



Optic chiasm in the species of order *Clupeiformes*, family *Clupeidae*: Optic chiasm of *Spratelloides gracilis* shows an opposite laterality to that of *Etrumeus teres*

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In most teleost fishes, the optic nerves decussate completely as they project to the mesencephalic region. Examination of the decussation pattern of 25 species from 11 different orders in Pisces revealed that each species shows a specific chiasmatic type. In 11 species out of the 25, laterality of the chiasmatic pattern was not determined; in half of the individuals examined, the left optic nerve ran dorsally to the right optic nerve, while in the other half, the right optic nerve was dorsal. In eight other species the optic nerves from both eyes branched into several bundles at the chiasmatic point, and intercalated to form a complicated decussation pattern. In the present study we report our findings that *Spratelloides gracilis*, of the order *Clupeiformes*, family *Clupeidae*, shows a particular laterality of decussation: the left optic nerve ran dorsally to the right ($n = 200/202$). In contrast, *Etrumeus teres*, of the same order and family, had a strong preference of the opposite (complementary) chiasmatic pattern to that of *S. gracilis* ($n = 59/59$), revealing that these two species display opposite left–right optic chiasm patterning. As far as we investigated, other species of *Clupeiformes* have not shown left–right preference in the decussation pattern. We conclude that the opposite laterality of the optic chiasm of these two closely related species, *S. gracilis* and *E. teres*, enables investigation of species-specific laterality in fishes of symmetric shapes.

Keywords: Optic nerve; Chiasma; Decussation; *Spratelloides gracilis*; *Etrumeus teres*; Clupeidae.

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In 1682, Isaac Newton predicted that the optic nerves might cross each other at the chiasmatic point (Jeffery, 2001; Sweeney, 1984). Then an anatomical chart of a human being, describing the existence of the optic chiasm, was published for the first time by John Taylor (Taylor, 1750). In Taylor's anatomical chart some optic nerve fibres crossed at the chiasmatic point and projected further to the contralateral side, while the remainder projected ipsilaterally without crossing. Today we know that this observation by Taylor was highly accurate. At present it is known that half of the optic nerves from each side cross at the chiasmatic point and project to the contralateral side, while the remainder project ipsilaterally, in mammals including humans (Fisher, 1986; Gray, 1918), monkeys (Fukuda, Sawai, Watanabe, Wakakuwa, & Morigiwa, 1989), cats (Cooper & Pettigrew, 1979), rabbits (Provis & Watson, 1981), and rodents (Baker & Jeffery, 1989; Colello & Guillery, 1990). In conditional knockout mice lacking *Exostoses 1*, which is indispensable for the synthesis of heparan sulphate, it is reported that after the optic nerves pass through the optic chiasm, most fibres are misrouted into the opposite eye; that is, projection towards the brain is inhibited (Inatani, Irie, Plump, Tessier-Lavigne, & Yamaguchi, 2003).

While avian adult optic nerves show total decussation (Drenhaus & Rager, 1992), a subset of fibres project ipsilaterally in the chick embryo during development (O'Leary, Gerfen, & Cowan, 1983). After an intraocular injection of monensin, which specifically inhibits membrane glycoprotein transport within the axon, axons from the injected eye are misrouted towards the opposite eye in chickens (Nakayama & Furuya, 1989). In *Xenopus laevis* tadpoles show total decussation of optic nerves, while adults show semi-decussation after metamorphosis (Hoskins, 1986; Hoskins & Grobstein, 1984; Nakagawa et al., 2000). Similarly, lamprey larvae show total decussation of the optic nerves, while adult lampreys show semi-decussation (Kennedy & Rubinson, 1977; Kosareva, 1980).

In fish, the decussation pattern at the optic chiasm has been studied in *Fundulus* embryos (Oppenheimer, 1950), goldfish (Fernald, 1982; Roth, 1979), and electric gymnotid fish (*Apteronotus leptorhynchus*; Sas & Maler, 1986). In these species the optic nerves cross at the chiasmatic point and show complete decussation. After formation of the optic chiasm, the optic nerves project to the mesencephalic region. Left–right asymmetry of the decussation pattern can occur in the optic chiasm of fishes, in that the optic nerve from one side can pass dorsally or ventrally to the nerve from the other side. While most lower vertebrates show total decussation of their optic nerves, many higher vertebrates show semi-decussation, an important difference when we consider the development and evolution of the brain and nervous system.

It has been reported that flatfish and flounder, which have remarkably asymmetric bodies, show left–right asymmetry of the optic chiasm (Parker, 1903; Policansky, 1982). The frontal region of flatfish develops to be sinistral

or dextral during metamorphosis, and shows remarkable asymmetry. The eyes also migrate to one side of the body concomitantly with the turning of the frontal region. This morphological asymmetry affects several functional asymmetries of the head and jaws during prey capture (Gibb, 1996). When flatfish larvae were treated with triiodothyronine prior to metamorphosis, the frontal bones were formed symmetrically, eye migration was inhibited, and the treated flatfishes developed symmetrically (Schreiber, 2006). This report proposes that the difference of the sensitivity for thyroid hormone between the left and right sides may determine the left–right asymmetry of the rotation of the frontal bone during metamorphosis. The mutant Japanese flounder (*Paralichthys olivaceus*) “reversed (*rev*)” displays facial left–right reversal due to randomised embryonic *pitx2* expression, which is normally left-handed (Hashimoto et al., 2002). The causative gene responsible for the *rev* locus has not yet been identified. It remains controversial whether flounder metamorphic asymmetry is determined by an unidentified genetic cascade in a manner independent of visceral asymmetry control.

The above molecular mechanism identified in the flatfishes may affect their chiasmic pattern. However, comparative studies of the chiasmic patterns in various fishes have been somewhat neglected, and here we query whether the laterality of the optic chiasm is predictable in fish with symmetric bodies, as it is in flatfish. Using a morphological approach we investigated the laterality of the optic chiasm in a number of freshwater and seawater fish, in order to discover an appropriate model animal for the study of left-right asymmetry in the optic chiasm.

METHOD AND MATERIALS

Patterns of optic chiasms of the adults were investigated in 25 species of 11 orders of freshwater and seawater fish (Figures 1 & 2). Dissections of the fish were performed in petri dishes filled with saline. After decapitation, aided by a stereoscopic microscope, the lower jaw and some additional tissue were removed to expose the optic chiasm. The optic chiasm was digitally photographed from the ventral side, and each was classified into one of the three types shown in Figure 2b.

Statistical analysis using a 2×2 contingency table (Fisher's direct probability test) was performed to examine whether there was a bias in the incidence of the type 1 chiasm compared to that of type 2.

RESULTS

The optic nerves crossed to form an optic chiasm in all individuals examined ($n = 988$ adults), across 25 species. Decussation was complete in all species

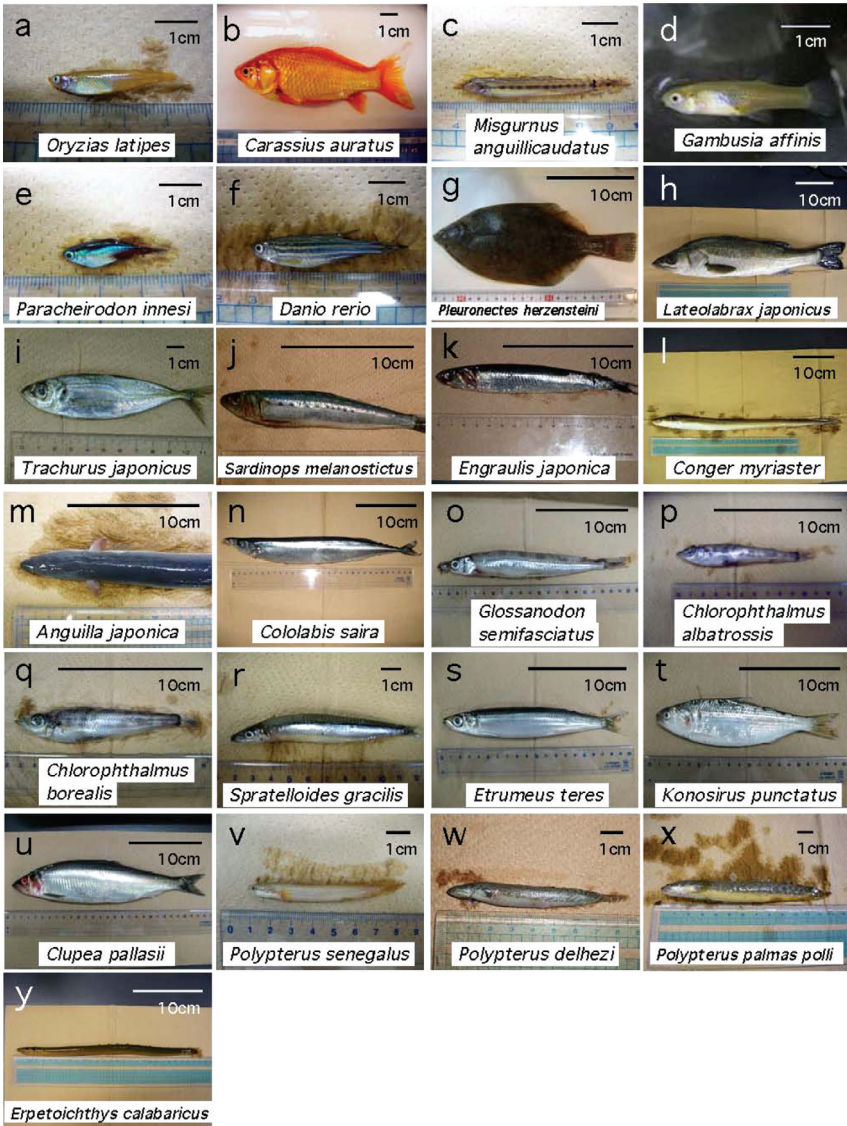


Figure 1. Photographs of the fish used in studies of the decussation pattern of the optic chiasm. Bars, 1 cm or 10 cm.

except the Polypteriformes. In each individual of the species showing total decussation, the pattern of decussation was classified into one of three types: type 1, in which the entire left optic nerve passed dorsally to the right optic nerve; type 2, in which the entire right optic nerve passed dorsally to the left;

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Oder	Family	Genus	Species name
Anguilliformes	Anguillidae	Anguilla	Anguilla japonica
Anguilliformes	Congridae	Conger	Conger myriaster
Argentiniiformes	Argentiniidae	Glossanodon	Glossanodon semifasciatus
Aulopiformes	Chlorophthalmidae	Chlorophthalmus	Chlorophthalmus albatrossis
Aulopiformes	Chlorophthalmidae	Chlorophthalmus	Chlorophthalmus borealis
Beloniformes	Adrianchthyidae	Oryzias	Oryzias latipes
Beloniformes	Scomberesocidae	Cololabis	Cololabis saira
Characiformes	Characidae	Paracheirodon	Paracheirodon innesi
Clupeiformes	Clupeidae	Clupea	Clupea pallasii
Clupeiformes	Clupeidae	Etrumeus	Etrumeus teres
Clupeiformes	Clupeidae	Konosirus	Konosirus punctatus
Clupeiformes	Clupeidae	Sardinops	Sardinops melanostictus
Clupeiformes	Clupeidae	Spratelloides	Spratelloides gracilis
Clupeiformes	Engraulidae	Engraulis	Engraulis japonica
Cypriniformes	Cobitidae	Misgurnus	Misgurnus anguillicaudatus
Cypriniformes	Cyprinidae	Carassius	Carassius auratus
Cypriniformes	Cyprinidae	Danio	Danio rerio
Cyprinodontiformes	Poeciliidae	Gambusia	Gambusia affinis
Perciformes	Carangidae	Trachurus	Trachurus japonicus
Perciformes	Percichthyidae	Lateolabrax	Lateolabrax japonicus
Pleuronectiformes	Pleuronectidae	Pleuronectes	Pleuronectes herzensteini
Polypteriformes	Polypteridae	Erpetoichthys	Erpetoichthys calabaricus
Polypteriformes	Polypteridae	Polypterus	Polypterus delhezi
Polypteriformes	Polypteridae	Polypterus	Polypterus palmas polli
Polypteriformes	Polypteridae	Polypterus	Polypterus senegalus

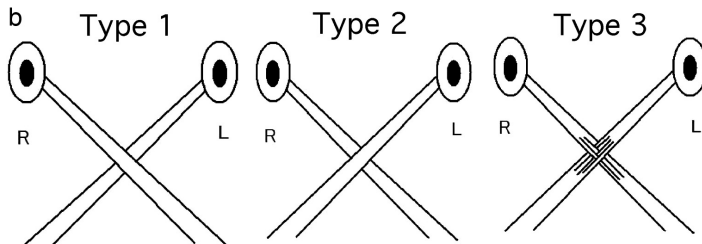


Figure 2. Taxonomic classification of the fishes used and definitions of the chiasmic patterns identified in this study. (a) List of the species examined in this report. Optic chiasmata were investigated in 25 species of 11 orders of freshwater and seawater fishes. (b) A type 1 chiasm is defined as a complete decussation in which the entire left optic nerve runs dorsally to the entire right optic nerve. A type 2 chiasm is that in which the entire right optic nerve runs dorsally to the entire left optic nerve (complementary to type 1 in its laterality). A type 3 chiasm is defined as that in which the optic nerves from both sides cross in a complex manner consisting of intercalated bundles. All the chiasmata shown are viewed from the ventral side in Figure 2-8 except for Figure 7.

or type 3, in which each optic nerve branched into several bundles and intercalated at the optic chiasm.

Among the 25 species examined, 11 species (*Oryzias latipes*, *Carassius auratus*, *Misgurnus anguillicaudatus*, *Gambusia affinis*, *Lateolabrax japonicus*,



Trachurus japonicus, *Sardinops melanostictus*, *Anguilla japonica*, *Conger myriaster*, *Cololabis saira*, and *Konosirus punctatus*) showed a type 1 or type 2 chiasmic pattern, with an apparently random, 40–60% occurrence of each pattern within each species (Figures 3 & 4; Table 1). Type 3 decussation was not observed in any of the species showing type 1 or 2 decussation. Statistical analyses using 2×2 contingency tables proved that, in these fishes, the choice between type 1 and type 2 chiasm had no significant bias at the 5% significance level (all the χ -values were below 3.24; $p > .05$).

Seven species (*Danio rerio*, *Paracheirodon innesi*, *Engraulis japonica*, *Glossanodon semifasciatus*, *Chlorophthalmus albatrossis*, *Chlorophthalmus borealis*, and *Clupea pallasii*) showed a type 3 decussation pattern. All of these species except *D. rerio* (zebrafish) showed type 3 decussation only, and no individual showed type 1 or 2 decussation (Figure 5, see Table 1). However, in zebrafish 12% of the specimens showed a type 1 or 2 decussation pattern, and the remaining 88% showed a type 3 pattern (Table 1).

Spratelloides gracilis from the family *Clupeidae*, order *Clupeiformes*, showed only a type 1 decussation pattern; in almost all specimens the left optic nerve ran dorsally to the right optic nerve ($n = 200/202$, Figure 6a, see Table 1). Regardless of the fishing ground (Kochi or Kagoshima, Japan), an absolute preference for type 1 decussation was conserved in *S. gracilis*. In contrast, *Etrumeus teres*, belonging to the same family of the same order as *S. gracilis*, showed an absolute preference for type 2 decussation ($n = 59/59$, Figure 6b, see Table 1). As with *S. gracilis*, there was no apparent effect of the different fishing grounds (Kagoshima or Shimane, Japan) on the pattern of decussation in *E. teres*.

The chiasmic pattern of decussation was highly varied across the order *Clupeiformes*. While *C. pallasii* and *E. japonica* showed type 3 chiasm (see Figure 5), *S. melanostictus* and *K. punctatus* showed both type 1 and type 2 chiasm at an equal frequency (see Figure 4). Although these species belong to the same family of the same order as *S. gracilis* and *E. teres*, the patterns of the optic chiasm varied among the species. While all of the species within this order have a symmetric body, only *S. gracilis* and *E. teres* (both of the subfamily *Dussumieriinae*) showed a particular laterality of the optic chiasm. These results suggest that a unique phenomenon occurred during the evolution of these fish, before or after the speciation of *S. gracilis* and *E. teres*.

Figure 3 (opposite). Fish showing both type 1 and type 2 chiasmic patterns in equal frequencies within one species. Scientific names of the fishes are indicated in the photographs. Of the 25 species examined, 11 species showed a random preference for a type 1 or type 2 chiasm within individuals (see also Figure 4). Left column, type 1; right column, type 2.

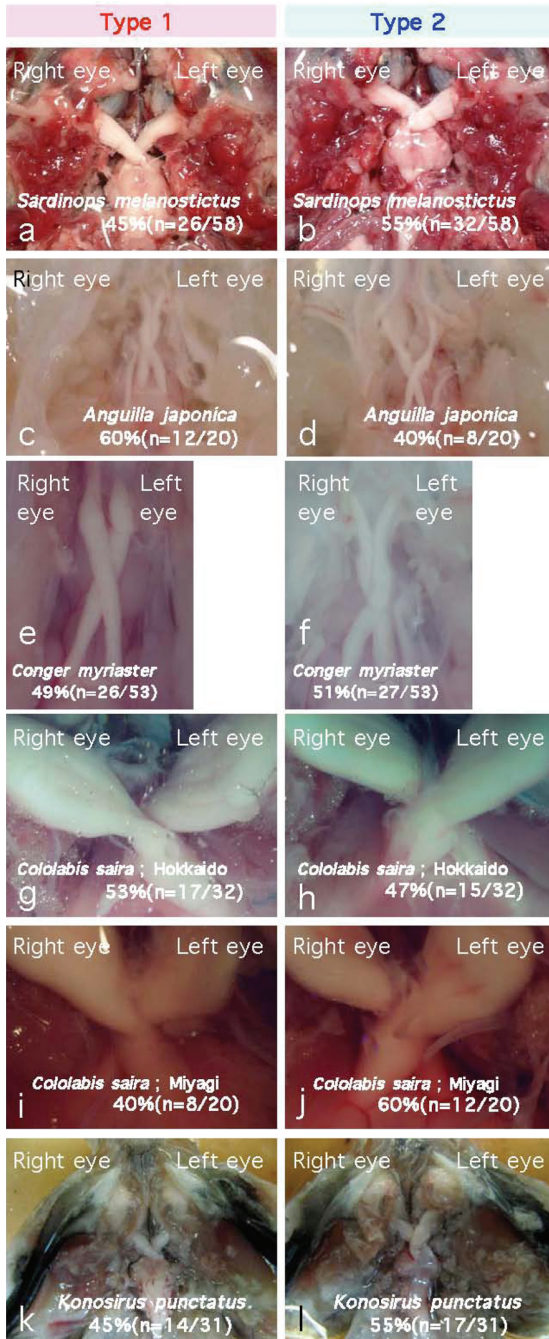


TABLE 1
Patterns of the optic chiasm of the fish investigated in this report

<i>Fish</i>	<i>Type 1</i>	<i>Type 2</i>	<i>Type 3</i>
Type 1			
<i>Spratelloides gracilis</i>	100% (n = 38/38)	0% (n = 0/38)	0% (n = 0/38)
<i>Spratelloides gracilis</i> (Kochi)	99% (n = 95/96)	1% (n = 1/96)	0% (n = 0/96)
<i>Spratelloides gracilis</i> (Kagoshima)	99% (n = 67/68)	1% (n = 1/68)	0% (n = 0/68)
<i>Pleuronectes herzensteini</i>	100% (n = 10/10)	0% (n = 0/10)	0% (n = 0/10)
Type 2			
<i>Etrumeus teres</i> (Kanagawa)	0% (n = 0/29)	100% (n = 29/29)	0% (n = 0/29)
<i>Etrumeus teres</i> (Shimane)	0% (n = 0/30)	100% (n = 30/30)	0% (n = 0/30)
Both Type 1 and Type 2			
<i>Oryzias latipes</i>	59% (n = 29/49)	41% (n = 20/49)	0% (n = 0/49)
<i>Carassius auratus</i>	53% (n = 16/30)	47% (n = 14/30)	0% (n = 0/30)
<i>Misgurnus anguillicaudatus</i>	53% (n = 27/51)	47% (n = 24/51)	0% (n = 0/51)
<i>Gambusia affinis</i>	53% (n = 27/51)	47% (n = 24/51)	0% (n = 0/51)
<i>Lateolabrax japonicus</i>	50% (n = 5/10)	50% (n = 5/10)	0% (n = 0/10)
<i>Trachurus japonicus</i>	40% (n = 16/40)	60% (n = 24/40)	0% (n = 0/40)
<i>Sardinops melanostictus</i>	45% (n = 26/58)	55% (n = 32/58)	0% (n = 0/58)
<i>Anguilla japonica</i>	60% (n = 12/20)	40% (n = 8/20)	0% (n = 0/20)
<i>Conger myriaster</i>	49% (n = 26/53)	51% (n = 27/53)	0% (n = 0/53)
<i>Cololabis saira</i> (Hokkaido)	53% (n = 17/32)	47% (n = 15/32)	0% (n = 0/32)
<i>Cololabis saira</i> (Miyagi)	40% (n = 8/20)	60% (n = 12/20)	0% (n = 0/20)
<i>Konosirus punctatus</i>	45% (n = 14/31)	55% (n = 17/31)	0% (n = 0/31)
Type 3			
<i>Danio rerio</i>	8% (n = 4/51)	4% (n = 2/51)	88% (n = 45/51)
<i>Paracheirodon innesi</i>	0% (n = 0/51)	0% (n = 0/51)	100% (n = 51/51)
<i>Engraulis japonica</i>	0% (n = 0/35)	0% (n = 0/35)	100% (n = 35/35)
<i>Glossanodon semifasciatus</i>	0% (n = 0/30)	0% (n = 0/30)	100% (n = 30/30)
<i>Chlorophthalmus albatrossis</i>	0% (n = 0/31)	0% (n = 0/31)	100% (n = 31/31)
<i>Chlorophthalmus borealis</i>	0% (n = 0/30)	0% (n = 0/30)	100% (n = 30/30)
<i>Clupea pallasii</i>	0% (n = 0/30)	0% (n = 0/30)	100% (n = 30/30)
<i>Fish</i>	<i>Type 1</i>	<i>Type 2</i>	<i>Other Type</i>
Other Type			
<i>Polypterus senegalus</i>	0% (n = 0/17)	0% (n = 0/17)	100% (n = 17/17)
<i>Polypterus delhezi</i>	0% (n = 0/3)	0% (n = 0/3)	100% (n = 3/3)
<i>Polypterus palmas polli</i>	0% (n = 0/5)	0% (n = 0/5)	100% (n = 5/5)
<i>Erpetoichthys calabaricus</i>	0% (n = 0/7)	0% (n = 0/7)	100% (n = 7/7)

Most species of symmetric shapes displayed either types 1 and 2 or type 3, except for *Spratelloides gracilis* and *Etrumeus teres*, which showed distinctive laterality in the chiasmatic pattern. In the fish species that showed a type 3 chiasm, neither a type 1 nor a type 2 chiasmatic pattern occurred, except for zebrafish (*Danio rerio*).

Figure 4 (opposite). These species also displayed a random occurrence of a type 1 or type 2 chiasm (see also Figure 3).

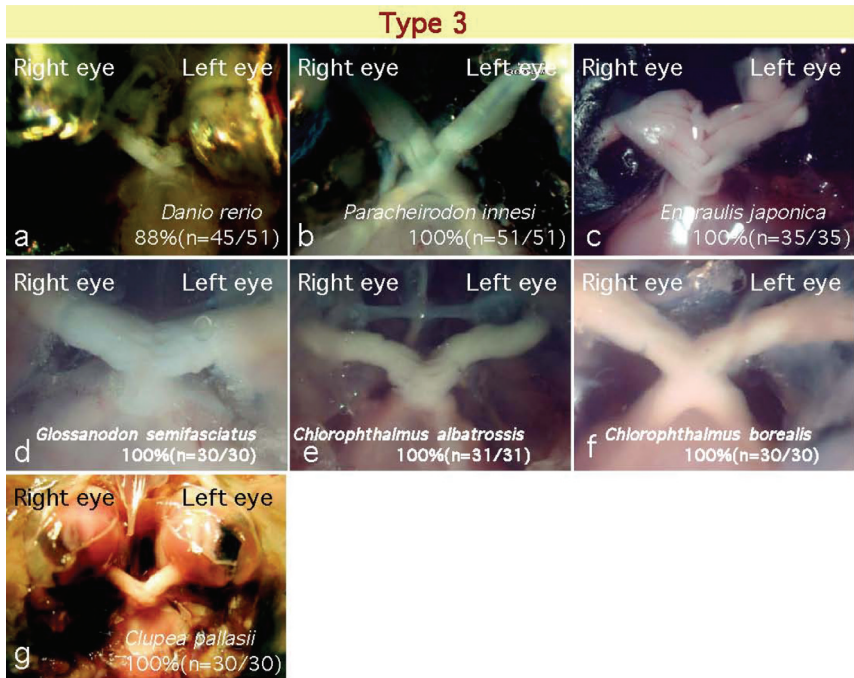


Figure 5. Of the 25 species examined, 7 species showed a type 3 chiasm. In these species, almost all individuals showed no laterality within the optic chiasm and the optic nerve fibres crossed in a complex manner.

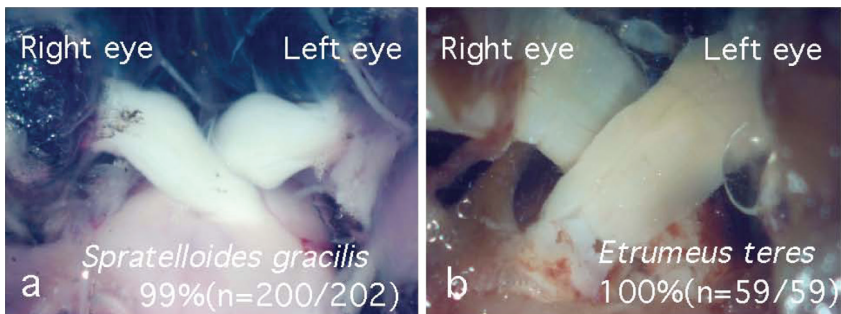


Figure 6. The optic chiasm of *Spratelloides gracilis* showed opposite laterality to that of *Etrumeus teres*, although these species belong to the same order *Clupeiformes* and the same family *Clupeidae*. (a) Optic chiasm of *S. gracilis*; left optic nerve invariably ran dorsally to the right optic nerve at the decussation. (b) Optic chiasm of *E. teres*; right optic nerve always ran dorsally to the left optic nerve.

The decussation pattern of the optic chiasm in flatfish, which have an asymmetric external morphology, has been studied using *Heterosomata* over a lengthy period (Parker, 1903; Policansky, 1982). Examination of the optic chiasm of *Pleuronectes herzensteini* (Japanese flounder), which has a remarkably asymmetric external morphology, revealed that the optic nerve from the left eye always runs dorsally to the right optic nerve (Tamura, 2005; $n = 10/10$; Figure 7).

Polypterus senegalus, of the order *Polypteriformes*, family *Polypteridae*, is an ancient fish belonging to *Brachiopterygii*. The optic chiasm of *P. senegalus* resembled a type 3 decussation pattern, but the optic nerves from both eyes branched into numerous bundles at the chiasmic point, and showed a fused decussation pattern ($n = 17/17$; Figure 8, see Table 1). *Polypterus delhezi* ($n = 3/3$) and *Polypterus palmas polli* ($n = 5/5$) in the same family of the same order also showed a “fused” chiasmic pattern. *Erpetoichthys calabaricus*, belonging to the order *Polypteriformes*, family *Polypteridae*, also showed a “fused” chiasm ($n = 7/7$). All of the ancient fishes investigated showed a similar chiasmic pattern.

Scores for all of the fish species examined in the present study can be found in Table 1. The chiasmic patterns of the fishes are labelled in the phylogenetic tree in Figure 9, in which the pattern of the optic chiasm in each species is indicated by a superscript. The evolutionarily ancient fishes showed a tendency towards a “fused” chiasmic pattern, and five species of higher orders belonging to superorder *Acanthopterygii* (*Gambusia affinis*, *Oryzias latipes*, *Cololabis saira*, *Lateolabrax japonicus*, and *Trachurus japonicus*) but not the *Pleuronectiformes* (flatfishes), showed both type 1 and type 2 patterns equally in each species (Figure 9, see Table 1).

DISCUSSION

Why might the optic chiasm in most fish show complete decussation? The answer to this question may relate partly to predator avoidance: lateral positioning of the eyes provides a wider visual field. Complete decussation facilitates the propagation of optical information to the contralateral side of the body, allowing efficient activation of motor neurons controlling the body wall muscle and aiding rapid escape from predators (Sarnat & Netsky, 1981). In the adult specimens of the 25 species investigated here, three patterns of decussation at the optic chiasm were observed (see Figure 3, Figure 4, Figure 5, and Table 1). Both types 1 and 2 consisted of a “simple chiasm” in that all of the optic nerves from one side wrapped over all of the counterpart. In contrast, type 3 consisted of a “meshed chiasm” in that the optic nerves from both eyes crossed in a more complicated manner. As such, these species appeared to have some as yet undetermined mechanism regulating the

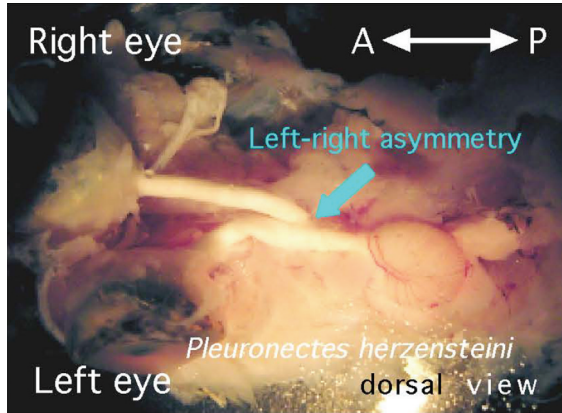


Figure 7. Decussation pattern within the optic chiasm of *Pleuronectes herzensteini* (Japanese flounder), which has a remarkably asymmetrical body. At the chiasm, the left optic nerve always ran dorsally to the right; all specimens showed definite laterality of the chiasmic pattern ($n = 10/10$). This figure is viewed from the dorsal side.

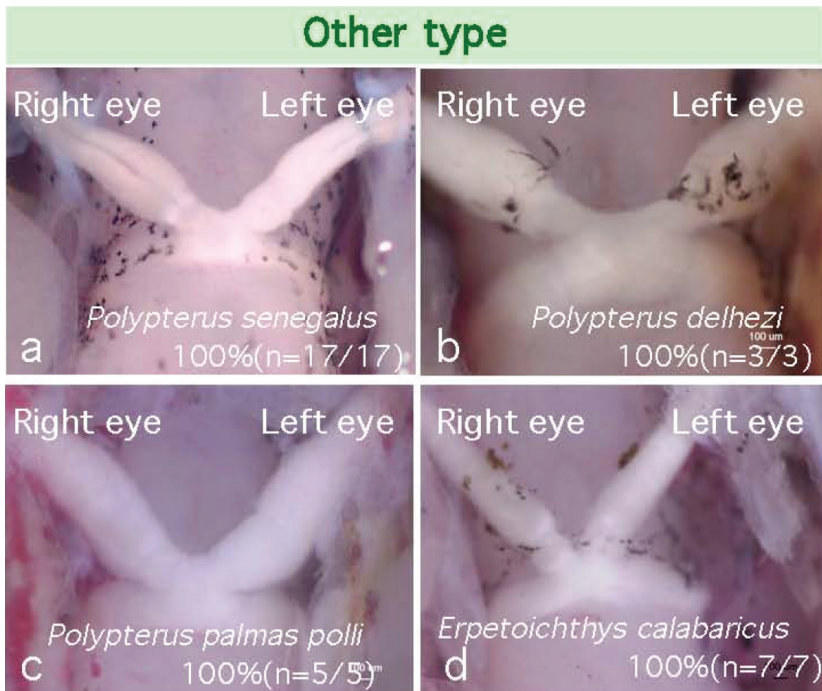


Figure 8. Ancestral fishes of subclass Branchiopterygii showed a “fused” type chiasm, in which no fasciculations of the optic nerves were observed at the chiasmic point.

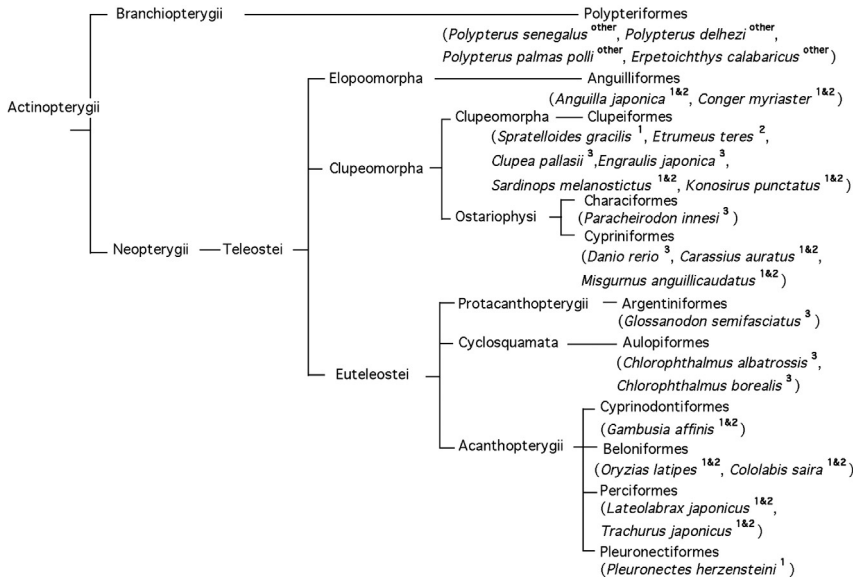


Figure 9. Phylogenetic tree of the fish investigated in this report, showing the chiasmic pattern for each species. The decussation pattern in the optic chiasm is indicated by a superscript (1, 2, and 3 indicate types 1, 2, and 3 chiasm respectively; 1 & 2 indicates a mixture of types 1 and 2, occurring in equal frequency).

formation of the chiasmic pattern during optic nerve projection to the tectum. Our investigations of *S. gracilis* and *E. teres* lead us to expect that the left–right patterning of the optic chiasm could constitute a useful indicator of the mechanism of neural development in teleosts.

If the timings of both the crossing and fasciculation of the optic nerves were determined randomly during embryogenesis, then all three chiasmic patterns (type 1, type 2, and type 3) should appear at equal frequencies in one species, or alternatively, only type 3 should appear in every specimen. However, our results revealed that in fish species showing type 1 and/or type 2 chiasmic patterns, type 3 specimens did not occur, while in fish with a type 3 chiasm, neither type 1 nor type 2 chiasmic patterns appeared (with the exception of zebrafish). These results lead us to hypothesise that an exclusive choice between types 1/2 vs type 3 chiasms could be exercised by an unknown mechanism during optic chiasm formation. To investigate this hypothesis further, it would be useful to perform genetic screens of a medaka (*Oryzias latipes*, of which all of the wild type specimens showed a type 1 or 2 chiasm) mutant frequently showing a type 3 chiasm, and to identify the causative gene(s). Alternatively, it would be useful to identify a zebrafish

(*Danio rerio*, of which most showed a type 3 chiasm) mutant showing only type 1 and 2 chiasms substituted for a type 3 chiasm.

P. senegalus, *P. delhezi*, *P. palmas polli*, and *E. calabaricus*, of the order *Polypteriformes*, family *Polypteridae*, are ancient fishes, and showed a type 3-like “fused” chiasm (see Figure 8 and Table 1). Considering that fishes of *Polypteridae* are ancestral ones, “fused” decussation with no fasciculate bundles at the chiasmic point may constitute the chiasmic prototype. To make type 1 and type 2 chiasms, an additional mechanism controlling the timing and/or dorsal/ventral positioning of the optic nerve projection might be required. *Polypteridae* fishes do not appear to possess a mechanism for allowing fasciculation of the optic fibres into thick bundle(s) at the chiasmic point.

S. gracilis and *E. teres*, of the order *Clupeiformes*, family *Clupeidae*, showed a strong laterality in the optic chiasm (see Figure 6 and Table 1). It is particularly interesting that *S. gracilis* and *E. teres* showed complementary laterality in the optic chiasm, even though the two species are the most closely related within the order *Clupeiformes* (Asanuma & Matsuoka, 2001; Lavoué, Miya, Saitoh, Ishiguro, & Nishida, 2007). While *C. pallasii* and *E. japonica* in the same family of the same order showed a type 3 chiasmic pattern (see Figure 5), *S. melanostictus* and *K. punctatus* showed type 1 and type 2 patterns (see Figure 4). Five extant species of *Spratelloides* are known, and three of them, *S. gracilis* (Temminck & Schlegel, 1846), *S. delicatulus* (Bennett, 1831), and *S. atrofasciatus* (Schultz, 1943), live in the warm Japanese ocean. *S. gracilis* specimens are widely distributed in tropical seas including coastal areas of West Japan and Australia, and *E. teres* are widely distributed in the warm oceans of West Japan and South China (Watanabe, Shirahuji, & Chimura, 2001).

The spawning seasons of *S. gracilis* and *E. teres* partially overlap. While *S. gracilis* spawns in the spring and early summer, when water temperatures are in the range of 23–27°C, *E. teres* spawns in autumn and spring, when water temperatures are in the range of 15–25°C (Watanabe et al., 2001). Since these two species partly share a spawning season, and both require warm water temperatures for embryogenesis, we suggest that complementary chiasmic patterns of these species are unlikely to be caused by differences in seasonal factors.

The opposite laterality of the optic chiasms of *S. gracilis* and *E. teres* will be useful for studying the establishment of the species-specific laterality of the central nervous system (CNS) in fishes of symmetric shapes. The brain lateralisation of human beings is thought to have originated from the functional asymmetries in the behaviour of lower vertebrates at both the individual and population level (for review see Bisazza, Rogers, &

Vallortigara, 1998; Vallortigara & Rogers 2005). Lateral asymmetry in the direction of turning during escape behaviour in a species of poeciliid fish, *Girardinus falcatus*, has been reported (Cantalupo, Bisazza, & Vallortigara, 1995). This asymmetry appears to be due to a heritable preferential use of the right eye (Bisazza, Dadda, & Cantalupo, 2005; Bisazza, Facchin, & Vallortigara, 2000; Facchin, Bisazza, & Vallortigara, 1999). In contrast, zebrafish fry use the left eye when they see their own reflection in a mirror (Sovrano & Andrew, 2006). At the neuroanatomical level, asymmetries of the parapineal complex in lampreys, teleosts, frogs, newts, and lizards have been reported (Concha & Wilson, 2001; Hendricks & Jesuthasan, 2007). Thus, studies on the laterality of the CNS might provide a deep insight into the evolution and speciation of the teleosts. Our findings show that laterality of the optic chiasm could be one new model system for studying teleost brain lateralisation.

Perissodus microlepis lives in Lake Tanganyika and is known as the “scale eater”. These fish have asymmetric bodies, and thus selectively eat scales only on one side of their prey (Hori, 1993; Nakajima, Matsuda, & Hori, 2004; Takahashi & Hori, 2008). The “left-handed” scale eater has its mouth open towards the right side and attacks the left side of its prey, whereas “right-handed” individuals have their mouth open towards their left side and attack the right side of their prey. We hypothesise that a similar survival-based bias governs the laterality of the decussation pattern in *S. gracilis* and *E. teres*. Ancestral species of both *S. gracilis* and *E. teres* might have originally had both type 1 and 2 chiasmatic patterns, following which one type might have been selected and the other eliminated during evolution. We anticipate that the laterality of the decussation pattern may be characterised by a small number of genes, because the two most closely related species examined (*S. gracilis* and *E. teres*) show completely opposite preferences in chiasmatic pattern. From a developmental viewpoint, during the embryonic stages a subset of optic neurons might be eliminated by apoptosis to simplify the overlap of optic nerves to form type 1 or type 2 decussation. Unfortunately, until recently it has been extremely difficult to cultivate *S. gracilis* and *E. teres* and to achieve their artificial insemination, and no reports on the embryogenesis of these species in the laboratory environment are available. As such, it is expected that an embryological approach to analyse the mechanism of optic chiasm formation in *S. gracilis* or *E. teres* will be very difficult. Instead, a genomic approach to analyse chiasmatic laterality might be suitable; detailed analyses of the homologues of optic chiasm-related genes and the corresponding cis-elements that are/will be identified in model fish such as zebrafish or medaka are needed. Otherwise,

techniques for the artificial breeding and fertilisation of *S. gracilis* and *E. teres* should be developed.

In zebrafish it has been reported that *cyclops* (*cyc*), *chameleon* (*con*), *you-too* (*yot*), *iguana* (*igu*), and *detour* (*dtr*) show no optic nerve decussation or show abnormal optic decussation patterning (Brand et al., 1996). The zebrafish *belladonna* (*bel*) mutant is defective in the axonal guidance factors, and neither the optic chiasm nor the forebrain commissural fibres are formed (Seth et al., 2006). Weak alleles of these genes are expected to provide useful information on the laterality of the chiasmatic pattern.

Using zebrafish, the laterality of the pineal complex and habenula in the dorsal diencephalon has been studied in detail, in the context of investigations into the left–right asymmetry of the brain (for review see Guglielmotti & Cristino, 2006; Halpern et al., 2003). The left nucleus of the habenulae (nuclei habenulae), the paired nuclei of the dorsal diencephalon neighbouring the pineal complex, is larger than the right nucleus and has more dense neuropil (Concha, Burdine, Russell, Schier, & Wilson, 2000; Halpern et al., 2003). Neural circuits connecting the pineal complex and the habenulae show left–right asymmetry in zebrafish. Projections from the dorsal habenulae to the interpeduncular nucleus (Fasciculus retroflexus [Meynert]) also show left–right differences (Aizawa et al., 2005; Gamse et al., 2005). These asymmetries are regulated by Nodal signalling (Aizawa et al., 2005; Concha et al., 2000, 2003; Long, Ahmad, & Rebagliati, 2003), Notch signalling (Aizawa, Goto, Sato, & Okamoto, 2007), Wnt signalling (Carl et al., 2007), and by neuropilin (a receptor for class III semaphorins) (Kuan, Yu, Moens, & Halpern, 2007).

Cyclops, one of the zebrafish *nodal*-related genes, is involved in left–right specification and is temporally expressed in the left side of the dorsal diencephalon and left-lateral plate mesoderm (Bisgrove, Essner, & Yost, 2000; Boggetti et al., 2000; Hashimoto et al., 2004). Zebrafish *southpaw*, which is highly homologous to *cyclops*, is also expressed in the left lateral plate mesoderm but not in the dorsal diencephalon (Long et al., 2003). Although *southpaw* is not expressed in the diencephalon, it has a significant effect on the left–right asymmetry of the brain (Long et al., 2003). It would be interesting to investigate the relationships among visceral left–right asymmetry, cerebral laterality, and the decussation pattern in *nodal*-related mutants. Such an approach will likely contribute to the identification of the genes governing the laterality of the chiasmatic pattern in *S. gracilis* and *E. teres*.

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