UC Irvine

UC Irvine Previously Published Works

Title

Pronounced genetic structure of mitochondrial DNA among populations of the circumglobally distributed grey mullet (*Mugil cephalus*)

Permalink

<https://escholarship.org/uc/item/92p8z442>

Journal

Journal of Fish Biology, 44(1)

ISSN

0022-1112

Authors

Crosetti, D
Nelson, WS
Avise, JC

Publication Date

1994

DOI

10.1111/j.1095-8649.1994.tb01584.x

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at

<https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

Pronounced genetic structure of mitochondrial DNA among populations of the circumglobally distributed grey mullet (*Mugil cephalus*)

D. CROSETTI*, W. S. NELSON† AND J. C. AVISE†

*ICRAM, Istituto Centrale per la Ricerca Scientifica e Tecnologica Applicata al Mare, V. L. Respighi 5, 00197 Rome, Italy and †Department of Genetics, University of Georgia, Athens, GA 30602 U.S.A.

(Received 18 January 1993, Accepted 9 March 1993)

Mitochondrial (mt) DNA genotypes of grey mullet (*Mugil cephalus*) from ocean basins around the world were analysed to estimate the amount of genetic differentiation in this cosmopolitan but mostly coastally-restricted species. Extensive genetic diversity was observed. Among 115 specimens from nine locales, 26 different haplotypes were detected using a battery of 13 restriction endonucleases. In phenetic analyses, these haplotypes grouped into seven distinct clusters whose members were in almost perfect accord with the geographic sources of the samples. Thus contemporary gene flow between the widespread collection locales must be absent or nearly so. Results contrast with the relative uniformity in mtDNA composition previously reported for populations of some circumglobally distributed pelagic fishes, and demonstrate that certain marine fishes with cosmopolitan distributions can exhibit pronounced population genetic structure even in the face of morphological conservatism.

Key words: population structure; gene flow; marine fishes.

I. INTRODUCTION

In the first molecular genetic study of a cosmopolitan marine fish based on mitochondrial (mt) DNA, Graves *et al.* (1984) reported a remarkably high degree of genetic similarity between samples of skipjack tuna (*Katsuwonus pelamis* L.) from the Atlantic and Pacific Oceans. Skipjack tuna are strong and active swimmers, lack discrete spawning areas, and produce larvae that are found circumtropically in oceanic waters. The absence of detected genetic differentiation was attributed to recent reproductive contact between Atlantic and Pacific populations, perhaps via gene flow around the Cape of Good Hope in South Africa where a seasonal sport fishery exists. A similar mtDNA survey of another pelagic tuna species [*Thunnus alalunga* (Bonnaterre)] similarly revealed 'no restriction sites which could distinguish an Atlantic from a Pacific albacore' (Graves & Dizon, 1989).

Such limited mtDNA differentiation on a global scale is impressive, particularly when contrasted with the dramatic mtDNA differentiation that characterizes many freshwater fishes and some coastal-restricted marine fishes over vastly smaller spatial scales (reviews in Avise *et al.*, 1987; Avise, 1992). For example, levels of mtDNA differentiation among freshwater populations in adjacent river drainages (Bermingham & Avise, 1986), or among some marine fishes from the east v. west coasts of Florida (Bowen & Avise, 1990), often greatly exceed those observed among tuna populations from opposite sides of the globe! These

findings underline how the varying natural histories of species, in conjunction with the physical structure of the environment, can influence magnitudes of genetic differentiation among conspecific populations. Nevertheless, differences in environmental patchiness and dispersal proclivity are unlikely to explain all of the variance among fish species in patterns of population structure. In theory and practice, historical demographic factors that no doubt are highly species-idiosyncratic must also have great impact on intraspecific genetic architectures (Bowen & Avise, 1990).

In phylogeographic studies, particularly enigmatic are species that appear to have rather limited dispersal capability relative to the extensive ranges occupied. For such taxa, several questions arise such as: (a) what is the level of gene flow (if any) connecting populations at the present time?; (b) how and over what timescales have such species achieved their widespread distributions?; and (c) how valid are the taxonomies that assign conspecificity to the widely scattered populations? In this brief report, we address these questions for the grey mullet (*Mugil cephalus* L.), a species that is distributed globally but normally is confined to near-coastal waters. In contrast to the observed mtDNA uniformity among widely separated collections of tuna, grey mullet exhibit pronounced population genetic structure on a global scale.

II. BACKGROUND

NATURAL HISTORY

The grey mullet inhabits coastal waters and estuaries of the tropical and subtropical seas of the world, roughly between 42° N and 42° S latitude (Fig. 1; Thomson, 1963). It is a euryhaline species, and often can penetrate lagoons and estuaries, migrating back to the sea to spawn. During the spawning season, ripe adults move in schools along beaches, then swim offshore to spawn in surface waters over the continental shelf. Eggs and larvae drift from the spawning grounds with ocean currents until the young are large enough to swim against tidal streams (at 2–3 months of age, or 20–30 mm length). Schools of young then enter lagoons and freshwater streams, attracted by low salinities and high trophic resources. As adults, permanent aggregations of mullet can be found in shallow seas, and sometimes occur well off-shore (180 fathoms). The freshwater phase for juveniles is not compulsory, as indicated by the presence of mullets around riverless islands remote from the coast (Thomson, 1963). The spawning migrations of mullet vary from only a few miles to 450 miles in one season (Thomson, 1963). Although most spawning probably occurs offshore, much of the migration route can be along shorelines, typically against prevailing coastal currents. Grey mullet play an important role in coastal fisheries and aquaculture in several regions of the world.

TAXONOMY

Mugil is one of 14 genera within Mugilidae, which altogether contains 64 species (Thomson, 1981). External morphology is highly conservative within the family, thus contributing to an uncertainty regarding evolutionary relationships at both generic and specific levels.

Within the currently-recognized entity *M. cephalus*, relationships and taxonomy also are debatable. Thomson (1963) reported 33 synonymies for this 'species', and several authors have suggested that in the final analysis *M. cephalus* may not be cosmopolitan (Schultz, 1949; DeSylva *et al.*, 1956). As stated by Briggs (1960), 'detailed morphological investigation might disclose *M. cephalus* to be a complex of species or subspecies'. For example, from differences in labial teeth structure, Ebeling (1961) considered the *M. cephalus*-like form on the Galapagos Islands to be a distinct species, *M. galapagensis*, but Thomson (1963, 1981) synonymized this species with *M. cephalus*; and from numbers of lateral line scales, Cadenat (1955; see also Delais, 1961) proposed that mullet of the west African coast warrant recognition as a distinct subspecies, *Mugil cephalus ashanteensis*. On the other hand, Thomson (1954) could not find significant morphological differences for specimens collected across major areas of the species' range. The general situation regarding *M. cephalus* is not unique: the basis for considering allopatric populations of many worldwide-distributed fishes to be conspecific normally involves morphological appraisals alone. Many of these 'species' occupy pelagic or bathypelagic environments, but some such as *M. cephalus* are restricted primarily to coastlines (Briggs, 1960).

Molecular assays should help clarify levels and patterns of genetic differentiation, particularly for such morphologically-conserved populations (Spain *et al.* 1980; Rosenblatt & Waples, 1986). For example, previous allozyme studies on several amphi-American tropical shore fishes have shown that minor morphological differences can be associated with genetic distances typically characteristic of distinct species (Gorman *et al.*, 1976; Gorman & Kim, 1977; Vawter *et al.*, 1980). Only preliminary genetic assays have been conducted on grey mullet thus far. Peterson & Shehadeh (1971) concluded from a study of eye lens proteins that genetic interchange between the nearby islands of Hawaii and Oahu was limited. Polymorphism in a serum protein was detected in populations from the Black Sea (Senkevich & Kulikova, 1970) and from the eastern (but not the western) coast of Australia (Hongskul, 1970). A survey of multiple allozyme loci in samples from Hawaii and southern California revealed a small genetic distance [$D=0.03 \pm 0.03$ (s.e.)] of magnitude normally observed among conspecific populations of other fish species (Rosenblatt & Waples, 1986). In a study of geographic variation in allozyme polymorphisms in the southeastern United States, Campton & Mahmoudi (1991) concluded that grey mullet collected from the eastern and western coasts of Florida appeared to reflect random draws from a single gene pool.

III. MATERIALS AND METHODS

Grey mullet ($n=115$ individuals) were collected from nine areas (Fig. 1): three sites in the Mediterranean Sea (eastern and western coasts of Italy, and Turkey); three sites in the Pacific Ocean (Taiwan, Hawaii, and Galapagos Islands); two sites in the Atlantic Ocean (United States and Mauritania); and one site in the Indian Ocean (South Africa). All fish were taken from nature, except for the samples from Taiwan which were farmed specimens previously collected nearby as fry in the wild.

FIG. 1. Geographic distribution of the grey mullet (after Thomson, 1963). Also shown are collection sites in the present study: B, North Carolina, U.S.A.; M, Nouakshott, Mauritania; F, Fiumicino, Italy; L, Lesina, Italy; Y, Bodrum, Turkey; S, South Africa, east coast; T, Taiwan, southwest coast; H, Oahu, Hawaii, U.S.A.; G, Isla Santa Cruz, Galapagos, Ecuador.

Fresh tissue samples for mtDNA analysis (ovary, liver and heart) were preserved in a buffer solution (MSB-EDTA) at 4°C. Mitochondrial DNA was isolated using a caesium-chloride centrifugation protocol (Lansman *et al.*, 1981). Small fishes from North Carolina were processed by an alkaline lysis method (Tamura & Aotsuka, 1988), where muscle and brain were also used. Each sample was digested with each of 13 restriction enzymes (Table I). Restriction fragments were end-labelled using Klenow and ^{35}S -dNTP(s), and revealed by autoradiography following electrophoresis through 0.9–1.4% agarose gels.

Fragment sizes in different profiles were compared against one another, and against a 1-kb ladder standard included in each gel. Only fragments larger than 0.4 kb were monitored. The number and complexity of different banding profiles (see later) precluded attempts to map restriction sites explicitly, and thus all analyses were confined to fragment comparisons. Implicit in such comparisons is the assumption that variation in the digestion profiles produced by separate enzymes is independent, as should be the case if point mutations (rather than mtDNA length mutations) are responsible. Although we cannot prove that such is the case with our data, no large and localized mtDNA length differences (that would be evidenced by concordant shifts in band positions across digestion profiles) were obvious. However, we cannot eliminate the possibility that smaller or scattered mtDNA size changes contributed to the variation in fragment digestion profiles observed, and thus to be conservative in interpretation, we will confine attention to analyses that do not rest heavily on independence assumptions. In other words, since the data were not scored as presence/absence of restriction sites, we prefer to avoid explicit attempts to recover phylogeny from the qualitative fragment data (e.g. by cladistic or parsimony approaches), and will focus instead on phenetic patterns and the geographic distributions of genotypes and their relevancy to the magnitude and pattern of 'population structure'.

Composite mtDNA haplotypes were summarized by a 13-letter code, each letter reflecting the digestion profile of one restriction enzyme. Estimates of nucleotide sequence divergence (p) between mtDNA clones were generated by the fragment approach of Nei & Li (1979). To emphasize the obvious spatial structure of genotypes, the resulting matrix was clustered using the UPGMA algorithm (Sneath & Sokal, 1973).

TABLE I. Descriptions of the mtDNA clones observed in the grey mullet

mtDNA clone	Genotypic description													No. fish (and location)
a	C	C	C	C	C	C	C	C	C	C	C	C	C	12 (Italy, east) 8 (Italy, west) 8 (Turkey)
b	C	C	C	C	C	C	C	C	C	C	C	C	B	1 (Italy, east) 1 (Italy, west) 1 (Turkey)
c	C	C	C	C	C	C	D	C	C	C	C	C	C	2 (Italy, east)
d	C	D	C	C	C	C	C	C	C	C	C	C	C	1 (Italy, east) 1 (Italy, west)
e	C	D	C	C	C	C	D	C	C	C	C	C	C	1 (Italy, east) 1 (Italy, west)
f	C	D	C	D	C	C	C	C	C	C	C	C	C	1 (Italy, west)
g	C	C	C	D	C	C	C	C	C	C	C	C	C	5 (Turkey)
h	—	W	C	B	C	B	A	C	D	D	B	C	C	3 (Mauritania)
i	—	W	C	B	C	B	A	C	E	D	B	C	C	1 (Mauritania)
j	—	Z	C	B	C	B	A	C	D	D	B	C	C	4 (Mauritania)
k	C	B	C	B	C	X	C	C	B	C	F	C	G	11 (South Africa)
l	C	B	C	B	C	X	C	C	B	C	H	C	G	2 (South Africa)
m	C	B	C	B	C	Z	C	C	B	C	F	C	G	2 (South Africa)
n	C	A	C	B	C	X	C	C	B	C	F	C	G	1 (South Africa)
o	C	B	C	B	C	X	C	C	F	C	F	C	G	1 (South Africa)
p	C	F	C	B	B	D	F	A	B	C	G	C	F	16 (Taiwan)
q	C	P	B	C	—	E	C	D	D	C	G	C	C	4 (Hawaii)
r	C	Q	B	C	C	E	C	D	D	C	G	C	C	11 (Hawaii)
s	C	S	B	C	—	E	C	D	D	C	G	C	C	2 (Hawaii)
t	C	Q	B	C	—	E	B	D	D	C	G	C	C	1 (Hawaii)
u	C	R	B	C	C	E	C	D	D	C	G	C	C	1 (Hawaii)
v	C	M	D	C	C	I	F	C	F	C	E	C	C	3 (N. Carolina)
w	C	L	D	C	—	I	F	—	—	C	E	C	C	1 (N. Carolina)
x	C	M	D	C	C	I	F	A	F	C	E	C	C	1 (N. Carolina)
y	C	P	C	C	C	E	C	—	A	C	A	C	B	2 (Galapagos)
z	C	P	C	C	C	E	C	—	A	C	B	C	B	5 (Galapagos)
Total 115*														

Letters (from left to right) designate multi-fragment gel profiles produced by digestion with *Ava*I, *Ava*II, *Bam*HI, *Bcl*II, *Hinc*II, *Hind*III, *Kpn*I, *Nde*I, *Pvu*II, *Spe*I, *Sst*II, and *Stu*I.

*Four additional specimens from Mauritania were scored for a subset of enzymes and on this basis belonged to clone i or j; similarly, three additional specimens from North Carolina that were scored for a subset of enzymes probably belonged to clone w or x.

IV. RESULTS

On average, more than 40 mtDNA restriction fragments were scored per individual, with a total of 123 fragments monitored overall. Grey mullet showed considerable mtDNA diversity. Among the 115 assayed specimens from nine locales 26 different haplotypes were observed (Table I). With the exception of *Ava*I and *Sst*II, all enzymes revealed multiple digestion profiles. With the exception of Taiwan, all sampled populations exhibited multiple mtDNA genotypes.

TABLE II. Estimates of mtDNA genetic variability within collections of the grey mullet (Mediterranean samples pooled)

Area	No. of individuals	Haplotype diversity	Nucleotide diversity
Mediterranean	43	0.83	0.0015
Mauritania	8	0.59	0.0022
South Africa	17	0.55	0.0012
Taiwan	16	0.00	0.0000
Hawaii	19	0.60	0.0014
North Carolina	5	0.56	0.0020
Galapagos Islands	7	0.41	0.0001
Total: 115		pooled*: 0.93	pooled*: 0.0200

*All 115 specimens considered as one pooled sample.

The lack of variation within the Taiwan sample cannot be explained by the fact that the fish were farm-cultured, because these specimens were derived from a sample of fry collected in the wild. Although the genetic structure of mullet fry assemblages is unknown, most individuals probably belong to different family units, as schools are formed when the young arrive near the coast. A comparable reduction of mtDNA variation also has been reported within Australian bluefish (*Pomatomus saltatrix* L.): only one of 18 individuals similarly assayed had a different haplotype (Graves *et al.*, 1992). These authors provisionally explained the observation as 'the result of a smaller effective population size of females, or as reflecting a longer period of isolation of the population'.

Over the entire mullet survey, the probability that randomly chosen pairs of individuals exhibited a differentiable mtDNA genotype (haplotype diversity) was 0.93 (Table II), and the mean sequence divergence estimated between such pairs (nucleotide diversity) was 0.020. The probability that randomly chosen mullet from separate locales (except within the Mediterranean—see later) exhibited a different genotype was 1.00, whereas the probabilities of genotypic non-identity within locales ranged from 0.00 (Taiwan) to 0.83 (Mediterranean) (Table II). Levels of sequence divergence between individuals ranged from 0.000–0.033. Within locales, such estimates invariably were less than $P=0.007$, whereas main genetic distances between locales were always greater than $P=0.014$ (Hawaii-Galapagos) and usually greater than $P=0.020$ (Fig. 2).

In the UPGMA phenogram (Fig. 2), the 26 genotypes resolved into seven distinct groups whose members align almost perfectly with geographic source. In other words, all fish from each collection locale clustered together genetically, to the exclusion of all individuals from any other site. The only exception to this pattern involved the Mediterranean collections (from Turkey, and the eastern and western coasts of Italy), where several genotypes were shared between locales, and clones were interspersed in the phenogram (Fig. 2; Table I). If these three collections are treated as a single sample representing the Mediterranean, then on a global scale the phenetic clusters composed of closely related genotypes correspond exactly with geographic location.

FIG. 2. UPGMA phenogram summarizing relationships among mtDNA haplotypes in *Mugil cephalus*.

V. DISCUSSION

The most interesting result of this mtDNA survey is the clear genetic differentiation among scattered mullet populations from around the world. The geographic coherence of clusters in the dendrogram is unambiguous, and reflects the locale-specific genetic constitutions of these fish. This pronounced population genetic structure was not necessarily anticipated, given the extensive migration of adults along coastlines, the lengthy planktonic larval stage (which conceivably could facilitate gene flow over large distances), the relative morphological uniformity of the species, and the fact that populations of other cosmopolitan marine fishes (e.g. tunas) previously had been reported to be nearly undifferentiated in mtDNA composition globally. Based on these genetic findings for the grey mullet, we shall now turn to the three questions raised in the introduction.

First, what is the level of contemporary gene flow between mullet populations? Within the Mediterranean, several mtDNA genotypes were present at more than one collection site, usually in similar frequencies [$G_{\text{het}}=1.044$, d.f.=2, N.S. (because of low cell numbers, the rare genotypes b–g were pooled in this statistical test for heterogeneity)]. The sample from Turkey did include one mtDNA clone (g) in frequency 0.36 that was not observed elsewhere in our limited collections, but the overall evidence (from shared mtDNA clones and the low magnitudes of sequence divergence between all Mediterranean genotypes) suggests considerable matrilineal exchange among sites, and/or recent historical connections among these Mediterranean populations. By contrast, no mtDNA genotypes were shared among any of the other sites assayed, and sequence divergence estimates among mtDNA haplotypes were much larger. Thus between the widely scattered locales monitored on the broader global scale, we conclude that little or no genetic exchange occurs at the present time.

How, then, and over what timescales, has the grey mullet achieved its cosmopolitan distribution? Available tagging data demonstrate that grey mullet are capable of long-distance movements along coasts (Kesteven, 1942, 1953; Thomson, 1955; Funicelli *et al.*, 1989), but to what extent the species can cross open oceans remains unknown. The spread of grey mullet to such remote islands as Hawaii and the Galapagos was hypothesized to have resulted either from nomadism by adults or passive drift of juveniles (Thomson, 1963). Although direct dispersal pathways across the Pacific are somewhat difficult to envision for the mullet, the eastern Pacific region does not appear to have been an absolute barrier for some other species of marine shore fishes (Briggs, 1960). Indeed, based on allozyme surveys, populations of 11 trans-Pacific shore fishes were found to be no more different genetically than were local populations of most other organisms (Rosenblatt & Waples, 1986).

If we adopt the assumption that the fragment profile changes in mullet were due mostly to point mutations (such that the statistical conversions to mtDNA sequence divergence are appropriate), rough estimates may be advanced for the times of evolutionary separation between the populations sampled. For these purposes, we calculated the *net* sequence divergence between each pair of populations (Mediterranean collections pooled) by subtracting the mean of the within-locale sequence divergence estimates (the nucleotide diversities in Table II) from the gross mean sequence divergence between each respective population pair. These corrected values were then converted to estimates of absolute separation time by assuming the 'conventional' mtDNA evolutionary rate of 2% sequence divergence per million years (see Avise *et al.*, 1992 and references therein). Resulting values for divergence times ranged from 0.63 mya (Hawaii–Galapagos) to 1.54 mya (Taiwan–Mauritania). Of course such results rely heavily on assumptions, and could represent serious over-estimates of true populational divergence times if mullet mtDNA evolves faster, and/or if the sequence divergence estimates themselves were inflated (as might be the case, for example, if unrecognized changes in mtDNA genomic size were responsible for much of the heterogeneity in digestion profiles). In any event, the mtDNA data available to date can be interpreted as consistent with the possibility that the global distribution of the grey mullet has been achieved by historical shifts

in range extending back in time perhaps over several hundred thousand generations.

The third question is especially difficult to address with current data: how valid is the current taxonomy which assigns conspecific status to 'grey mullet' populations around the world? In principle, no arbitrary magnitude of mitochondrial (or nuclear) divergence can establish an absolute dividing line demarcating biological species, particularly when assayed populations are allopatric and their reproductive compatibilities untested in nature. Nonetheless, genetic divergence estimates between widely separated grey mullet populations ($P \approx 0.02-0.03$) are of a magnitude not infrequently reported between well-differentiated conspecific populations of other vertebrates, including fishes (Bermingham & Avise, 1986; Gonzales-Villaseñor & Powers, 1990; Ovenden, 1990; Billington & Hebert, 1991), and are somewhat lower than most estimates for congeneric vertebrate species [where usually, $P \gg 0.05$ (Avise *et al.*, 1987; Moritz *et al.*, 1987), but see Meyer *et al.* (1990) for dramatic exceptions]. On the other hand, the overall magnitude of mtDNA nucleotide diversity in the grey mullet is considerably greater than that previously reported for several other widely distributed marine taxa (Palumbi & Wilson, 1990; Bowen *et al.*, 1992; Ovenden *et al.*, 1992), as well as for another circumglobal species, *Homo sapiens* (Cann *et al.*, 1987). Based solely on such appraisals of comparative mtDNA divergence levels, assayed samples of the 'grey mullet' appear to fall in the 'grey zone' between well-differentiated conspecific samples and closely related species, as was predicted many years ago by Briggs (1960)!

Results of this preliminary survey of the grey mullet are significant in two regards. First, they demonstrate that a relative morphological uniformity among populations is not necessarily indicative of a lack of significant population genetic structure. Second, the results indicate that at least some recognized species of cosmopolitan marine fishes will prove to exhibit considerable population genetic differentiation on a global scale. Indeed, recent mtDNA surveys of another globally-distributed fish (the blue marlin, *Makaira nigricans* Lacepède) led to a similar conclusion, although the population structure reported did not appear as pronounced as that described here for the mullet (Finnerty & Block, 1992). Any future genetic studies of global population structure in mullet probably should involve more extensive coverage of geographic populations, and include nuclear as well as mitochondrial genes. In particular, direct nucleotide sequencing should help to circumvent the difficulties we encountered in interpreting the particular molecular changes responsible for the complex diversity of mtDNA digestion profiles revealed.

The authors wish to express their gratitude to all those who permitted the collection of mullet samples around the world: Dr J. C. Kuo and the Hanaqua fish farm, Taiwan; Dr C. S. Lee and C. S. Tamaru, Hawaii; G. F. Boots, North Carolina, U.S.A.; Dr P. Villani, the Cooperativa Pescatori Lago di Sabaudia, Coipa s.r.l., and M. Migliori, Italy; Dr E. Ciccotti, Turkey; Dr L. Sola, R. Mosquera, and the Charles Darwin Research Station of the Galapagos National Park, Galapagos, Ecuador; Dr G. Contrafatto, Dr R. van der Elst and S. Chater, South Africa; and M. Hatti, Mauritania. DC wishes to thank Dr G. Venturini for advice and help in molecular techniques in Rome. Research was supported by a grant from the NSF to JCA, and collections were financed by a grant from the National Geographic Society.

References

- Avise, J. C. (1992). Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. *Oikos* **63**, 62–76.
- Avise, J. C., Arnold, J., Ball, R. M. Jr., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A. & Saunders, N. C. (1987). Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* **18**, 489–522.
- Avise, J. C., Bowen, B. W., Lamb, T., Meylan, A. B. & Bermingham, E. (1992). Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the Testudines. *Molecular Biology and Evolution* **9**, 457–473.
- Bermingham, E. & Avise, J. C. (1986). Molecular zoogeography of freshwater fishes in the southeastern United States. *Genetics* **113**, 939–965.
- Billington, N. & Hebert, P. D. (1991). Mitochondrial DNA diversity in fishes and its applications for introductions. *Canadian Journal of Fisheries and Aquatic Sciences* **48** (suppl. 1), 80–94.
- Bowen, B. W. & Avise, J. C. (1990). Genetic structure of Atlantic and Gulf of Mexico populations of sea bass, menhaden, and sturgeon: influence of zoogeographic factors and life-history patterns. *Marine Biology* **107**, 371–381.
- Bowen, B. W., Meylan, A. B., Ross, J. P., Limpus, C. J., Balazs, G. H. & Avise, J. C. (1992). Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. *Evolution* **46**, 865–881.
- Briggs, J. C. (1960). Fishes of worldwide (circumtropical) distribution. *Copeia* **1960**, 171–180.
- Cadenat, J. (1955). Sur les mulets de la côte occidentale d'Afrique. *Rapports Conseil International pour l'Exploration de la Mer* **37**, 59–62.
- Campton, D. E. & Mahmoudi, B. (1991). Allozyme variation and population structure of striped mullet (*Mugil cephalus*) in Florida. *Copeia* **1991**, 485–492.
- Cann, R. L., Stoneking, M. & Wilson, A. C. (1987). Mitochondrial DNA and human evolution. *Nature* **325**, 31–36.
- DeSylva, D. P., Stearns, H. B. & Tabb, D. C. (1956). Populations of the black mullet (*Mugil cephalus* L.) in Florida. *Florida Board Conservation Technical Series* No. 19.
- Delais, M. (1961). Comparaison entre *Mugil cephalus* L. de la côte Méditerranée et sa sous-espèce de l'ouest africain, *M. cephalus* L. s.sp. *ashanteensis* Blkr. *Rapports Commission Internationale pour la Mer Méditerranée* **16**, 853–854.
- Ebeling, A. W. (1961). *Mugil galapagensis*, a new mullet from the Galapagos Islands. *Copeia* **1961**, 295–304.
- Finnerty, J. R. & Block, B. A. (1992). Direct sequencing of mitochondrial DNA detects highly divergent haplotypes in blue marlin (*Makaira nigricans*). *Molecular Marine Biology and Biotechnology* **1**, 206–214.
- Funicelli, N. A., Meineke, D. A., Bryant, H. E., Deway, M. R., Ludwig, G. M. & Mengel, L. S. (1989). Movements of striped mullet, *Mugil cephalus*, tagged in Everglades National Park, Florida. *Bulletin of Marine Science* **44**, 171–178.
- Gonzales-Villaseñor, L. I. & Powers, D. A. (1990). Mitochondrial DNA restriction site polymorphisms in the teleost *Fundulus heteroclitus* support secondary intergradation. *Evolution* **44**, 27–37.
- Gorman, G. C. & Kim, Y. J. (1977). Genotypic evolution in the face of phenotypic conservatism: *Abudefduf* (Pomacentridae) from the Atlantic and Pacific sides of Panama. *Copeia* **1977**, 694–697.
- Gorman, G. C., Kim, Y. J. & Rubino, R. (1976). Genetic relationships of three species of *Bathygobius* from the Atlantic and Pacific sides of Panama. *Copeia* **1976**, 361–364.
- Graves, J. E. & Dizon, A. E. (1989). Mitochondrial DNA sequence similarity of Atlantic and Pacific albacore tuna (*Thunnus alalunga*). *Canadian Journal of Fisheries and Aquatic Sciences* **46**, 870–873.

- Graves, J. E., Ferris, S. D. & Dizon, A. E. (1984). Close genetic similarity of Atlantic and Pacific skipjack tuna (*Katsuwonus pelamis*) demonstrated with restriction endonuclease analysis of mitochondrial DNA. *Marine Biology* **79**, 315–319.
- Graves, J. E., Beardley, A. M., McDowell, J. R. & Scoles, D. R. (1982). Stock structure in the bluefish, *Pomatomus saltatrix*, along the mid-Atlantic coast. *Fishery Bulletin* **90**: 703–710.
- Hongskul, V. (1970). Serum polymorphism in grey mullet (*Mugil cephalus* Linn.). *Proceedings of the Indo-Pacific Fisheries Council* **13**, 107–112.
- Kesteven, G. L. (1942). Studies in the biology of the Australian mullet. 1. An account of the fishery and preliminary statement of the biology of *Mugil dobula* Gunther. *Bulletin of the Australian Council for Scientific and Industrial Research* **157**, 1–147.
- Kesteven, G. L. (1953). Further results of tagging sea mullet, *Mugil cephalus* Linnaeus, on the eastern Australian coast. *Australian Journal of Marine and Freshwater Research* **4**, 251–306.
- Lansman, R. A., Shade, R. O., Shapira, J. F. & Avise, J. C. (1981). The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. *Journal of Molecular Evolution* **17**, 214–226.
- Meyer, A., Kocher, T. D., Basasibwaki, P. & Wilson, A. C. (1990). Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature* **347**, 550–553.
- Moritz, C., Dowling, T. E. & Brown, W. M. (1987). Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annual Review of Ecology and Systematics* **18**, 269–292.
- Nei, M. & Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Science U.S.A.* **76**, 5269–5273.
- Ovenden, J. R. (1990). Mitochondrial DNA and marine stock assessment: a review. *Australian Journal of Marine and Freshwater Research* **41**, 835–853.
- Ovenden, J. R., Brasher, D. J. & White, R. W. G. (1992). Mitochondrial DNA analysis of the rock lobster *Jasus edwardsii* supports an apparent absence of population subdivision throughout Australia. *Marine Biology* **112**, 319–326.
- Palumbi, S. R. & Wilson, A. C. (1990). Mitochondrial DNA diversity in the sea urchins *Strongylocentrotus purpuratus* and *S. droebachiensis*. *Evolution* **44**, 403–415.
- Peterson, G. L. & Shehadeh, Z. H. (1971). Subpopulations of the Hawaiian striped mullet *Mugil cephalus*: analysis of variations of nuclear eye-lens protein electropherograms and nuclear eye-lens weights. *Marine Biology* **11**, 52–60.
- Rosenblatt, R. H. & Waples, R. S. (1986). A genetic comparison of allopatric populations of shore fish species from the eastern and central Pacific Ocean: dispersal or vicariance? *Copeia* **1986**, 275–284.
- Schultz, L. P. (1949). A further contribution to the ichthyology of Venezuela. *Proceedings of the United States Natural History Museum* **99**, 1–211.
- Senkevich, N. K. & Kulikova, N. I. (1970). Intraspecific differentiation of serum proteins in the blood of Black sea mullet. *Journal of Ichthyology* **10**, 538–544.
- Sneath, P. H. A. & Sokal, R. R. (1973). *Numerical Taxonomy*. San Francisco, CA: W. H. Freeman.
- Spain, A. V., Grant, C. J. & Sinclair, D. F. (1980). Phenotypic affinities of 11 species of Australian mullet (Pisces: Mugilidae). *Australian Journal of Marine and Freshwater Research* **31**, 69–83.
- Tamura, K. & Aotsuka, T. (1988). Rapid isolation of animal mitochondrial DNA by the alkaline lysis procedure. *Biochemical Genetics* **26**, 815–819.
- Thomson, J. M. (1954). The Mugilidae of Australia and adjacent seas. *Australian Journal of Marine and Freshwater Research* **5**, 70–131.
- Thomson, J. M. (1955). The movements and migrations of mullet (*Mugil cephalus* L.). *Australian Journal of Marine and Freshwater Research* **6**, 328–347.

- Thomson, J. M. (1963). Synopsis of biological data on the grey mullet (*Mugil cephalus* Linnaeus 1758). *Division of Fisheries and Oceanography, CSIRO, Australia, Fisheries Synopsis* 1.
- Thomson, J. M. (1981). The taxonomy of grey mullets. In *Aquaculture of Grey Mulletts* (Oren, O. H., ed.), pp. 1–16. Cambridge: Cambridge University Press.
- Vawter, A. T., Rosenblatt, R. H. & Gorman, G. C. (1980). Genetic divergence among fishes of the eastern Pacific and Caribbean: support for the molecular clock. *Evolution* **34**, 705–711.