ft. Pierce, FL	FTP	April 1996	31	25	56	Atlantic
West Palm Beach, FL	WPB	May 1998	29	25	54	Atlantic
Florida Keys:						
Key West, FL	KEY[1]	March 1996	41	10	51	Gulf
Key West, FL	KEY[2]	March 1997	--	--	29	Gulf
Key West, FL	KEY[3]	January 1999	29	19	48	Gulf
Gulf of Mexico (west coast):						
Marco Island, FL	MCI	April 1996	29	26	55	Atlantic
Boca Grande, FL	BCG	April 1996	31	4	35	Gulf
Sarasota, FL	SAR[1]	April 1996	39	5	44	Gulf
Sarasota, FL	SAR[2]	November 1996	60	0	60	Gulf
Sarasota, FL	SAR[3]	April 1997	55	2	57	Gulf
Sarasota, FL	SAR[4]	April 1998	68	2	70	Gulf
Sarasota, FL	SAR[5]	November 1998	62	6	68	Gulf
Panama City, FL	PCY	October 1996	25	25	50	Gulf

* Assignment to Atlantic or Gulf migratory group based on the two migratory unit hypothesis where boundaries change seasonally. See text for further details.

Table. 2. Summary of variation in seven microsatellites among king mackerel (*Scomberomorus cavalla*) sampled from the east and west coasts of Florida and the Florida Keys.

Microsatellite	Repeat sequence	# Alleles	Average heterozygosity ± S.E.	P_{HW}*
Sca 14	$(CA)_6$ TA $(CA)_{13}$	5	0.474 ± 0.016	0/20
Sca 23	$(CA)_4$ AAC $(AG)_{12}$	24	0.803 ± 0.105	4/20†
Sca 37	$(TG)_8$ AG $(TG)_4$ AG $(TG)_4$	9	0.509 ± 0.014	0/20
Sca 44	$(CTCG)_2$ CTAT $(CTGT)_5$	8	0.677 ± 0.014	0/20
Sca 49	$(TG)_{17}$	15	0.656 ± 0.018	0/20
Sca 61	$(CA)_6$ TGTA $(CA)_8$	6	0.311 ± 0.018	0/20
Sca 65	$(TG)_{13}$	24	0.798 ± 0.016	0/20

* Proportion of samples where $P < 0.05$, following Bonferroni correction.

† Probability values for three samples (SEB^2, KEY^1, and SAR^4) were 0.000. The probability value for SAR^1 was 0.006 (adjusted α was 0.003).

Table 3. Probability of genotype equilibrium (pairwise comparisons) among seven microsatellite loci in king mackerel (*Scomberomorus cavalla*) sampled from the east and west coasts of Florida and the Florida Keys.

Locus	*Sca* 14	*Sca* 23	*Sca* 37	*Sca* 44	*Sca* 49	*Sca* 61	*Sca* 65
Sca 14	---	0.389	0.684	0.900	0.665	0.977	0.906
Sca 23		---	0.022	1.000	0.805	0.603	0.194
Sca 37			---	0.724	0.876	0.910	0.430
Sca 44				---	0.328	0.000	0.020
Sca 49					---	0.295	0.998
Sca 61						---	0.873
Sca 65							---

Corrected α (for initial test) = 0.002

Table 4. Probability values from exact tests of homogeneity of allele distributions between males and females for seven microsatellites among king mackerel (*Scomberomorus cavalla*) sampled from the east and west coasts of Florida and the Florida Keys.

Sample*	*Sca* 14	*Sca* 23	*Sca* 37	*Sca* 44	*Sca* 49	*Sca* 61	*Sca* 65
JKV[2]	0.419	0.951	0.757	0.119	0.021	0.590	0.091
NSB	0.679	0.044	0.433	0.653	0.277	0.450	0.080
CCN	0.154	0.661	0.644	0.665	0.514	0.752	0.412
SEB[1]	0.250	0.243	0.035	0.931	0.447	0.510	0.544
SEB[2]	0.795	0.079	0.762	0.437	0.619	0.536	0.359
SEB[3]	0.051	0.927	0.974	0.155	0.176	0.405	0.199
FTP	0.695	0.177	0.580	0.561	0.304	0.916	0.449
WPB	0.089	0.581	0.153	0.648	0.455	0.288	0.466
KEY[1]	1.000	0.569	0.689	0.591	0.314	1.000	0.131
KEY[3]	0.553	0.349	0.808	0.278	0.512	0.727	0.112
MCI	0.858	0.773	0.774	0.943	0.975	0.049	0.023
BCG	1.000	0.784	0.054	0.520	0.350	0.836	0.574
SAR[1]	1.000	0.961	0.398	0.305	0.530	0.566	0.517
SAR[3]	0.337	0.402	0.052	0.635	0.191	1.000	0.240
SAR[4]	0.659	0.511	1.000	0.188	0.168	0.120	0.809
SAR[5]	0.788	0.972	0.704	0.530	0.032	0.368	0.136
PCY	0.847	0.031	0.790	0.058	0.464	0.207	0.519
Atlantic[a]	0.336	0.915	0.238	0.586	0.025	0.272	0.672
Keys[b]	0.630	0.700	0.968	0.405	0.435	0.814	0.101
Gulf[c]	0.677	0.717	0.355	0.064	0.456	0.844	0.416
All[d]	0.354	0.823	0.164	0.494	0.972	0.188	0.321

* No sex data were available for KEY[2], and samples JCK[1] and SAR[2] contained only ♀♀.

[a] Atlantic: JKV[2], NSB, CCN, SEB[1] - SEB[3], FTP, and WPB (pooled).
[b] Keys: KEY[1] and KEY[3] (pooled).
[c] Gulf: MCI, BCG, SAR[1] - SAR[5], and PCY (pooled).
[d] All: all samples (pooled).

Table 5. Probability values from exact tests of homogeneity of allele distributions among year classes (cohorts) for seven microsatellites in king mackerel (*Scomberomorus cavalla*) sampled from the east and west coasts of Florida and the Florida Keys.

Sample	*Sca* 14	*Sca* 23	*Sca* 37	*Sca* 44	*Sca* 49	*Sca* 61	*Sca* 65
JKV[1]	0.163	0.056	0.938	0.717	0.176	0.758	0.328
NSB	0.644	0.476	0.136	0.216	0.352	0.580	0.934
CCN	0.203	0.535	0.139	0.206	0.950	0.189	0.544
SEB[1]	0.033	0.430	0.186	0.603	0.146	0.431	0.264
SEB[2]	0.400	0.050	0.956	0.784	0.932	0.037	0.962
SEB[3]	0.472	0.616	0.197	0.650	0.696	0.145	0.211
FTP	0.493	0.582	0.597	0.947	0.511	0.059	0.858
WPB	0.757	0.421	0.482	0.987	0.571	0.418	0.459
KEY[1]	0.513	0.636	0.325	0.190	0.144	0.355	0.810
KEY[3]	0.645	0.130	0.074	0.493	0.188	0.897	0.745
MCI	0.269	0.057	0.012	0.705	0.669	0.376	0.919
BCG	0.919	0.528	0.708	0.293	0.185	0.037	0.285
SAR[1]	0.449	0.638	0.299	0.573	0.914	0.974	0.486
SAR[2]	0.410	0.421	0.438	0.127	0.534	0.235	0.496
SAR[4]	0.166	0.000	0.157	0.193	0.030	0.004	0.400
SAR[5]	0.476	0.323	0.475	0.785	0.099	0.873	0.448
PCY	0.387	0.441	0.142	0.970	0.754	0.356	0.891
Atlantic[a]	0.868	0.266	0.188	0.809	0.094	0.257	0.988
Keys[b]	0.438	0.096	0.173	0.705	0.199	0.878	0.983
Gulf[c]	0.560	0.117	0.203	0.654	0.189	0.050	0.669
All[d]	0.404	0.149	0.337	0.445	0.114	0.128	0.953

Age data were not available for the following samples: JKV[2], KEY[2], and SAR[3]

[a] Atlantic: JKV[1], NSB, CCN, SEB[1] - SEB[3], FTB, and WPB (eight samples total)
[b] Keys: KEY[1] and KEY[3]
[c] Gulf: MCI, BCG, SAR[1], SAR[2], SAR[4], SAR[5], and PCY (seven samples total)
[d] All: all 17 samples for which age data were available

Table 6a. Results of tests for spatial homogeneity in allele distribution of seven microsatellites among king mackerel (*Scomberomorus cavalla*) sampled from the east and west coasts of Florida and the Florida Keys.

Test group	All samples[a]		Atlantic localities[b]		Florida Keys[c]		Gulf localities[d]	
	P_{EXACT}	P_{RB}	P_{EXACT}	P_{RB}	P_{EXACT}	P_{RB}	P_{EXACT}	P_{RB}
Microsatellite								
Sca 14	0.465	0.447	0.845	0.826	0.510	0.503	0.697	0.668
Sca 23	0.026	0.106	0.250	0.416	0.076	0.068	0.175	0.140
Sca 37	0.431	0.388	0.592	0.498	0.038	0.024	0.592	0.619
Sca 44	0.084	0.073	0.558	0.508	0.111	0.161	0.187	0.136
Sca 49	0.230	0.472	0.487	0.746	0.591	0.529	0.112	0.112
Sca 61	0.278	0.428	0.531	0.563	0.065	0.084	0.273	0.296
Sca 65	0.611	0.588	0.457	0.611	0.910	0.957	0.411	0.169

P_{EXACT} = probability based on Fisher's exact tests, with 1,000 permutations
P_{RB} = probability based on 1,000 bootstrapped replicates (after Roff and Bentzen 1987)

[a] All: all twenty samples.
[b] Atlantic: JKV^1 - JKV^2, NSB, CCN, SEB^1 - SEB^3, FTP, and WPB (nine samples total).
[c] Keys: KEY^1 - KEY^3 (three samples total).
[d] Gulf: MCI, BCG, SAR^1 - SAR^5, and PCY (seven samples total).

Table 6b. Results of tests for spatial homogeneity in allele distribution of seven microsatellites between and among pooled samples of king mackerel (*Scomberomorus cavalla*) from the east and west coasts of Florida and the Florida Keys.

Comparison	Atlantic vs Gulf				Atlantic vs Keys vs Gulf			
	P_{EXACT}	P_{RB}	Φ_{CT}	P	P_{EXACT}	P_{RB}	Φ_{CT}	P
Microsatellite								
Sca 14	0.009	0.006	0.005	0.010	0.020	0.029	0.004	0.031
Sca 23	0.010	0.007	0.001	0.125	0.026	0.081	0.000	0.256
Sca 37	0.426	0.443	–0.001	0.588	0.009	0.001	–0.001	0.794
Sca 44	0.060	0.056	0.004	0.023	0.008	0.004	0.003	0.024
Sca 49	0.506	0.491	–0.001	0.912	0.580	0.535	–0.001	0.707
Sca 61	0.391	0.382	0.001	0.169	0.520	0.689	–0.001	0.398
Sca 65	0.625	0.645	0.004	0.556	0.775	0.508	0.004	0.145

P_{EXACT} = probability based on Fisher's exact tests, with 1,000 permutations
P_{RB} = probability based on 1,000 bootstrapped replicates (after Roff and Bentzen 1987)
Φ_{CT} = Estimate of population subdivision based on AMOVA; P is the probability that Φ_{CT} differs significantly from zero (5000 permutations)

Atlantic: JKV[1] - JKV[2], NSB, CCN, SEB[1] - SEB[3], FTP, and WPB (nine samples total)
Keys: KEY[1] - KEY[3] (three samples total)
Gulf: MCI, BCG, SAR[1] - SAR[5], and PCY (seven samples total)

Table 7. Allele frequencies at *Sca* 14 and *Sca 23* for king mackerel (*Scomberomorus cavalla)* from the Atlantic, Florida Keys, and Gulf.

Microsatellite (allele)	Atlantic	Florida Keys	Gulf
Sca 14			
18	0.017	0.004	0.006
19	0.053	0.058	0.034
20	0.670	0.667	0.735
21	0.233	0.240	0.200
22	0.029	0.031	0.027
Sca 23			
12	0.063	0.054	0.052
13	0.028	0.050	0.028
14	0.221	0.250	0.241
15	0.012	0.008	0.005
16	0.131	0.092	0.121
17	0.026	0.008	0.015
18	0.146	0.108	0.132
19	0.185	0.177	0.149
20	0.008	0.011	0.015
21	0.100	0.111	0.109
22	0.011	0.011	0.011
23	0.009	0.011	0.012
24	0.001	0.000	0.001
25	0.015	0.027	0.016
26	0.002	0.004	0.009
27	0.002	0.004	0.000
28	0.009	0.019	0.018
29	0.017	0.019	0.039
30	0.009	0.015	0.010
31	0.002	0.008	0.009
32	0.002	0.008	0.001
33	0.001	0.004	0.002
34	0.002	0.000	0.002
*Sca*44			
8	0.004	0.015	0.009
12	0.084	0.046	0.090
14	0.030	0.019	0.041
16	0.366	0.308	0.295
18	0.398	0.465	0.438
20	0.104	0.131	0.118
22	0.012	0.011	0.010
24	0.000	0.004	0.000

Table 8a. Analysis of molecular variation (AMOVA) among microsatellites of king mackerel (*Scomberomorus cavalla*): comparison of Atlantic vs Gulf (excluding the Florida Keys).

Microsatellite Comparison	*Sca* 14	*Sca* 23	*Sca* 37	*Sca* 44	*Sca* 49	*Sca* 61	*Sca* 65
Variance component							
Among regions:							
Variance	0.001252	0.000279	– 0.000172	0.001296	– 0.000436	0.000210	0.001691
% total	0.54	0.07	– 0.07	0.37	– 0.13	0.13	0.43
Φ_{CT}	0.005	0.001	– 0.001	0.004	– 0.001	0.001	0.004
P	0.010	0.125	0.588	0.023	0.912	0.169	0.556
Among samples within regions:							
Variance	– 0.000346	0.000206	– 0.000021	0.000621	0.001440	0.000173	– 0.000390
% total	– 0.15	0.05	– 0.01	0.18	0.44	0.11	– 0.10
Φ_{SC}	–0.002	0.000	– 0.000	0.002	0.004	0.001	– 0.001
P	0.674	0.347	0.436	0.203	0.042	0.319	0.697
Within samples:							
Variance	0.228952	0.433085	0.248991	0.346410	0.327415	0.158543	0.387968
% total	99.61	99.88	100.08	99.45	99.69.	99.82	99.67
Φ_{ST}	0.004	0.001	– 0.001	0.006	0.003	0.002	0.003
P	---	---	---	---	---	---	---

Table 8b. Analysis of molecular variation (AMOVA) among microsatellites of king mackerel (*Scomberomorus cavalla*): comparison of Atlantic vs Florida Keys vs Gulf.

Microsatellite Comparison	*Sca* 14	*Sca* 23	*Sca* 37	*Sca* 44	*Sca* 49	*Sca* 61	*Sca* 65
Variance component							
Among regions:							
Variance	0.00000939	0.000151	−0.000245	0.001118	−0.000313	−0.000029	0.001489
% total	0.40	0.03	−0.10	0.32	−0.10	−0.13	0.38
Φ_{CT}	0.004	0.000	−0.001	0.003	−0.001	−0.001	0.004
P	0.031	0.256	0.794	0.024	0.707	0.398	0.145
Among samples within regions:							
Variance	−0.000363	0.000391	−0.000166	0.000452	0.001173	0.000561	−0.000579
% total	−0.16	0.09	−0.07	0.13	0.36	0.35	−0.15
Φ_{SC}	−0.002	0.001	−0.001	0.003	0.004	0.003	−0.002
P	0.683	0.248	0.521	0.275	0.067	0.112	0.806
Within samples:							
Variance	0.231380	0.433104	0.249003	0.345131	0.326178	0.159850	0.385873
% total	99.75	99.87	100.17	99.55	99.70.	99.67	99.76
Φ_{ST}	0.002	0.001	−0.002	0.005	0.003	0.003	0.002
P	---	---	---	---	---	---	---

Table 9. Results of tests for homogeneity in allele distributions of seven microsatellites among currently recognized migratory units (stocks) of king mackerel (*Scomberomorus cavalla*) sampled from the east and west coasts of Florida and the Florida Keys.

Test group	Atlantic stock vs Gulf stock		Atlantic stock vs Gulf stock^{A}		Atlantic stock vs Gulf stock^{G}	
Microsatellite	P_{EXACT}	P_{RB}	P_{EXACT}	P_{RB}	P_{EXACT}	P_{RB}
Sca 14	0.586	0.600	0.669	0.685	0.172	0.113
Sca 23	0.879	0.833	0.781	0.780	0.475	0.545
Sca 37	0.447	0.404	0.463	0.491	0.506	0.485
Sca 44	0.101	0.108	0.077	0.101	0.155	0.111
Sca 49	0.171	0.260	0.154	0.181	0.253	0.260
Sca 61	0.329	0.288	0.653	0.675	0.125	0.114
Sca 65	0.965	0.977	0.956	0.951	0.679	0.728

Atlantic stock: JCK^{1}, JCK^{2}, NSB, FTP, WPB, and MCI.

Gulf stock: CCN, SEB^{1} - SEB^{3}, KEY^{1} - KEY^{3}, BCG, SAR^{1} - SAR^{5}, and PCY

Gulf stock^{A}: CCN, SEB^{1} - SEB^{3}, and KEY^{1} - KEY^{3}

Gulf stock^{G}: BCG, SAR^{1} - SAR^{5}, and PCY

Table 10. Rogers' genetic distance between samples of king mackerel (*Scomberomorus cavalla*) from the east and west coasts of Florida and the Florida Keys.

Sample	JCK[2]	NSB	CCN	SEB[1]	SEB[2]	SEB[3]	FTP	WPB	KEY[1]	KEY[2]	KEY[3]	MCI	BCG	SAR[1]	SAR[2]	SAR[3]	SAR[4]	SAR[5]	PCY
JCK[1]	0.071	0.076	0.074	0.072	0.087	0.082	0.067	0.072	0.076	0.087	0.072	0.088	0.078	0.079	0.068	0.065	0.060	0.052	0.077
JCK[2]		0.085	0.096	0.083	0.116	0.113	0.073	0.082	0.102	0.099	0.107	0.114	0.106	0.102	0.088	0.103	0.094	0.090	0.100
NSB			0.084	0.080	0.097	0.081	0.064	0.076	0.089	0.093	0.088	0.101	0.102	0.093	0.082	0.082	0.085	0.074	0.074
CCN				0.081	0.086	0.084	0.066	0.073	0.070	0.082	0.075	0.093	0.101	0.057	0.072	0.081	0.075	0.076	0.070
SEB[1]					0.121	0.109	0.069	0.068	0.089	0.095	0.093	0.087	0.086	0.096	0.081	0.073	0.094	0.082	0.090
SEB[2]						0.083	0.090	0.103	0.097	0.095	0.086	0.107	0.122	0.093	0.097	0.109	0.092	0.092	0.096
SEB[3]							0.081	0.091	0.061	0.091	0.077	0.083	0.096	0.077	0.072	0.091	0.073	0.057	0.072
FTP								0.057	0.080	0.090	0.085	0.089	0.098	0.079	0.080	0.086	0.080	0.055	0.069
WPB									0.075	0.084	0.081	0.095	0.097	0.076	0.079	0.092	0.086	0.066	0.074
KEY[1]										0.093	0.076	0.076	0.077	0.062	0.060	0.076	0.071	0.064	0.070
KEY[2]											0.089	0.094	0.115	0.077	0.082	0.100	0.086	0.089	0.082
KEY[3]												0.105	0.110	0.081	0.086	0.091	0.085	0.075	0.085
MCI													0.084	0.087	0.090	0.079	0.092	0.078	0.083
BCG														0.099	0.081	0.071	0.092	0.083	0.100
SAR[1]															0.074	0.083	0.065	0.071	0.067
SAR[2]																0.071	0.053	0.068	0.077
SAR[3]																	0.074	0.073	0.082
SAR[4]																		0.059	0.068
SAR[5]																			0.064

Table 11. Cavalli-Sforxa and Edwards (1967) chord (genetic) distance (x 10^{-1}) between samples of king mackerel (*Scomberomorus cavalla*) from the east and west coasts of Florida and the Florida Keys.

Sample	JCK[2]	NSB	CCN	SEB[1]	SEB[2]	SEB[3]	FTP	WPB	KEY[1]	KEY[2]	KEY[3]	MCI	BCG	SAR[1]	SAR[2]	SAR[3]	SAR[4]	SAR[5]	PCY
JCK[1]	0.092	0.109	0.093	0.081	0.090	0.083	0.072	0.079	0.105	0.134	0.084	0.110	0.115	0.075	0.104	0.061	0.078	0.057	0.105
JCK[2]		0.098	0.136	0.105	0.139	0.123	0.111	0.139	0.134	0.162	0.160	0.132	0.146	0.132	0.117	0.108	0.101	0.109	0.146
NSB			0.134	0.104	0.112	0.095	0.107	0.112	0.130	0.143	0.129	0.110	0.136	0.135	0.099	0.092	0.104	0.100	0.115
CCN				0.098	0.109	0.099	0.080	0.104	0.130	0.155	0.103	0.124	0.127	0.118	0.117	0.090	0.099	0.084	0.112
SEB[1]					0.113	0.109	0.059	0.069	0.144	0.145	0.096	0.095	0.117	0.124	0.109	0.077	0.101	0.075	0.121
SEB[2]						0.103	0.092	0.100	0.148	0.123	0.120	0.108	0.136	0.156	0.113	0.104	0.120	0.095	0.141
SEB[3]							0.074	0.106	0.093	0.109	0.089	0.093	0.124	0.110	0.084	0.096	0.084	0.076	0.107
FTP								0.067	0.099	0.134	0.091	0.089	0.128	0.107	0.083	0.074	0.084	0.049	0.102
WPB									0.129	0.127	0.096	0.125	0.129	0.119	0.114	0.083	0.113	0.078	0.103
KEY[1]										0.155	0.132	0.112	0.123	0.102	0.087	0.113	0.090	0.100	0.119
KEY[2]											0.145	0.135	0.173	0.175	0.143	0.135	0.136	0.137	0.131
KEY[3]												0.127	0.133	0.131	0.127	0.103	0.116	0.073	0.111
MCI													0.128	0.130	0.111	0.093	0.086	0.095	0.103
BCG														0.150	0.121	0.103	0.121	0.099	0.154
SAR[1]															0.129	0.103	0.083	0.083	0.118
SAR[2]																0.094	0.082	0.087	0.121
SAR[3]																	0.077	0.066	0.087
SAR[4]																		0.070	0.090
SAR[5]																			0.066

Table 11. Cavalli-Sforza and Edwards (1967) chord (genetic) distance (x 10^{-1}) between samples of king mackerel (*Scomberomorus cavalla*) from the east and west coasts of Florida and the Florida Keys.

Sample	JCK[2]	NSB	CCN	SEB[1]	SEB[2]	SEB[3]	FTP	WPB	KEY[1]	KEY[2]	KEY[3]	MCI	BCG	SAR[1]	SAR[2]	SAR[3]	SAR[4]	SAR[5]	PCY
JCK[1]	0.092	0.109	0.093	0.081	0.090	0.083	0.072	0.079	0.105	0.134	0.084	0.110	0.115	0.075	0.104	0.061	0.078	0.057	0.105
JCK[2]		0.098	0.136	0.105	0.139	0.123	0.111	0.139	0.134	0.162	0.160	0.132	0.146	0.132	0.117	0.108	0.101	0.109	0.146
NSB			0.134	0.104	0.112	0.095	0.107	0.112	0.130	0.143	0.129	0.110	0.136	0.135	0.099	0.092	0.104	0.100	0.115
CCN				0.098	0.109	0.099	0.080	0.104	0.130	0.155	0.103	0.124	0.127	0.118	0.117	0.090	0.099	0.084	0.112
SEB[1]					0.113	0.109	0.059	0.069	0.144	0.145	0.096	0.095	0.117	0.124	0.109	0.077	0.101	0.075	0.121
SEB[2]						0.103	0.092	0.100	0.148	0.123	0.120	0.108	0.136	0.156	0.113	0.104	0.120	0.095	0.141
SEB[3]							0.074	0.106	0.093	0.109	0.089	0.093	0.124	0.110	0.084	0.096	0.084	0.076	0.107
FTP								0.067	0.099	0.134	0.091	0.089	0.128	0.107	0.083	0.074	0.084	0.049	0.102
WPB									0.129	0.127	0.096	0.125	0.129	0.119	0.114	0.083	0.113	0.078	0.103
KEY[1]										0.155	0.132	0.112	0.123	0.102	0.087	0.113	0.090	0.100	0.119
KEY[2]											0.145	0.135	0.173	0.175	0.143	0.135	0.136	0.137	0.131
KEY[3]												0.127	0.133	0.131	0.127	0.103	0.116	0.073	0.111
MCI													0.128	0.130	0.111	0.093	0.086	0.095	0.103
BCG														0.150	0.121	0.103	0.121	0.099	0.154
SAR[1]															0.129	0.103	0.083	0.083	0.118
SAR[2]																0.094	0.082	0.087	0.121
SAR[3]																	0.077	0.066	0.087
SAR[4]																		0.070	0.090
SAR[5]																			0.066

Table 12. Assignment (in percent) of individuals from each of 20 samples of king mackerel (*Scomberomorus cavalla*) from the east and west coast of Florida and the Florida Keys to either an 'Atlantic' or 'Gulf' grouping.

Sample	Assigned Group	
	Atlantic	Gulf
Atlantic Ocean:		
JCK[1]	51.8	48.2
JCK[2]	62.5	37.5
NSB	58.0	42.0
CCN	46.0	54.0
SEB[1]	58.0	42.0
SEB[2]	62.0	38.0
SEB[3]	52.0	48.0
FTP	53.6	46.4
WPB	41.8	58.2
Avg.	54.0	46.0
Florida Keys:		
KEY[1]	37.2	62.8
KEY[2]	58.6	41.4
KEY[3]	64.0	36.0
Avg.	53.3	46.7
Gulf of Mexico:		
MCI	49.1	50.9
BCG	45.7	54.3
SAR[1]	46.8	53.2
SAR[2]	53.3	46.7
SAR[3]	53.6	46.4
SAR[4]	41.1	58.6
SAR[5]	54.3	45.7
PCY	46.0	54.0
Avg.	48.8	51.2

Appendix Table A1. Nuclear microsatellites employed for king mackerel (*Scomberomorus cavalla*).

Microsatellite	Primer sequence (5' → 3') (forward and reverse, respectively)	Length (base pairs)	Annealing temperature (^{0}C)
Sca 14	ATT CCC CAA ACA ATA CAC AC AGT GGA CGA CCC ATT CTA C	93	56
Sca 23	AGC CCT CTT ACA ATC TGC TAC CC AAA CCT TTA AGG CCT CAA GTA AAG	145	58
Sca 37	GCG CCG TGA CTT TTT ATT GCT C CAA CAA TTA GTC GCA GCC CTA G	154	58
Sca 44	ATG GCC AAA TGG CAC ATA ATC A GGG CAG CTC CAT GGG TCT GAG T	169	58
Sca 49	AGA TGT GAC AAC AGT GGG ATG GCA GCA GTA ATA AAG	157	56
Sca 61	GGT ACT GTC GGG AGA ATG AGA T TGA ATT TTA TAT GGA GGG TCT G	228	56
Sca 65	AGC TGC TGC CAT GAT TTG TT TCC TCC ACT GCC CCT TTC TT	129	52

Appendix Table B1. Allele frequencies at microsatellites in king mackerel (*Scomberomorus cavalla*).

Sample / Microsatellite (allele)	JKV[1]	JKV[2]	NSB	CCN	SEB[1]	SEB[2]	SEB[3]	FTP	WPB	KEY[1]
Sca 14										
18	0.000	0.031	0.040	0.030	0.010	0.030	0.000	0.009	0.009	0.010
19	0.046	0.063	0.070	0.040	0.041	0.070	0.050	0.063	0.037	0.030
20	0.741	0.719	0.650	0.630	0.735	0.590	0.670	0.643	0.667	0.680
21	0.185	0.188	0.220	0.250	0.184	0.280	0.250	0.259	0.259	0.260
22	0.028	0.000	0.020	0.050	0.031	0.030	0.030	0.027	0.028	0.020
Sca 23										
12	0.046	0.016	0.060	0.080	0.090	0.030	0.060	0.091	0.073	0.029
13	0.019	0.032	0.030	0.050	0.020	0.000	0.060	0.027	0.018	0.039
14	0.231	0.210	0.120	0.250	0.230	0.230	0.210	0.236	0.264	0.265
15	0.009	0.016	0.000	0.020	0.020	0.000	0.010	0.018	0.018	0.000
16	0.102	0.129	0.140	0.100	0.140	0.170	0.140	0.127	0.136	0.147
17	0.019	0.048	0.030	0.000	0.060	0.010	0.010	0.036	0.027	0.020
18	0.176	0.161	0.140	0.180	0.120	0.190	0.100	0.155	0.100	0.088
19	0.241	0.177	0.180	0.120	0.180	0.280	0.150	0.145	0.191	0.098
20	0.009	0.016	0.000	0.010	0.010	0.000	0.000	0.018	0.009	0.020
21	0.111	0.113	0.130	0.110	0.060	0.050	0.140	0.064	0.091	0.118
22	0.019	0.016	0.020	0.020	0.000	0.000	0.010	0.000	0.018	0.020
23	0.000	0.016	0.020	0.000	0.010	0.010	0.030	0.000	0.000	0.000
24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000
25	0.009	0.016	0.030	0.010	0.000	0.030	0.020	0.009	0.009	0.039
26	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.009	0.000
27	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.009	0.000	0.010
28	0.009	0.016	0.010	0.020	0.000	0.000	0.010	0.018	0.000	0.039
29	0.000	0.016	0.060	0.010	0.030	0.000	0.010	0.018	0.009	0.029
30	0.000	0.000	0.020	0.000	0.010	0.000	0.010	0.018	0.018	0.010
31	0.000	0.000	0.000	0.010	0.010	0.000	0.000	0.000	0.000	0.010
32	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.010
33	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.010
34	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
35	0.000	0.000	0.000	0.010	0.010	0.000	0.000	0.000	0.000	0.000
Sca 37										
12	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
14	0.000	0.031	0.030	0.000	0.000	0.010	0.010	0.000	0.000	0.020
15	0.667	0.688	0.600	0.694	0.720	0.630	0.540	0.623	0.630	0.637
16	0.009	0.016	0.040	0.010	0.030	0.010	0.030	0.019	0.019	0.000
17	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
18	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
19	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
20	0.296	0.266	0.320	0.286	0.240	0.330	0.400	0.330	0.286	0.333
21	0.019	0.000	0.000	0.010	0.010	0.020	0.020	0.028	0.010	0.010

Sca 44										
8	0.000	0.000	0.000	0.010	0.000	0.000	0.010	0.018	0.000	0.020
10	0.066	0.078	0.110	0.120	0.060	0.080	0.090	0.098	0.056	0.069
12	0.038	0.047	0.030	0.020	0.030	0.040	0.010	0.027	0.037	0.020
14	0.387	0.469	0.390	0.300	0.390	0.310	0.290	0.420	0.370	0.275
16	0.396	0.281	0.380	0.420	0.410	0.470	0.500	0.357	0.333	0.471
18	0.104	0.109	0.090	0.110	0.100	0.090	0.070	0.071	0.194	0.147
20	0.009	0.016	0.000	0.020	0.010	0.010	0.030	0.009	0.009	0.000
22	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sca 49										
8	0.009	0.000	0.000	0.000	0.000	0.000	0.010	0.009	0.000	0.000
9	0.046	0.109	0.060	0.000	0.043	0.020	0.020	0.036	0.037	0.067
10	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011
11	0.546	0.547	0.490	0.590	0.435	0.690	0.580	0.518	0.481	0.533
12	0.167	0.203	0.150	0.130	0.207	0.100	0.170	0.188	0.167	0.178
13	0.019	0.031	0.060	0.030	0.033	0.030	0.030	0.018	0.046	0.000
14	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
15	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
16	0.009	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
17	0.046	0.031	0.090	0.070	0.076	0.060	0.080	0.063	0.093	0.089
18	0.130	0.063	0.130	0.170	0.196	0.090	0.090	0.161	0.148	0.111
19	0.009	0.016	0.000	0.010	0.011	0.010	0.010	0.009	0.009	0.011
20	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.009	0.000
21	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000
22	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000
Sca 61										
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010
12	0.083	0.078	0.100	0.041	0.071	0.102	0.130	0.074	0.046	0.088
13	0.139	0.063	0.050	0.112	0.061	0.071	0.090	0.065	0.083	0.137
14	0.769	0.859	0.850	0.847	0.857	0.827	0.780	0.861	0.861	0.765
15	0.009	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.009	0.000
Sca 65										
7	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010
10	0.380	0.422	0.360	0.430	0.440	0.350	0.360	0.384	0.464	0.438
11	0.028	0.047	0.040	0.050	0.030	0.080	0.040	0.063	0.036	0.063
12	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.010
13	0.065	0.016	0.130	0.060	0.080	0.090	0.130	0.036	0.036	0.073
14	0.037	0.094	0.030	0.030	0.020	0.000	0.030	0.018	0.000	0.042
15	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.009	0.009	0.000
16	0.241	0.219	0.200	0.170	0.150	0.280	0.200	0.223	0.209	0.219
17	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000
18	0.000	0.000	0.000	0.010	0.010	0.010	0.000	0.018	0.018	0.000
19	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000
20	0.009	0.000	0.000	0.010	0.020	0.010	0.000	0.009	0.018	0.010
22	0.093	0.047	0.150	0.080	0.080	0.070	0.150	0.080	0.109	0.063
23	0.028	0.016	0.010	0.020	0.020	0.010	0.010	0.018	0.009	0.021
24	0.009	0.016	0.020	0.040	0.030	0.030	0.020	0.045	0.018	0.000
25	0.046	0.016	0.020	0.000	0.040	0.020	0.020	0.045	0.027	0.031

26	0.037	0.031	0.020	0.050	0.010	0.020	0.020	0.036	0.027	0.010
27	0.019	0.016	0.010	0.030	0.020	0.020	0.010	0.009	0.000	0.000
28	0.000	0.016	0.000	0.020	0.010	0.000	0.000	0.000	0.000	0.000
29	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
30	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010
31	0.000	0.016	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000
32	0.009	0.031	0.010	0.000	0.010	0.000	0.010	0.000	0.000	0.000
34	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Legend to samples:

JKV[1]: Jacksonville, Florida (July 1996)
JKV[2]: Jacksonville, Florida (July, 1998)
NSB: New Smryna Beach, Florida (July 1996)
CCN: Cape Canaveral, Florida (December 1998)
SEB[1]: Sebastian, Florida (March 1997)
SEB[2]: Sebastian, Florida (March 1998)
SEB[3]: Sebastian, Florida (December 1998)
FTP: Ft. Pierce, Florida (April 1996)
WPB: West Palm Beach, Florida (May 1998)
KEY[1]: Key West, Florida (March 1996)

Appendix Table B2. Allele frequencies at microsatellites in king mackerel (*Scomberomorus cavalla*).

Sample Microsatellite (allele)	KEY[2]	KEY[3]	MCI	BCG	SAR[1]	SAR[2]	SAR[3]	SAR[4]	SAR[5]	PCY
Sca 14										
18	0.000	0.000	0.009	0.014	0.000	0.009	0.009	0.000	0.007	0.000
19	0.069	0.080	0.036	0.057	0.033	0.018	0.063	0.021	0.036	0.020
20	0.724	0.620	0.745	0.771	0.685	0.764	0.741	0.771	0.714	0.680
21	0.190	0.250	0.173	0.143	0.250	0.200	0.170	0.186	0.221	0.230
22	0.017	0.050	0.036	0.014	0.033	0.009	0.018	0.021	0.021	0.070
Sca 23										
12	0.086	0.060	0.018	0.057	0.096	0.033	0.027	0.036	0.096	0.060
13	0.017	0.080	0.027	0.029	0.032	0.017	0.027	0.043	0.029	0.020
14	0.224	0.250	0.309	0.257	0.266	0.217	0.200	0.239	0.250	0.200
15	0.017	0.010	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.020
16	0.069	0.050	0.145	0.171	0.064	0.183	0.118	0.109	0.103	0.080
17	0.000	0.000	0.018	0.000	0.032	0.008	0.009	0.029	0.007	0.010
18	0.121	0.120	0.155	0.043	0.160	0.108	0.173	0.130	0.132	0.130
19	0.207	0.240	0.136	0.157	0.117	0.142	0.164	0.174	0.176	0.110
20	0.000	0.010	0.000	0.029	0.011	0.017	0.009	0.007	0.022	0.030
21	0.086	0.120	0.045	0.157	0.117	0.117	0.127	0.109	0.096	0.130
22	0.017	0.000	0.000	0.000	0.021	0.008	0.027	0.007	0.000	0.030
23	0.052	0.000	0.027	0.014	0.000	0.033	0.000	0.014	0.000	0.010
24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000
25	0.034	0.010	0.000	0.029	0.000	0.033	0.018	0.014	0.022	0.010
26	0.017	0.000	0.009	0.014	0.000	0.000	0.009	0.007	0.000	0.040
27	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
28	0.000	0.010	0.027	0.014	0.011	0.025	0.009	0.014	0.015	0.030
29	0.017	0.010	0.036	0.014	0.064	0.025	0.036	0.036	0.037	0.060
30	0.017	0.020	0.018	0.000	0.000	0.017	0.009	0.007	0.015	0.010
31	0.000	0.010	0.027	0.014	0.000	0.000	0.009	0.007	0.000	0.020
32	0.017	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000
33	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000
34	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.007	0.000	0.000
35	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sca 37										
12	0.069	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
14	0.034	0.000	0.009	0.000	0.000	0.017	0.009	0.007	0.000	0.023
15	0.603	0.653	0.627	0.700	0.628	0.642	0.679	0.600	0.579	0.593
16	0.000	0.010	0.018	0.043	0.021	0.025	0.036	0.007	0.007	0.000
17	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000
18	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
19	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
20	0.276	0.327	0.327	0.257	0.319	0.292	0.250	0.343	0.379	0.349
21	0.017	0.010	0.018	0.000	0.032	0.017	0.027	0.043	0.036	0.035

Sca 44										
8	0.034	0.000	0.009	0.014	0.000	0.017	0.000	0.029	0.000	0.000
10	0.069	0.010	0.056	0.100	0.096	0.133	0.089	0.114	0.072	0.050
12	0.052	0.000	0.037	0.014	0.032	0.008	0.098	0.036	0.051	0.040
14	0.276	0.360	0.259	0.314	0.255	0.300	0.268	0.293	0.355	0.300
16	0.431	0.480	0.546	0.443	0.436	0.408	0.455	0.393	0.413	0.430
18	0.121	0.120	0.093	0.100	0.170	0.133	0.080	0.129	0.087	0.160
20	0.017	0.020	0.000	0.014	0.011	0.000	0.009	0.007	0.022	0.020
22	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sca 49										
8	0.000	0.000	0.009	0.000	0.011	0.010	0.009	0.000	0.000	0.000
9	0.069	0.070	0.055	0.043	0.054	0.031	0.054	0.043	0.036	0.020
10	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.007	0.000	0.000
11	0.621	0.580	0.464	0.457	0.576	0.594	0.455	0.614	0.529	0.560
12	0.121	0.100	0.218	0.200	0.076	0.156	0.152	0.114	0.200	0.180
13	0.017	0.040	0.009	0.029	0.011	0.010	0.027	0.014	0.014	0.060
14	0.000	0.000	0.009	0.014	0.000	0.000	0.000	0.007	0.000	0.000
15	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000
16	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
17	0.052	0.080	0.100	0.129	0.076	0.063	0.089	0.021	0.071	0.050
18	0.086	0.130	0.118	0.129	0.141	0.135	0.196	0.171	0.143	0.130
19	0.034	0.000	0.018	0.000	0.000	0.000	0.009	0.007	0.000	0.000
20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
21	0.000	0.000	0.000	0.000	0.022	0.000	0.009	0.000	0.000	0.000
22	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000
Sca 61										
4	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000
5	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000
12	0.052	0.130	0.073	0.086	0.054	0.138	0.127	0.107	0.100	0.070
13	0.017	0.100	0.055	0.143	0.098	0.078	0.100	0.114	0.121	0.070
14	0.931	0.760	0.873	0.757	0.837	0.776	0.755	0.779	0.771	0.860
15	0.999	0.010	0.000	0.014	0.000	0.000	0.018	0.000	0.007	0.000
Sca 65										
7	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010
10	0.483	0.460	0.364	0.357	0.436	0.446	0.375	0.357	0.386	0.340
11	0.017	0.030	0.036	0.100	0.064	0.045	0.071	0.086	0.043	0.060
12	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
13	0.052	0.080	0.055	0.057	0.032	0.054	0.063	0.057	0.021	0.060
14	0.000	0.020	0.027	0.000	0.064	0.036	0.018	0.071	0.029	0.020
15	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
16	0.207	0.160	0.273	0.300	0.245	0.179	0.205	0.193	0.243	0.190
17	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.010
18	0.000	0.010	0.000	0.000	0.000	0.009	0.009	0.007	0.007	0.000
19	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.010
20	0.017	0.010	0.000	0.014	0.000	0.036	0.009	0.000	0.007	0.000
22	0.103	0.110	0.073	0.029	0.053	0.080	0.080	0.100	0.114	0.090
23	0.034	0.020	0.036	0.057	0.021	0.000	0.018	0.014	0.029	0.020
24	0.017	0.040	0.055	0.000	0.000	0.009	0.018	0.014	0.021	0.040
25	0.034	0.030	0.018	0.029	0.021	0.036	0.054	0.029	0.029	0.090

[*Sca* 65]

26	0.017	0.020	0.018	0.014	0.032	0.027	0.045	0.021	0.029	0.050
27	0.017	0.010	0.018	0.014	0.011	0.018	0.027	0.014	0.029	0.010
28	0.000	0.000	0.000	0.014	0.000	0.000	0.009	0.014	0.000	0.000
29	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.007	0.000	0.000
30	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
31	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000
32	0.000	0.000	0.000	0.014	0.021	0.000	0.000	0.014	0.007	0.000
34	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000

Legend to samples:

- KEY[2]: Key West, Florida (March 1997)
- KEY[3]: Key West, Florida (January 1999)
- MCI: Marco Island, Florida (April 1996)
- BCG: Boca Grande, Florida (April 1996)
- SAR[1]: Treasure Island, Florida (April 1996)
- SAR[2]: Treasure Island, Florida (November 1996)
- SAR[3]: Treasure Island, Florida (April 1997)
- SAR[4]: Treasure Island, Florida (April 1998)
- SAR[5]: Treasure Island, Florida (November 1998)
- PCY: Panama City, Florida (October 1996)

Appendix Table C1. Summary statistics for each of seven microsatellites among samples of king mackerel (*Scomberomorus cavalla*).

Microsatellite	JKV[1]	JKV[2]	NSB	CCN	SEB[1]	SEB[2]	SEB[3]	FTP	WPB	KEY[1]
Sca 14										
n	54	32	50	50	49	50	50	56	54	50
H_{DC}	0.481	0.375	0.460	0.500	0.408	0.600	0.420	0.571	0.537	0.520
P_{HW}	0.710	0.297	0.141	0.354	0.333	0.431	0.440	0.203	0.070	0.103
Sca 23										
n	54	31	50	50	50	50	50	55	55	51
H_{DC}	0.833	0.806	0.780	0.840	0.820	0.600	0.800	0.855	0.800	0.765
P_{HW}	0.571	0.094	0.121	0.618	0.654	0.000	0.141	0.085	0.483	0.000
Sca 37										
n	54	32	50	49	50	50	50	53	54	51
H_{DC}	0.500	0.531	0.540	0.449	0.380	0.520	0.560	0.585	0.463	0.608
P_{HW}	0.039	0.306	0.903	0.193	0.607	1.000	0.784	0.291	0.293	0.069
Sca 44										
n	53	32	50	50	50	50	50	56	54	51
H_{DC}	0.604	0.719	0.720	0.680	0.720	0.620	0.660	0.643	0.722	0.569
P_{HW}	0.584	0.965	0.911	0.518	0.583	0.618	0.719	0.875	0.095	0.095
Sca 49										
n	54	32	50	49	46	50	50	56	54	45
H_{DC}	0.611	0.656	0.820	0.680	0.739	0.540	0.620	0.696	0.704	0.600
P_{HW}	0.722	0.543	0.871	0.509	0.290	0.753	0.313	0.958	0.943	0.571
Sca 61										
n	54	32	50	49	49	49	50	54	54	51
H_{DC}	0.407	0.281	0.280	0.306	0.224	0.306	0.360	0.204	0.278	0.392
P_{HW}	1.000	1.000	0.709	1.000	0.152	0.306	0.703	0.093	1.000	0.851
Sca 65										
n	54	32	50	50	50	50	50	56	55	48
H_{DC}	0.870	0.813	0.900	0.900	0.820	0.800	0.780	0.839	0.727	0.729
P_{HW}	0.975	0.388	0.647	0.985	0.777	0.242	0.917	0.978	0.479	0.838

Appendix Table C2. Summary statistics for each of seven microsatellites among samples of king mackerel (*Scomberomorus cavalla*).

Microsatellite (allele)	KEY[2]	KEY[3]	MCI	BCG	SAR[1]	SAR[2]	SAR[3]	SAR[4]	SAR[5]	PCY
Sca 14										
n	29	50	55	35	46	55	56	70	70	50
H_{DC}	0.517	0.500	0.473	0.371	0.543	0.345	0.429	0.414	0.500	0.520
P_{HW}	0.760	0.143	0.246	0.042	0.562	0.403	0.690	0.540	0.824	0.336
Sca 23										
n	29	50	55	35	47	60	55	69	68	50
H_{DC}	0.897	0.760	0.745	0.886	0.723	0.800	0.855	0.783	0.838	0.880
P_{HW}	0.416	0.106	0.032	0.947	0.006	0.039	0.846	0.000	0.647	0.365
*Sca*37										
n	29	50	55	35	47	60	55	69	68	50
H_{DC}	0.552	0.469	0.491	0.486	0.447	0.617	0.446	0.571	0.543	0.419
P_{HW}	0.092	0.647	0.384	0.851	0.079	0.431	0.817	0.499	0.785	0.235
Sca 44										
n	29	50	54	35	47	60	56	70	70	50
H_{DC}	0.828	0.560	0.611	0.686	0.723	0.633	0.696	0.714	0.739	0.700
P_{HW}	0.686	0.219	0.953	0.722	0.353	0.495	0.335	0.813	0.132	0.050
Sca 49										
n	29	50	55	35	46	48	56	70	70	50
H_{DC}	0.586	0.580	0.727	0.771	0.630	0.625	0.714	0.500	0.686	0.640
P_{HW}	0.667	0.055	0.679	0.153	0.098	0.806	0.303	0.246	0.847	0.856
Sca 61										
n	29	50	55	35	46	58	55	70	70	50
H_{DC}	0.138	0.480	0.218	0.343	0.326	0.379	0.327	0.314	0.371	0.280
P_{HW}	1.000	0.486	0.390	0.073	1.000	0.348	0.016	0.105	0.364	1.000
Sca 65										
n	29	50	55	35	47	56	56	70	70	50
H_{DC}	0.690	0.800	0.727	0.743	0.681	0.696	0.839	0.843	0.886	0.880
P_{HW}	0.581	0.656	0.317	0.615	0.250	0.425	0.526	0.879	0.183	0.942

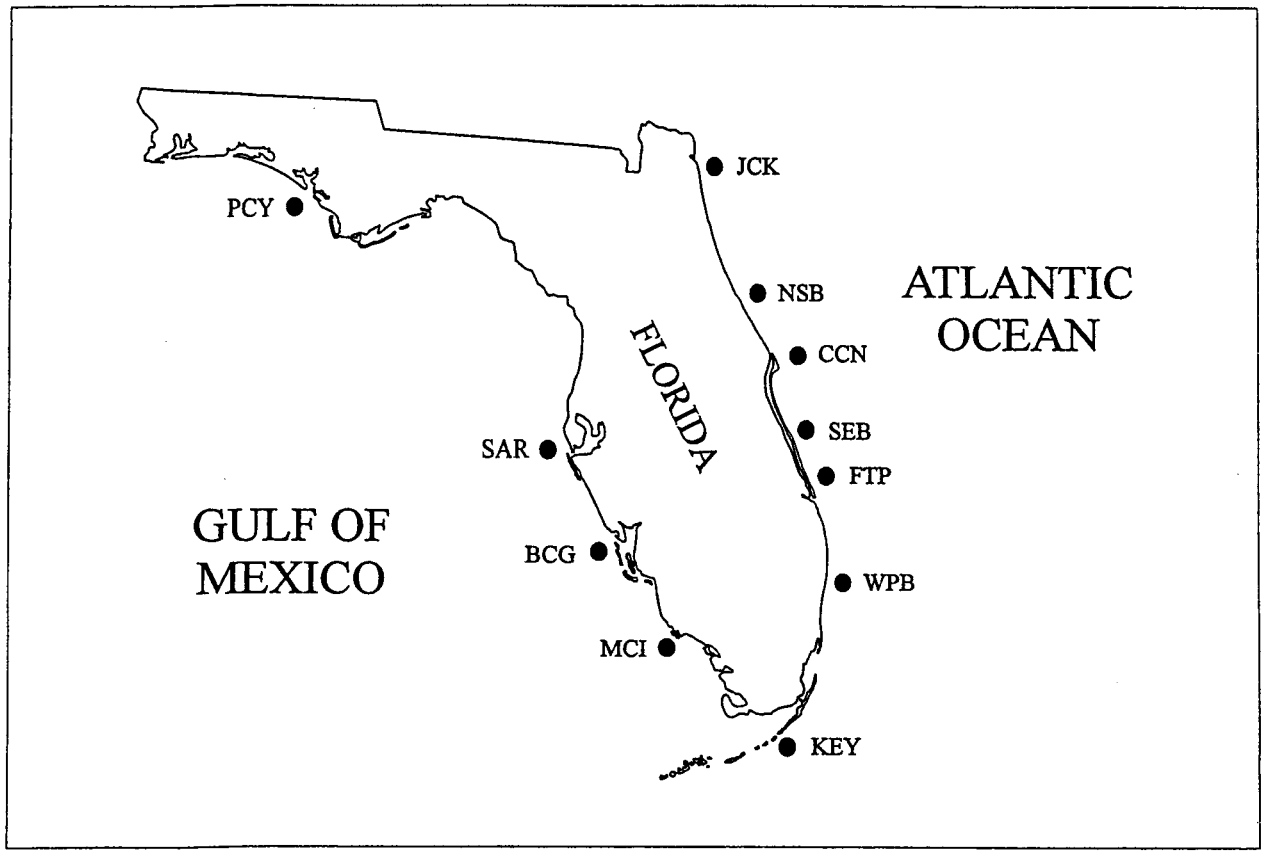
FIGURE LEGENDS

Figure 1. Sampling localities for king mackerel (*Scomberomorus cavalla*). Acronyms for each locality are defined in Table 1.

Figure 2. Neighbor-joining tree generated from a matrix of intersample genetic distances (after Rogers 1972).

Figure 3. Neighbor-joining tree generated from a matrix of intersample chord distances (after Cavalli-Sforza and Edwards 1967.) Numbers at nodes are the percentage of time that a node was supported in 500 bootstap replicates.

Figure 4. Correlograms based on spatial autocorrelation analysis of alleles at seven microsatellites in king mackerel (*Scomberomorus cavalla*). Abscissa: distance classes (left to right) based on equal number of pairwise comparisons per distance class. Ordinate: mean autocorrelation coefficients (Moran's I values) for each distance class. Bars about each mean represent one standard error on either side of a mean. Dotted line represents expected values when no correlation exists.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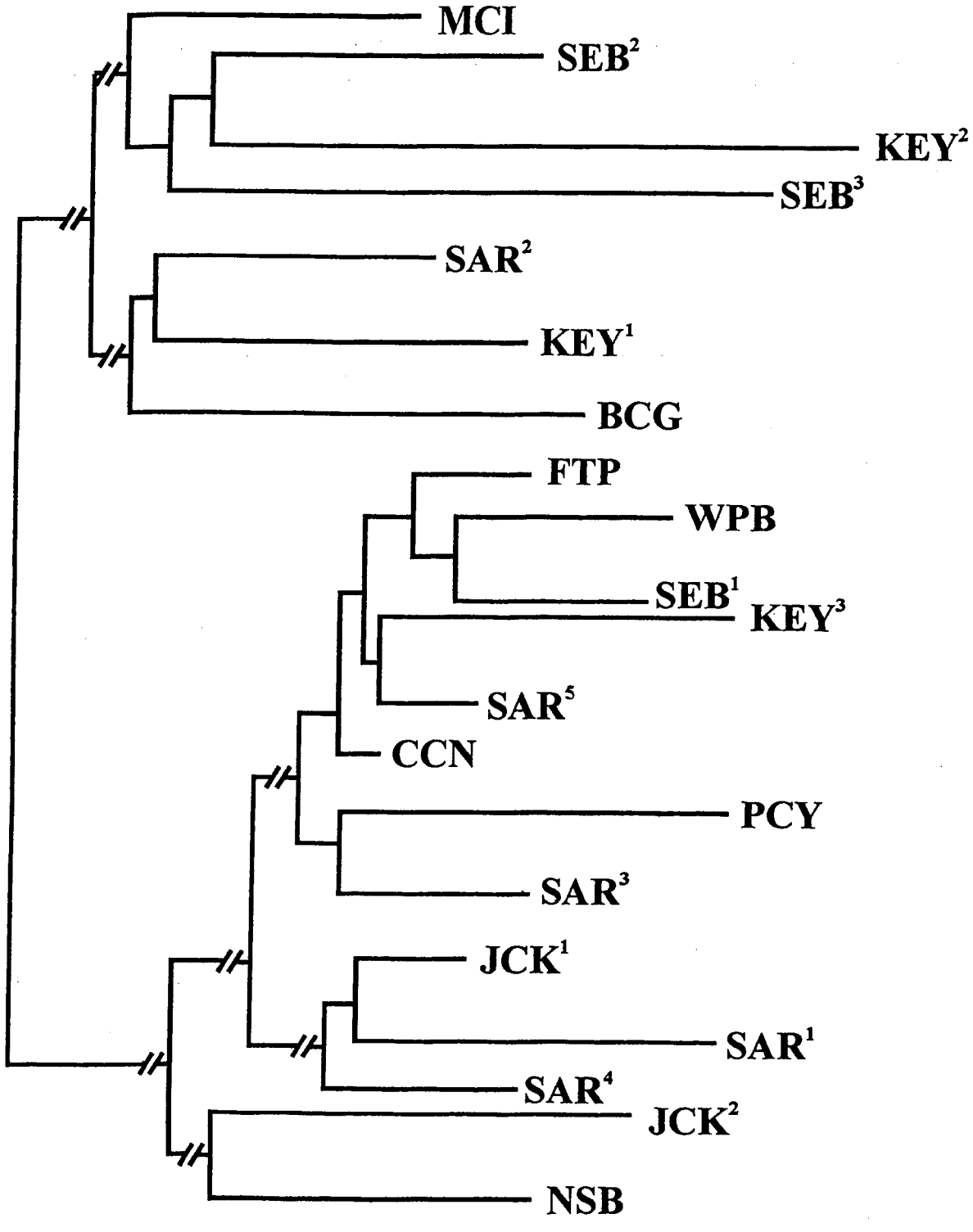
MCI
KEY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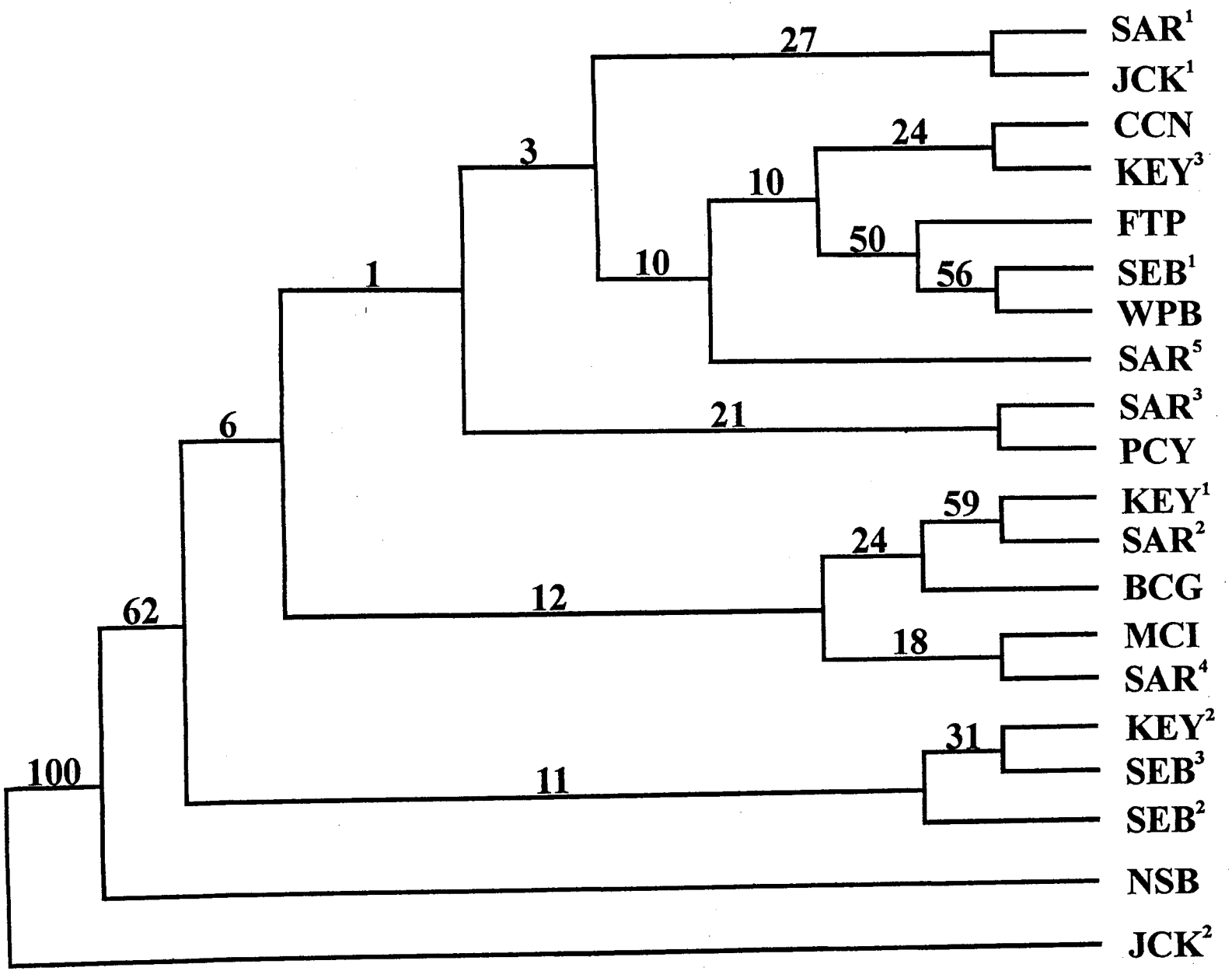
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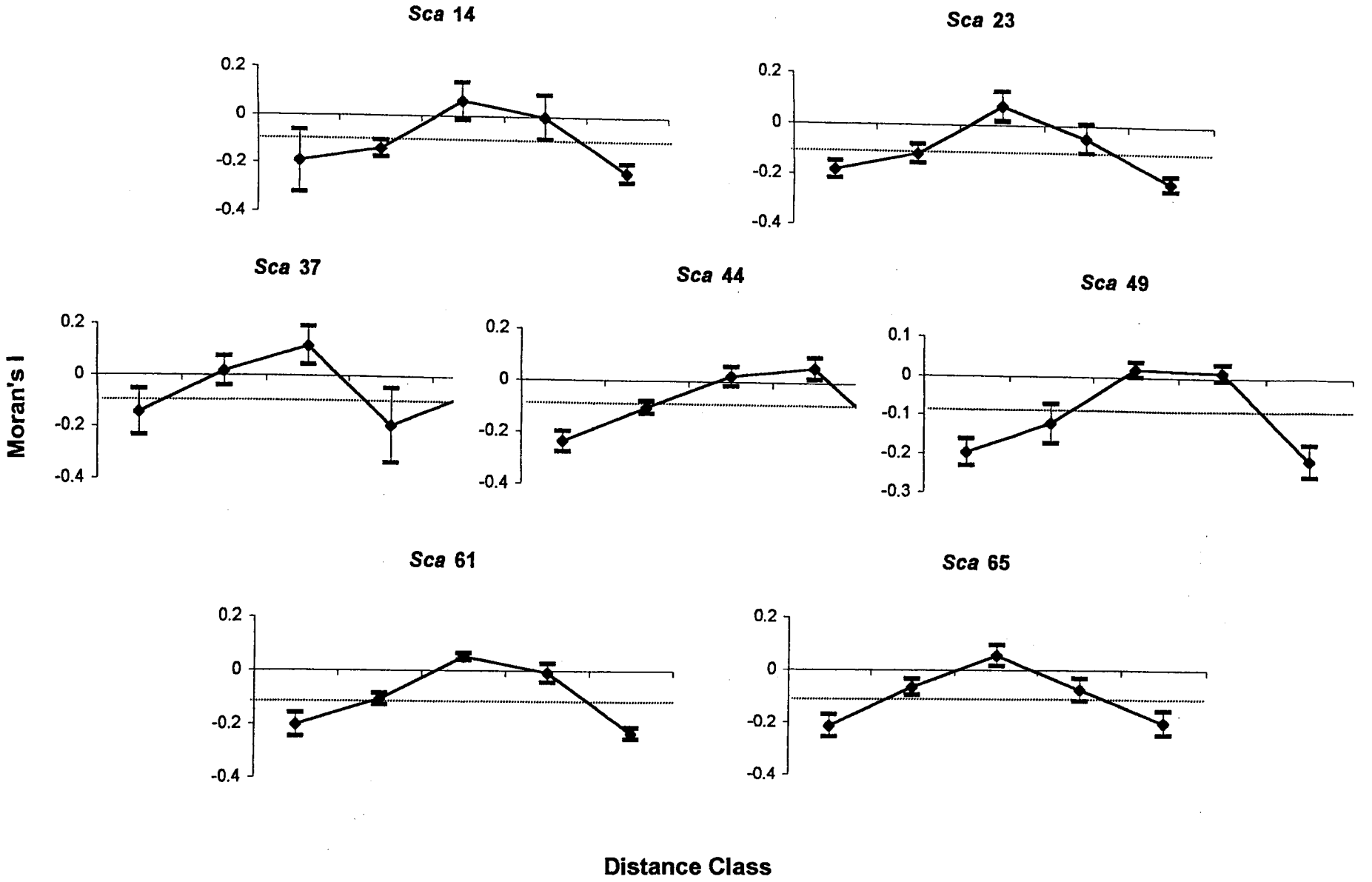
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Sca 49
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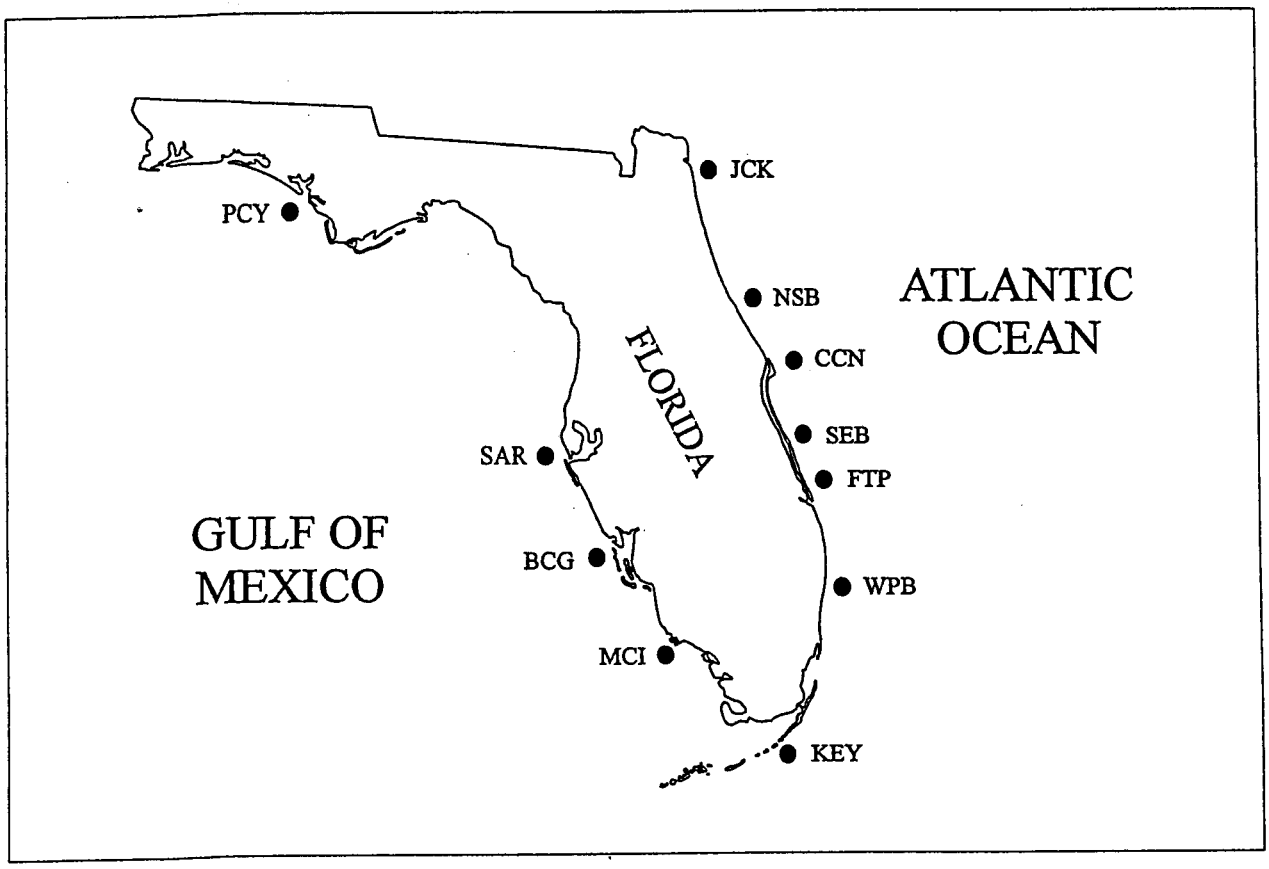
Moran's I
Distance Class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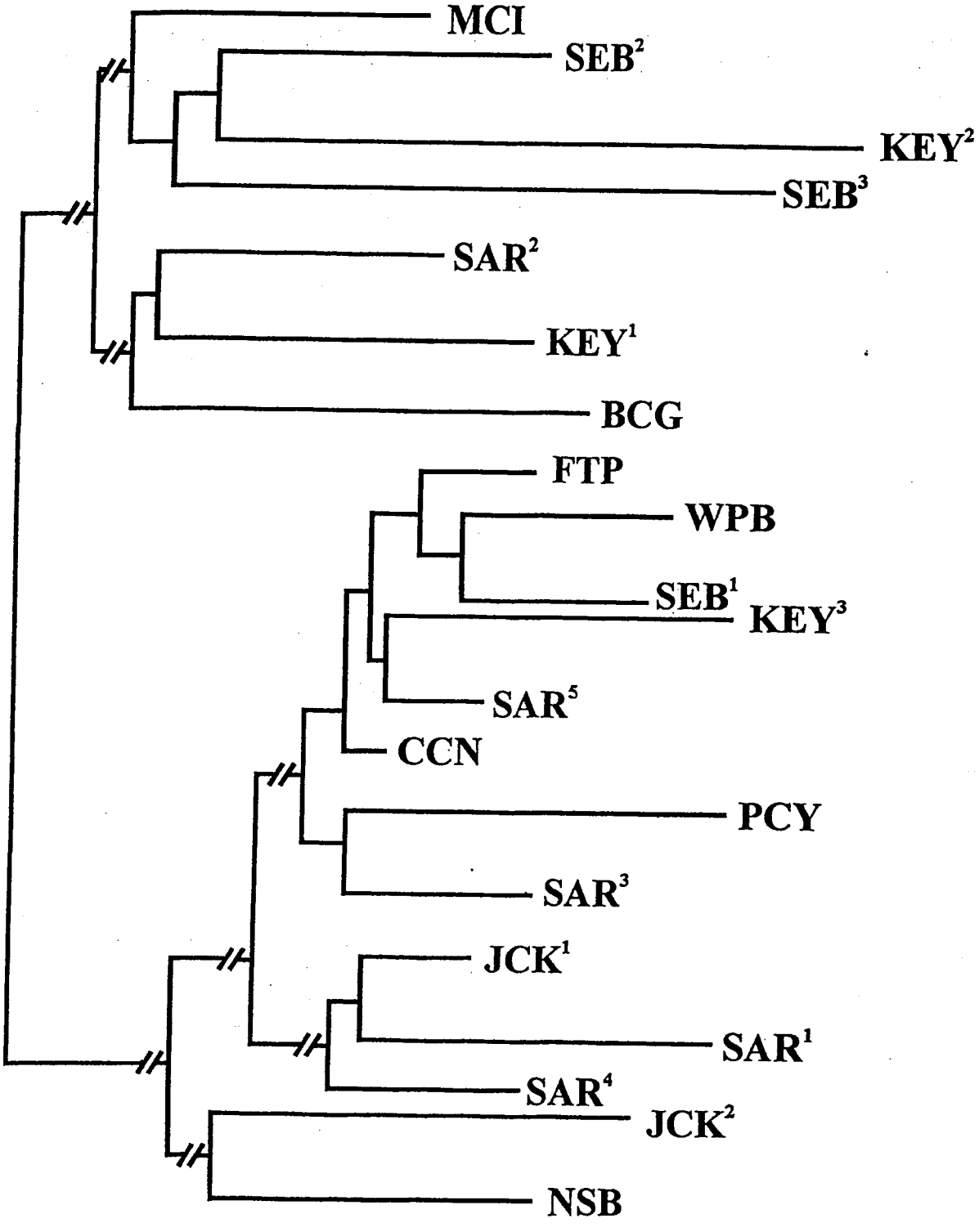
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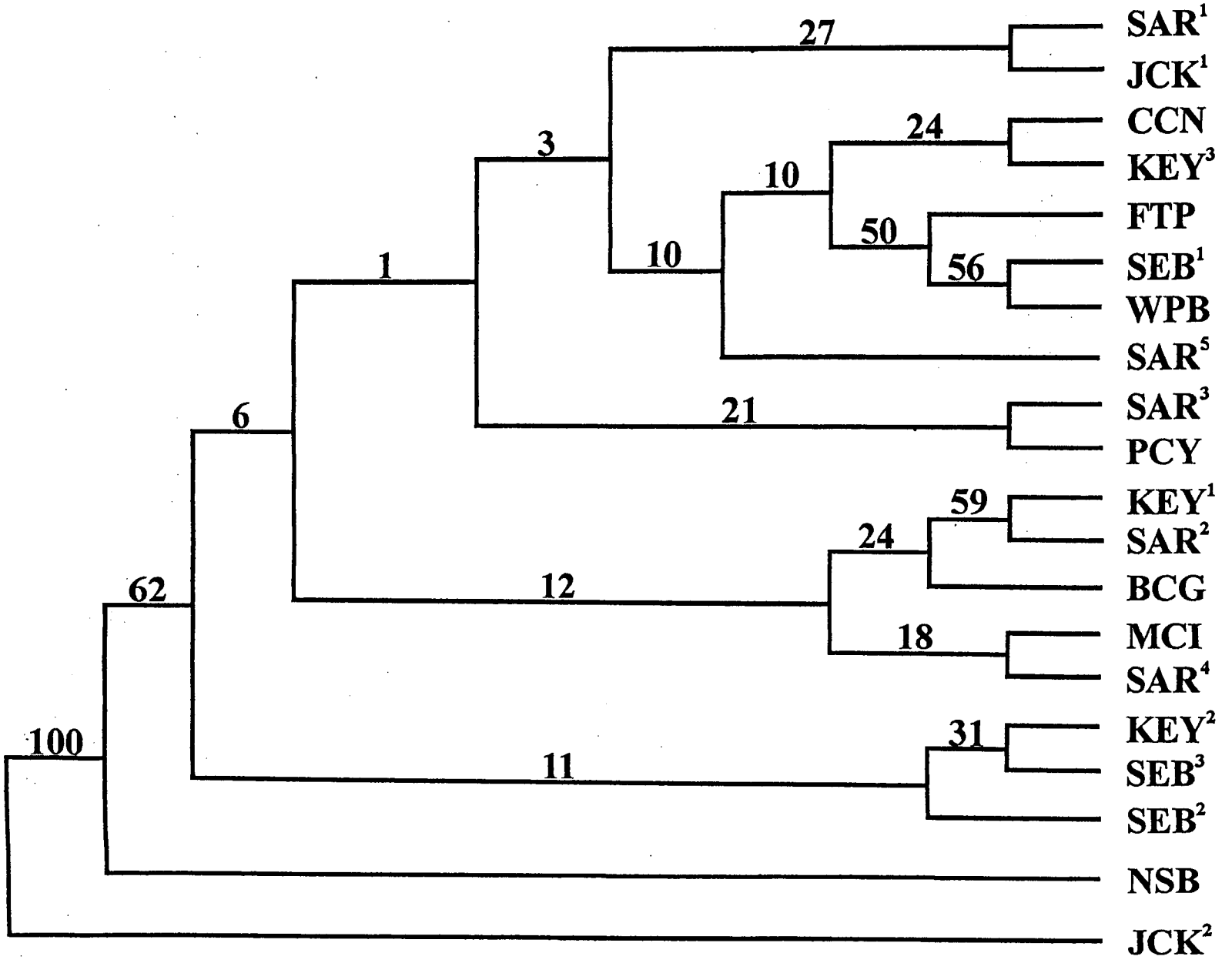
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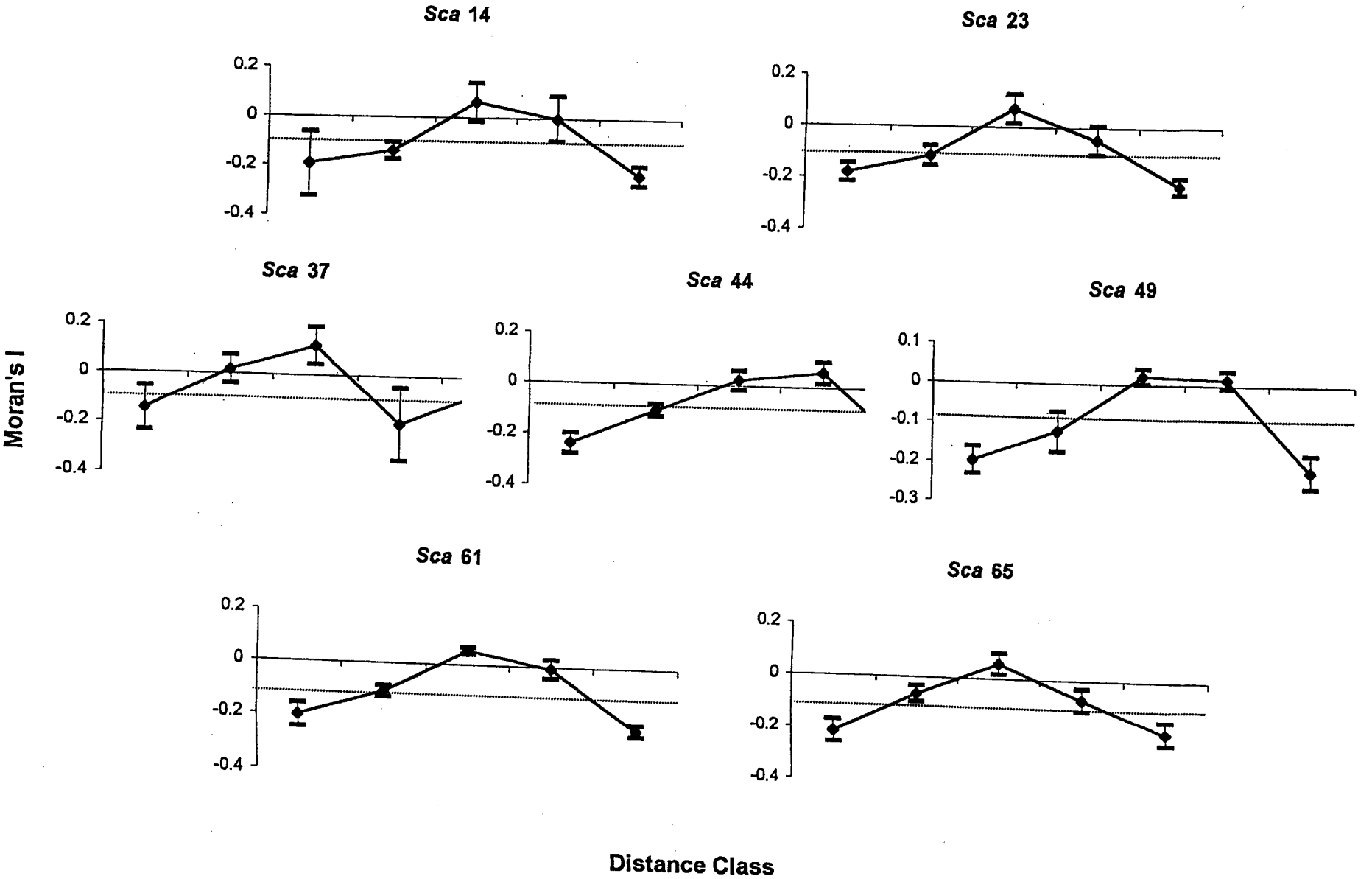
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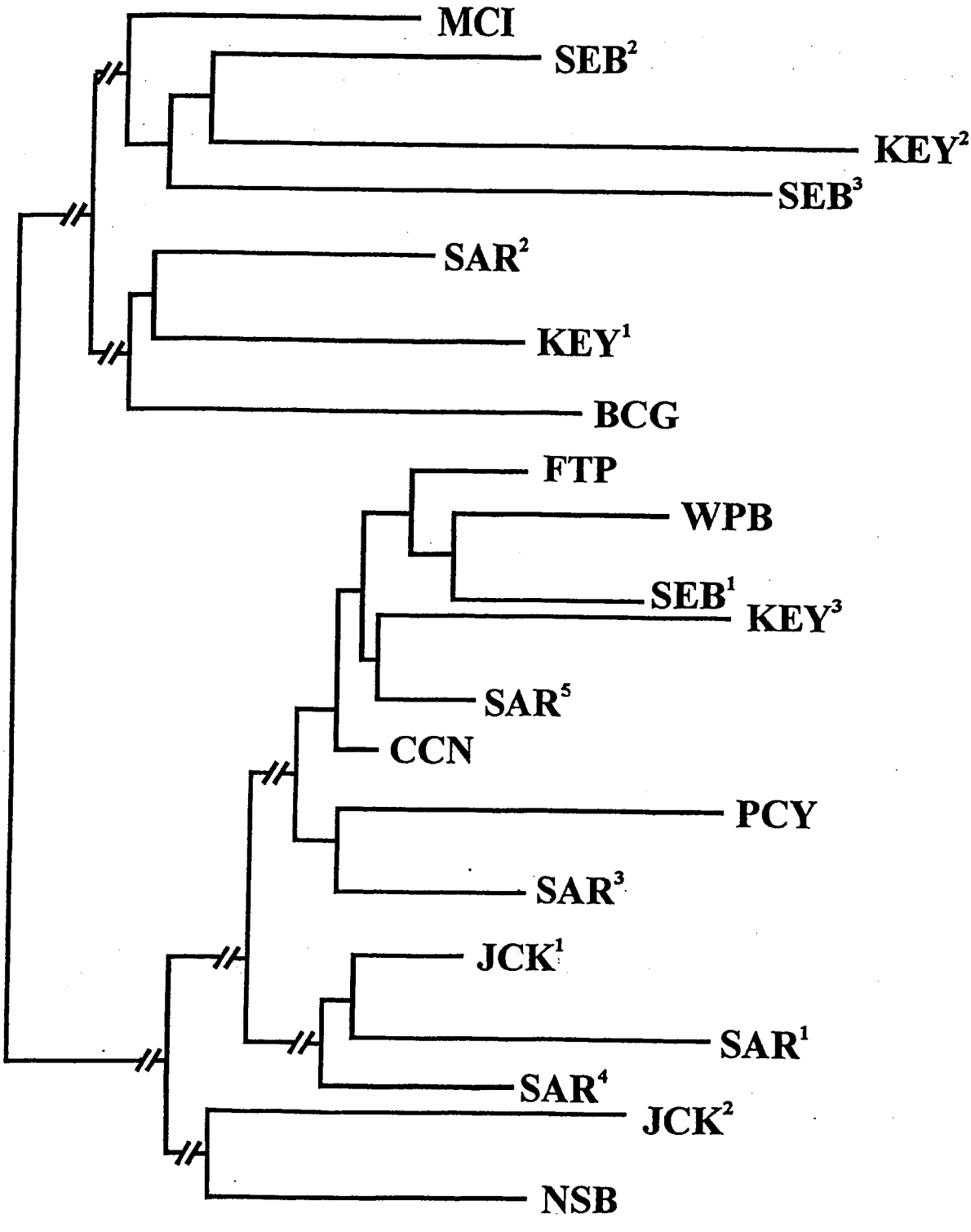
Moran's I
Distance Class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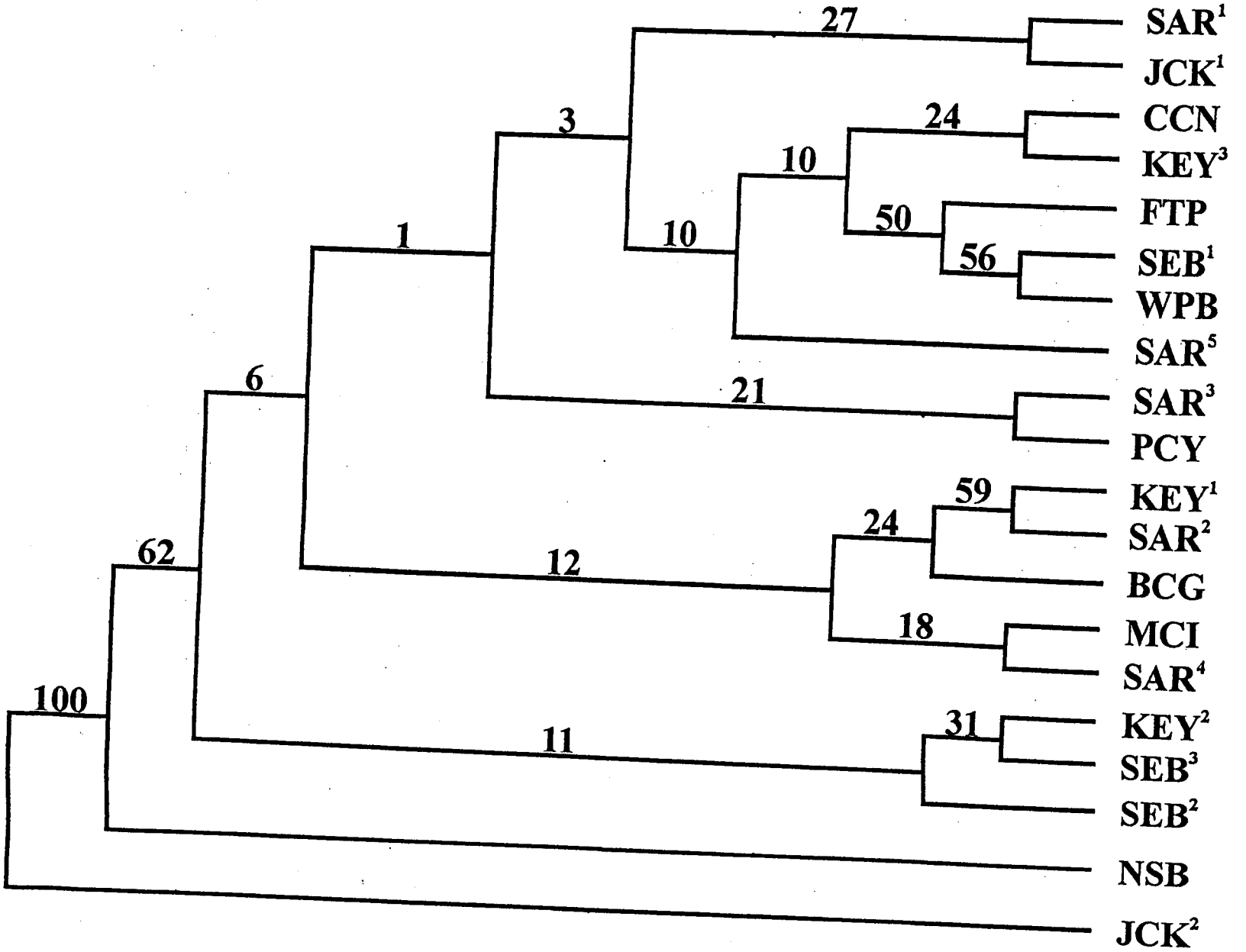
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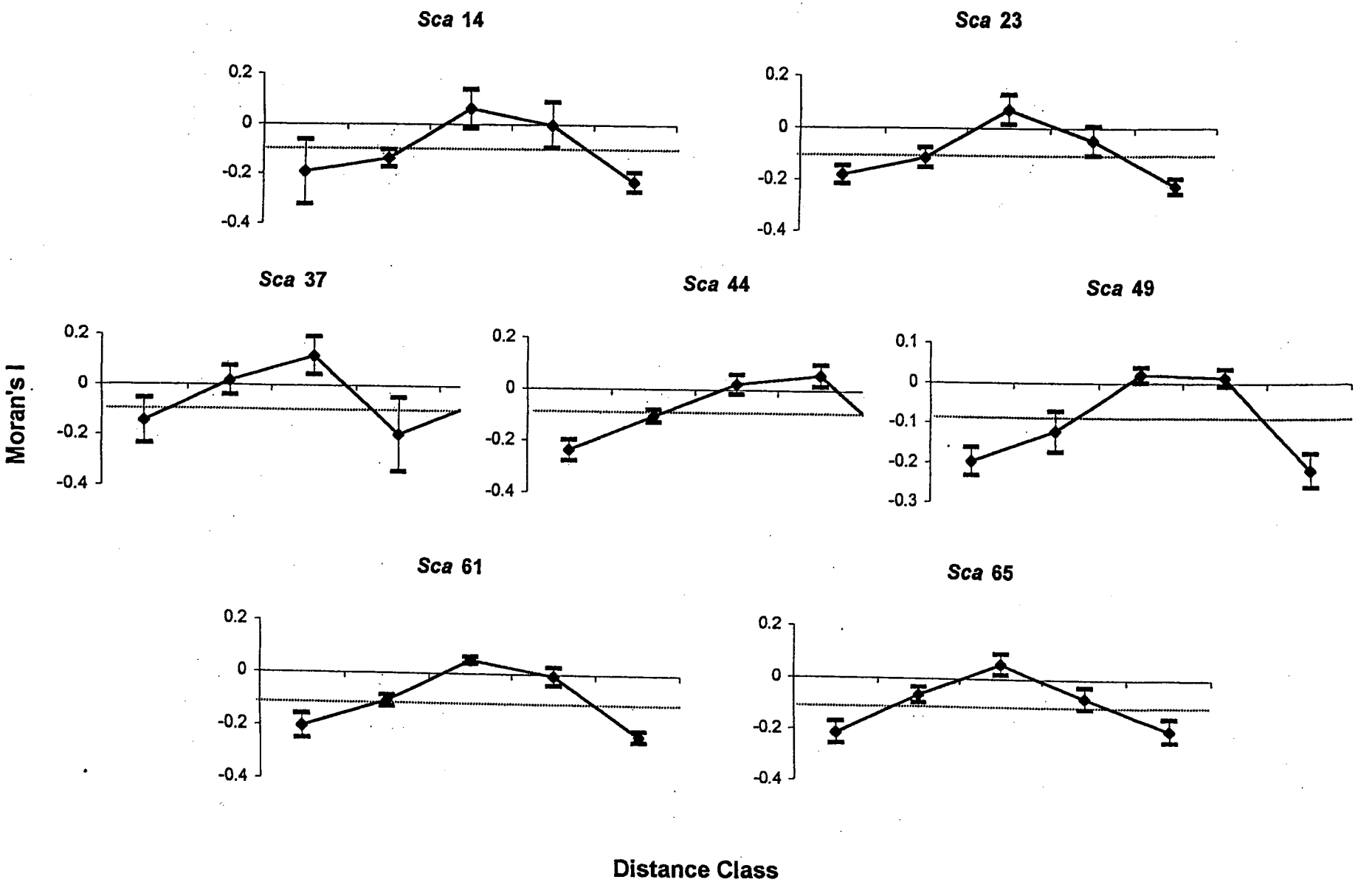
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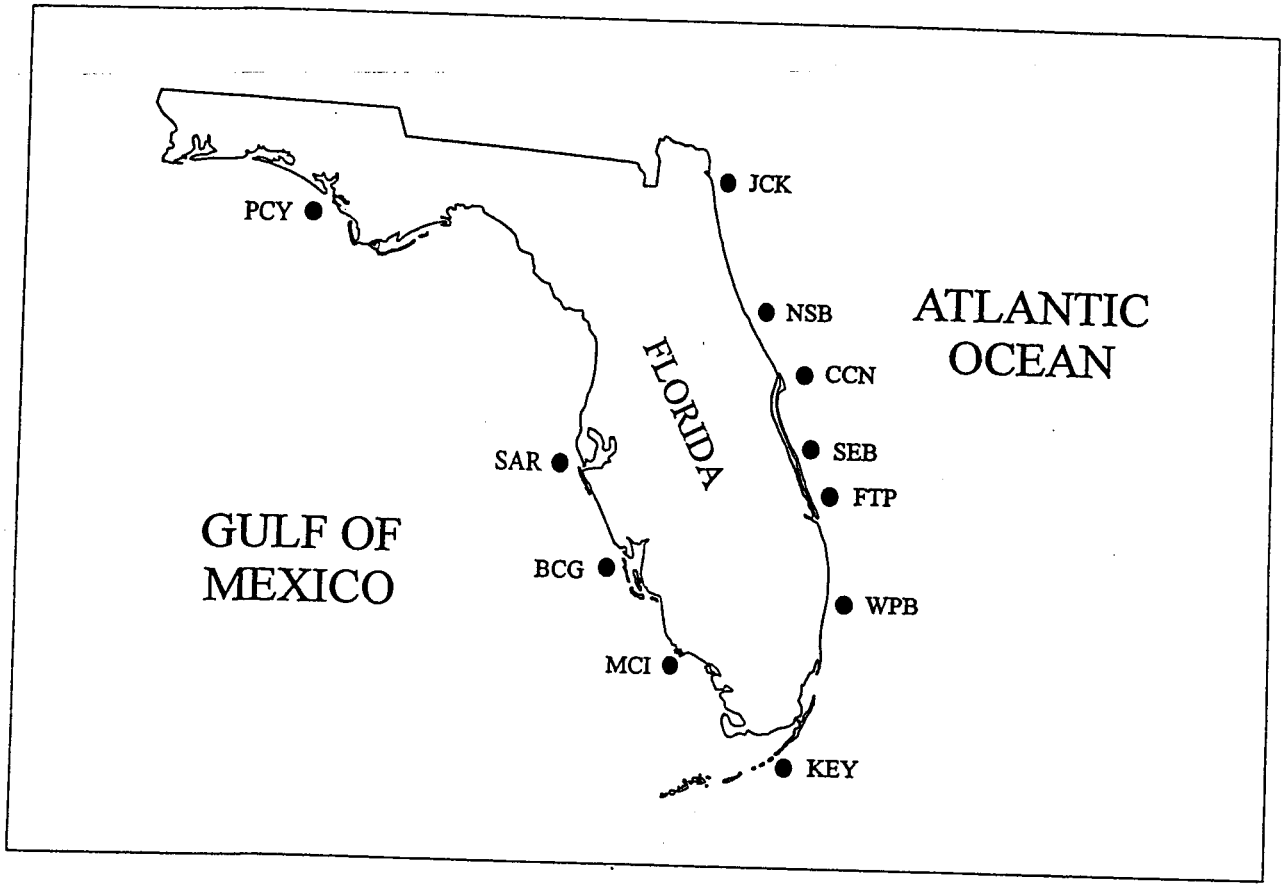
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