J Mol Evol (1994) 39:282–295

Journal of Molecular Evolution

On the Evolution of Protamines in Bony Fish: Alternatives to the "Retroviral Horizontal Transmission" Hypothesis

Núria Saperas,[1,2] Juan Ausio,[3] Domènec Lloris,[2] Manel Chiva[1,4]

[1] Departament d'Enginyeria Química ETSEIB, UPC, Diagonal 647, Barcelona E-08028, Spain
[2] Institut de Ciències del Mar, CSIC, Passeig Nacional s/n, Barcelona E-08039, Spain
[3] Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia, Canada V8W 3P6
[4] Departament de Ciències Fisiologiques Humanes i de la Nutrició, Universitat de Barcelona, Diagonal, s/n, Barcelona E-08028, Spain

Received: 3 October 1993 / Revised and accepted: 4 January 1994

Abstract. Fish protamines are highly specialized molecules which are responsible for chromatin condensation during the last stages of spermatogenesis (spermiogenesis). However, not all fish contain protamines in their sperm nuclei; rather, there seems to be a random distribution of protamines within this group. The origin of this sporadic presence of protamines in the sperm and its significance have not yet been precisely determined. In this paper we have conducted an exhaustive survey of the literature available on the different types of nuclear protein composition of the sperm of teleost fish in order to try to correlate these data with what is presently known about the taxonomy of this group. The results of this analysis have allowed us to make the following observations. The divergence between protamines and histones has occurred several times during the evolution of the bony fish. However, the relative frequency of this divergence is almost negligible during the differentiation of genera and species (intrafamily variation) and is very small during the differentiation of families (interfamily variation). Nevertheless, the divergence is very noticeable among the different orders. It is therefore possible to conclude from all this that the sporadic distribution of protamines in bony fish is not a random event as initially believed. Furthermore, such a heterogeneous distribution of protamines cannot be easily accounted for by a mechanism of horizontal retroviral transmission through repeated and independent acquisition of a protamine gene as has been recently proposed (Jankowski, Stater, Dixon (1986) *J Mol Evol* 23:1–10). Rather, it could possibly be explained by a repeated and independent loss of the expression of the protamine gene (or loss of the gene itself) which mainly occurred during the diversification of the orders of this group.

Key words: Protamines — Bony fish — Retroviral horizontal transmission

Correspondence to: J. Ausio

Introduction

About Protamines

The protamines of the Teleost fish, in particular those found in Salmonids, have often been taken as a representative of this "family" of sperm-specific nuclear proteins. For this reason they have been thoroughly characterized both at the protein and gene level (see Oliva and Dixon 1991 for a recent review on protamines).

Protamines are small proteins (25–80 amino acids) which are highly arginine rich (≥50% arginine) (type P). The basic function of these proteins is to displace and replace the somatic-type histones of the stem cells in the last stages of the cell differentiation process of spermatogenesis (spermiogenesis). The molecular mechanisms by which the histone displacement occurs are as yet poorly understood. In several instances it involves the coordinate interplay of many metabolic modifications of the proteins involved such as acetylation of the

histones to be displaced and phosphorylation-dephosphorylation of the protamine molecules during and after deposition onto the DNA. However, this spermatogenic pattern, although widespread in vertebrates, is not universal. In fact, different organisms within the same taxonomic group may exhibit completely different strategies. An extremely opposed situation is that in which the mature spermatozoa end product does not contain protamines but only somaticlike histones (type H) which often contain sperm-specific histone variants (types H + H1, H + A; see legend to Fig. 3 for a more precise definition of these two particular types). In other instances the somatic histones present at the onset of spermatogenesis are replaced, to a different extent, by proteins with an intermediate composition between protamines and histones (protaminelike proteins) (type PL). It is not yet clear, however, whether or not this replacement involves the same mechanisms of acetylation of the histones and phosphorylation-dephosphorylation turnover of the PL proteins as in the case of the protamine type.

The evolutionary link between these three types of spermatogenesis is still unclear. However, the trend seems to be that within a given taxonomical group, the protamine type is usually found in those organisms that are considered phylogenetically more evolved within this taxon. This fact taken together with the aforementioned enzymatic complexity associated with the protamine displacement of histones in the organisms of type P has led to the conclusion that this type represents the most evolved of the spermatogenic processes.

From this perspective, the presence of histones in some species and of protamines in other species in the mature spermatozoa of closely related groups within the same taxon, such as in teleost fishes (to be discussed later), represents a puzzle from the evolutionary point of view.

About the Origin of Protamines

In deuterostomes, protamines first appear in teleost fish and are absent from other chordate groups (Saperas et al. 1993a). Fish protamines are usually small and contain very few amino acids, ranging from 27 in sturgeon protamines (Yulikova et al. 1976, 1979) to 34 in tuna fish (Bretzel 1972, 1973) (Fig. 1). The highly abundant arginine residues are usually present in clusters which are flanked by discrete groups of one to four amino acids containing residues amenable to posttranslational phosphorylation such as serine and threonine. (See Oliva and Dixon 1991 for a recent review.) Because of the small number of amino acids and the high arginine content of these proteins, it is very difficult to establish their true homology to other proteins by using a comparative analysis with the known protein sequences available in the different data banks. (See Doolittle 1986; Doolittle et al. 1986; and references therein.) Despite this difficulty, during the last 25 years several interesting hypotheses have been proposed regarding the origin of these molecules. For instance, early on, Black and Dixon (1967) proposed the existence of an ancestral pentapeptide Ala-Arg-Arg-Arg-Arg which could have led to the fish protamines (clupeines) through four successive partial gene duplications and a few amino acid mutations. More recently Krawetz et al. (1987) have expanded this hypothesis and suggested that this initial protein domain is not only responsible for the origin of fish protamines but also for the origin of the central protein core of the protamines from mammals and other vertebrates. An additional aspect of this hypothesis is that the basic repetitive unit could intrinsically operate as a nuclear targeting sequence. These kinds of sequences are essential for the transport of proteins from the cytoplasm to the nucleus and have a consensus sequence consisting of a cluster of two basic residues, a spacer region, and a basic cluster consisting at least three basic amino acids (Dingwall and Laskey 1991; Hanover 1992). Some of these latter basic clusters are similar to the clusters proposed by Krawetz et al. (1987).

An alternative hypothesis was proposed by von Holt et al. (1984) based on their studies of the basic nuclear proteins of the sperm form echinoderms. The sperm of echinoderms does not contain protamines but instead contains histones (type H). In several instances (i.e., sea urchins) the sperm contains highly specialized (sperm-specific) histone H1 and H2B variants (types H + H1 and H + A). The N-terminal regions of these histone variants are very different from their somatic counterparts (von Holt et al. 1984). These differences are mainly due to the presence of repetitive penta or tetrapeptides with the consensus sequence Pro-basic-basic-basic-Ser (where the basic residue could either be lysine or arginine) in the sperm variants. Von Holt et al. (1984) propose that protamines could be considered to be arrays of tetra- or pentapeptides, similar to those found in the spermatic forms of histone H1 and H2B, but lacking the hydrophobic core of these histones. In this context, protamines could have arisen through the following evolutionary pathway: histones (somaticlike) → specialized sperm histones (echinoderms) → protamines (Fish). At present, the consensus sequence of von Holt's tetrapentapeptides of the sperm of echinoderms has been redefined as a tetrapeptide with the consensus sequence Ser-Pro-basic-basic (known as the SPKK motif) (Poccia 1991; Poccia and Green 1992). Nevertheless, this reassignment of the repetitive motif does not alter the content of the aforementioned hypothesis.

About the Random Distribution of Protamines in the Sperm of Teleosts

The problem of the sporadic distribution of the fish protamines can be briefly defined as follows. The sperm nuclei of most of the species of teleost fish analyzed thus

1 ACIPENSERIFORMES				
Acipenser guldenstadti [Caspian sturgeon]	sturine B	(1)	ARRRRR SS RPQRRRRR RR HGRRRR GRR
Acipenser stellatus	stelline A	(2)	ARRRRRH ASTKLKRRRRR RR HGKKSHK
2 CLUPEIFORMES			
Clupea pallasii [Pacific herring]	clupeine YI	(3)	ARRRR SSSRPIRRRR PRRRTTRRRR AGRRRR
	clupeine YII	(3)	PRRRTRR AS RPVRRRR PRR VSRRRR A RRRR
	clupeine Z	(3)	ARRRRSRRAS RPVRRRR PRR VSRRRR A RRRR
3 SALMONIFORMES			
3a *Salmo irideus* [Rainbow trout]	iridine 1a	(4)	PRRRR ASRR VRRRRRPR VS RRRRRGGRRRR
	iridine 1b	(4)	PRRRR ASRR IRRRRRPR VS RRRRRGGRRRR
	iridine 2a	(5)	PRRRR SSSRPVRRRRRAR VSRRRRRRGGRRRR
	iridine 2b	(5)	PRRRR SSSRPVRRRRRPR VSRRRRRRGGRRRR
	iridine 2c	(5)	PRRRR SSRRPVRRRRRPR VSRRRRRRGGRRRR
	iridine 3a	(5)	PRRRRR SSSRPIRRRRRPR VS RRRRRGGRRRR
	iridine Ia	(5)	PRRRR SSSRPVRRRRRPRR VSRRRRRRGGRRRR
	iridine II	(4)	PRRRR SSSRPVRRRR ARR VSRRRRRRGGRRRR
	iridine Ib	(5)	PRRRRR SSSRPIRRRR PRR VSRRRRR GGRRRR
	iridine CI	(5)	PRRRRR ASRR VRRRRRPR VSRRRRR GGRRRR
	iridine CIII	(5)	PRRRR ASR PVRRRR PRR VSRRRRR GGRRRR
	iridine TP14	(5)	PRRRR SSRPPVRRRRRPR VSRRRRRRGGRRRR
	iridine pTp4	(5)	PRRRR ASRR IRRRRRPR VSRRRR GGRRRRR
3b *Oncorhynchus keta* [Chum salmon]	salmine A1	(4)	PRRRR SSSRPVRRRRRPR VSRRRRRRGGRRRR
	Peak 1	(6)	PRRRRR SSSRPIRRRRRPR ASRRRR GGRRRR
	Peak 2	(6)	PRRRR SSRRPVRRRRRPR VSRRRRRRGGRRRR
	Peak 4	(6)	PRRRR ASRR IRRRRRPR VSRRRRR GGRRRR
3c *Esox lucius* [Pike]		(7)	PRRRRA SSGRPVRRRRRPKMS RRRRRGGRRRR (S)
4 PERCIFORMES			
4a *Thunnus thynnus* [Tuna]	thynnine Y1	(8)	PRRRR EAS RPVRRRRRYRRSTAARRRRRVVRRRR
	thynnine Y2	(9)	PRRRR QAS RPVRRRRRYRRSTAARRRRRVVRRRR
	thynnine Z1	(10)	PRRRRR SS RPVRRRRRYRRSTVARRRRRVVRRRR
	thynnine Z2	(11)	PRRRRR SS RPVRRRRRYRRSTAARRRRRVVRRRR
4b *Sarda orientalis* [Striped bonito]	sardaine Z1	(12)	PRRRRR SS RPVRRRRRYRRSTAARRRRRVVRRRR
	sardaine Z2	(12)	PRRRRR AS RPVRRRRRYRRSTAARRRRRVVRRRR
4c *Mugil japonicus* [Formosan grey mullet]	mugiline M6	(13)	PRRRR ETS RPIRRRRRARRAPMRRRRR VVRRRR
	mugiline M7	(13)	PRRRR QTS RPIRRRRRARRAPMRRRRR VVRRRR
4d *Perca flavescens* [Yellow perch]		(14)	PRRRR HA ARPVRRRRRTRRSSRVHRRRRAVRRRR
4e *Dicentrarchus labrax*		(15)	PRRRR QAS RPVRRRRRTRRSTAERRRRRVVRRRR

Fig. 1. Comparison of the sequences of the protamines from teleost fish. The sequences have been aligned to obtain maximum similarity and have been arranged according to the taxonomic classification of this group as shown in Table 2. 1: order Acipenseriformes (family Acipenseridae); 2: order Clupeiformes (family Clupeidae); 3: order Salmoniformes (3a, 3b: family Salmonidae; 3c: family Esocidae); 4: order Perciformes (4a, 4b: family Scombridae; 4c: family Mugilidae; 4d: family Percidae; 4e: family Percichthyidae). References are as follows: (12) Yulikova et al. (1976); (2) Yukilova et al. (1979); (3) Ando et al. (1973); (4) Ando and Watanabe (1969); (5) Oliva and Dixon (1991); (6) Hoffman et al. (1990); (7) Speckert et al. (1983); (8) Bretzel (1972b); (9) Bretzel (1972a); (10) Bretzel (1973a); (11) Bretzel (1973b); (12) Okamoto et al. (1992); (13) Okamoto et al. (1987); (14) Chao and Davies (1992); and (15) Saperas et al. (1993c).

far contain either protamines (type P) and/or protaminelike proteins (type PL) or histones (type H). At first sight the distribution of these different molecule types does not apparently have any correlation with the taxonomy of the group (Saperas 1992; Saperas et al. 1993b) but rather seems to be partially randomly distributed throughout the different taxonomical groups.

Three different hypotheses have been proposed in order to explain the uneven distribution of these protein groups in bony fish. The first of them, by Nandi et al. (1979), suggests that this heterogeneous distribution of these sperm proteins can be related to the physicochemical parameters (i.e., salinity) of the environment in which the fish reproduction takes place. According to this, seawater fish (marine or saline-resistant) would contain protamines whereas freshwater species would have histones in their sperm nuclei. Although this explanation seems quite attractive, recent experimental evidence (Lemke 1985; see also the previous survey by Kasinsky 1989 and Saperas et al. 1993b) shows that there are far too many exceptions to this hypothesis.

A second hypothesis proposed by Kasinsky and his collaborators (Mann et al. 1982; Kasinsky et al. 1978, 1985a,b), suggests the existence of a clear correlation between the biology of reproduction (i.e., internal vs external fecundation) and the sperm protein type. Accordingly those organisms with internal fertilization would contain protamines and those with external fertilization would retain histones in their sperm nuclei. The third and more recent hypothesis is that by Jankowsky et al. (1986). These authors noticed that the flanking regions of the protamine genes from rainbow trout (Salmo gairdneri) exhibit a large extent of identity with the long-terminal repeat (LTR) sequences of avian retroviruses. Based on this observation they proposed several possible explanations to account for the sporadic distribution of protamines, which can be summarized as follows: (1) The protamine gene has been rendered silent or has been lost in some species. (2) The absence of the protamine gene would represent the usual pattern in fish and only certain groups of fish would have acquired this gene as a result of horizontal transmission through retroviral infection. The second alternative could be complemented with a prokaryote origin of the basic pentapeptide discussed earlier.

In the present work we analyze the problem of the sporadic distribution of protamines in teleost fish. Several extensive and comprehensive attempts to classify the protein composition of the sperm chromatin of this group have been performed during the last decade

Table 1. Distribution of the different types of nuclear sperm proteins (see Fig. 3) amongst different orders and families of the bony fish (class: Osteichthyes subclass: Actinopterygii)[a]

Order	Family	NSP
Acipenseriformes	Acipenseridae	P
Lepisosteiformes	Lepisosteidae	P
Amiiformes	Amiidae	H
Osteoglossiformes	Notopteridae	P
Elopiformes	—	—
Notacanthiformes	Notacanthidae	H + A
Anguilliformes	Congridae	P
Clupeiformes	Clupeidae	P
	Engraulidae	P
Gonorynchiformes	—	—
Cypriniformes	Cyprinidae	H
	Cobitidae	H
Characiformes	—	—
Siluriformes	Bagridae	H
	Clariidae	H
	Heteropneustidae	H
Gymnotiformes	—	—
Salmoniformes	Salmonidae	P
	Esocidae	P
	Alepocephalidae	P
Stomiiformes	—	—
Aulopiformes	—	—
Myctophiformes	Myctophidae	H + A
Percopsiformes	—	—
Gadiformes	Moridae	H + A
	Gadidae	H + A
	Merlucciidae	H + A
Ophidiiformes	Bythitidae	H + A
Batrachoidiformes	Batrachoididae	H
Lophiiformes	Lophiidae	P
Gobiesociformes	—	—
Cyprinodontiformes	Exocoetidae	P
	Hemiramphidae	P
	Belonidae	P
Atheriniformes	—	—
Lampriformes	—	—
Beryciformes	Berycidae	P
Zeiformes	—	—
Gasterosteiformes	Gasterosteidae	P
Indostomiformes	—	—
Pegasiformes	—	—
Syngnathiformes	—	—
Dactylopteriformes	—	—
Synbranchiformes	—	—
Scorpaeniformes	Scorpaenidae	P
	Triglidae	H
	Cyclopteridae	P
Perciformes	Centropomidae	P
	Percichthyidae	P
	Percidae	P
	Sillaginidae	P
	Pomatomidae	P
	Carangidae	P
	Emmelichthyidae	P
	Lutjanidae	P
	Sparidae	H + H1
	Sciaenidae	P
	Mullidae	PL
	Pentacerotidae	P
	Nandidae	P
	Cichlidae	P
	Mugilidae	P
	Polynemidae	P
	Labridae	P
	Notothenidae	P
	Harpagiferidae	P
	Channichthyidae	P
	Trachinidae	P
	Uranoscopidae	P
	Blenniidae	H
	Gobiidae	P
	Gempylidae	P
	Trichiuridae	H + H1
	Scombridae	P
	Xiphiidae	P
	Centrolophidae	P
	Nomeidae	P
	Stromateidae	P
	Anabantidae	P
	Channidae	H
Pleuronectiformes	Pleuronectidae	PL
Tetraodontiformes	Tetraodontidae	P

[a] The classification followed is according to Nelson (1984). NSP = nuclear sperm proteins (P, PL, H, H + H1, H + A as in legend to Fig. 3)

(Kasinsky 1989; Daisley 1980). However, an attempt to analyze the evolutionary problem using these data has not yet been carried out. We have revised here all the information available on the composition of the sperm-protein types (H, PL, and P) of the bony fish characterized to date and have arranged the species taxonomically. The analysis of this information has been proven very useful in assessing the validity of the above-mentioned evolutionary hypotheses.

Analysis of the Data Available on Nuclear Sperm Proteins from Different Bony Fish

In Fig. 3 we show the electrophoretic pattern of the nuclear sperm proteins from a few selected fish that can be taken as representative of the different protein types (H, H + H1, PL, P) found in the teleost fish.

Table 1 and Table 2 show a comprehensive and exhaustive analysis of the nuclear-sperm protein composition of different species from 22 different orders of teleost fish.

In 14 of these orders only one family has been studied in each. In 7 of them, 2–3 families have been characterized in each. In contrast, 33 families have been analyzed within the order Perciformes. There is also a large variability in the number of species studied within each family.

Nonrandom Distribution of Protamines and Histones Within Each Family

Tables 1 and 2 show the nuclear sperm protein composition of species from 66 families of teleost fish. The da-

Table 2. Compilation of the information available on the nuclear-sperm protein composition of the fish species characterized to date: the different species presented here have been taxonomically grouped according to Nelson (1984)[a]

Taxonomic arrangement	NSP	Reference	Cited by
Order: Acipenseriformes			
Family: Acipenseridae			
Acipenser guldenstadti Brandt, 1833	P	Yulikova et al. 1976	
Acipenser stellatus Pallas, 1771	P	Yulikova et al. 1979	
Acipenser sturio Linnaeus, 1758	P	Kossel 1896	A
Acipenser huso = *Huso huso* (Linnaeus, 1758)	P	Lisitzuin and Aleksandrovskaya 1933	A
Order: Lepisosteiformes			
Family: Lepisosteidae			
Lepisosteus osseus (Linnaeus, 1758)	P	Daisley 1980	
Order: Amiiformes			
Family: Amiidae			
Amia calva Linnaeus, 1766	H	Daisley 1980	
Order: Osteoglossiformes			
Family: Notopteridae			
Notopterus chitala Gray (?)	P	Nandi et al. 1979	
Order: Elopiformes	(?)		
Order: Notacanthiformes			
Family: Notacanthidae			
Notacanthus sexspinis Richardson, 1846	H + A	Saperas et al. 1993b	
Order: Anguilliformes			
Family: Congridae			
Muraenesox cinereus (Forsskål 1775)	P	Nandi et al., 1979	
Order: Clupeiformes			
Family: Clupeidae			
Amblygaster immaculatus = *Sardinella gibbosa* (Bleeker, 1849)?	P	Yamakawa and Ibuka 1926	A
Clupea harengus Linnaeus, 1758	P	Kossel 1897; Kossel and Dakin 1904	A
Clupea pallasii Valenciennes, 1847	P	Yamakawa and Yoshimoto 1926	A
		Daisley 1980	K
Gonialosa manminna = *G. manmina* (Hamilton-Buchanan, 1822)	P	Nandi et al. 1979	
Hilsa ilisha = *Tenualosa ilisha* (Hamilton-Buchanan, 1822)	P	Nandi et al. 1979	
Konosirus punctatus (Temminck and Schlegel, 1846)	P	Yamakawa et al. 1923	A
Sardina pilchardus (Walbaum, 1792)	P	Saperas et al. 1993b	
Sardinella fimbriata (Valenciennes, 1847)	P	Nandi et al. 1979	
Sardinia coerulea = *Sardinops caeruleus* (Girard, 1854)	P	Dunn 1926	A
Family: Engraulidae			
Setipinna phasa (Hamilton-Buchanan, 1822)	P	Nandi et al. 1979	
Order: Gonorynchiformes	(?)		
Order: Cypriniformes			
Family: Cyprinidae			
Abramis brama (Linnaeus, 1758)	H	Kadura et al. 1988	
Blicca bjoerkna (Linnaeus, 1758)	H	Kadura et al. 1988	
Carassius auratus (Linnaeus, 1758)	H	Muñoz-Guerra et al. 1982	
Catla catla (Hamilton, 1822)	H	Nandi et al. 1979	
Ctenopharyngodon idella (Valenciennes, 1844)	H	Kadura et al. 1983	
Cyprinus carpio Linnaeus, 1758	H	Nandi et al. 1979	
		Kadura et al. 1988	
*Labeo rohita**	H	Nandi et al. 1979	
Rutilus rutilus (Linnaeus, 1758)	H	Kadura et al. 1988	
Tinca tinca (Linnaeus, 1758)	H	Vendrely and Vendrely 1966	K
Family: Cobitidae			
Misgurnus fossilis (Linnaeus, 1758)	H	Kadura et al. 1988	
Order: Characiformes	(?)		
Order: Siluriformes			
Family: Bagridae			
Mystus vittatus (Bloch 1797)	H	Nandi et al. 1979	
Family: Clariidae			
Clarias batrachus Bleeker, 1865	H	Nandi et al. 1979	
Family: Heteropneustidae			
*Heteropneustes fossilis**	H	Nandi et al. 1979	
Order: Gymnotiformes	(?)		
Order: Salmoniformes			
Family: Salmonidae			
Coregonus albus = *C. albula* (Linnaeus, 1758)	P	Kossel 1913	A
Coregonus lavaretus (Linnaeus, 1758)	P	Waldschmidt-Leitz and Gutermann 1961	A

Table 2. Continued

Taxonomic arrangement	NSP	Reference	Cited by
*Coregonus macrophthalmus**	P	Kossel and Staudt 1926	A
Oncorhynchus keta (Walbaum, 1792)	P	Watanabe (Ph.D. thesis) 1969	A
Oncorhynchus kisutch (Walbaum, 1792)	P	Ingles (Ph.D. thesis) 1968	A
Oncorhynchus nerka (Walbaum, 1792)	P	Yamakawa and Nokata 1923	A
Oncorhynchus tschawytscha = *O. tshawytscha* (Walbaum, 1792)	P	Callanan et al. 1957	A
Prosopium williamsoni (Girard, 1856)	P	Moir (Ph.D. thesis) 1987	
Salmo clarki = *Oncorhynchus clarki* (Richardson, 1836)	P	Moir (Ph.D. thesis) 1987	
Salmo fontinalis = *Salvelinus fontinalis* (Mitchill, 1815)	P	Hammarsten 1924	A
Salmo gairdneri = *Oncorhynchus mykiss* (Walbaum, 1792)	P	Ingles (Ph.D. thesis) 1968	A
Salmo irideus = *Oncorhynchus mykiss* (Walbaum, 1792)	P	Ando and Watanabe 1969	K
Salmo lacustris = *Salmo trutta lacustris* (Linnaeus, 1758)	P	Klezkowski 1946	A
Salmo salar Linnaeus, 1758	P	Miescher 1874; Felix et al. 1952	A
Salmo trutta fario (Linnaeus, 1758)	P	Waldschmidt-Leitz 1961	A
Salmo trutta lacustris (Linnaeus, 1758)	P	Waldschmidt-Leitz 1961	A
Salvelinus alpinus (Linnaeus, 1758)	P	Waldschmidt-Leitz and Gutermann 1961	A
Salvelinus fontinalis (Mitchill, 1815)	P	Waldschmidt-Leitz and Gutermann 1961	A
Salvelinus namaycush (Walbaum, 1792)	P	Kossel and Edlbacher 1913	A
Thymallus arcticus (Pallas 1776)	P	Moir (Ph.D. thesis) 1987	
Thymallus thymallus (Linnaeus, 1758)	P	Moir (Ph.D. thesis) 1987	
Family: Esocidae			
Esox lucius (Linnaeus, 1758)	P	Daisley (Ph.D. thesis) 1980	
Family: Alepocephalidae			
Alepocephalus rostratus Risso, 1820	P	Saperas (Ph.D. thesis) 1992	
Order: Stomiiformes	(?)		
Order: Aulopiformes	(?)		
Order: Myctophiformes			
Family: Myctophidae			
Lampanyctus crocodilus (Risso, 1810)	H + A	Saperas (Ph.D. thesis) 1992	
Symbolophorus veranyi (Moreau, 1888)	H + A	Saperas (Ph.D. thesis) 1992	
Order: Percopsiformes	(?)		
Order: Gadiformes			
Family: Moridae			
Mora moro (Risso, 1810)	H + A	Saperas et al. 1993b	
Family: Gadidae			
Gadus morhua Linnaeus, 1758	H	Bloch 1969	
Lota vulgaris = *Molva molva* (Linnaeus, 1758)	H	Bloch 1969	
Micromesistius poutassou (Risso, 1826)	H + A	Saperas (Ph.D. thesis) 1992	
Phycis blennoides (Brünnich, 1768)	H + A	Saperas et al. 1993b	
Family: Merlucciidae			
Merluccius capensis Castelnau, 1861	H + A	Saperas et al. 1993b	
Merluccius merluccius (Linnaeus, 1758)	H + A	Saperas et al. 1993b	
Order: Ophidiiformes			
Family: Bythitidae			
Cataetyx laticeps Koefoed, 1927	H + A	Saperas et al. 1993b	
Order: Batrachoidiformes			
Family: Batrachoididae			
Opsanus beta (Goode and Bean, 1885)	H	Bloch 1976	
Opsanus tau (Linnaeus, 1766)	H	Casas et al. 1981	
Order: Lophiiformes			
Family: Lophiidae			
Lophius budegassa Spinola, 1807	P	Saperas et al. 1993b	
Order: Gobiesociformes	(?)		
Order: Cyprinodontiformes			
Family: Exocoetidae			
Cypselurus agoo = *Cheilopogon agoo agoo* (Temminck and Schlegel, 1846)	P	Yamakawa et al. 1923	A
Family: Hemiramphidae			
*Hemiramphus unifasciatus**	P	Nandi et al. 1979	
Family: Belonidae			
*Xenentodon cancila**	P	Nandi et al. 1979	
Order: Atheriniformes	(?)		
Order: Lampriformes	(?)		
Order: Beryciformes			
Family: Berycidae			

Table 2. Continued

Taxonomic arrangement	NSP	Reference	Cited by
Beryx splendens Lowe, 1834	P	Kadura et al. 1988	
Order: Zeiformes	(?)		
Order: Gasterosteiformes			
Family: Gasterosteidae			
Order: Dactylopteriformes	(?)		
Order: Synbranchiformes	(?)		
Order: Scorpaeniformes			
Family: Scorpaenidae			
Helycolenus dactylopterus dactylopterus (Delaroche 1809)	P	Saperas et al. 1993b	
Family: Triglidae			
Trigla lucerna Linnaeus, 1758	H	Saperas et al. 1993b	
Family: Cyclopteridae			
Gasterosteus aculeatus Linnaeus, 1758	P	Lemke (M.Sc. thesis) 1985	
Order: Indostomiformes	(?)		
Order: Pegasiformes	(?)		
Order: Syngnathiformes	(?)		
Cyclopterus lumpus Linnaeus, 1758	P	Markowin 1899	A
Order: Perciformes			
Suborder: Percoidei			
Family: Centropomidae			
Lates calcarifer (Bloch, 1790)	P	Nandi et al. 1979	
Family: Percichthyidae			
Dicentrarchus labrax (Linnaeus, 1758)	P	Saperas et al. 1993c	
Lateolabrax japonicus (Cuvier, 1828)	P	Yamakawa et al. 1916	A
Morone americanus = *M. americana* (Gmelin, 1788)	P	Daisley (Ph.D. thesis) 1980	
Stereolepis ishinagi = *S. gigas* Ayres, 1859	P	Yamakawa et al. 1916	A
Family: Percidae			
*Luciperca sandra**	P	Lisitzuin and Aleksandrovskaya 1933	A
Lucioperca lucioperca = *Stizostedion lucioperca* (Linnaeus, 1758)	P	Kadura et al. 1988	
Perca flavescens (Mitchill, 1814)	P	Daisley (Ph.D. thesis) 1980	
Perca fluviatilis (Linnaeus, 1758)	P	Kadura et al. 1988	
Stizostedion vitreum (Mitchill, 1818)	P	Kossel 1913	A
Family: Sillaginidae			
Sillago sihama (Forsskål, 1775)	P	Nandi et al. 1979	
Family: Pomatomidae			
Scombrops boops (Houttuyn, 1782)	P	Yamakawa et al. 1916	A
Family: Carangidae			
Caranx fulvoguttatus = *Carangoides fulvoguttatus* (Forsskål, 1775)	P	Kadura et al. 1988	
Decapterus kiliche (Valenciennes, 1839)	P	Kadura et al. 1988	
Seriola aureovittata Temminck and Schlegel (1842)	P	Yamakawa et al. 1916	A
Family: Emmelichthyidae			
Emmelichthys nitidus Richardson, 1845	P	Kadura et al. 1988	
Plagiogenenion rubiginosus (Hutton, 1875)	P	Kadura et al. 1988	
Family: Lutjanidae			
Lutianus vitta = *Lutjanus vitta* (Quoy and Gaimard, 1824)	P	Yamakawa et al. 1923	A
Family: Sparidae			
Diplodus annularis (Linnaeus, 1758)	H + H1	Saperas et al. 1993b	
Diplodus sargus sargus (Linnaeus, 1758)	H + H1	Saperas et al. 1993b	
Lithognathus mormyrus (Linnaeus, 1758)	H + H1	Saperas et al. 1993b	
Pagellus acarne (Risso, 1826)	H + H1	Saperas et al. 1993b	
Sparus aurata Linnaeus, 1758	H + H1	Saperas et al. 1993b	
Family: Sciaenidae			
Sagenichthys ancylodon = *Macrodon ancylodon* (Bloch and Schneider, 1801)	P	Kossel and Staudt 1927	A
Sciaena schlegeli = *Argyrosomus argentatus* (Houttuyn, 1782)	P	Yamakawa et al. 1916	A
Family: Mullidae			
Mullus barbatus Linnaeus, 1758	PL	Saperas et al. 1993b	
Mullus surmuletus Linnaeus, 1758	PL	Saperas et al. 1993b	
Family: Pentacerotidae			
Pentaceros richardsoni Smith, 1849	P	Kadura et al. 1988	
Family: Nandidae			
Nandus nandus Valenciennes, 1836	P	Nandi et al. 1979	
Family: Cichlidae			
*Sarothedon mossambica**	P	Moav et al. 1974	K

Table 2. Continued

Taxonomic arrangement	NSP	Reference	Cited by
Tilapia mossambica (Peter, 1852)	P	Nandi et al. 1979	
Suborder: Mugiloidei			
Family: Mugilidae			
Mugil cephalus Linnaeus, 1758	P	Yamakawa and Nokata 1926	A
*Mugil japonicus**	P	Ota et al. 1966	A
Mugil parsia = *Liza parsia* (Hamilton-Buchanan), 1822	P	Nandi et al. 1979	
Mugil tade = *Liza tada* (Forsskål, 1775)	P	Nandi et al. 1979	
Suborder: Polynemoidei			
Family: Polynemidae			
Eleutheronema tetradactylum (Shaw, 1804)	P	Nandi et al. 1979	
Polynemus paradiseus Linnaeus, 1758	P	Nandi et al. 1979	
Suborder: Labroidei			
Family: Labridae			
Crenilabrus pavo = *Symphodus* (*Crenilabrus*) *tinca* (Linnaeus, 1758)	P	Kossel 1910	A
Symphodus (*Crenilabrus*) *ocellatus ocellatus* (Forsskål, 1775)	P	Saperas et al. 1993b	
Suborder: Nototheniidei			
Family: Nototheniidae			
Eleginops maclovinus (Valenciennes, 1830)	P	Saperas (Ph.D. thesis) 1992	
Family: Harpagiferidae			
Harpagifer sp.	P	Saperas (Ph.D. thesis) 1992	
Family: Channichthyidae			
Champsocephalus esox (Günther, 1861)	P	Saperas (Ph.D. thesis) 1992	
Suborder: Trachinoidei			
Family: Trachinidae			
Trachinus draco Linnaeus, 1758	P	Saperas et al. 1993b	
Family: Uranoscopidae			
Uranoscopus scaber Linnaeus, 1758	P	Saperas et al. 1993b	
Suborder: Blennioidei			
Family: Blenniidae			
Lipophrys trigloides (Valenciennes, 1836)	H	Saperas (Ph.D. thesis) 1992	
Suborder: Gobioidei			
Family: Gobiidae			
Glossogobius giuris (Buchanan-Hamilton, 1877)	P	Nandi et al. 1979	
Suborder: Scombroidei			
Family: Gempylidae			
Thyrsites atun (Euphrasen, 1791)	P	Saperas et al. 1993b	
Thyrsitoides marleyi Fowler, 1929	P	Kadura et al. 1988	
Family: Trichiuridae			
Lepidopus caudatus (Euphrasen, 1788)	H + H1	Saperas et al. 1993b	
Family: Scombridae			
Gymnosarda vagaus = *Katsuwonus pelamis* (Linnaeus, 1758)	P	Yamakawa and Nokata 1923	A
Pelamys sarda = *Sarda sarda* (Bloch, 1793)	P	Kossel 1913	A
Scomber sp.	P	Saperas et al. 1993b	
Scomber japonicus Houttuyn, 1782	P	Kadura et al. 1988	
Scomber scombrus Linnaeus, 1758	P	Kurajeff 1899	A
Scomberomorus niphonius (Cuvier, 1831)	P	Yamakawa et al. 1916	A
Thunnus alalunga (Bonnaterre, 1788)	P	Kossel and Staudt 1927	A
Thunnus thynnus (Linnaeus, 1758)	P	Bretzel 1973	K
Family: Xiphiidae			
Xiphias gladius Linnaeus, 1758	P	Kossel 1913	A
Suborder: Stromateoidei			
Family: Centrolophidae			
*Hyperoglyphe antarctica**	P	Kadura et al. 1988	
Schedophilus ovalis (Valenciennes, 1833)	P	Kadura et al. 1988	
Family: Nomeidae			
*Cubiceps coeruleus**	P	Kadura et al. 1988	
*Cubiceps brevimanus**	P	Kadura et al. 1988	
Family: Stomateidae			
Stromateus argenteus = *Pampus argenteus* (Euphrasen, 1788)	P	Nandi et al. 1979	
Suborder: Anabantoidei			
Family: Anabantidae			
Anabas testudineus Valenciennes, 1836	P	Nandi et al. 1979	
Suborder: Channoidei			

Table 2. Continued

Taxonomic arrangement	NSP	Reference	Cited by
Family: Channidae			
Channa punctatus or *Ophicephalus punctatus**	H	Nandi et al. 1979	
Order:	Pleuronectiformes		
Family: Pleuronectidae			
Hippoglossus hippoglossus (Linnaeus, 1758)	PL	Moir (Ph.D. thesis) 1987	
Pseudopleuronectes americanus (Walbaum, 1792)	PL	Kennedy and Davies 1980	
Order: Tetraodontiformes			
Family: Tetraodontidae			
*Sphoeroides pardalis**	P	Yamakawa et al. 1923	A
*Sphoeroides rubripes**	P	Yamakawa et al. 1923	A

[a] The nomenclature of the different species has been obtained from the following sources: Whitehead et al. (1984–1986) (N.E. Atlantic and Mediterranean Sea). Whitehead (1985) (clupeoidei from other geographic distribution). Hart (1973) (fishes of the Pacific coast of Canada). Leim and Scott (1966) (fishes of the Atlantic coast of Canada). Scott and Crossman (1973) and Maitland and Linsell (1980) (freshwater fishes). Lindberg and Legeza (1969) and Lindberg and Krasyukova (1971) (fishes of the Sea of Japan and neighboring regions). In order to facilitate the use of this table for future research, the name of the author responsible for the assignment of the species' name has also been included in this table. The names of the species have been updated in several cases. In these instances, two names are shown in the table. The first designates the name used in the original publication and the second designates the updated name according to the current nomenclature. Those species for which an updated nomenclature could not be found have been labeled with an asterisk. When the data pertaining to the protein composition could be obtained from the original source, the reference has been quoted in the list of references given at the end of our paper. In those cases when this information was obtained from previous reviews the review source has been quoted in the column "Cited by." A: Ando et al. (1973) and K = Kasinsky (1989). NSP = nuclear sperm protein

ta presented in these tables represent the compilation results obtained to date by different researchers in this field. In at least 25 of the families analyzed, data from several different species and/or genera are available. In these families—Acipenseridae, Clupeidae, Cyprinidae, Salmonidae, Myctophidae, Gadidae, Merlucciidae, Batrachoididae, Percichthyidae, Percidae, Carangidae, Emmelichthyidae, Sparidae, Sciaenidae, Nullidae, Cichlidae, Nugilidae, Polynemidae, Labridae, Gempylidae, Scombridae, Centrolophidae, Nomeidae, Pleuronectidae and Tetraodontidae—the species analyzed in each of them all have proteins that belong to the same protein type. Thus, the most noticeable aspect of Tables 1 and 2 is that the protein type remains constant within each family and that there is a nonrandom distribution of protamines and histones within each family. We believe that the number of representative species reported in Table 2 is large enough to bestow a generalized acceptance of this conclusion. However, the possibility that with more extensive analysis some exceptions could be found to this rule cannot be completely disregarded.

The Distribution of the Sperm-Protein Types is Uniform Within Each Order with Two Exceptions

The same considerations discussed above at the taxonomic level of family can also be applied at the level of order. Unfortunately, no information can be gathered about the internal protein variation from those 14 orders for which only a single family has been characterized. Within the remaining orders, two of them (Clupeiformes and Cypriniformes) have several species that have been studied within each of two different families. In this case, the analysis is consistent with a nonrandom distribution of the protein types within these orders. Thus, whereas all the representative species of Clupeiformes belong to type P, all the organisms analyzed to date from the Cypriniformes contain histones (H). In four other orders (Siluriformes, Salmoniformes, Gadiformes, and Cyprinodontiformes) three families have been analyzed within each of them. Furthermore, in this case the families within each order all exhibit the same protein type: Protamine type (P) in Salmoniformes and Cyprinodontiformes and histones type (H) in Siluriformes and Gadiformes. In the last two orders, Scorpaeniformes and Perciformes, the three families of the former that have been characterized have been found to exhibit protein type variability. Thus while the families Scorpaenidae and Cyclopteridae exhibit the protamine type (P) the nuclear sperm proteins of the Triglidae belong to the histone type (H). The order Perciformes is the one that has been more extensively characterized and thus deserves special consideration. Although a large number of species are presently grouped within this order it seems very likely that this is a polyphyletic taxonomic group (Nelson 1984). In the absence of a general consensus amongst zoologists and taxonomists, the Perciformes is presently considered to be a bundle of families that exhibit a closer evolutionary relationship amongst themselves than with other families from unrelated orders. The nuclear sperm proteins of 33 families have been characterized in this order. Twenty-eight of them belong

to the protamine type (P), one of them belongs to the protaminelike type (PL), and four of them contain histones (type H) in their sperm. Therefore, although this group has protein type variability, the different protein types do not exhibit a complete random distribution. The major protein type of this order is the protamine type (P), and organisms with the (H) type are only present at a much lower frequency. In our opinion, such unbalance (a few families containing histones within a group with a clear protamine background) may be important in order to provide an interpretation of the different hypotheses about the random distribution of the different protein types that will be discussed later.

Random Distribution of the Sperm-Protein Types Within the Class Osteichthyes

Out of 22 orders studied to date, 10 of them contain nuclear sperm proteins that belong to the protamine type (P), 2 of them (Scorpaeniformes and Perciformes) contain mainly protamines (type P), 1 of them (Pleuronectiformes) contains sperm proteins from the protaminelike type (PL), and 9 of them contain histones in their sperm (type H). Although there is a subtle imbalance toward the protamine type, it is obvious from this analysis that the nuclear sperm proteins are quite evenly distributed amongst the two extreme types (P and H) with no dominance of a single type.

General Conclusions

In Fig. 2 we have superimposed the nuclear-sperm protein type on two different phylogenetic representations (Moyle and Cech 1982; Nelson 1984). The first one was used by Kasinsky (1989) in his revision of the nuclear sperm protein diversity of the animal kingdom. Neither of these two representations (nor any others that we have been able to find) allows one to establish the existence of different evolutionary trends that can be ascribed to the different sperm protein types. Therefore from the analysis presented, we conclude that in the teleost fish:

1. The divergence amongst protamines and histones has occurred repeatedly in different evolutionary lines.
2. The relative frequency of this divergence is negligible at the branching point of genera into species, is very small during the formation and/or separation of different families, and is extremely large during the separation formation of different orders (approximately 50%).

These conclusions are consistent with the idea that the divergence between histones and protamines must have mainly occurred early in the evolution of fish (as has been discussed by Daisley 1980), yet the presence of histones in a species of teleost fish does not necessarily indicate a primitive character (Kasinsky 1989). The above conclusions partially agree with the idea that "if a particular species of fish has either somatic histones or protamines in sperm, then all the other species of fish in that taxonomic order will have the same characteristics" (Bloch 1969, 1976; Nandi et al. 1979; Daisley 1980; also discussed in Kasinsky 1989). However, our data completely disagree with the statement that in bony fish "only a limited number of suborders, notably the salmoniform and cluperiform-fishes, express the small arginine-rich protamines" (Krawetz and Dixon 1988).

Discussion of the Models Proposed to Explain the Sporadicity of the Nuclear-Sperm Protein Type in Bony Fish

Two main alternatives can be postulated to account for the sporadicity of the protamine and histone distribution in the sperm of teleost fish. (1) In their evolutionary origin bony fish ancestors had histones in their sperm and they have sporadically acquired the protamine later on. (2) The protamine type of nuclear sperm protein was already present in the early ancestors of bony fish but they have sporadically expressed the histone type in their sperm nuclei.

The first alternative is the one most widely accepted at present (Daisley 1980; Jankowsky et al. 1986; Krawetz and Dixon 1988; Moir 1987; Moir and Dixon 1988a,b; Oliva and Dixon 1991). In support of this alternative a model based on horizontal transmission via infection by retroviruses has been recently proposed (Jankowsky et al. 1986). This hypothesis is fundamentally based on the observation made by Jankowsky et al. (1986) that the protamine genes from the rainbow trout are flanked by nucleotide sequences very similar to the long terminal repeats (LTR) from avian retrovirus.

The second alternative was also discussed by Jankowsky et al. (1986). It was proposed "that the gene has been rendered silent or has been lost in some species independently among them." This hypothesis does not involve acquisition of an exogenous gene and therefore we shall refer to it with the name of "internal transmission" or "internal evolution."

The analysis of the information presented in this paper does not allow us to distinguish unequivocally between the two above-mentioned alternatives. However, it provides better support for a process of internal transmission than for an evolutionary pathway involving horizontal transmission, such as suggested by Jankowsky et al. (1986). There are several reasons for this conclusion. First, the nuclear-sperm protein type is highly conserved within the different orders. As previously mentioned, the only order with an exception to this rule is the heterogeneous order Perciformes in which the protamine type (P) is the dominant one, and only a

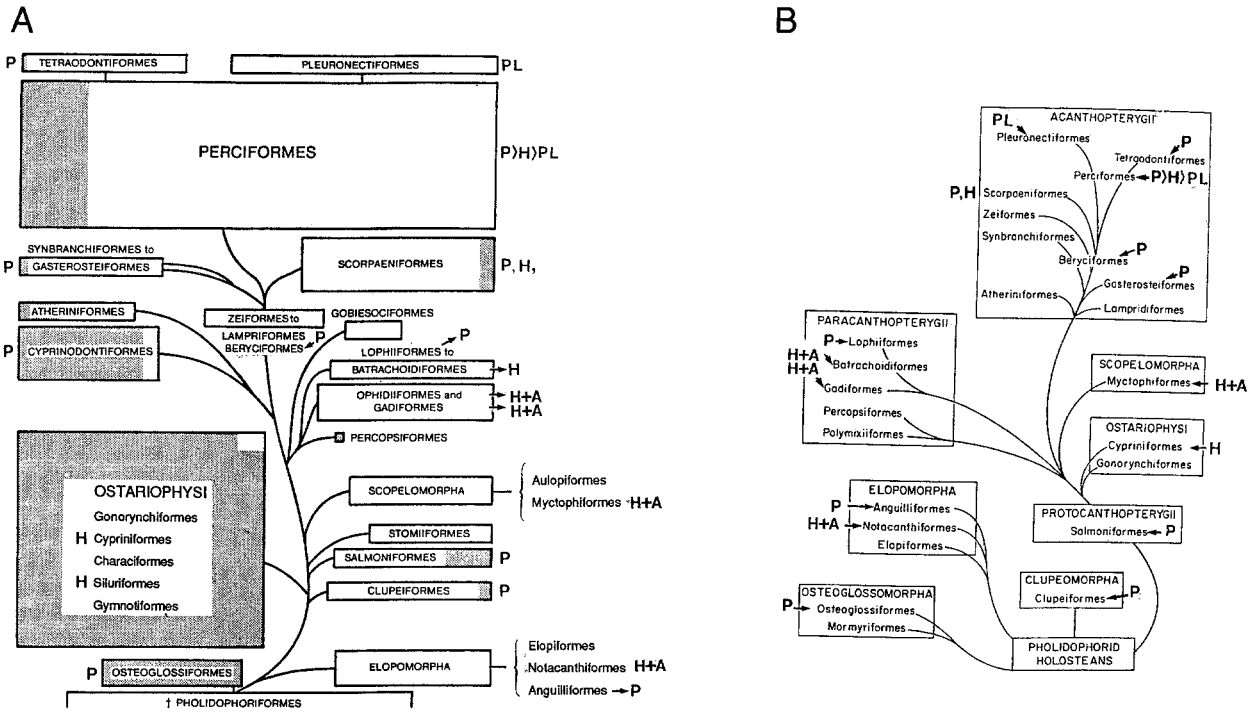

Fig. 2. Distribution of the different types of nuclear sperm proteins among the main groups of teleost fish. The phylogenetic trees have been taken from Nelson (1984) A and from Moyle and Cech (1982) B. See Fig. 3 for a definition of the different protein types.

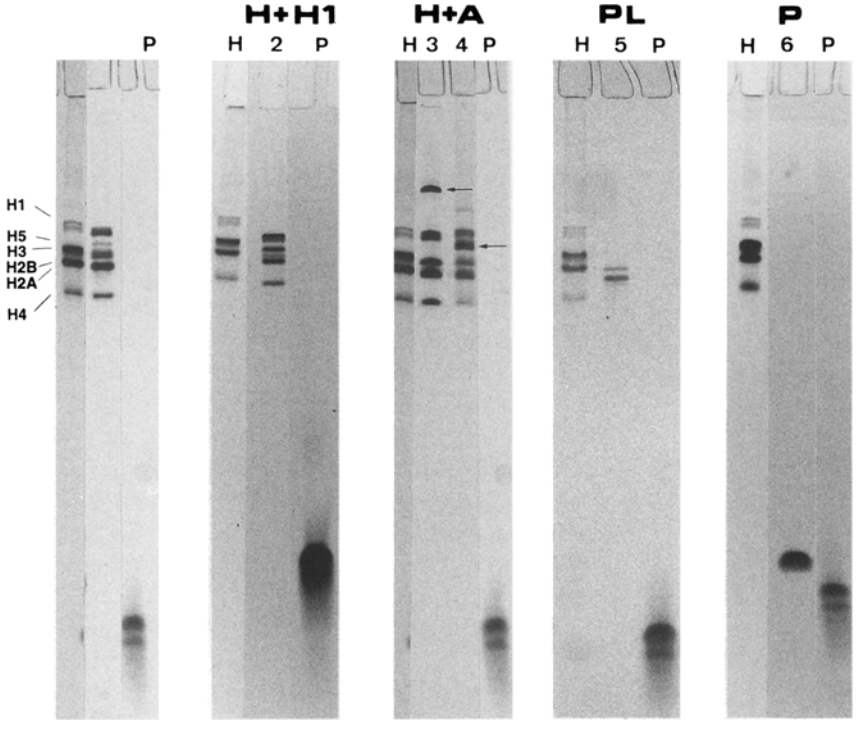

Fig. 3. Different types of nuclear sperm proteins of the bony fish. H: histone type. In this case the nuclear-sperm protein composition consists of histones similar to those found in the nucleus of somatic cells. In some instances (type H + H1) these histones may contain higher stoichiometric amounts of linker histones as occurs in the nucleated erythrocytes from chicken (van Holde, 1988), or they may be associated to additional sperm-specific proteins (*arrows*) (type H + A). In the protaminelike type PL the nuclear sperm chromatin is organized by protaminelike proteins such as those found in the sperm of invertebrates (Ausio 1986; Saperas et al. 1992). P = protamine type. 1: *Trigla lucerna* (family Triglidae); 2: *Pagellus acarne* (family Sparidae), 3: *Merluccius capensis* (family Merlucciidae); 4: *Cataetyx laticeps* (family Bythitidae); 5: *Mullus surmuletus* (family Mullidae); 6: *Dicentrarchus labrax* (family Percichthyidae); H: chicken erythrocyte histones; and P: commercial salmine, used as protein markers.

small number of families exhibit the H type. In these later instances it is possible that the species belonging to the H type have lost the protamine gene and/or its expression. In addition part of the disagreement could possibly be accounted for and/or explained by the intrinsic heterogeneity of this taxonomic group. Second, the changes in the relative frequency of the divergence between the histone type (H) and the protamine type (P) (high in orders, relatively small in families, and negligible at the species level) are more consistent with the genetic changes of an internal evolutionary process than with a random acquisition of certain (protamine) genes. In such an instance the relative frequencies of the protein-type divergence would be expected to be very sim-

ilar, independent of the taxonomic level considered. Finally, a sporadic and repeated independent retroviral acquisition of a "protamine gene" cannot explain the strong correlation existing (within the P type) between the protamine amino acid sequence diversity and the extent of divergency of the species in which these protamines are found (Fig. 1). In other words, within the frame of the retroviral model there is no reason why protamines from different species within the same order would necessarily have to be more similar among themselves than among other protamines from different orders. (i.e., protamines from the order Perciformes should be as different from those of the order Salmoniformes as from those of the order Accipenseriformes and this is not indeed the case.) In contrast, in an internal evolutionary process, protamines from closely related species are expected to be more similar than protamines from evolutionary distant groups, as is indeed observed (Fig. 1). Unfortunately, the intrinsic characteristics of these molecules (short sequences and a high arginine content) do not allow a cladistic approach (Doolittle 1986; Doolittle et al. 1986). Figure 1 summarizes the amino acid sequences of the protamines of the bony fish known to date as well as the taxonomic classification of the species from which they have been obtained.

Given the difficulty in applying the mathematical algorithms to establish the evolutionary relatedness of these protein sequences, a hint into the evolution of these small molecules can only be obtained by comparison of their encoding genes. Despite the fact that very little information is presently available on the protamine genes, the hybridization studies of Daisley (Daisley 1980; Daisley and Davies 1982) using cDNA vectors from trout and yellow perch are most illustrative. Daisley's studies unambiguously show that the extent of hybridization by the genomic DNA and/or the mRNA follows the trend of the taxonomic classification of the species analyzed. The percentage of hybridization increases in the following way: intrafamily > interfamily (intrasuborder) > intersuborder (intraorder) > interorder. These results are again more consistent with a process of internal evolution of fish protamines than with a horizontal pathway with random distribution. Similar results to those obtained by Daisley have also been reported by Moir (1987) and Oliva and Dixon (1991).

The idea of internal evolution in the bony fish has been repeatedly utilized in the past. It has been used implicitly to explain the intraspecific differences of the six different protamines from the rainbow trout (McKay et al. 1986a). It has also been explicitly used to account for the inter-specific variation. Moir and Dixon (1988b) proposed that the protamine genes from Oncorynhus keta and Salmo gairdneri (both from the *Salmonidae* family) arose from a common ancestral gene. At a higher taxonomic level (order), Okamoto et al. (1987) have concluded that the protamines from Mugil japonicus (order Perciformes) are more similar to the protamines from other members of this order than to species from more evolutionary distant orders (see Fig. 1) (See also Okamoto et al. 1992).

In conclusion, our analysis is also fully consistent with an internal model for the evolution of the nuclear sperm proteins in teleost fish. Although it does not rule out the model of a retroviral origin for a bony fish protamine ancestor, it clearly contradicts the model of a repeated and independent acquisition of the protamine gene via retroviral infection as the cause for the sporadic distribution of protamines in fish. Instead, it seems possible that the presence of histones in the sperm nuclei of fish could be due to an independent loss of expression of the protamine gene (or be due to the loss of the gene itself). The functional implications of this event have been discussed earlier (Saperas 1992, 1993c). Although in a different context, this phenomenon could be related to that observed in the heterogeneous distribution of protamine P2 in mammals. Protamine P2 is found in the sperm of mouse, hamster, stallion, humans, and rhesus monkey (Bellvé et al. 1988; Corzett et al. 1987; Pirhonen et al. 1990; McKay et al. 1986b; Balhorn 1989) but is absent from the sperm nucleus of other mammals studied to date such as the rat. In some of these latter mammals it has been possible to show that the protamine P2 gene exhibits mutations that affect the viability of its transcription and/or translation (Maier et al. 1990). In addition it has been possible to show in the rat (Rattus norvegicus) the existence of small amounts of the protamine P2 precursor in spermatogenic cells but not in mature sperm (Stanker et al. 1992).

Acknowledgments. We thank Dr. Harold Kasinsky for his helpful discussions, Nieves Forcada for her skillful assistance in preparing Fig. 1; Corinne Rocchini for carefully reading the manuscript, and Marian Goodman for her careful typing. This work was supported by grant PB90-0605 from CICYT (Spain) and also by NSERC grant OGP 0046 399 and NATO grant CRG 930717 to J. Ausio.

References

Ando T, Watanabe S (1969) A new method for fractionation of protamines and the amino acid sequences of salmine and three components of iridine. Int J Protein Res 1:221–224

Ando T, Yamasaki M, Suzuki K (1973) Protamines. Isolation, characterization, structure and function. Springer-Verlag, Berlin

Ausio J (1986) Structural variability and compositional homology of the protamine-like components of the sperm from bivalve molluscs. Comp Biochem Physiol 85B:439–449

Balhorn R (1989) Mammalian protamines—structure and molecular interactions. In: Adolph KW (ed) Molecular biology of chromosome function. Springer-Verlag, New York, pp 366–395

Bellvé AR, McKay DJ, Renaux BS, Dixon GH (1988) Purification and characterization of mouse protamines P1 and P2. Amino acid reference of P2. Biochemistry 27:2890–2897

Black JA, Dixon GH (1967) Evolution of protamine: a further example of partial gene duplication. Nature 216:152–154

Bloch DP (1969) A catalog of sperm histones. Genetics (Suppl) 61: 93–111

Bloch DP (1976) Histones of sperm. In: King RC (ed) Handbook of genetics, vol. 5. Plenum Press, New York, pp 139–167

Bretzel G (1972a) Über Thynnin, das Protamine des Thunfisches. Die Vollstaendige Aminosaurensequenz von Thynnin Y2. XI. Mitteilung über die Struktur der Untersuchsreike von E. Wald Schmidt-Leitz und Mitarbeitern. Hoppe Seyler's Z Physiol Chem 353:933–943

Bretzel G (1972b) Über Thynnin, das Protamin des Thunfisches. Die Sequenz der Komponente Y1. XII. Mitteilung über die Struktur der Protamine in der Untersunchgugsreike von E. Waldschmidt-Leitz and Mitarbeitern. Hoppe Seyler's Z Physiol Chem 353:1362–1364

Bretzel G (1973a) Über Thynnin, das Protamin des Thunfisches. Die Aminosauresequenz von Thynnin Z1. XIII. Mitteilung über die Struktur der Protamine in der Untersunchungsreike von E. Waldschmidt-Leitz und Mitarbeitern. Hoppe Seyler's Z Physiol Chem 354:312–320

Bretzel G (1973b) Über Thynnin, das Protamin des Thunfisches. Die Aminosauresequenz von Thynnin Z1. XIII. Mitteilung über die Struktur der Protamine in der Untersunchungsreike von E. Waldschmidt-Leitz und Mitarbeitern. Hoppe Seyler's Z Physiol Chem 354:543–549

Casas MT, Muñoz-Guerra S, Subirana JP (1981) Preliminary report on the ultrastructure of chromatin in the histone containing spermatozoa of a teleost fish. Biol Cell 40:87–92

Chao H, Davies PL (1992) Amino acid sequence of the unique protamine from the yellow perch. FEBS Lett 299:166–168

Corzett M, Blacher R, Mazrimas JA, Balhorn R (1987) Analysis of hamster protamines—primary sequence and species distribution. J Cell Biol 105:151a

Daisley St L (1980) Protamine gene evolution in fish. PhD Thesis, Queens University, Kingston, Ontario

Daisley St L, Davies PL (1982) Divergence of protamine gene sequences in fish. Biochim Biophys Acta 698:271–279

Dingwall C, Laskey RA (1991) Nuclear targeting sequences—a consensus? Trends Bichem Sci 16:478–481

Doolittle RF (1986) Of urfs and orfs. A primer on how to analyze derived amino acid sequences. University Science Books, Mill Valley, CA, USA

Doolittle RF, Feng DF, Johnson MS, McClure MA (1986) Relationships of human protein sequences to those of other organisms. Cold Spring Harb Symp Quant Biol 51, Pt:1:447–455

Hanover JA (1992) The nuclear pore: at the crossroads. FASEB J 6: 2288–2295

Hart JL (1973) Pacific fishes of Canada. Fisheries Research Board of Canada, Bulletin 180, Ottawa

Hoffmann JA, Chance RE, Johnson MG (1990) Purification and analysis of the major components of Chum salmon protamine contained in insulin formulations using high performance liquid chromatography. Protein Expr Purif 1:127–133

Jankowsky JM, States JC, Dixon GH (1986) Evidence of sequences resembling avian retrovirus long terminal repeats flanking the trout protamine. Gene J Mol Evol 23:1–10

Kadura SN, Khrapumor SN, Aleeksenko VR (1988) Three types of sperm proteins in eukaryotes (Russian). Ukr Biokim Zh 60:14–19

Kadura SN, Khrapunov SN, Chabanny, Berdyshev GD (1983) Changes in chromatin basic proteins during male gametogenesis of grass carp. Comp Biochem Physiol 74B:343–350

Kasinsky HE (1989) Specificity and distribution of sperm basic proteins. In: Hnilica LS, Stein GS, Stein JL (eds) Histones and other basic nuclear proteins. CRC Press, Boca Ratón, FL, pp 73–163

Kasinsky HE, Huang SY, Kwauk S, Mann M, Sweeney MAJ, Yee B (1978) On the diversity of sperm histones in the vertebrates. III. Electrophoretic variability of testis-specific histone patterns in Anura contrasts with relative constancy in Squamata. J Exp Zool 203:109–126

Kasinsky HE, Huang SY, Mann M, Roca J, Subirana JA (1985a) On the diversity of sperm histones in the vertebrates: IV. Cytochemical and amino acid analysis in Anura. J Exp Zool 234:33–46

Kasinsky HE, Mann M, Lemke M, Huang SY (1985b) Diversity of sperm basic chromosomal proteins in the vertebrates: a phylogenetic point of view. In: Reecks GR, Goodwin GH, Puigdoménech P (eds) Chromosomal proteins and gene expression. Plenum Press, New York, pp 335–352

Kennedy BP, Davies PL (1980) Acid-soluble nuclear proteins of the testis during spermatogenesis in the winter flounder: loss of the high mobility group proteins. J Biol Chem 255:2533–2539

Krawetz SA, Dixon GH (1988) Sequence similarities of the protamine genes: implications for regulation and evolution. J Mol Evol 27: 291–297

Krawetz SA, Connor W, Dixon GH (1987) Cloning of bovine P1 protamine cDNA and the evolution of vertebrate P1 protamines. DNA 6:47–57

Leim AH, Scott WB (1966) Fishes of the Atlantic coast of Canada. Fisheries Research Board of Canada, Bulletin 155, Ottawa

Lemke MJ (1985) Variability of testis-specific proteins in *Gasterosteus aculeatus* L. and related species. MSc thesis, University of British Columbia, Vancouver

Lindberg GU, Krasyukova ZV (1971) Fishes of the Sea of Japan and the adjacent areas of the Sea of Okhotsk and the Yellow Sea. Part 3. Teleostomi. XXIX. Perciformes. Academy of Sciences of the Union of Soviet Socialist Republics, Leningrad

Lindberg GU, Legeza MI (1969) Fishes of the Sea of Japan and the adjacent areas of the Sea of Okhotsk and the Yellow Sea. Part 2. Teleostomi. XII. Acipenseriformes–XXVIII. Polynemiformes. Academy of Sciences of the Union of Soviet Socialist Republics, Moskva-Leningrad

Maier WN, Nussbaum G, Domenjoud L, Klemm U, Engel W (1990) The lack of protamine 2 (P2) in boar and bull spermatozoa is due to mutations within the P2 gene. Nucleic Acids Res 18:1249–1254

Maitland PS, Linsell K (1980) Guía de los peces de agua dulce de Europa. Editorial Omega, Barcelona

Mann M, Risley MS, Eckhardt RA, Kasinsky HE (1982) Characterization of spermatid/sperm basic chromosomal proteins in the genus *Xenopus* (Anura, Pipidae). J Exp Zool 222:173–186

McKay DJ, Renaux BS, Dixon GH (1986a) Rainbow trout protamines. Amino acid sequences of six distinct proteins from a single testis. Eur J Biochem 158:361–366

McKay DJ, Renaux BS, Dixon GH (1986b) Human sperm protamines amino-acid sequences of two forms of protamine P2. Eur J Biochem 156:5–8

Moir RD (1987) Structure of several multigene families in salmonid fishes. PhD thesis, Department of Medical Science, University of Calgary, Alberta, Canada

Moir RD, Dixon GH (1988a) A repetitive DNA sequence in the salmonid fishes similar to a retroviral long repeat. J Mol Evol 27: 1–7

Moir RD, Dixon GH (1988b) Characterization of a protamine gene from the Chum salmon (*Oncorhynchus keta*). J Mol Evol 27:8–16

Moyle PB, Cech JJ Jr (1982) Fishes: an introduction to ichthyology. Prentice-Hall, Englewood Cliffs, NJ

Muñoz-Guerra S, Azorin F, Casas MT, Marcet X, Maristany MA, Roca J, Subirana JA (1982) Structural organization of sperm chromatin from the fish *Carassius auratus*. Exp Cell Res 137:47–53

Nandi AK, Chandhuri A, Mandal RK (1979) Nature and evolutionary significance of basic proteins in fish spermatozoa. Indian J Biochem Biophys 16:6–10

Nelson JS (1984) Fishes of the world, 2nd ed. John Wiley and Sons, New York

Okamoto Y, Kuno K, Motohiro T, Nishi N, Muta E, Ota S (1992) Primary structures of sardaines Z1 and Z2, protamines isolated from striped bonito (*Sarda orientalis*). J Biochem 111:157–161

Okamoto Y, Muta E, Ota S (1987) Primary structures of M6 and M7 of mugiline β (*Mugil japonicus*). J Biochem 101:1017–1024

Oliva R, Dixon GH (1991) Vertebrate protamine genes and the histone-to-protamine replacement reaction. Progr Nucleic Acid Res Mol Biol 40:25–94

Pirhonen A, Valtonen P, Linnala-Kankkunen A, Heiskanen N, Haenpaa P (1990) Primary structures of two protamine P2 variants (St2a and St2b) from stallion spermatozoa. Biochem Biophys Acta 1039:177–180

Poccia DL (1991) Sp histones and chromatin structure in male germ line nuclei and male pronuclei of the sea urchin. In: Baccetti B (ed) Comparative spermatology 20 years after, vol 75. Serono Symposia Publications from Raven Press, pp 61–65

Poccia DL, Green GR (1992) Packaging and unpackaging the sea urchin sperm genome. Trends Biochem Sci 17:223–227

Saperas N (1992) Distribució i caracterització de les proteïmes espermàtiques bàsiques en peixos, agnats i procordats. PhD thesis, Universitat Politècnica de Catalunya, Barcelona, Spain

Saperas N, Chiva M, Ausio J (1992) Purification and characterization of the protamines and related proteins from the sperm of a tunicate, *Styela plicata*. Comp Biochem Physiol 106B:9690–9974

Saperas N, Chiva M, Ribes E, Kasinsky H, Rosenberg H, Youson JH, Ausio J (1994) Chromosomal proteins of the sperm of a Cephalochordate (*Branchiostoma floridae*) and an Agnathan (*Petromyzon marinus*). Compositional variability of the nuclear sperm proteins of deuterostomes. Biol Bull 186:101–114

Saperas N, Lloris D, Chiva M (1993b) Sporadic appearance of histones, histone-like proteins, and protamines in sperm chromatin of bony fish. J Exp Zool 265:575–586

Saperas N, Ribes E, Buesa C, Garcia-Hegardt F, Chiva M (1993c) Differences in chromatin condensation during spermatogenesis in two species of fish with distinct protamines. J Exp Zool 265:185–194

Scott WB, Crossman EJ (1973) Freshwater fishes of Canada. Fisheries Research Board of Canada, Bulletin 184, Ottawa

Speckert W, Kennedy B, Daisley St L, Davies P (1983) Primary structure of protamine from the northern pike *Esox lucius*. Eur J Biochem 136:283–289

Stanker LH, McKeown C, Balhorn R, Lee C, Mazrimas J, Goralka M, Wyrobek A (1992) Immunological evidence for a P2 protamine precursor in mature rat sperm. Mol Reprod Dev 33:481–488

van Holde KE (1988) Chromatin. Springer Verlag, Berlin

von Holt C, De Groot P, Schwager S, Brandt WF (1984) The structure of sea urchin histones and considerations on their function. In: Stein GS, Stein JL, Marzluff WF (eds) Histone genes: structure, organization and regulation. John Wiley, New York, pp 65–105

Whitehead PJP (1985) Clupeoid fishes of hte world (Suborder clupeoidei). Part 1, Chirocentridae, Clupeidae and Pristigasteridae. FAO Species Catalogue, vol 7. FAO Fisheries Synopsis No. 125, Rome

Whitehead PJP, Bauchot ML, Hureau JC, Nielsen J, Tortonese E (eds) (1984–86) Fishes of the Northeastern Atlantic and the Mediterranean, 3 vols. UNESCO, Paris

Yulikova EP, Evseenko LK, Baratova L, Belyamova LP, Rybin VK, Silaev AB (1976) The primary structure of sturine B, a protamine from Caspian sturgeon. Bioorg Khim 2:1613–1618

Yulikova EP, Rybin VK, Silaev AB (1979) The primary structure of stellin A. Bioorg Khim 5:5–10