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Karyotypes and DNA Values for Members of the Suborder *Esocoidei* (*Osteichthyes*: *Salmoniformes*)*

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Abstract. Karyotypes prepared from tissues of gills, gonads, fins, liver, and spleen, yielded the following diploid numbers: all *Esox* spp. 50, all *Umbra* spp. (excluding *U. krameri*) 22, *Novumbra hubbsi* 48, *Dallia pectoralis* 78. DNA measurements from Feulgen stain content of erythrocytes, indicated that the species could be divided into two categories: 30–39% of human-*Esox* spp., *Dallia pectoralis*, *Novumbra hubbsi*: 70% of human-*Umbra* spp. Chromosomal polymorphism was not observed among the various tissues examined nor was it observed among specimens from different habitats. Both the karyotypes and the DNA values indicated that the present tendency to combine the genera *Umbra*, *Novumbra*, and *Dallia* in the single family Umbridae may be ill advised.

Introduction

In a research programme involving the biology, distribution, and systematics of the esocoid fishes an attempt has been made to utilize those biochemical and cytological techniques which might yield information on relationships, phylogeny, and evolution of these fishes. The results of karyotype studies and DNA measurements will be outlined here.

The suborder *Esocoidei*, a group of primary freshwater fishes, contains the pikes and pickerels (*Esox*), the mudminnows (*Umbra* and *Novumbra*), and the Alaska blackfish (*Dallia*). The members of these 4 genera have been considered to represent, depending on point of view, 2–4 families and 10–13 living species. The only major differences of opinion today are the placement of *Dallia pectoralis* Bean, the Alaska blackfish and *Novumbra hubbsi* Schultz, the Olympic mudminnow. They were considered sole representatives of the families *Dalliidae* and *Novumbridae* but are now usually included, as distinct genera, in the family Umbridae.

The family *Esocidae* consists of 6 species as follows: *Esox lucius* Linnaeus, the pike or northern pike, which is circumpolar in the northern hemisphere; *E. reicherti* Dybowski, the Amur pike which occurs naturally only in the Amur River in the U.S.S.R (and China ?) but has been introduced in Pennsylvania, U.S.A.; *E. masquinongy* Mitchill, the muskellunge; *E. niger* Lesueur, the chain pickerel; *E. a. americanus* Gmelin, the redfin pickerel; and *E. a. vermiculatus* Lesueur, the grass pickerel. The last four occur naturally only in eastern and central North America, but have been introduced elsewhere in North America.

The family *Umbridae* now consists of the following species: the European mudminnow or hundfish (known variously as *Umbra krameri* Walbaum, *Umbra lacustris* Grossinger, or *Umbra umbra* Berg) in the Danube and Dniester river systems of central Europe; *U. pygmaea* DeKay, the eastern mudminnow, restricted to the U.S.A. east of the Appalachian Mountains; *U. limi* (Kirtland), the central mudminnow, restricted to central North America; *Novumbra hubbsi* Schultz, the Olympic mudminnow which occurs only in Washington State U.S.A.; and *Dallia pectoralis* Bean, the Alaska blackfish which is restricted to a limited area of Alaska and Siberia.

Karyotypes of three esocoids have been reported in the literature. Using sections of testes, Foley (1926) determined a chromosome complement of 22 metacentrics for *Umbra limi*. Recently, McGregor (1970) using kidney tissue reported consistent counts of 50 acrocentric chromosomes for *Esox masquinongy*. Several European authors (Svärdson and Wickbom, 1939; Lieder, 1953, 1956, 1959; Prakken *et al.*, 1955; Nygren *et al.*, 1968) reported diploid complements ranging from 18 to 50 for *Esox lucius*. The results of the first five studies were briefly reviewed by Nygren *et al.*, (1968) who concluded that although the determination of $2n = 18$ was probably in error, the determinations ranging from 46 to 50 might be the result of real chromosome variation among tissues or among specimens from different habitats. Ohno *et al.*, (1965) have reported that different tissues of the rainbow trout *Salmo irideus* (= *S. gairdneri*) yielded different karyotypes. Hence specimens of *Esox lucius* were examined from two localities in Ontario and one in England in an attempt to determine if variation in karyotypes occurred between individuals of the same species from different habitats. Also the possibility of intertissue chromosomal polymorphism was investigated for *Esox americanus* and *Esox reicherti* by examining squash preparations from different tissue. Finally, it was hoped to learn what DNA values, chromosome number, and chromosome morphology might contribute to the understanding of the relationship of the various species and species groups.

Materials and Methods

Chromosome squashes were prepared using a modification of the technique of McPhail and Jones (1966). For each 10 g of body weight 0.1 cc of 0.1% colcemid or colchicine was injected into the dorsal musculature. The injected fish was placed in a well-aerated aquarium and killed after 1 to 4 hours, depending on species and condition. In general, individuals recently obtained from the field yielded suitable preparations after 2 hours. Placing specimens in a well-aerated aquarium several hours prior to injection produced better squashes.

After the injection period, all gill arches were removed and carefully cleansed of blood. Cleansed arches were put in double distilled water in a dialysis bag and bag plus arches immersed in double distilled water for 50 to 65 minutes. Double distilled water around the bag was replaced after 30 minutes. Gill arches were removed from the bag, excess water removed by touching the raker side to filter paper and the arches were then dropped into 60% aceto-orcein stain for 20 to 40 minutes. Aceto-orcein was prepared by refluxing, for 30 minutes, 4 g of synthetic orcein with 60 cc of glacial acetic acid and 40 cc of double distilled water. Stain was filtered prior to use.

A stained arch was agitated in a drop of 60% acetic acid on a glass slide. Three slides were made from each gill arch. The resulting slurry of tissue was squashed by thumb pressure and the cover slip temporarily sealed with clear nail polish. Squashes of other tissues were prepared in a similar manner.

Most squashes were examined and photographed in this state using bright field or phase contrast microscopy. In some cases photographs were taken directly from permanent mounts. Permanent mounts were prepared by dissolving away the nail polish in acetone (about 60 seconds), exposing the slide to the air for 5 minutes and then inverting it on dry ice. A new coverslip was mounted using the freeze dry technique of Conger and Fairchild (1953). To obtain satisfactory permanent mounts the slide was left on dry ice for at least 48 hours. Faster, and better quality, permanent mounts were obtained by immersing the slides in liquid nitrogen for several seconds rather than inverting them on dry ice.

Relative DNA values were obtained by measuring the Feulgen stain content of erythrocyte nuclei with an integrating microdensitometer (Barr and Stroud, Type GN. 2). Air-dried blood smears, prepared as species became available, were simultaneously fixed in a 3:1 solution of ethanol-glacial acetic acid for 30 minutes, hydrolized in 5 N HCl at room temperature for 50 minutes, and stained in Feulgen for 1 hour. Slides were then washed 3 times in SO_2 water, dehydrated and mounted in euparal.

Human buccal epithelial cells and erythrocyte smears of goldfish *Carassius auratus* (Linnaeus) were used as controls.

For each species a minimum of 2 preparations were examined, usually 20 cells from each, with the mean of 3 readings for each cell taken as the absorption value. Estimation of the absolute amount of DNA per cell was made using a value of 7.0×10^{-9} mg DNA/cell as the absolute amount of DNA of placental mammals (Atkin *et al.*, 1965; Ohno and Atkin, 1966).

Karyotypes and DNA values were obtained for all living representatives of the suborder except the now rare *Umbra krameri*.

Sources of living material were as follows: *Esox lucius*, two localities in Ontario, Canada and Lake Windermere, England; *E. reicherti*, eggs from Amur River, U.S.S.R, reared in a hatchery of the Pennsylvania Fish Commission; *E. masquinongy*, a single locality in Ontario; *E. niger*, a single locality in North Carolina, U.S.A.; *E. a. americanus* from Quebec, Canada and North Carolina; *E. a. vermiculatus*, a

single locality in Ontario; *Umbra pygmaea*, a single locality in North Carolina; *U. limi*, three localities in Ontario; *Novumbra hubbsi*, a single locality in Washington State, U.S.A.; *Dallia pectoralis*, a single locality in Alaska, U.S.A. Tissue from gill, fin, gonad liver, and spleen were examined for *E. americanus*.

Results

Karyotypes

Counts of chromosomes obtained for each species are shown in Table 1. Representative karyotypes are presented in Figs. 1–6. Karyotypes of *E. lucius* and *E. masquinongy* were indistinguishable from those previously recorded by Nygren *et al.*. (1968) and McGregor (1970) respectively. Mitotic plates prepared from gill tissue for *E. masquinongy* and *E. a. americanus* are shown in Figs. 7 and 8.

Squash preparations from all *Esox* species produced predominantly counts of 50 acrocentric chromosomes (Table 1). All *Esox* karyotypes were similar and no distinctions between species or sexes were apparent. Similarly no evidence of chromosomal polymorphism was found geographically for *E. lucius* or among different tissues for *E. americanus* and *E. reicherti* (Table 1, Figs. 8 and 9). Counts of less than $2n = 50$ were attributed primarily to chromosome loss during squashing. In the few cases where counts of 51 and 52 chromosomes were obtained, it was felt that the extra chromosomes resulted from separation of the metaphase chromatids during squashing. In general, when preparations were of the quality shown in Figs. 7–9 a diploid complement of 50 acrocentrics was found.

Umbra limi and *U. pygmaea* (Fig. 6) possessed similar karyotypes of 22 large metacentric and submetacentric chromosomes. Little difficulty in preparing or interpreting squashes was encountered, and thus only a few cells were scored. Karyotypes from *U. limi* appeared identical to those described by Foley (1926).

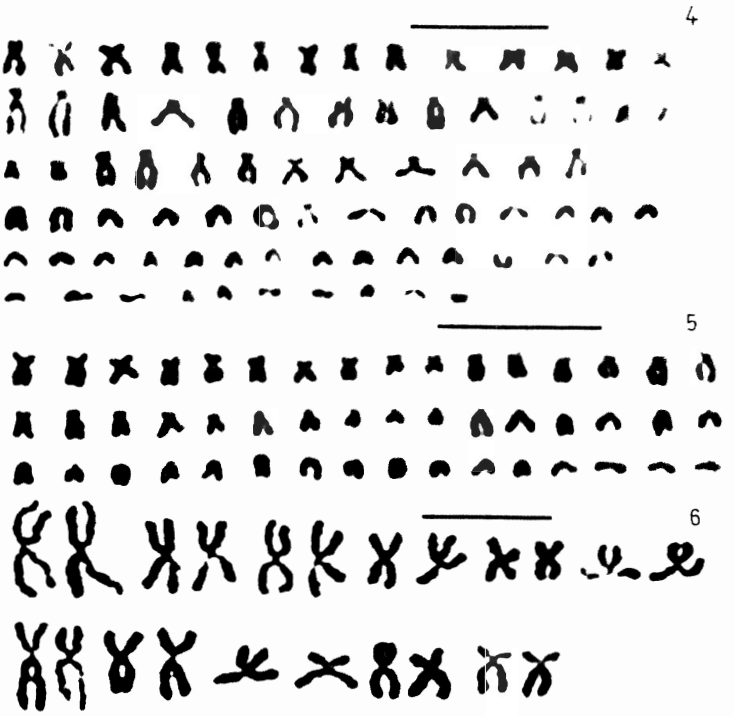
The diploid complement of *Novumbra hubbsi* consisted of 48 metacentric, submetacentric, and acrocentric chromosomes (Fig. 5). Good quality preparations (Fig. 10) were more difficult to obtain in this species and there was a greater variation in counts (Table 1). Counts of $2n = 47$ were obtained for both male and female fish. A study of six karyotypes showed no evidence of polymorphism.

Dallia pectoralis possessed 78 acrocentric, metacentric, and submetacentric chromosomes (Fig. 4). Interpretation of the karyotype was complicated by the large number of chromosomes and the extremely small size of many of the acrocentric chromosomes. Counts of $2n = 77$ were obtained for both sexes. Some well-squashed metaphase plates produced counts higher than $2n = 78$ but the small size of the acrocentric

Table 1. *Summary of chromosome counts*

Species	Total No. of specimens	Tissue	No. of cells scored	2*n* No.	Frequency of chromosome counts												Morphology
					42	43	44	45	46	47	48	49	50	51	52		
Esox lucius	5	gill	37	50	0	0	2	0	1	1	6	6	21	0	0		all acrocentric
Esox reicherti	6	gill	93	50	2	1	1	1	4	1	2	13	68	0	0		all acrocentric
		spleen	7	50	0	0	0	0	0	0	0	1	6	0	0		
Esox masquinongy	1	gill	36	50	0	0	1	0	0	1	3	1	29	1	0		all acrocentric
Esox niger	3	gill	76	50	0	1	1	0	2	6	5	10	48	2	1		all acrocentric
Esox	5	gill	68	50	0	0	1	0	2	4	2	4	55	0	0		all acrocentric
a. americanus		fin	19	50	0	0	0	0	0	2	1	2	14	0	0		
		gonad	12	50	0	0	1	0	0	0	1	0	10	0	0		
		liver	8	50	0	0	0	0	0	2	0	0	6	0	0		
		spleen	1	50									1				
Esox	7	gill	92	50	1	0	2	1	2	5	6	2	73	0	0		all acrocentric
a. vermiculatus		fin	9	50						1		1	7				
		liver	7	50									7				
		spleen	2	50									2				
Novumbra hubbsi	7	gill	50	48	3	1	4	2	5	10	19	2	1	2	1		many acrocentrics, a few metacentrics and submetacentrics
					20	21	22	23	24								
Umbra pygmaea	2	gill	39	22	3	3	33	0	0								all large metacentrics
Umbra limi	5	gill	16	22	1	1	14	0	0								all large metacentrics
					70	71	72	73	74	75	76	77	78	79	80	81	82	
Dallia pectoralis	12	gill	110	78	3	8	8	7	6	6	17	21	19	6	6	2	1	a mixture of acrocentrics metacentrics and submetacentrics

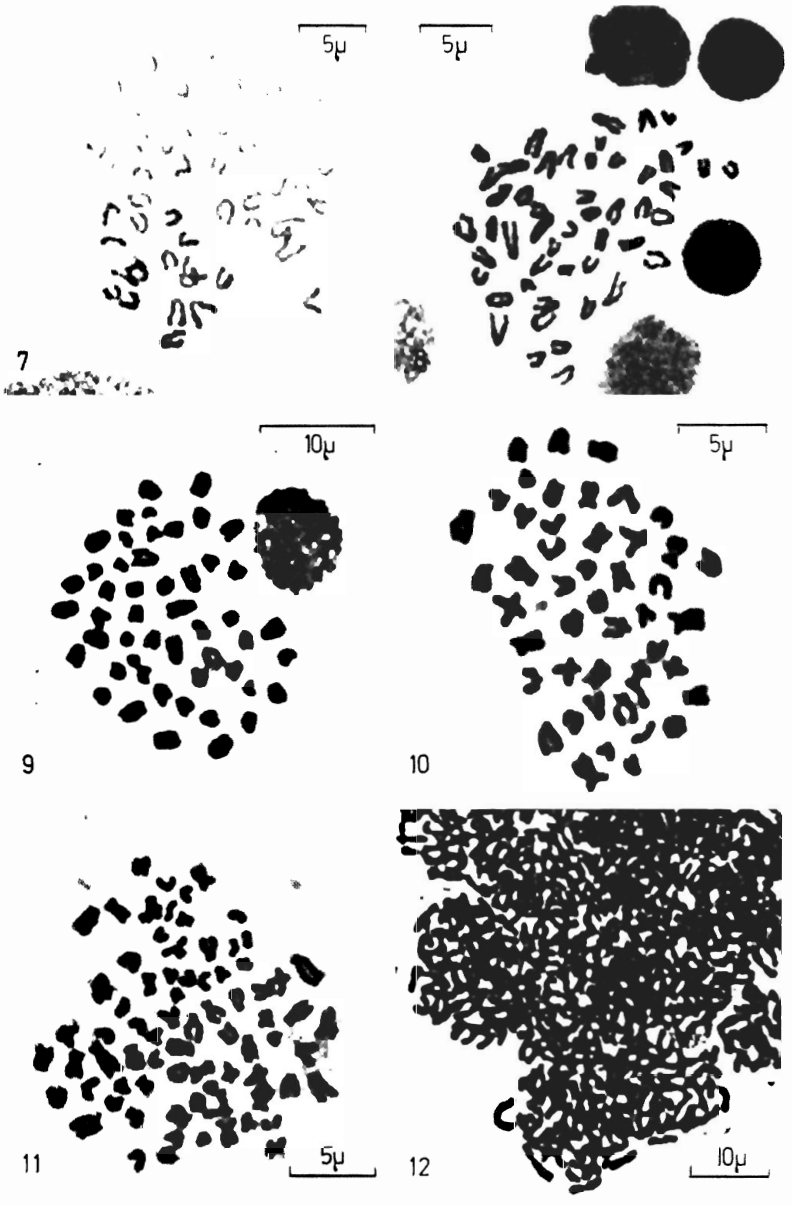
Figs. 1–3. *Esox* species. The bars represent 10 μ. Fig. 1. *E. reicherti*, karyotype prepared from gill tissue. Fig. 2. *E. niger*, karyotype prepared from gill tissue. Fig. 3. *E. a. vermiculatus*, karyotype prepared from gill tissue



Figs. 4–6. The bars represent 10 μ. Fig. 4. *Dallia pectoralis*, karyotype prepared from gill tissue. Fig. 5. *Norumbra hubbsi*, karyotype prepared from gill tissue. Fig. 6. *Umbra pygmaea*, karyotype prepared from gill tissue

chromosomes made it difficult to determine whether the additional chromosomes were the result of breakages or were part of the complement. There were insufficient, good quality preparations to make a positive statement concerning chromosomal polymorphism. Four karyotypes, from squashes showing no overlap and not overly contracted (Fig. 11), were similar and yielded a diploid complement of $2n = 78$.

Tissue from testes was examined for *Umbra limi*, *Dallia pectoralis*, and *Esox a. americanus*. Areas containing large numbers of chromosomes were observed in the testes preparations from all three species (Fig. 12). However, if tissue was only lightly squashed, it was apparent that many cells were in synchronous division and would have become confluent if more pressure had been exerted.



Figs. 7–12. The bars represent 10 μ. Fig. 7. *Esox masquinongy*, squash preparation of gill tissue. Fig. 8. *Esox a. americanus*, squash preparation of gill tissue. Fig. 9. *Esox a. vermiculatus*, squash preparation of fin epithelium. Fig. 10. *Novumbra hubbsi*, squash preparation of gill tissue. Fig. 11. *Dallia pectoralis*, squash preparation of gill tissue. Fig. 12. *Umbra limi*, squash preparation of testes

Relative DNA Measurements

Relative DNA values, and the estimated absolute values of DNA per cell, are summarized in Table 2. The relative value for goldfish of 49% that of human epithelial cells is in close agreement with the value obtained by Ohno and Atkin (1966). They obtained a value of 52% of human lymphocytes which, when corrected for the 10% deficiency of lymphocyte stain content, compared with epithelial cells (Atkin and Richards, 1956; Hale, 1963; Atkin *et al.*, 1965), gives a value of approximately 48% that of human epithelial cells.

Table 2. *Relative and estimated absolute amounts of DNA/mg/cell for members of suborder Esocoidei*

Species	No. cells measured	Relative DNA and standard error	Estimated absolute DNA/mg/cell
Esox lucius	40	0.388 ± 0.006	2.72×10^{-9}
Esox reicherti	40	0.365 ± 0.006	2.56×10^{-9}
Esox masquinongy	40	0.365 ± 0.006	2.56×10^{-9}
Esox niger	40	0.338 ± 0.004	2.37×10^{-9}
E. a. americanus	40	0.338 ± 0.004	2.37×10^{-9}
E. a. vermiculatus	40	0.321 ± 0.004	2.25×10^{-9}
Umbra pygmaea	40	0.688 ± 0.013	4.82×10^{-9}
Umbra limi	40	0.719 ± 0.010	5.03×10^{-9}
Novumbra hubbsi	40	0.297 ± 0.004	2.08×10^{-9}
Dallia pectoralis	40	0.361 ± 0.006	2.53×10^{-9}
Carassius auratus	60	0.490 ± 0.044	3.43×10^{-9}
Human epithelial cells	40	1.000 ± 0.008	7.0×10^{-9}

Variation due to time prior to fixing was tested (t test) and showed no significant difference at the 0.05 level.

The DNA values can be grouped into two categories. The five *Esox* species, *Dallia pectoralis*, and *Novumbra hubbsi* have DNA values ranging from 30–39%, and *Umbra limi* and *Umbra pygmaea* 68–72%, of human buccal epithelial cells.

Discussion

The chromosome complements and DNA values have been determined for all but one species (*Umbra krameri*) of the suborder *Esocoidei*.

The 2n karyotypes of this small group of specialized salmoniform fishes can be divided into four groups: *Dallia pectoralis* with 78 varied chromosomes; *Novumbra hubbsi* with 48 less varied chromosomes; *Umbra* spp. (excluding *U. krameri*) with 22 large, metacentric chromosomes; and

Esox spp. with 50 acrocentric chromosomes. The DNA values can be grouped in two categories: those with 30–39% of human-*Esox* spp., *Dallia pectoralis*, *Novumbra hubbsi*; and those with 68–72% of human-*Umbra* spp.

The karyotypes obtained from gill tissue for *E. lucius* and *E. masquinongy* were similar to those found from kidney tissue by Nygren *et al.*, (1968) and McGregor (1970) respectively. Similarly, Foley (1921) studying *U. limi*, and Nygren *et al.*, (1968) studying *E. lucius* gave karyotypes from testes that were identical to our results from gill tissue. In the present study, no distinctions were apparent in the karyotypes produced from fin, gonad, spleen, liver, and gill from the subspecies of *E. americanus*. Therefore chromosomal polymorphism among different tissues was not observed in this study. Nor was it evident in the results of the other workers mentioned above. Further, it was felt that this type of chromosomal polymorphism was not common in the animals within the suborder.

Nygren *et al.*, (1968) claimed that polyploid mitotic and meiotic cells existed in the testes of *E. lucius* and polyploid mitotic cells were also present in kidney tissue. In some preparations Nygren *et al.*, claimed that "The number of chromosomes in these polyploid mitotic cells may amount to over one thousand". In our study, examination of the testes from *Umbra limi*, *Dallia pectoralis*, and *Esox americanus vermiculatus* did reveal areas containing large numbers of chromosomes. However, we found no evidence that these large numbers belonged to a single nucleus. It was felt that chromosomes became confluent during squashing, and once this occurred it was no longer possible to separate chromosomes from the individual nuclei. Also, measurements of red blood cell DNA from all esocoid species yielded no suggestion of polyploid nuclei. Hence polyploid mitotic cells were not considered to be an important factor.

Karyotypes reported in the past for *Esox lucius* in North America and in Europe have differed. Our results show the number in this species in Canada and England to be similar and agree with those of Nygren *et al.*, (1968) for Sweden. We suggest that $2n = 50$ is the number not only for this species over the whole of its circumpolar distribution, but for all species in the family *Esocidae*. If the numbers reported by Svärdson and Wickbom (1939), Prakken *et al.*, (1955), and by Lieder (1953, 1956, and 1959) are real, then this variation is not apparently common within the species *Esox lucius*.

It is difficult to speculate on evolutionary history within the suborder. If the theories of Ohno *et al.*, (1965) are correct, that the ancestral vertebrates contained 48 acrocentrics, then the species of *Esox* (with 50 acrocentrics) would not appear to be chromosomally too

far removed from the ancestral type and it is conceivable that the species of *Umbra* may be derived from a primitive esocoid ancestor by a series of Robertsonian fusions between acrocentrics, followed or preceded by a change in the DNA. Whereas it is conceivable that the 48 "varied" chromosomes of *Novumbra* might be derived from the ancestral complement by pericentric inversions or centromere shifts, no such simple relationship can even be postulated for *Dallia*, and it is only the DNA value that ties it to the esocids.

It is perhaps prudent at this time to conclude only that both the karyotypes and DNA values suggest that the present tendency to combine the genera, *Umbra*, *Novumbra*, and *Dallia* in the single family Umbridae might be ill advised.

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