Genet. Res., Camb. (1978), **32**, *pp.* 37–46
With 3 plates
Printed in Great Britain

Gross karyotypic change and evolution in North American cyprinid fishes*

By J. R. GOLD, W. D. WOMAC, F. H. DEAL and J. A. BARLOW, Jr.

Genetics Section, Texas A & M University, College Station, Texas 77843, U.S.A.

(*Received* 23 *January* 1978)

SUMMARY

We have examined karyotypes of five species from five genera of cyprinid fishes endemic to the central and southeastern United States: *Campostoma anomalum*, *Hybognathus hayi*, *Hybopsis aestivalis*, *Phenacobius mirabilis*, and *Pimephales vigilax*. All five have a diploid chromosome number of 50. Variation in chromosome arm number among the five species is slight, and may be due to measurement error or technique difficulties. The karyotypes of 40 North American cyprinids are now known. All but five species have 50 (diploid) chromosomes. Variation in chromosome arm number also appears minimal; one-armed chromosomes (centromeres subterminal to terminal) normally comprise only a small fraction of the karyotype, and each species has roughly the same number of chromosomes with median and submedian centromeres. The conservatism of gross chromosomal evolution among these fishes is not in accord with recent hypotheses which suggest that progressive evolution of organisms may depend to a large degree on gene rearrangement brought about by gross chromosomal restructuring. Cyprinids are a highly speciose group in North America, and there is relatively strong morphological differentiation among species. The present data suggest that gross chromosomal restructuring may play only a minor role in the speciation and evolution of these fishes.

INTRODUCTION

Recent surveys of karyotypes of cyprinid fishes (minnows) endemic to North America have revealed a striking homogeneity among species in both chromosome number and gross chromosome structure. The overwhelming majority of species karyotyped have diploid complements of 50 chromosomes, and variation in fundamental chromosome arm number appears to be minimal. In any species, centromere positions typically form a continuous series from median to terminal, and chromosomes with very short second arms comprise only a small fraction of the karyotype (Gravell & Malsberger, 1965; McPhail & Jones, 1966; Legendre & Steven, 1969; Lieppman & Hubbs, 1969; Denton & Howell, 1969; Greenfield & Greenfield, 1972; Uyeno & Smith, 1972; Campos & Hubbs, 1973; Greenfield *et al.* 1973; Uyeno & Miller, 1973; Howell & Villa, 1976; Gold & Avise, 1977).

* Number V in the series 'Cytogenetic studies in North American Minnows (Cyprinidae)'.

The pattern of chromosomal evolution in these fishes is of interest in view of recent studies in both plants and animals which have shown positive correlations between karyotypic evolution, organismal evolution, and speciation (Wilson, Maxson & Sarich, 1974*a*; Wilson, Sarich & Maxson, 1974*b*; Wilson *et al.* 1975; Levin & Wilson, 1976; Bush *et al.* 1977). Many taxa which have undergone rapid speciation and organismal evolution seem also to have undergone rapid chromosomal evolution. Cyprinids are highly speciose in North America (250 species in 35–40 genera), but are not known from fossil remains prior to the Miocene (Miller, 1959, 1965; Kimmel, 1975; Smith, 1975). Further, although taxonomic problems within the group are numerous, the average cyprinid species in North America is relatively well-differentiated morphologically. Thus, these fishes appear to have undergone relatively rapid speciation and morphological evolution in the absence of extensive gross chromosomal rearrangement.

In this paper, karyotypes of five cyprinid species belonging to five different genera are described. Chromosomal evolution in North American Cyprinidae is discussed in relation to speciation rate and degree of morphological evolution.

MATERIALS AND METHODS

The five taxa were collected from the following localities in Texas: *Campostoma anomalum* (Salado Cr., Bell Co.); *Hybognathus hayi* (Little Cypress Cr., Upshur Co.); *Hybopsis aestivalis* (Brazos R., Brazos Co.); *Phenacobius mirabilis* (Village Cr., Hardin Co.); and *Pimephales vigilax* (Brazos R., Brazos Co.). All specimens were collected by seining and transported live to Texas A & M University.

The method of chromosome preparation was after Gold (1974). Counts of chromosome numbers were made from negatives, and those showing the best-spread metaphases were developed into positives, cut-out, and arranged into karyograms. Chromosome arm lengths were measured using precision callipers, and classification of chromosomes by centromere position followed Levan, Fredga & Sandberg (1964).

RESULTS

All five species have a diploid chromosome number of 50 (Table 1). Counts of 50 were obtained in over 60 % of all cells examined (12–29 cells per individual), and with the exception of *Phenacobius*, one male and one female were examined per species. No sex chromosomes were identified. Most cells not yielding modal counts were short by one or more chromosomes; cells with less than 45 chromosomes were not included. These hypomodal counts probably result from loss during preparation, chromosome overlap, or miscounting. Hypermodal counts were infrequent (< 2 % of all cells counted) and were presumed to result from premature chromatid separation or miscounting. In all well-formed spreads, 50 chromosomes were readily apparent. Modal karyograms of the five species are illustrated in Figs. 1–5 (Plates 1–3).

Karyotypes of the five species were difficult to distinguish from one another. Each contained a graded series of small chromosomes (*c.* 4–9 microns), with measured centromere placements ranging from almost median to nearly terminal.

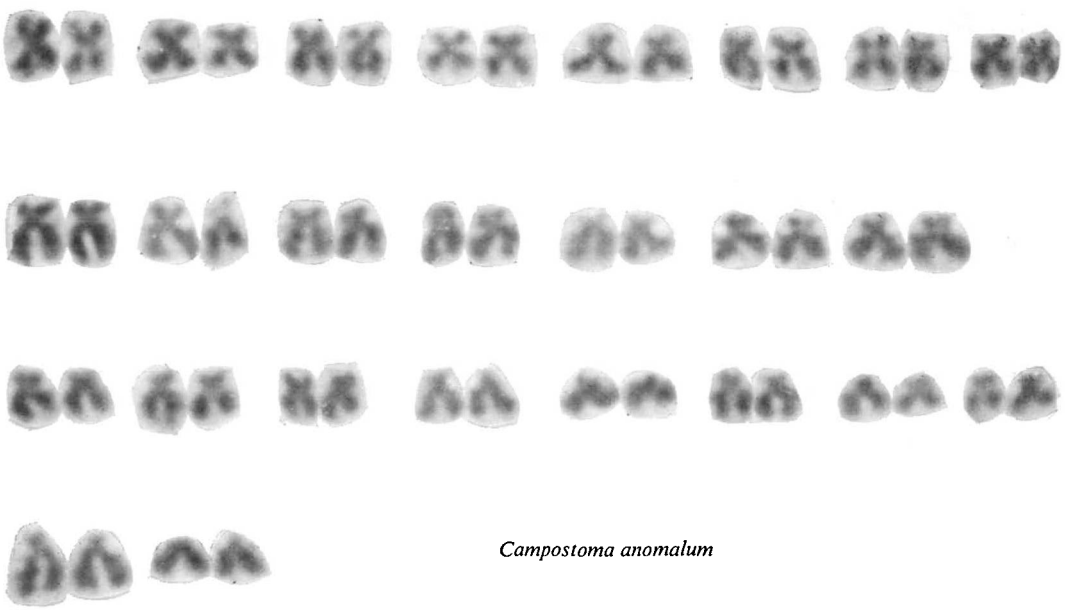


Fig. 1. Somatic metaphase chromosomes (from kidney) of *Campostoma anomalum*, $2n = 50$.

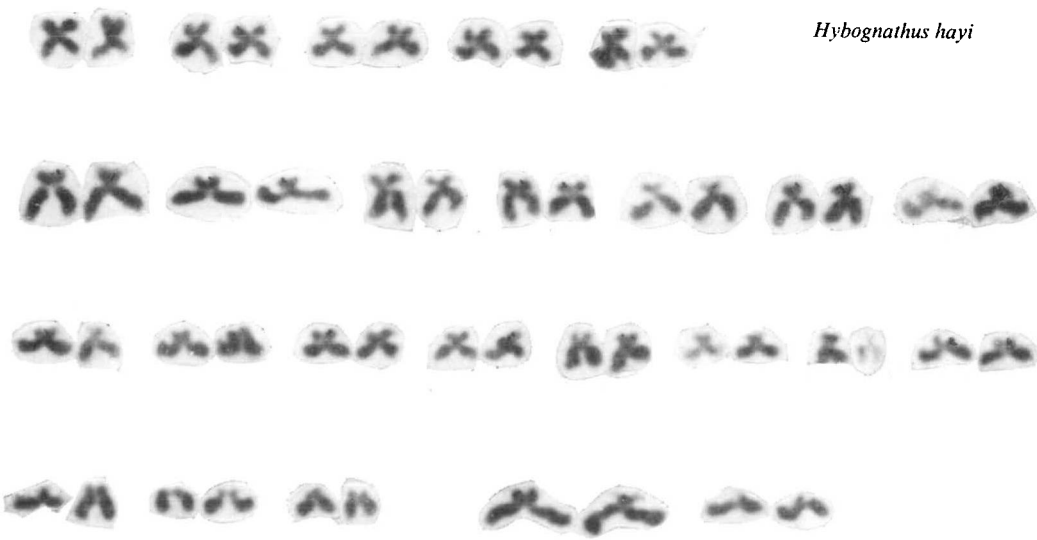
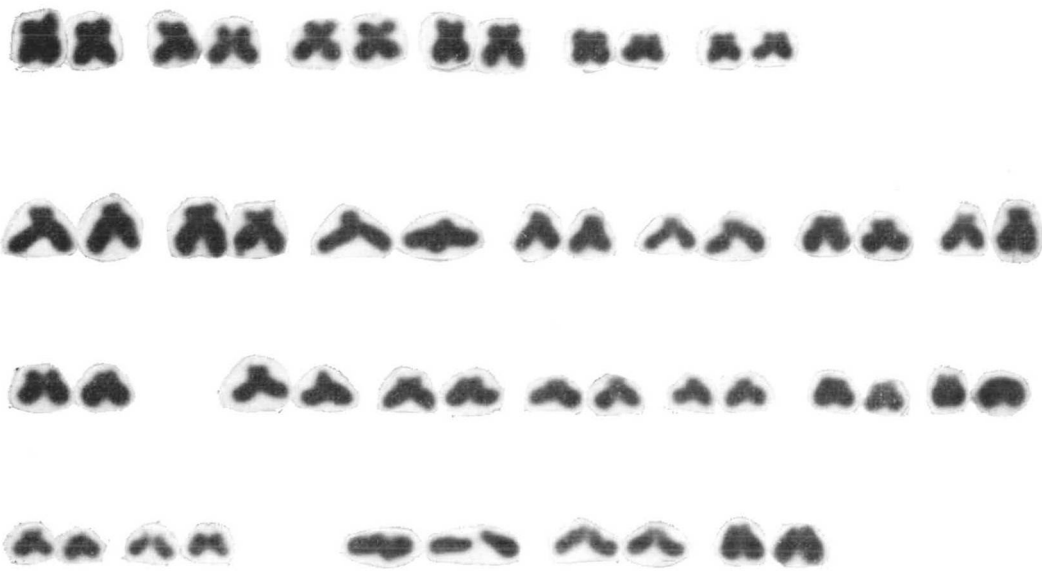
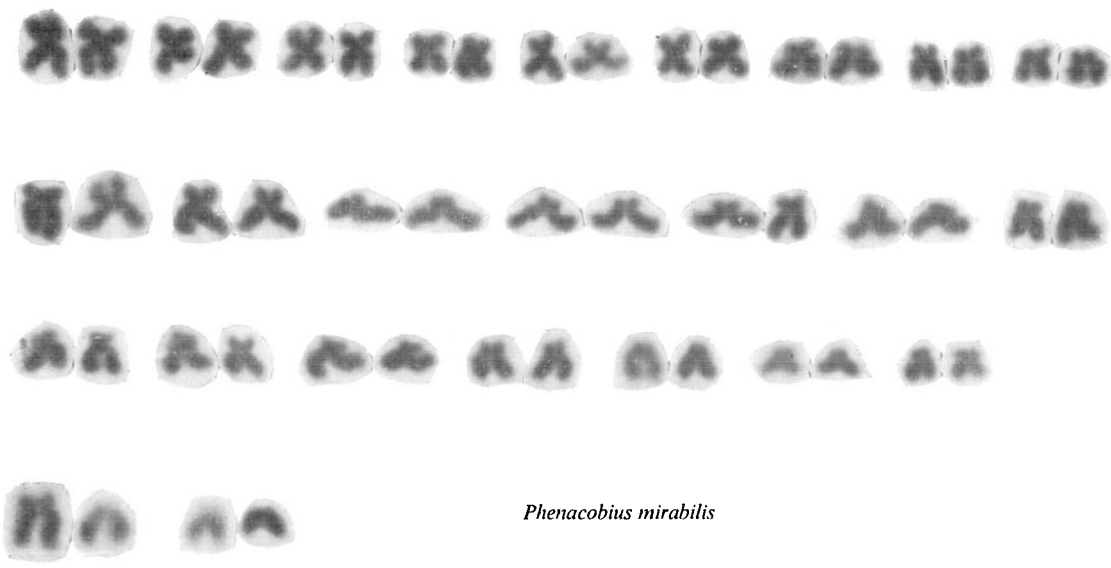


Fig. 2. Somatic metaphase chromosomes (from kidney) of *Hybognathus hayi*, $2n = 50$.



Hybopsis aestivalis

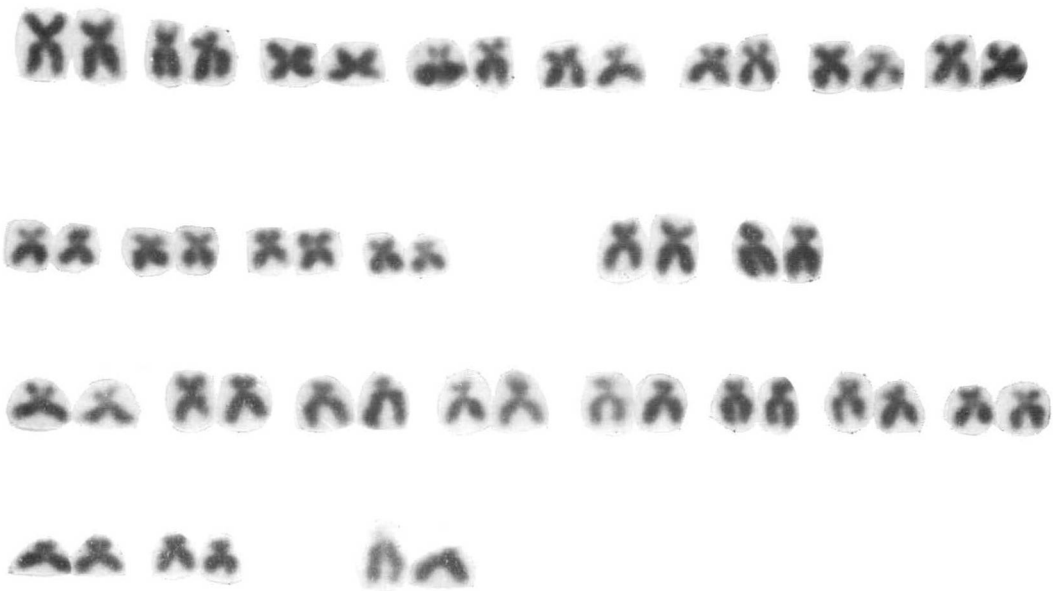
Fig. 3. Somatic metaphase chromosomes (from kidney) of *Hybopsis aestivalis*, $2n = 50$.



Phenacobius mirabilis

Fig. 4. Somatic metaphase chromosomes (from kidney) of *Phenacobius mirabilis*, $2n = 50$.

https://doi.org/10.1017/S0016672300018504 Published online by Cambridge University Press



Pimephales vigilax

Fig. 5. Somatic metaphase chromosomes (from kidney) of *Pimephales vigilax*, $2n = 50$.

https://doi.org/10.1017/S0016672300018504 Published online by Cambridge University Press

Table 1. *Chromosome number counts from five species (genera) of North American Cyprinidae*

Taxon	Number of individuals	Number of cells examined	% Modal counts	Modal 2*n* number
Campostoma anomalum	3	65	75	50
Hybognathus hayi	4	66	62	50
Hybopsis aestivalis	3	78	72	50
Phenacobius mirabilis	1	29	61	50
Pimephales vigilax	5	83	67	50

Table 2. *Estimated centromere positions of chromosome pairs and arm numbers of five species (genera) of North American cyprinidae*

Taxon	Chromosome formula*	Questionable M-SM	Estimated arm number (haploid)
Campostoma anomalum	8–15–2	2	48
Hybognathus hayi	5–18–2	3	48
Hybopsis aestivalis	6–16–3	4	47
Phenacobius mirabilis	9–14–2	2	48
Pimephales vigilax	12–12–1	5	49

* M-SM-A (metacentric–submetacentric–acrocentric).

Since arm attenuation (from spreading) and contraction (during mitosis) were undoubtedly variable from chromosome to chromosome and cell to cell, the pairing of homologues was at best partially subjective. Our attempts to classify chromosomes of these five species by centromere location within size groupings were unsuccessful.

In Table 2, our estimates of centromere position and (haploid) chromosome arm number for the five species are shown. Differences among the species in the number of metacentric (M) and submetacentric (SM) chromosomes were measured, but between 2–5 pairs per species fell on the M-SM border and could not be unambiguously classified to a discrete category. These 'questionable M-SM' chromosomes were included in the SM category in Table 2. Considering their number, all five species could have identical complements of M and SM chromosomes.

The number of acrocentric (A) chromosome pairs (centromeres subterminal to terminal) appeared to vary: *H. aestivalis* was scored as having three A pairs; *C. anomalum*, *H. hayi*, and *P. mirabilis* two A pairs each; and *P. vigilax* one A pair. Haploid arm number estimates, generated by scoring M and SM chromosomes as bi-armed and A chromosomes as uni-armed, varied from 47 to 49, a range typical for most North American cyprinids (Uyeno & Miller, 1973; Howell & Villa, 1976; Gold & Avise, 1977). We interpret the variation in arm number conservatively. Several A chromosomes were close to the SM-A border and were

difficult to measure. Further, some A chromosomes have short arms which are at least as long as the short arms of many SM chromosomes (compare chromosome pair no. 4, row 4, of the *H. hayi* karyotype in Plate 1, fig. 2 with any SM chromosome pair in row 3). That A chromosomes with relatively long short arms should be scored as uni-armed seems inappropriate. In any event, we consider the gross karyotypes of these five species as extremely similar, if not identical.

DISCUSSION

Karyotypic evolution in North American Cyprinidae. Chromosome numbers of 40 North American cyprinid species (22 genera) are now published. These are listed by genus in Table 3: 35 species (87·5 %) have 50 diploid chromosomes; two species (5 %) have 48 chromosomes; and three species (7·5 %) have 52 chromosomes. The proportion of species with 50 chromosomes may be greater. We recently have completed chromosome counts from an additional 15 cyprinid species, all of which had 50 chromosomes. One of these was *Semotilus atromaculatus* which previously was reported to have 52 chromosomes (Legendre & Steven, 1969). Chromosome number evolution in these fishes has been remarkably conservative, and those chromosomal rearrangements which bring about changes in chromosome number (Robertsonian fusions/fissions, etc.) apparently have occurred only infrequently.

On the other hand, the general asymmetry (White, 1973) of North American cyprinid karyotypes suggests that rearrangements which affect centromere position may have occurred in the separate histories of different species. Most cyprinids examined typically show a graded series of chromosomes with centromere positions varying from median to submedian to subterminal to terminal; chromosomes scored as uni-armed (A) normally comprise only a small fraction (usually $< 20\,\%$) of the karyotype (Avise & Gold, 1977). Rearrangements of the non-fusion/fission type which affect centromere position are usually inferred from differences in chromosome arm number, and there is evidence that some North American cyprinids differ in arm number. Most cyprinids appear to have 46–49 haploid arms (Uyeno & Miller, 1973; Howell & Villa, 1976; Gold & Avise, 1977; this paper), but one species, *Richardsonius egregius*, was scored as having 43 arms (Gold & Avise, 1977), and another species, *Notropis umbratilis*, recently karyotyped in our laboratory, had 50 arms.

We do not know how repeatable these differences in arm number are, and whether cyprinid species with 50 diploid chromosomes truly differ in gross karyotype. With present staining techniques, it is virtually impossible to ascertain chromosomal homologies across species, and the small size of most minnow chromosomes probably precludes accurate centromere placement. Nevertheless, at least some of the reported differences undoubtedly are real, and it may be assumed that non-fusion/fission rearrangement has played a role in North American cyprinid evolution. On the whole, however, the present evidence indicates that arm number evolution also has been conservative.

Chromosomal evolution, speciation, and morphological evolution in North American Cyprinidae. In many animal and plant taxa, progressive organismal evolution and speciation apparently depend to a large degree on gene rearrangement brought about by gross chromosomal restructuring. Rapidly evolving groups such as placental mammals seem to have undergone considerably more chromosomal evolution than slowly evolving groups such as frogs or birds (Wilson, 1976; Prager & Wilson, 1975; Wilson *et al.* 1975; Levin & Wilson, 1976; Bush *et al.* 1977), and rates of organismal evolution (morphology, behaviour, etc.) and speciation are positively correlated with rates of gross karyotypic evolution. It has been suggested that the 'genetic revolutions' important to speciation may occur through gene rearrangement rather than through changes in structural genes.

Using the equations in Bush *et al.* (1977), we have estimated rates of chromosome number change and speciation within living genera of North American cyprinids. Rate of cyprinid chromosome arm number change could not be estimated from the available data. Ages for cyprinid genera were based on the first appearance of a genus in the fossil record (Miller, 1965; Kimmel, 1975; Smith, 1975), and absolute ages for geological times were taken from Savage (1975). Only genera for which karyotype data were available (Table 3) were used, and extinct species (from living genera) were included in estimating speciation events per lineage (genus). The latter was done to compensate for the high number of monotypic cyprinid genera, but did not appreciably affect the estimate of cyprinid speciation rate.

Our estimates of chromosome number evolution and speciation rate within living North American cyprinid genera appear in Table 4; evolutionary rates of other lower vertebrates and of some mammals are shown for comparison. Chromosome number change in cyprinids has been slow. The average rate of 0·012 changes per lineage per million years is roughly the same as that in frogs and snakes, about half that in lizards and bats, and considerably less than that in higher mammals. In contrast, speciation rate in cyprinids (0·7 new species per lineage per million years) has been relatively rapid, about twice that in other lower vertebrates and approximately the same as that in bats. The ratios between rates of chromosome number change and speciation (Table 4) reveal that in cyprinids fewer chromosome number changes occur per speciation event than in most vertebrates. Clearly many speciations in North American cyprinids occur in the absence of gene rearrangements which alter chromosome number. The relatively rapid speciation exhibited by cyprinids does not seem comensurate with their slow chromosome number evolution.

Reliable data on cyprinid arm number evolution may reveal that rearrangements affecting centromere position, but not chromosome number, are exceedingly frequent. At present this seems unlikely. We have examined over 33 cyprinid species and found only seven possible arm number changes; most species are very similar in arm number. Further, although rates of arm number evolution in most vertebrates are usually higher than rates of chromosome number evolution, the difference is small (1·3–2x) and only in a few cases (e.g. snakes) do arm number changes far exceed chromosome number changes (Bush *et al.* 1977). Finally, the

Table 3. *Genera of North American Cyprinidae for which chromosome numbers are available*

Genus	No. of species with diploid chromosome number of 48	50	52
Campostoma[1]	—	1	—
Chrosomus[3, 5]	—	3	—
Exoglossum[3]	1	—	—
Gila[2, 11]	—	2	—
Hesperoleucus[2]	—	1	—
Hybognathus[1]	—	1	—
Hybopsis[1, 3]	—	2	—
Lavinia[2]	—	1	—
Lepidoma[4]	—	3	—
Meda[4]	—	1	—
Mylopharodon[2]	—	1	—
Notemigonus[2]	—	1	—
Notropis[5, 6, 9, 10]	—	6	—
Opsopoeodus[6]	1	—	—
Phenacobius[1]	—	1	—
Pimephales[1, 3, 7]	—	2	1
Plagopterus[4]	—	1	—
Pogonichthys[2]	—	1	—
Ptychocheilus[2, 12]	—	2	—
Richardsonius[2]	—	1	—
Rhinichthys[8, 13]	—	3	—
Semotilus[3]	—	1	2

References: [1] this paper; [2] Gold & Avise (1977); [3] Legendre & Steven (1969); [4] Uyeno & Miller (1973); [5] Greenfield *et al.* (1973); [6] Campos & Hubbs (1973); [7] Gravell & Malsberger (1965); [8] McPhail & Jones (1966); [9] Denton & Howell (1969); [10] Lieppman & Hubbs (1969); [11] Greenfield & Greenfield (1972); [12] Uyeno & Smith (1972); [13] Howell & Villa (1976).

Table 4. *Rates of chromosome number evolution and speciation within genera of North American Cyprinidae and other vertebrates*

Group	Chromosome number changes/ lineage/ Myr*	New species/ lineage/ Myr	Chromosome number changes/ speciation event
N.A. cyprinids	0·012	0·7	0·017
Mammals†			
Primates	0·333	2·6	0·128
Rodents	0·178	1·6	0·111
Bats	0·028	0·7	0·040
Lower vertebrates†			
Lizards	0·027	0·3	0·090
Snakes	0·007	0·4	0·017
Frogs	0·011	0·2	0·055

* Myr = Million years. † From Bush *et al.* (1977).

https://doi.org/10.1017/S0016672300018504 Published online by Cambridge University Press

rates of both types of gross karyotypic change are highly correlated among vertebrates ($r = 0{\cdot}938$, from Bush *et al.* 1977); i.e. groups which have had rapid chromosome number evolution tend also to have had rapid arm number evolution. In short, the present evidence does not indicate a direct relationship between rate of chromosomal evolution and rate of speciation among North American cyprinids.

A second feature of chromosomal reorganization among organisms is that groups which have experienced rapid and extensive karyotypic change tend also to have experienced rapid and extensive organismal change (Wilson, 1976; Wilson *et al.* 1974*b*; King & Wilson, 1975). Anatomically conservative lineages such as frogs have experienced far less chromosomal change than rapidly evolving lineages such as placental mammals. A positive relationship between chromosomal and organismal evolution predicts that karyotypically conservative taxa should show relatively less organismal change as compared to taxa which have undergone rapid karyotypic change.

In Table 5, several morphological features which serve to identify the five species (genera) examined in this study are listed. These five taxa were chosen for study on the basis of availability and broad distribution, and may represent an average degree of morphological evolution among North American Cyprinidae. Many of these characteristics (e.g. mouth shape, dentition) are likely under polygenic control and subject to epistatic interactions, and hence indicative of major evolutionary adaptations (Kluge & Farris, 1969).

Morphological divergence among the five species (genera) appears to have been fairly extensive (Table 5). Several characteristics are unique to each species, and a few are shared by only two or three species. Significant and possibly adaptive differences may include those involving intestine shape and length, mouth shape and position, and pharyngeal tooth morphology. The three species with virtually identical gross karyotypes (*Campostoma*, *Hybognathus*, and *Phenacobius*) are well differentiated morphologically. Qualitatively, the degree of morphological evolution among these five species appears far greater than the degree of karyotypic evolution. The same is probably true for most North American cyprinids.

This study complements and extends that of Avise & Gold (1977): speciation and morphological evolution among North American cyprinids appear to have been considerably more rapid and extensive than gross chromosomal evolution. Progressive evolution in these fishes may thus depend on genetic changes other than gross chromosomal restructuring. Certainly, those events which produce visible changes in karyotype are rare relative to events responsible for speciation.

The nature of the molecular events which underlie speciation in cyprinids is unknown. Recent studies by Avise (1977*a*, *b*) suggest that structural gene (allozyme) changes among cyprinids are independent of speciation events, related perhaps to elapsed time. In the future, rates of other modes of organismal evolution among cyprinids need to be quantified and compared to rates of speciation and karyotypic change. It will also be important to consider carefully biological and genetic factors which could contribute to the differing rates of evolution.

Table 5. *Some major morphological differences among five species (genera) of North American Cyprinidae.* +, *present or common*; −, *absent or rare*

Character	*C. anomalum*	*H. hayi*	*H. aestivalis*	*P. mirabilis*	*P. vigilax*
Pronounced dorsal spot	−	−	−	−	+
Caudal spot	+	−	−	+	+
Crossbar on dorsal and anal fins	+	−	−	−	−
Cross-hatch markings	−	+	−	−	−
Heavily speckled body	−	−	+	−	−
Fleshy side lobes on lower jaws	+	−	−	+	−
Maxillary barbels	−	−	+	−	−
Cartilaginous ridge on lower jaw	+	−	−	−	−
Chest scaled	+	+	−	−	−
Pre-dorsal scales small, crowded	−	−	−	−	+
Membrane between 1st two dorsal rays	−	−	−	−	+
Peritoneum	Black	Black	Silvery	White	Silvery
Lateral line scales	*c.* 53	35–41	35–43	43–52	40–43
Body shape	Slightly compressed	Moderately compressed	Cylindrical	Slender	Slender
Intestine	Very long, in coils and loops	Long*	Short, one anterior loop	Short, one anterior loop	Short, no transverse loop
Sexual dimorphism (colours, tubercles)	Very pronounced	Slight	None noticeable	None noticeable	Pronounced
Mouth	Horizontal, sub-terminal	Crescent-shaped, terminal	Horizontal, sub-terminal	Inferior, sucker-like	Oblique, nearly terminal
Pharyngeal teeth	4–4, with grinding surfaces	4–4, with grinding surfaces	4–4, with hooks	4–4, with hooks	4–4, with hooks

* Intestine of *H. hayi* is long, but is somewhat shorter than in other *Hybognathus* species.

We thank J. W. Bickham and H. J. Price for their comments on the manuscript. Work was supported by funds administered through the Dean's Office of the College of Agriculture and The Texas Agricultural Experiment Station of Texas A & M University.

REFERENCES

AVISE, J. C. (1977*a*). Genic heterozygosity and rate of speciation. *Paleobiology* **3**, 422–432.

AVISE, J. C. (1977*b*). Is evolution gradual or rectangular? Evidence from living fishes. *Proceedings of the National Academy of Sciences* (*U.S.A.*) **74**, 5083–5087.

AVISE, J. C. & GOLD, J. R. (1977). Chromosomal divergence and rates of speciation in two families of North American fishes. *Evolution* **31**, 1–13.

BUSH, G. L., CASE, S. M., WILSON, A. C. & PATTON, J. L. (1977). Rapid speciation and chromosomal evolution in mammals. *Proceedings of the National Academy of Sciences* (*U.S.A.*) **74**, 3942–3946.

Campos, H. H. & Hubbs, C. (1973). Taxonomic implications of the karyotype of *Opsopoeodus emiliae*. *Copeia*. (1973), 161–163.

Denton, T. E. & Howell, W. M. (1969). A technique for obtaining chromosomes from the scale epithelium of teleost fishes. *Copeia* (1969), 392–393.

Gold, J. R. (1974). A fast and easy method for chromosome karyotyping in adult teleosts. *The Progressive Fish – Culturalist* **36**, 169–171.

Gold, J. R. & Avise, J. C. (1977). Cytogenetic studies in North American minnows (Cyprinidae). I. Karyology of nine California genera. *Copeia* (1977), 541–549.

Gravell, M. & Malsberger, R. G. (1965). A permanent cell line from the fathead minnow (*Pimephales promelas*). *Annals of the New York Academy of Sciences* **126**, 555–565.

Greenfield, D. W. & Greenfield, T. (1972). Introgressive hybridization between *Gila orcutti* and *Hesperoleucus symmetricus* (Pisces: Cyprinidae) in the Cuyama River basin, California: I. Meristics, morphometrics, and breeding. *Copeia* (1972), 849–859.

Greenfield, D. W., Abdel-Hameed, F., Deckert, G. D. & Flinn, R. R. (1973). Hybridization between *Chrosomus erythrogaster* and *Notropis cornutus* (Pisces: Cyprinidae). *Copeia* (1973), 54–59.

Howell, W. M. & Villa, J. (1976). Chromosomal homogeneity in two sympatric cyprinid fishes of the genus *Rhinichthys*. *Copeia* (1976), 112–116.

Kimmel, P. G. (1975). Fishes of the Miocene – Pliocene Deer Butte formation, southeast Oregon. *University of Michigan Museum of Paleontology Papers on Paleontology* **14**, 69–87.

King, M. C. & Wilson, A. C. (1975). Evolution at two levels in humans and chimpanzees. *Science* **188**, 107–116.

Kluge, A. G. & Farris, J. S. (1969). Quantitative phyletics and the evolution of anurans. *Systematic Zoology* **18**, 1–32.

Legendre, P. & Steven, D. M. (1969). Denombrement des chromosomes chez quelques cyprins. *Naturaliste Canada* **96**, 913–918.

Levan, A., Fredga, K. & Sandberg, A. A. (1964). Nomenclature for centromeric position on chromosomes. *Hereditas* **52**, 201–220.

Levin, D. L. & Wilson, A. C. (1976). Rates of evolution in seed plants: Net increase in diversity of chromosome numbers and species numbers through time. *Proceedings of the National Academy of Sciences* (*U.S.A.*) **73**, 2086–2090.

Lieppman, M. & Hubbs, C. (1969). A karyological analysis of two cyprinid fishes. *Notemigonus crysoleucas* and *Notropis lutrensis*. *Texas Reports on Biology and Medicine* **27**, 427–435.

McPhail, J. D. & Jones, R. L. (1966). A simple technique for obtaining chromosomes from teleost fishes. *Journal of the Fisheries Research Board of Canada* **23**, 767–768.

Miller, R. R. (1959). Origin and affinities of the freshwater fish fauna of Western North America. Zoogeography. *American Association for the Advancement of Science Publication* **51**, 187–222.

Miller, R. R. (1965). Quaternary freshwater fishes of North America. In *The Quaternary of the United States* (ed. H. E. Wright, Jr. and D. G. Frey), pp. 569–581. New Jersey: Princeton University Press, Princeton.

Prager, E. M. & Wilson, A. C. (1975). Slow evolutionary loss of the potential for interspecific hybridization in birds: a manifestation of slow regulatory evolution. *Proceedings of the National Academy of Sciences* (*U.S.A.*) **72**, 200–204.

Savage, D. E. (1975). Cenozoic – the primate episode. In *Approaches to Primate Paleobiology* (ed. F. S. Szalay). Contributions to *Primatology* **5**, 2–27.

Smith, G. R. (1975). Fishes of the Pliocene Glenns Ferry formation, southeast Idaho. *University of Michigan Museum of Paleontology Papers on Paleontology* **14**, 1–68.

Uyeno, T. & Miller, R. R. (1973). Chromosomes and the evolution of the Plagopterin fishes (Cyprinidae) of the Colorado River system. *Copeia* (1973), 776–782.

Uyeno, T. & Smith, G. R. (1972). Tetraploid origin of the karyotype of Catostomid fishes. *Science* **159**, 644–646.

White, M. J. D. (1973). *Animal Cytology and Evolution*. Cambridge University Press, William Clowes and Sons, Ltd, London.

Wilson, A. C. (1976). Gene regulation in evolution. In *Molecular Evolution* (ed. F. J. Ayala), pp. 225–234. Sunderland, Mass.: Sinauer Assoc., Inc.

WILSON, A. C., MAXSON, L. R. & SARICH, V. M. (1974*a*). Two types of molecular evolution. Evidence from studies of interspecific hybridization. *Proceedings of the National Academy of Sciences* (*U.S.A.*) **71**, 2843–2847.

WILSON, A. C., SARICH, V. M. & MAXSON, L. R. (1974*b*). The importance of gene rearrangement in evolution: evidence from studies on rates of chromosomal, protein, and anatomical evolution. *Proceedings of the National Academy of Sciences* (*U.S.A.*) **71**, 3028–3030.

WILSON, A. C., BUSH, G. L., CASE, S. M. & KING, M. C. (1975). Social structuring of mammalian populations and rate of chromosomal evolution. *Proceedings of the National Academy of Sciences* (*U.S.A.*) **72**, 5061–5065.