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CHROMOSOMAL DIVERGENCE AND SPECIATION IN TWO FAMILIES OF NORTH AMERICAN FISHES[1]

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Cladogenesis, or splitting, refers to the formation of independently evolving lineages from a single ancestral line. A fundamental cladogenetic process is speciation. Rates of cladogenesis vary greatly among different groups or phylads. Occasionally, rates of speciation may appear very different even among closely related phylads living in similar macroenvironments.

Theoretical models have recently been proposed which predict relative levels of genetic divergence between species belonging to species-rich (speciose) versus species-poor (depauperate) phylads (Avise and Ayala, 1975). The predictions of these models depend upon the relationship between rates of speciation and amount of genetic or molecular divergence. The qualitative results are as follows: (1) when genetic distance between species is a function of time since they last shared a common ancestor, the mean level of genetic divergence between living species will be approximately the same in speciose as in depauperate phylads of similar evolutionary age; (2) when genetic distance between species is a function of the number of speciation events in the history of a phylad, living members of speciose phylads are much more distinct genetically than members of depauperate phylads, on the average, and the ratio of mean genetic distances increases as the frequency of speciations in one group relative to the other becomes greater.

Avise and Ayala (1976) have tested these hypotheses at the *genic* level by comparing divergence in proteins encoded by a number of genetic loci in representative minnow and sunfish species. As described elsewhere (Avise and Ayala, 1976), North American minnows (Cyprinidae) and sunfish (Centrarchidae) have apparently had very different rates of speciation in their evolutionary histories. Yet the preliminary results indicate that the speciose minnows are not genetically more distinct

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than the depauperate sunfish; the mean level of genic differentiation among minnows appears very similar to that among members of the sunfish genus *Lepomis*. These results, if confirmed, are inconsistent with the thesis that speciations in these phylads have been accompanied by substantial *genic* changes; time since splitting appears to be a much more important predictor of genic distance.

Predictions of the models may be tested at other levels as well. In this paper, we examine amounts of *chromosomal* divergence between representative minnows, relative to that among sunfish. There are reasons to suspect that chromosomal rearrangements might conceivably play an extremely important role in many speciations, and hence that species groups characterized by faster rates of speciation would show greater karyotypic diversity. We have examined the karyotypes of species belonging to nine different genera of minnows. We have also reviewed the literature of karyotypic configurations of other minnow species. The levels of karyotypic diversity in North American minnows will be compared to previously published data on the chromosomal configurations of sunfish (Roberts, 1964) as tests of the hypotheses about possible relationships between chromosomal divergence and rates of speciation in these fishes.

Materials and Methods

Minnows and Sunfish

Most of the 40 genera and 250 species of North American minnows (Cyprinidae) are thought to belong to a single subfamily, the Leuciscinae, and to have been derived from one or a few ancestral stocks which crossed into North America perhaps in the Miocene (Miller, 1959, 1965; Gosline, 1974). We have examined the karyotypes of species belonging to nine genera of cyprinids. Karyotype confirgurations are also available for at least 16 other species of Leuciscinae belonging to seven additional genera, including six members of the large genus *Notropis*. Taken altogether, these species should permit a reasonable assessment of the amount and type of karyotypic divergence in the highly speciose North American minnows.

The sunfish family Centrarchidae, containing only 9 genera and 30 living species, is native to North America and first appears in the fossil record early in the Cenozoic (Schlaikjer, 1937; Romer, 1966; Branson and Moore, 1962). The most diverse centrarchid genus is *Lepomis* with 11 species; it first appears in the fossil record at the Miocene-Pliocene boundary.

Roberts (1964) made a thorough study of karyotypes of 20 species belonging to eight centrarcid genera. We shall utilize these data to compare levels of chromosomal diversity in sunfish versus minnows. The most relevant comparison for tests of the theoretical models may involve the speciose North American Leuciscinae versus the depauperate *Lepomis*. However, it is possible the *Lepomis* species are somewhat younger than minnow species, on the average, and hence that *Lepomis* is a biased underestimate of the amount of differentiation in the depauperate phylad according to the time-divergence model. The Centrarchidae as a whole permit another comparison with the Leuciscinae; if anything, this comparison is likely to be somewhat biased towards an overestimate of differentiation in a depauperate phylad. But as we shall see, the conclusions on levels of karyotypic diversity in sunfish versus minnows remain the same whichever comparison is employed.

Chromosome Techniques

Fish were collected by seine and transported live to the laboratory where they were injected intramuscularly with a colchicine solution. Chromosomes were prepared from kidney tissue according to the technique of Gold (1974). Basically, this technique involves gently homogenizing the cells, treating with hypotonic KCl, and fixing overnight in a 3:1 methanol: acetic acid mixture. The fixed cells are dropped from 2–3 feet onto microscope slides which

TABLE 1. *Species of native North American Cyprinidae for which karyotype data are available.*

Species	Number of individuals	Number of cells examined	Modal *2n* number	# terminal + subterminal chromosomes	Source
Hesperoleucus symmetricus	8	99	50	8	Present study
Lavinia exilicauda	3	75	50	8	Present study
Ptychocheilus grandis	3	64	50	8	Present study
Mylopharodon conocephalus	3	80	50	6	Present study
Gila bicolor	4	135	50	6	Present study
Richardsonius egregius	5	100	50	14	Present study
Pogonichthys macrolepidotus	2	99	50	6	Present study
Orthodon microlepidotus	2	60	50	6	Present study
Notemigonus crysoleucus	4	74	50	6	Present study
Lepidomeda albivallis	10	25	50	—	Uyeno & Miller, 1973
Lepidomeda mollispinis	4	10	50	—	Uyeno & Miller, 1973
Lepidomeda vittata	6	48	50	—	Uyeno & Miller, 1973
Meda fulgida	13	66	50	—	Uyeno & Miller, 1973
Plagopterus argentissimus	5	20	50	—	Uyeno & Miller, 1973
Notropis callistius	—	—	50	—	Denton & Howell, 1969
Notropis stilbius	—	—	50	—	Denton & Howell, 1969
Notropis lutrensis	—	—	50	—	Lieppman & Hubbs, 1969
Pimephales promelas	—	—	50	—	Gravell & Malsberger, 1965
Opsopoeodus (= *Notropis*) *emiliae*	5	—	48	—	Campos & Hubbs, 1973
Notropis venustus	7	—	50	—	Campos & Hubbs, 1973
Notropis texanus	6	—	50	—	Campos & Hubbs, 1973
Rhinichthys evermanni	—	—	50	—	McPhail & Jones, 1966
Gila orcutti	—	—	50	—	Greenfield & Greenfield, 1972
Notropis cornutus	5	7	50	—	Greenfield et al., 1973
Chrosomus erythrogaster	5	17	50	—	Greenfield et al., 1973

are then air-dried and stained with undiluted Giemsa. Well spread metaphase preparations were photographed, and enlargements were studied for centromere placement and arm-ratios. For each species, we counted chromosome numbers in a minimum of 60 cells.

RESULTS

Karyotypic Diversity in North American Cyprinids

The California minnows examined in this study are listed in Table 1. Counts of $2n = 50$ were obtained in over 70 percent of all cells. Most cells not yielding modal counts were short by one or a few chromosomes, undoubtedly because some chromosomes were occasionally lost during cell preparation, were obscured by overlap, or were miscounted. Furthermore, in virtually all exceptionally well formed spreads, 50 chromosomes were readily apparent. Each of these nine species of California minnows possesses $2n = 50$ chromosomes.

The karyotypes of most of the nine genera of California minnows were extremely difficult, if not impossible, to distinguish from one another. Each karyotype consists of a graded series of chromosomes with centromere placement ranging from median to nearly terminal. Chromosomal lengths in each species varied from about 4 to 9 microns. Sex chromosomes were not identifiable. We have carefully measured arm ratios (r) in well spread karyotypes, and classified each pair of chromosomes as follows: median, $r = 1.0$–1.7; submedian, $r = 1.7$–3.0; subterminal, $r = 3.0$–7.0; terminal, $r = 7.0$–∞ (terminology after Levan et al., 1964). Based on our estimates, two species, *Hesperoleucus symmetricus* and *Lavinia exilicauda*, appear to to be the most similar karyotypically, exhibiting virtually the same numbers of all

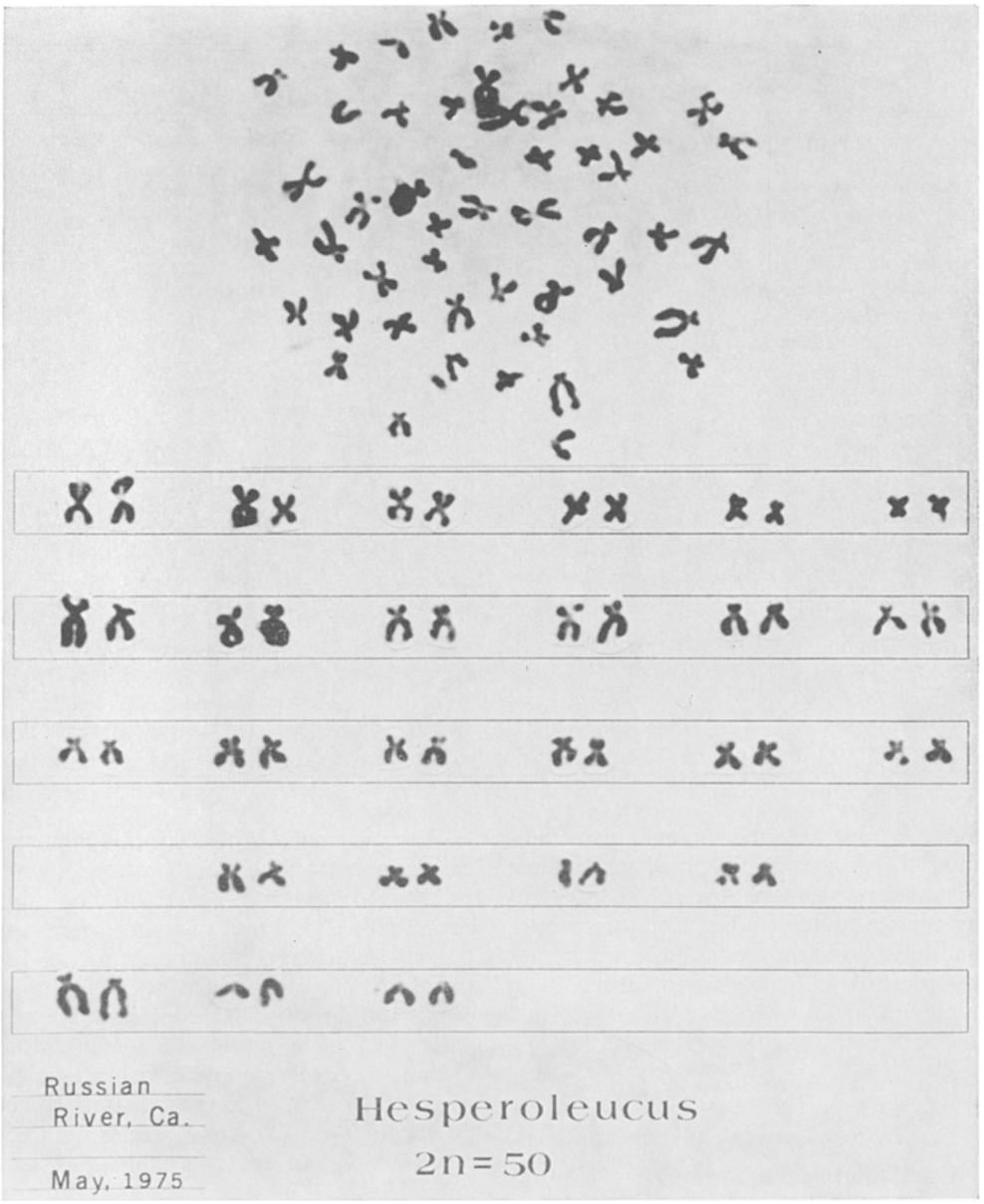

FIG. 1. Somatic metaphase karyotype from kidney cell of *Hesperoleucus symmetricus*, $2n = 50$.

chromosome categories (Figs. 1 and 2). These two species are biochemically also the most similar of the California minnows (Avise et al., 1975). However, we have little confidence in our ability to distinguish reliably the karyotypes of these two species from those of most of the other California minnows. One species, *Richardsonius egregius*, exhibited a karyotype somewhat different from the other species. While the other species showed either 6 or 8 terminal plus subterminal chromosomes

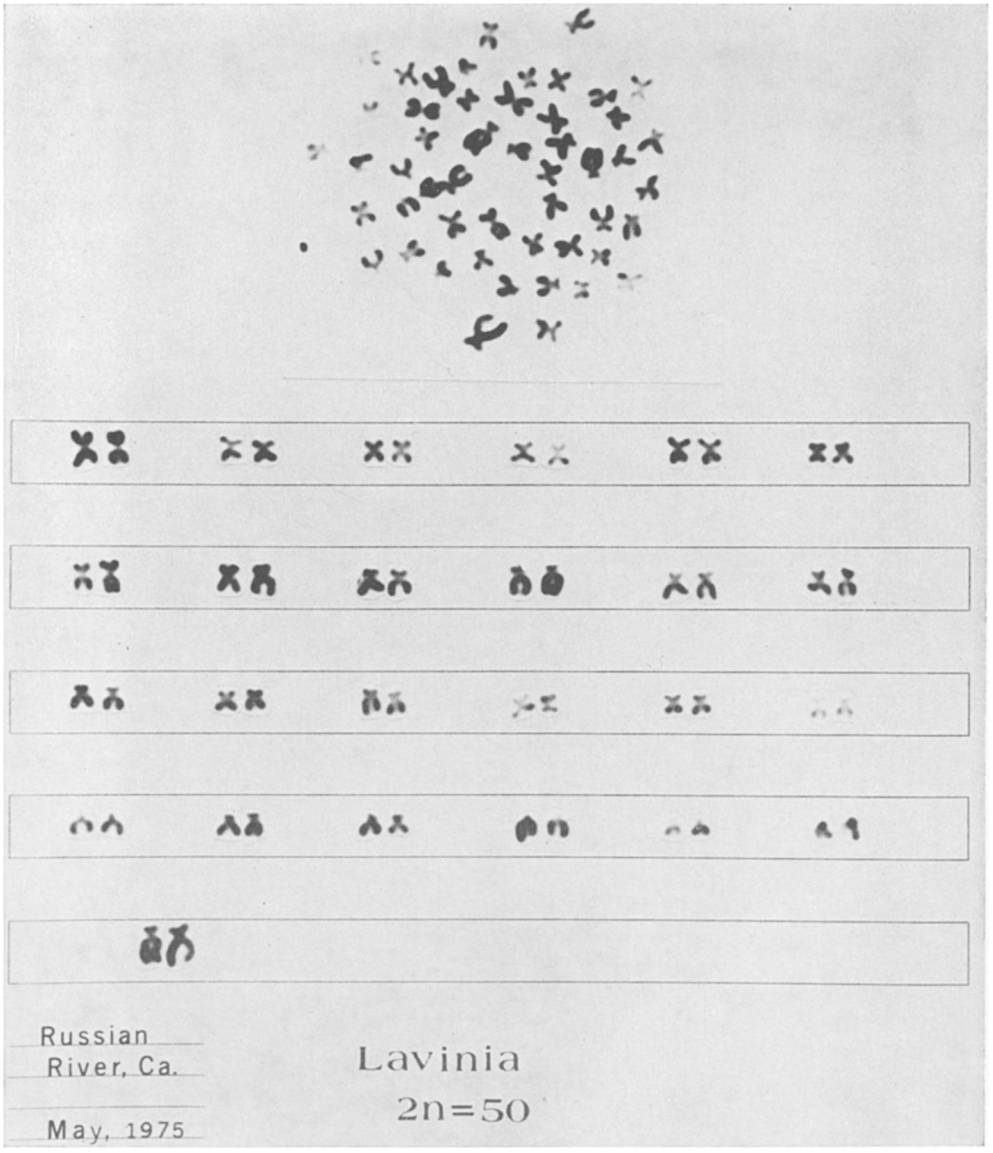

FIG. 2. Somatic metaphase karyotype from kidney cell of *Lavinia exilicauda*, $2n = 50$.

(Table 1), *Richardsonius* appeared to have 14 (Fig. 3).

Chromosome homologies across species could not be determined and hence we cannot be sure of the number of centromere placement changes in these minnows. Nonetheless, each species did possess one pair of exceptionally large terminal or subterminal chromosomes. Centromere placement in this pair of chromosomes was clearly different in different species, ranging from $r = 2.7$–3.3 in various karyograms of *Lavinia exilicauda* to $r \gg 7.0$ in *Ptychocheilus grandis* and *Pogonichthys macrolepidotus*. Thus despite the outward similarity of karyotypic configurations in the California minnows, and the constancy of chromosome numbers, there is evidence

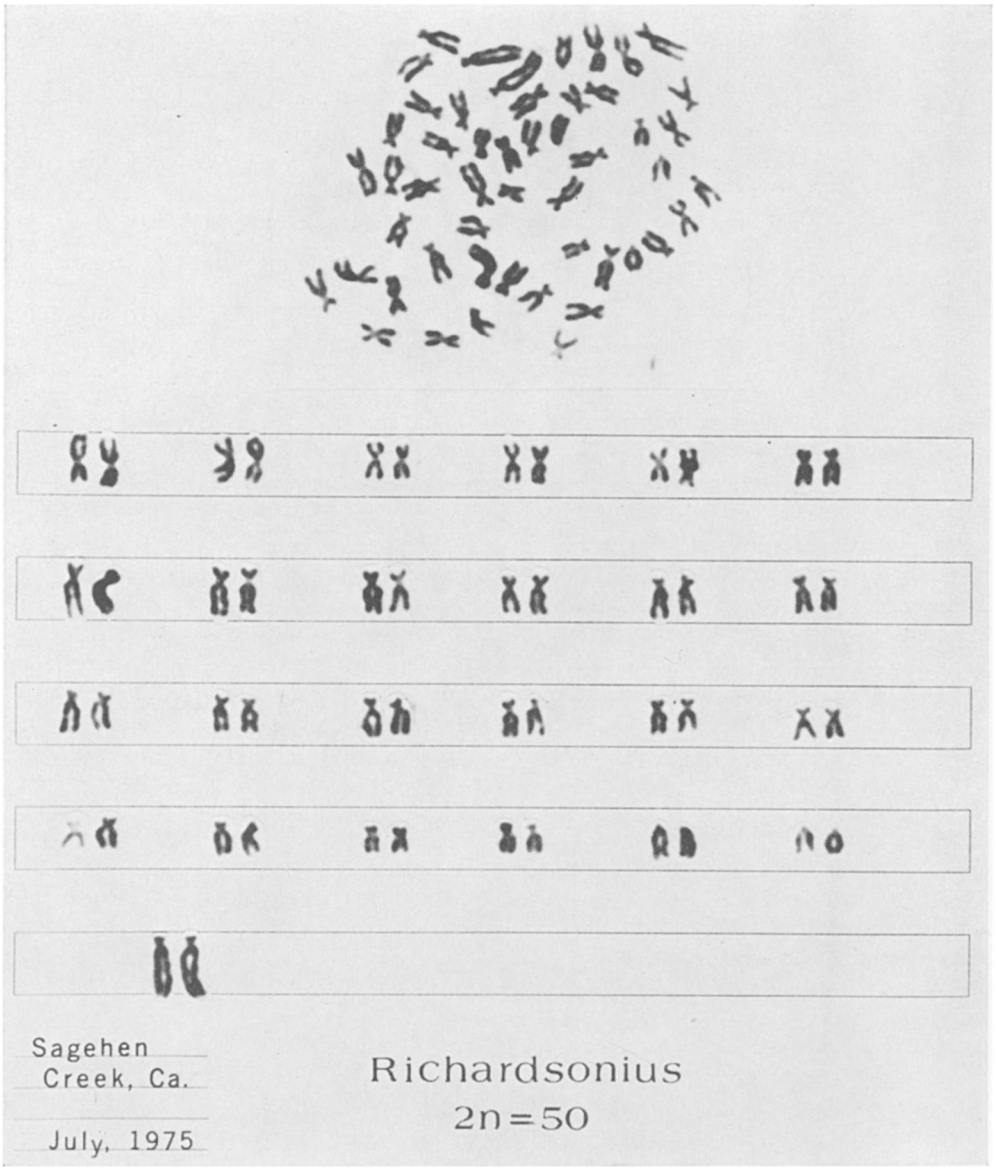

FIG. 3. Somatic metaphase karyotype from kidney cell of *Richardsonius egregius*, $2n = 50$. The karyotype of this species was the most distinctive of species belonging to nine genera of California minnows.

that a number of centromeric shifts have occurred in their separate evolutionary histories. For a more complete description of these karyotypes see Gold and Avise (1976).

The amount and pattern of karyotypic diversity among the California minnows appears typical of that observed among all North American minnows examined to date. Uyeno and Miller (1973) report diploid numbers of 50 in five species and three genera in the minnow group Plago-

pterini. The karyotypes were very similar, though distinguishable, and the number of metacentric chromosomes (centromere strictly median) varied from a low of 12 in *Lepidomeda albivinis* and *L. mollispinis* to 18 in *Plagopterus argentissimus* and *Meda fulgida*. McPhail and Jones (1966) found $2n = 50$ in *Rhinichthys evermanni*; again centromere position graded from median to terminal and the general configuration was similar to that among the Plagopterini and the California minnows.

Karyotypes have been described in six species of *Notropis*, a minnow genus which includes about 125 of the 250 species of North American minnows. Each *Notropis* species examined has $2n = 50$. Denton and Howell (1969) picture karyotypes which appear nearly identical in *Notropis callistius* and *N. stilbius*; each includes a number of median, submedian, subterminal, and terminal chromosomes. Lieppman and Hubbs (1969) found 5 pairs of metacentric, 16 pairs of submetacentric and subtelocentric, and 4 pairs of telocentric chromosomes in *Notropis lutrensis*. They indicate that this karyotype differs from that in *Notemigonus crysoleucus* by centromeric shifts in several chromosomes. Campos and Hubbs (1973) report that *Notropis venustus* and *N. texanus* have slightly different numbers of metacentric, submetacentric, and acrocentric chromosomes, although the karyotypes are again extremely difficult to distinguish. A third species examined by Campos and Hubbs, *Opsopoeodus* (= *Notropis*) *emiliae*, contains $2n = 48$. In addition to the published karyotypes of North American minnows, the number of chromosomes in about 60 other North American minnow species is typically 50 (unpublished data—see Uyeno, 1971; Uyeno and Miller, 1973; Uyeno and Smith, 1972; Campos and Hubbs, 1973).

In summary, gross chromosomal evolution in the North American minnows appears to have been remarkably conservative. Almost all species exhibit $2n = 50$ chromosomes. The chromosome complement of any species consists of a series more or less continuous in size and in centromere placement. Karyotypes typically consist of a number of median, submedian, subterminal, and terminal chromosomes. The terminal and nearly terminal chromosomes usually comprise less than 20 percent of the total chromosome complement. Sex chromosomes have not been identified. With currently applied methods of fish chromosome cytology, it is not possible to determine unambiguously which pairs of chromosomes are homologous in different species of minnows. Many species are difficult if not impossible to distinguish karyologically.

Nonetheless, there is considerable evidence that centromeric shifts have repeatedly occurred in the history of North American minnows. Changes in centromere placement without change in chromosome number can result from pericentric inversions, from non-reciprocal or unequal reciprocal translocations or from regional duplications. A number of species appear to differ considerably in numbers of median and submedian, and terminal and subterminal chromosomes. It is not yet clear how repeatable these observations are, but at least some of the reported differences are probably real. Some authors (i.e. Lieppman and Hubbs, 1969) have been impressed more with the differences than with the similarities of karyotypes of North American minnows.

Karyotypic Diversity in North American Centrarchids

Roberts (1964) has carefully analyzed the karyotypes of 20 of the 30 living species of Centrarchidae, including nine species of *Lepomis* and representatives of seven other genera (see Table 2). The following account is taken from that study.

Analyses of an average of more than 50 cells per species demonstrated that 16 of the 20 species had diploid chromosome numbers of 48. Three species (*Micropterus salmoides*, *M. dolomieui* and *Lepomis humilis*) had diploid numbers of 46, and one species (*Lepomis cyanellus*) appeared to

TABLE 2. *Species of North American Centrarchidae for which karyotype data are available. All data are from Roberts (1964).*

Species	Number of individuals	Number of cells with modal number	Modal 2n number
Lepomis gulosus	2	34	48
Lepomis macrochirus	42	33	48
Lepomis cyanellus (North Carolina)	4	35	48
Lepomis cyanellus (West Virginia)	39	51	46
Lepomis auritus	2	56	48
Lepomis microlophus	9	59	48
Lepomis gibbosus	36	88	48
Lepomis humilis	7	58	46
Lepomis marginatus	3	6	48
Lepomis megalotis	4	4	48
Pomoxis nigromaculatus	7	62	48
Pomoxis annularis	2	16	48
Micropterus salmoides	10	153	46
Micropterus dolomieui	5	59	46
Ambloplites rupestris	5	47	48
Centrarchus macropterus	10	80	48
Enneacanthus gloriosus	7	69	48
Enneacanthus obesus	5	19	48
Enneacanthus chaetodon	4	53	48
Acantharchus pomotis	3	4	48
Elassoma zonatum	7	20	48

be polymorphic for chromosome numbers 46 and 48. The variation in chromosomal numbers among species was probably due to Robertsonian fusions or fissions, since each of the species with $2n = 46$ exhibited a pair of large submedian chromosomes not observed in the other species. Assuming that $2n = 48$ is the ancestral condition, the 46 chromosome compliment of *L. cyanellus* is most likely the result of a single centric fusion which subsequently became fixed in that population.

With the exception of the pair of large submedian chromosomes in four species, the chromosome compliments of 19 of the 20 species were made up entirely of terminal chromosomes, or subterminal chromosomes with very short arms. According to Roberts (1964) "No karyotypic differences could be detected among (these) . . . . species with diploid numbers of 48." In most cases, it was impossible to determine unambiguously which chromosomes had short arms. Although measurements of arm ratios indicate some differences in centromere placement among species, the differences were no greater than those appearing in replicate cells from the same species, and hence may have been due to experimental error.

The karyotype of one species (*Elassoma zonatum*) was distinctive, and consisted of a small pair of submedian chromosomes and numerous subterminal chromosomes with well defined second arms. Roberts (1964) felt that the karyotype of *Elassoma* is sufficiently distinct to indicate independent origin. Morphologically (Branson and Moore, 1962) and biochemically (Avise et al., 1976), *Elassoma* is the most distinct of all centrarchid genera. Jordan (1877) first described *Elassoma* as a member of the primary South American family Cichlidae.

In summary, gross karyotypic evolution in Centrarchidae also appears to have been conservative. With the exception of at most three or four independent centric fusions, chromosome number among the centrarchids has remained at $2n = 48$. Sex chromosomes are not distinguishable. Karyotypes typically consist solely of terminal chromosomes, or subterminal chromosomes with very short arms. With the exception of a single genus (*Elassoma*), centromere placement was not demonstrably heterogeneous among any of the species.

DISCUSSION

Chromosomal Divergence and Reproductive Isolation

Two features of chromosomal reorganization may be important to the process of speciation. First, chromosomal rearrangements *per se* could underlie reproductive isolation and hence speciation. Hybrids of parents homozygous for different translocations, pericentric or paracentric inversions, or centric fusions or fissions, would likely be partially sterile due to the production of duplication and deficiency

gametes. Such chromosome changes lead to partial sterility in hybrids of some organisms (White, 1973*a*, *b*).

It is well known that many species and genera within both the Centrarchidae and Cyprinidae hybridize in the laboratory and in nature (Hubbs, 1955). Hybrids between a number of species of Centrarchidae are fertile (Lagler and Steinmetz, 1957; Childers, 1967; West, 1970; Whitt et al., 1971, 1973; Metcalf et al., 1972). All species of Centrarchidae which are known to produce fertile hybrids have karyotypes which were indistinguishable by Roberts (1964). Hybrids between *Lepomis gulosus* and *Micropterus salmoides*, which differ in chromosome number, are sterile (West, 1970). Less solid information is available on fertility of cyprinid hybrids. Some hybrids are at least partially fertile, since introgressive hybridization has been reported (Greenfield and Deckert, 1973; Hubbs and Miller, 1943; Miller, 1945; Avise et al., 1975). In particular, there is strong evidence for introgressive hybridization between two of the species examined karyotypically in this study, *Hesperoleucus symmetricus* and *Lavinia exilicauda* (Avise et al., 1975). The karyotypes of *Hesperoleucus* and *Lavinia* were virtually indistinguishable.

If chromosomal reorganizations *per se* have repeatedly contributed to reproductive isolation and hence to speciation in the evolutionary histories of the North American minnows, we would expect to find a great deal more karyotypic diversity among the speciose minnows than among the depauperate sunfish. Although there is some evidence for greater chromosomal diversity, and perhaps greater mean difference in chromosome configuration among minnow species, the evidence at present is not compelling. If anything, there appears to have been a somewhat greater rate of Robertsonian fusion or fission among the centrarchids than among the North American cyprinids, although the cyprinids may be characterized by a higher rate of centromeric rearrangement. In the future, it will be valuable to quantify fertility of hybrids between a number of cyprinid and centrarchid species of known karyotype, as well as to examine patterns of chromosome pairing during meiosis in these hybrids.

Chromosomal Divergence and Genetic Regulation

A second feature of chromosomal reorganization which may be important to cladogenetic processes has been discussed by Wallace (1963), Stebbins (1969), Davidson and Britten (1973), Wilson et al. (1974*a*, *b*) and others. They point out that changes in the genetic regulatory apparatus may play a crucial role in evolution; rearrangements in the genetic material, rather than point mutations themselves, could be effective means of achieving new patterns of genetic regulation. Such rearrangements in the positioning of genetic material might be visible at the level of the chromosome karyotype. As applied to the speciation process, it has been postulated that "genetic revolutions" may occur through gene rearrangement rather than through changes in structural genes.

At the level of gross chromosomal organization, we have little evidence that chromosomal divergence has proceeded at a much more rapid rate among minnows than among sunfish. Both groups appear to be characterized by general conservatism in karyotype evolution. To the extent that changes in genetic regulation are reflected in chromosomal rearrangements, the rates of regulatory evolution do not appear more rapid among the minnows.

The conservative nature of karyotypic evolution among North American centrarchids and cyprinids is by no means characteristic of all groups of fishes. Among the true trouts, *Salmo*, chromosome number counts range from 54 to 80, and Robertsonian changes are common (Roberts, 1967, 1968, 1970; Ohno et al., 1965; Gold and Gall, 1975). Among 20 of the 25 living species of killifish (*Fundulus*) diploid numbers range from 32 to 48; Robertsonian changes, as well as pericentric in-

versions and loss of chromosome segments are probably responsible for the differences in karyotypes (Chen, 1971). In the pleuronectiform fishes, the karyotypes of 15 species representing 13 genera and 5 families were found to vary from $2n = 28$–48, the differences presumably arising from both centric fusions and pericentric inversions (Le Grande, 1975). On the other hand, the second most speciose genus of North American fishes, *Etheostoma* (80 species), may be characterized by fewer chromosomal changes. Five species studied exhibit $2n = 48$; numbers of acrocentric chromosomes ranged from 42 to 46 (Ross, 1973). (Nonetheless, Ross concluded "morphological diversity within *Etheostoma* is paralleled by karyological diversity.") At present, evidence does not indicate a positive relationship between rates of karyotypic evolution and rates of cladogenesis among fishes.

What molecular events then are associated with differing rates of cladogenesis among groups of fishes? Preliminary results suggest that protein distances are not greater among minnows than among sunfish, and that time since divergence, rather than the number of speciations in the history of a phylad, is a more important predictor of mean levels of structural gene divergence. One possible explanation is that speciation events in these fishes typically involve changes in only a small proportion of the genome. Another possibility is that changes in genetic regulation are more important than changes in structural genes in determining rates of cladogenesis (Wilson et al., 1975*a*). Results of the present study indicate that genetic regulatory evolution, *as reflected in gross karyotype evolution,* has not proceeded at a much more rapid rate in minnows than in sunfish. We certainly cannot exclude the possibility that important changes in the genome have occurred beyond the power of resolution of current electrophoretic or light microscopic techniques. For example, despite great similarity in karyotypic configuration of six Eurasian cyprinid species, DNA content apparently can vary by as much as 100% (Muramoto et al., 1968; Wolf et al., 1969; Ohno, 1970). Applications of higher resolution techniques (such as *G* and *C* banding of chromosomes) are to be encouraged.

A third possibility is that rate of speciation in minnows has not in fact been greater than in sunfish. The fossil record is far from complete, and the proposed time of origin of these phylads is likely to change somewhat with new fossil discoveries. We cannot altogether rule out the possibility that different rates of extinction, or different times of origin, are responsible for the greater number of living minnows. Finally, we do not expect that the same molecular or other processes will be responsible for speciation events in all organisms. For these reasons, tests of hypotheses concerning rates of cladogenesis and rates of molecular and organismal evolution should be conducted in a wide variety of animal groups.

Summary

Some evolutionary groups or phylads are characterized by different rates of speciation. Theoretical models have previously been formulated which predict molecular consequences of different speciation rates depending upon biologically realistic assumptions. When genetic or molecular distance between species is a function of time since they last shared a common ancestor, the mean level of genetic divergence between living species will be approximately the same in species-rich as in species-poor phylads of similar evolutionary age; when genetic distance between species is a function of the number of speciation events in the history of a phylad, living members of species-rich phylads are much more distinct than members of species-poor phylads, on the average.

Judging from the fossil record and from the number of living species, two phylads of North American fishes of comparable evolutionary age are marked by different rates of speciation. Speciation events apparently occurred much more frequently in

the evolutionary histories of North American Cyprinidae than in Centrarchidae. In this paper we continue our search for molecular correlates of rates of speciation in these fishes by examining chromosomal cytologies of representative minnows and sunfish.

We have examined karyotypes of species belonging to nine genera of Cyprinidae, and surveyed the literature of previously published cyprinid karyotypes. Diversity among cyprinid karyotypes is compared to that among representative centrarchids (Roberts, 1964). Karyotypic evolution in both groups is rather conservative. Most North American minnows exhibit $2n = 50$; centromere positions in any species typically show a continuous series from median to submedian to subterminal to terminal. Chromosomes with very short second arms normally comprise less than 20 percent of the karyotype. Nonetheless, centromeric shifts have repeatedly occurred in the evolutionary histories of these minnows. Most centrarchid species exhibit a karyotype of $2n = 48$, composed entirely of terminal chromosomes or subterminal chromosomes with very short second arms. Three species have $2n = 46$, in each case presumably the result of a single centric fusion which subsequently became fixed in that species.

The levels of chromosomal divergence in minnows versus sunfish are discussed in the context of current concepts about the relationships between chromosomal evolution, the development of reproductive isolation, and genetic regulation.

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SYMPOSIUM: The South American Herpetofauna: Its Origin, Evolution, and Dispersal, with Implications for its Preservation is the title for a symposium involving 24 participants from 11 countries to be hosted by The University of Kansas on 11–13 August 1977. The symposium is part of the joint annual meetings of the Society for the Study of Amphibians and Reptiles and the Herpetologists' League. For additional information and registration forms, write to William E. Duellman, Museum of Natural History, University of Kansas, Lawrence, Kansas 66045.