THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

A COMPARATIVE CHROMOSOME STUDY IN THE PUPFISH GENUS CYPRINODON (TELEOSTEI: CYPRINODONTIDAE)

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

MICHAEL MALOY STEVENSON

Norman, Oklahoma

1975

A COMPARATIVE CHROMOSOME STUDY IN THE PUPFISH GENUS

CYPRINODON (TELEOSTEI: CYPRINODONTIDAE)

APPROVED BY

DISSERTATION COMMITTEE

TABLE OF CONTENTS

FORWARD

Systematic studies in the last few years have become more multi-disciplinary and synthetic in approach as new and more powerful analytical methods have become available. This is certainly the case in phenetic analyses where now large amounts of comparative morphological data can be treated by rapid computerized techniques. Additionally, comparative ecological, behavioral and physiological studies are also enjoying an increased importance in systematic work through the incorporation of new tools and concepts. Furthermore, there has been an increase in more sophisticated genetic analyses, ranging from hybridization studies to the elucidation, by electrophoresis and chromatography, of allelic variability. Also, at the genetic level, additional data of taxonomic significance have come from the comparative analysis of chromosome complements.

It has been since the elucidation of the human karyotype that a phenomenal increase in mammalian chromosome studies has occured. However, those reports have only recently been stimulating to workers dealing with other vertebrate classes. Moreover, the results obtained within the latter groups have not been as spectacular, especially in the fishes. Nevertheless, with technical improvements in the area of cytological preparation, karyology has become a significant tool in many vertebrate groups for studying the relationships between taxa.

Chromosome complements are now often used as a unit systematic character (as in this report), because they not only have characteristics of their own, but they also determine the phenotypic characters of the

carrier plus its genetic interaction with other individuals. For example, structural differences within a chromosome can result in an immediate pairing discontinuity between genotypes whether they are genetically differentiated or not. However, as cytological data have usefullness only as indicators of genetic interactions (and thus relationships), they lose special significance when other genetic data are available. In other words, comparative cytology has relevance in systematics only among organisms that have not been subjected to genetic experiment but might otherwise be expected to hybridize. This implies, however, that one can reasonably conclude that the other characters used to determine relationships reflect a common ancestry rather than convergence. Thus, cytological traits can only be compared between closely related organisms and cannot have greater significance that other phenotypic characters used to evaluate them.

It has become increasingly apparent that karyotypic evolution is often an isolated set of evolutionary events and, thus, the determination of ancestral versus derived chromosome states may have nothing to do with the phylogenetic ancestry or advancement of taxa. On the other hand, there are cases where the comparison of chromosome number and/or structure between forms may have certain utility. This is most likely in distinguishing between phenotypically similar populations (such as found in Cyprinodon) as to which forms might be more or less karyotypically related and/or specialiaed and possibly separating those that are conspecific from those that are not. However, this implies as noted above, that we know what a species is.

A COMPARATIVE CHROMOSOME STUDY IN THE PUPFISH GENUS *CYPRINODON* (TELEOSTEI: CYPRINODONTIDAE)

CHAPTER I

INTRODUCTION

Within the pupfish genus *Cyprinodon*, the majority of the usually allopatric and morphologically distinct populations are regarded as nominal species. However, despite extensive alpha taxonomic work, there are but few comparative systematic studies involving these forms. And in those that are available, emphasis has been placed on species west of the Continental Divide (Miller, 1948, 1950; Turner, 1974).

In review, Miller (1948) used exclusively external morphological characters and drew several conclusions concerning the evolutionary history of certain Death Valley pupfish forms. He postulated similar and possibly contiguous populations of *Cyprinodon* (and *Empetrichthys*) in that region during the post-Pleistocene pluvial, but envisioned rather rapid morphological change ("speciation") with isolation of those populations during the subsequent drying up of stream and lacustrine areas. Further data come from Turner (1974) who found that certain protein systems demonstrated the genetic distinctness of the five species and four of the six subspecies discussed by Miller (1948). However, a separation at only 10 % of the loci he examined between forms was less divergence than predicted by the morphological evidence. On a broader scale, Liu (1969) compared the courtship behaviors of 13 of the 26 nominal and unnamed *Cyprinodon* species and drew

several systematic inferences from those and additional hybridization data. He proposed four behavioral groups (designated superspecies) equivalent to the east to west recognized morphological species complexes of Miller (mostly unpublished material).

In general, the species of Cyprinodon east of the Continental Divide suffer from a paucity of descriptive taxonomic work as revealed by the several undescribed forms (R. R. Miller, pers. comm.). Also, other than Liu's (1969) work, these geographically placed species lack comparative studies in regard to evolutionary relationships (but see Miller, 1968; Echelle and Miller, 1974; Miller and Echelle, 1975). Also, since most species are allopatric from other Cyprinodon, species boundries are not adequately tested in nature. Therefore, the available information suggests that definitive relationships within Cyprinodon are yet to be resolved. I began this study under the assumption that comparative karyological studies might provide additional insight into the systematics of this problematic group.

CHAPTER II

MATERIALS AND METHODS

MATERIALS

All specimens utilized in this study were collected with one-quarter inch mesh seins. Specimens were transported to laboratory facilities alive in five gallon styrofoam ice chests. They were retained in these chests or placed and maintained in four or six foot diameter plastic pools until processing.

Specimens of Cyprinodon variegatus Lacepede were collected from brackish water ditches and tide-pools along the Gulf Coast of Texas near Galveston, Galveston Co. (C. variegatus gibbosus); from Lake Balmorhea, a fresh water impoundment 2 mi. S of Balmorhea, Reeves Co., TX.; from brackish water ditches in Fort Clinch State Park (C. v. variegatus), Fernandina Beach, Nassau Co., Fla.; and from tidal lagoons on the outskirts of Progresso, Yucatan, Mex. (C. variegatus artifrons). Material of Cyprinodon hubbsi Carr was obtained from along the shore of Lake Eustis at the town of Eustis, Lake Co., Fla. Specimens of Cyprinodon rubrofluviatilis Fowler were collected from the Elm Fork of the Red River, 4.5 mi. N of Reed, Greer Co., OK; Prairie Dog Town Fork of the Red River, 7.5 mi. S of Hollis, Harman Co., OK and 10 mi. N of Childress, Childress Co., TX; Oscar Creek, a tributary of the Red River, 7 mi. E of Ryan, Jefferson Co., OK; and the Salt Fork of the Brazos River, 13 mi. N of Aspermont, Stonewall Co., TX. Cyprinodon sp. ("pecosensis") was collected from the Pecos River, 19 mi.

S of Monahans, Ward Co., TX and from an irrigation channel near Grandfalls, Pecos Co., Tx. Specimens of Cyprinodon bovinus Baird and Girard (see Echelle and Miller, 1974) were taken from Diamond Y Spring and Leon Creek, 11 mi. N of Ft. Stockton, Pecos Co., TX. Cyprinodon elegans Baird and Girard was collected from road-side irrigation ditches flowing from San Solomon Springs along U. S. Hwy. 290 between Balmorhea and Toyahvale, Reeves Co., TX. Specimens of Cyprinodon eximius Girard were collected from Alamito Creek, 5 mi. E of Presidio, Presidio Co., TX. Cyprinodon atrorus Miller was collected from an alkalai playa lake (Laguna Grande) about one mile off Federal Hwy. 30, 18 mi. SW of Cuatro Cienegas, Cohuila, Mex. Cyprinodon bifasciatus Miller was also collected in this same area from Pozos de la Becerra, a thermal spring about 15 mi. SW of Cuatro Cienegas. Specimens of Cyprinodon beltrani Alverez came from Lake Chichankanab, Yucatan, Mex.

At each locality, approximately a dozen males and females were taken and transported to either Norman, Okla., Houston, TX, or New Orleans, LA. for processing. In one case, however, specimens of C. bifasciatus were processed at the site of collection due to their unusual sensitivity to thermal change (Miller, 1968).

METHODS

Two techniques were used in this study to obtain chromosome spreads from gill epithelia. In both, the initial treatment of the fish was similar to that of McPhail and Jones (1966). The fish was given a 0.01-0.05 ml injection of 0.05 % colchicine solution in the hypaxial muscle mass and allowed to incubate in aged tap water at room temperature with vigorous aeration for 2-6 hours. After injection and some recovery time, the gill filaments were brushed with a stainless steel probe to moderately disrupt

epithelial cells in order to stimulate mitotic activity. This seemed to produce a higher mitotic index, especially in fish that were in poor physical condition. It was generally found, however, that unhealthy fish produced poor results, even with gill disruption.

After incubation, the fish was killed by pithing. All branchial arches were removed and placed either directly into glass distilled water for hypotonic treatment or first placed in a 0.1 M KCN solution or 20-90 seconds and then into the distilled water. The latter technique presumably "killed" the osmoregulatory mechanism of the cells and allowed more rapid and consistant swelling of the tissue during hypotonic treatment (Stewart and Levin, 1968). The duration of the hypotonic treatment varied from 15 to 45 minutes, depending on when the filaments became swollen at which time they would cling to the surface layer of the distilled water. Following hypotonic treatment, the gills were placed in 3:1 methanol-acetic acid (Carnoy's fixative) for one hour.

Following fixation, two techniques were used to obtain the chromosome spreads. In the first, the gills were transferred to 60 % acetic acid to loosen the epithelial cells. After 3 min., the gills were then gently agitated to slough off swollen cells. After removing the gill arches, the solution was pipetted into a centrifuge tube and hand centrifuged (ca. 700 rpm) for 5 min. The supernatent was removed and fresh Carnoy's added slowly to the button to wash and then recentrifuged for 2-4 min. The supernatent was again removed and approximately 1-2 ml of Carnoy's added making a suspension of cells. This suspension was then dropped on slides (chilled in a methanol-water solution) from an arms length distance above the slide, the height greatly increasing the spread of the cells on impact. The slides were then allowed to dry for 24 hours, avoiding dust.

The second technique used in this analysis follows closely that described by Stock et al. (1972) for mouse testicular tissue. In this study the gills were placed in 0.5 ml of the 60 % acetic acid solution in a deep well slide and rather vigorously agitated, following the initial treatment described above. The cell suspension in the solution surrounding the gills was then removed with a microcapillary pipette (Drommond style). A drop of the cell suspension was placed on a slide warmed on a hot plate to near 60 C and then quickly withdrawn back into the barrel of the pipette so that only a thin circular film remained on the slide surface. This circle contained cells which did not return into the pipette; a process that can be repeated over again so that many such circles are made on a single slide. The slides were then removed from the heat and protected from dust until staining. In both techniques, slides were stained with pinacyanol chloride as described Klinger and Hammond (1971).

To provide chromosome spreads of sufficient clarity and appropriate arm position for Giemsa banding techniques, I used a tissue culture technique similar to that of Chen (1970). Gonadal and occasionally gill tissues were used to produce short term cultures. Gibco's McCoy 5a media (Hsu modification) was used for all cultures, which were supplemented with 20 % fetal bovine serum and incubated at 30 C for 3-5 days. Cultures were harvested as reported by Chen (1970) and slides were made by the arms-distance drop method on slides wetted with distilled water and afterwards left to air-dry. C-bands were produced following the technique of Pardue and Gall (1970) as modified by Arrighi and Hsu (1971). The G-bands were produced either with the trypsin method of Seabright (1971) or the urea treatment of Shiraishi and Yosida (1972). However, neither technique produced definitive bands for comparative purposes.

CHAPTER III

FISH KARYOLOGY:

Some Concepts From Recent Work

Only within the last ten years, has chromosome analysis in fishes become a major tool in their systematics. However, less than 2 % of the teleosts have been karyotyped (Ebling and Chen, 1970). In fact, for this group of vertebrates, the development and utilization of reproducible chromosome techniques have come quite recently. These methods range from the squash technique of McPhail and Jones (1968) to the gonadal tissue culture method of Chen (1970). Susequently, these improved methods have allowed comparative chromosome studies within several groups of fishes: for example, the catostomids (Uyeno and Smith, 1972), cyprinids (Lieppman and Hubbs, 1970), fudulins (Chen, 1971), rivulins (Scheel, 1972), gambusiins (Campos and Hubbs, 1971), gasterosteids (Chen and Reismann, 1973) and percids (Ross, 1973).

With this recent interest in fish karyology, several morphometric features of the teleost chromosome complement, plus cytological evidence of sex determination, have been revealed. The former has led to some nomenclatorial problems with reference to the position of the centromere and changes in that position. The latter, has produced a reevaluation of the previously assumed primitiveness of the fish genome.

From recent studies, two phenomena of chromosome arm rearrangement also seem important in fishes by modifying both the chromosome number and the morphometrics of the karyotype. The first is called centric or

Robertsonian fusion (Robertson, 1916). It entails the fusion of two acrocentrics (terminal centromere elements) into a single element with the centromere near the center, resulting in a biarmed chromosome. This presumably occurs through some type of translocation with the loss of a centromere (White, 1973). Barring further change, the resulting chromosome is a metacentric element if both newly fused arms are similar in length. This phenomenon results in a reduction in chromosome number but does not produce a change in the arm number [Nombre Fundamentale (NF), Matthey, 1964]. Presumably, little chromatin material is disturbed. This process has been described frequently in fishes and is used to explain changes in chromosome number without changes in arm number. Many of the above cited workers discuss this phenomenon as do several workers dealing with the Salmonidae (e.g., Boothroyd, 1959; Simon and Dollar, 1963; Chernenko, 1969).

A second structural change that seems important in fish chromosomal evolution is the pericentric inversion. This alteration can produce metacentric, submetacentric or subtelocentric elements with no resulting change in chromosome number but often with a change in the NF. The latter depends on how one describes the short arm of the last two element types. Again, a comparative survey between closely related fish species often demonstrates a difference in the number of chromosome elements that are submeta- or subtelocentric as opposed to acrocentrics. It is usually presumed that the latter are changed to the former via pericentric inversions. On the other hand, paracentric inversions are difficult to detect in somatic metaphase spreads, and are rarely investigated unless a chromosome marker system is available. Examples of both fusion and inversion phenomena and discussions as to their relation to fish taxonomy are found in many of the previously cited works.

Some evidence of interpopulational variation in the chromosome characteristics described above has been reported in the Atlantic salmon (Salmo salar) by Boothroyd (1959), the rainbow trout (Salmo gairdneri) by Ohno et al. (1965) and the green sunfish (Lepomis cyanellus) by Roberts (1964) and Ohno and Atkin (1966). Interpopulational variation in the karyotype is also suspected in some allopatric populations of Fundulus parvipinnis, F. chrysotus and F. notti (Chen, 1971) and in many cases within the Old World Rivulinae (Scheel, 1972). Post-zygotic intraindividual karyotypic variation has also been observed in the rainbow trout (Ohno et al., 1965) and green sunfish (Becak et al., 1966a). However, due to the general paucity of examined material in fishes, the frequency of occurence of either of these phenomena cannot be determined.

Chromosome Nomenclature

In examining the karyotypic literature in other animal groups besides fishes, it is apparent that the terminology used to describe arm morphometrics and the position of the centromere are without precise definition. For example, the term telocentric has been generally accepted to describe the centromere at the extreme end of the chromosome (i.e., there is no short arm beyond the attachment of the spindle fiber) (Levan et al., 1965). However, many cytologists follow Navashin (1932) who claimed that no truly one-armed chromosomes existed in nature, that is, no centromere can be located terminally. White (1973) has been the most vociferous in promoting this idea and has introduced the term acrocentric (in lieu of telocentric) for chromosomes in nature where the centromere appears to be at the tip. However, a second arm is always assumed no matter if invisible to the observer. If a second arm is visible, the chromosome then becomes a sub-

acrocentric. White also coined the term metacentric in which the centromere is near the center of the chromosome dividing it approximately into two halves. These terms, in addition to the submetacentric condition, have gained wide acceptance in animal karyology. But again, there is little standardization in determining those positions, thus, reducing the comparative value of karyotypic studies by different workers.

Levan, Fredge and Sandberg (1965) reviewed the lack of standardization in chromosome nomenclature and introduced a new terminology that is based on measured values for determining centromeric regions. These authors also took exception to the idea of the non-terminal centromere. Similar arguments can be found in John and Lewis (1968) and DuPraw (1970). Therefore, telocentric chromosomes are held to be a reality by these authors but the term acrocentric is retained when a "short" arm is visible beyond the centromere. However, the terminology of Levan et al. (1965) demands adequate resolution of the chromosome elements to accurately delimit the centromeric region and rather straight arms to determine ratios. To implement their system, these authors present two separate calculations: 1) the difference between long (l) and short (s) arms (d=l-s) or 2) the ratio of short to long arm (r=l/s). Using these measurements, they have divided the chromosome into four equal sized regions: the median, submedian, subterminal and terminal with d values of 0.0-2.5, 2.5-5.0, 5.0-7.5, 7.5-10 or r values of 1.0-1.7, 1.7-3.0, 3.0-7.0, 7.0-infinity, respectively.

For fish material, the clarity of figures needed for this type of analysis is rarely realized. And even when it is, most workers are content with direct observation for their determinations of chromosome nomenclature. However, exception are found in Chen and Ruddle (1970) and later Chen (1971). These authors introduced additional terminology for discussing total chro-

mosome lengths and to differentiate acrocentrics between several species of Fundulus. They retained the common metacentric, submetacentric and acrocentric nomenclature but classified "normal" sized chromosomes as "fundamental" (F) while those that were twice the length of the fundamental elements as "long" (L). the L-types were always metacentric or nearly so and were assumed to be the result of Robertsonion fusion of two rather equal sized F-type acorcentrics. Those species which possessed L chromosomes always had less than 2n = 48, the most common diploid number in the genus. Additionally, there was not an attempt by those authors to delimit a subacrocentric region in the Fundulus material, but rather acrocentrics were classified as either short short-armed (SSA) or long short-armed (LSA). A SSA was determined when the ratio of the long are length to total chromosome length was more than 0.8, a LSA when it was less. This is complementary to the centromeric index of the Denver report (1960) when the short arm is the one measured. However, the choice of this particular break point (0.8) is not sufficiently explained. Furthermore, the lack of clarity in many of their figures does not lend itself to such preciseness.

Levan et al. (1965) have presented an abbreviated nomenclature from their original four arm regions that I have found usefull in this paper. This includes combined median-submedian (msm) regions and subterminal-terminal (sst) regions but also includes a median point (d value = 0, r value = 0) and a strictly terminal point (d value = 10, r value = infinity).

Sex Chromosomes

Extensive studies on sex determination in fishes have been conducted chiefly within the cyprinodonts Xiphophorus (Gordon, 1954), Rivulus (Harrington, 1971) and Oryzias (Yamamoto, 1969). From these and other related works, most teleosts appear to be gonochorist and exhibit sexual

dimorphism, although protogynous and protandry hermaphroditism and even gynogensis are exhibited (Atz, 1964). However, the majority of species are monoecious. Furthermore, as the separation of sexes is generally considered advantageous to the process of adaptation (Alston, 1967), and if heteromorphic sex chromosome pairs insure that dimorphism (Ohno, 1967), it follows, and is in fact the case, that the diverse teleosts should show an equal diversity of cytological sex-determining mechanisms (Ebeling and Chen, 1970). Detailed discussions of the occurrence and evolution of sex chromosomes in fishes and vertebrates in general are given by Ohno (1967) and Mittwoch (1967).

The teleost groups enjoying the most intensive work on sex determination have been the cyprinodontids and the poeciliids, as cited above. In these fishes, sex determining mechanisms may vary both within a species, such as in Xiphophorus (Gordon, 1954; Kallman, 1965) and Poeciliopsis (Schultz, 1961) or between closely related species and genera, such as Fundulus (Chen and Ebeling, 1968) and certain other cyprinodontids (Uyeno and Miller, 1971). From the observed variability in those fishes, Ebeling and Chen (1970) made the following summary observations: 1) the differentiation of sex chromosomes in many fishes may be concealed; 2) the members of a sex pair may in fact by largely homologous, even though crossing-over is restricted; 3) relatively few genes may determine sex in some gonochorists and 4) both the internal and external environment can drastically alter sexual expression.

Chromosomal heterogamety has been demonstrated in several deep-sea fishes (Chen, 1969) but seems rare in most fresh water groups. Within the Atheriniformes (following Rosen, 1964), Chen and Ebeling (1968) have reported the occurrence of female heterogamety (ZZ-ZW) in the mosquito fish, Gambusia

affinis and Chen and Ruddle (1969) have confirmed male heterogamety (XX-XY) in Fundulus diaphinus and F. parvipinnis. In both studies, however, the reduced clarity of their figures raises some doubt as to the distinctness of the heteromorphic pairs. Campos and Hubbs (1971), in examining six species of Gambusia (only females reported) found heteromorphic chromosomes (a single metacentric element) in only three of the species examined. Those three species are more closely related to each other than to G. affinis and the three species that did not show heteromorphic chromosomes. Therefore, if these heteromorphic elements are sex chromosomes in Gambusia, the metacentric element has evolved at least twice or become acrocentric twice through inversion. Additionally, the marked difference between species in size of the metacentric suggests that these are not homologous elements.

There are two known species with heteromorphic sex elements within the subfamily Cyprinodontinae. The monotypic genus Megupsilon (Miller and Walters, 1972), a close relative of Cyprinodon, apparantly possesses multiple sex chromosomes rendering it unique among fishes (Uyeno and Miller, 1971). The female of this species has a diploid number of 48 with a pair of metacentrics, 18 pairs of submeta-subtelocentrics and 5 pairs of acrocentrics. The male has but 47 chromosomes including the metacentric pair, 16 pairs of submeta-subtelocentrics but only 4 pairs of acrocentrics. Additionally, the male contains an unpaired metacentric element that is three to five times longer than any other chromosome, Presumably, the karyotype of the female has 44 autosomes plus $X_1X_1X_2X_2$ acrocentric sex chromosomes, whereas the male has 44 autosomes and X_1X_2 acrocentric sex chromosomes plus the large element designated a Y. The origin of this karyotype is postulated from a primitive (covert) X_1X_1-X_1Y system where an autosome fused with the primitive acrocentric Y to form the oversized metacentric element. The

homolog of the fused autosome, by definition (Matthey, 1965) became an additional sex chromosome, X_2. These authors also demonstrated the presence of a very large trivalent amon 22 bivalents in spermatogonial diakinesis. The latter seems to indicate translocation phenomena with genetic similarity between the three paired elements.

In a similar study, Levin and Foster (1972) examined the karyotypes of two additional cyprinodontids, Jordanella floridae and Garmanella pulchra, where a second case of multiple sex chromosomes was discovered in the latter species. Again, the female had 2n = 48 while the male only 47. However, the presumed X_1X_2Y chromosome in the male was not grossly out of proportion to the remaining elements, yet was clearly a trivalent in male diakinesis. The oversized nature of that chromosome in Megupsilon seems to suggest additional DNA, possibly via a saltatory addition of repeated DNA sequences along the chromosome length.

In the genus Cyprinodon there has been no published material indicating the presence of sex chromosomes, while in fact there have been no published accounts of any pupfish karyotypes save that of C. tularosa (Miller and Echelle, 1975).

Chromosome Number and DNA

Many authors have concluded that the so-called basic or generalized teleostean karyotype contains a diploid number of 48 one-armed (acrocentric) chromosomes (NF = 48) (Post, 1965; Roberts, 1967; Chen, 1969; Ohno et al., 1968). This particular count and configuration occurs in species from widely diverse and orders and classes (e.g., the hagfish, Eptatretus stoutii (Agnatha), the flunder, Pleuronichthys verticalis) with many representatives in groups in between these two extremes of fish phylogeny

(Ohno, 1970). At the same time, the DNA content of these "basic" karyotypes varies widely with the jawless hagfish having 78 % of the cellular DNA of mammals while the flounder has but 20 %. This latter value is also found in several cypriniform and atheriniform groups and is the lowest value encountered in fish material thus far examined.

It is this same variability of chromosome number and DNA value within many fish taxa that is striking. Within the Agnatha, the lampreys of the order Petromyzoniformes are reported to have 94-96 minute chromosomes (Nogusa, 1960) and DNA values of 38 % that of mammals (Atkin and Ohno, 1967). On the other hand, the hagfish has diploid count of 48 but with approximately 78 % of mammalian DNA values (Ohno, 1970). Regarding other non-teleosts, data are available for the shovelnose sturgeon, Scaphirhynchus platyrhynchus (a chondrostean) which has 2n = 112 elements, with nearly half being microchromosomes, and a DNA level near 50 % that of mammalian values (Ohno et al., 1969b). These same authors also found micro-elements in the holocephalan ratfish (Hydrolagus colliei) and the holostean spotted gar (Lepisosteus oculatus) with 2n = 58 and 68, respectively. Another holostean, the bowfin (Amia calva), had no microchromosomes with 2n = 46. The values found in the former species were considered relictual by those authors and representative of those possibly found in ancient crossopterygian progenitors of terrestrial vertebrates. On the other hand, a modern crossopterygian, the South American lungfish (Lepidosiren) has a diploid number of only 2n = 38, with no micro-elements, but holds the current record for DNA values having 3,540 % that of mammals (Ohno and Atkin, 1966).

As with the Agnatha, considerable variability exists within the Salmonidae, a group considered to be primitive among teleosts. Chromosome numbers range from 2n = 52-84. The higher counts occur within Coregonus

and Prosopium (Booke, 1968), while the lower values occur in Oncorhynchus (Simon, 1963) and Salmo (Simon and Dollar, 1963; Svardson, 1945). The latter two genera show a great deal of variation between species, however. Although salmonid species exhibit extensive NF variation, presumably through arm rearrangements and fusion, they all have high DNA values (80-90 % that found in placental mammals). Despite wide variability among primitive and advanced fishes as demonstrated above, the basic chromosome number of 2n = 48 and a DNA value 20 % that of mammals are held to be ancestral (Ohno, 1970).

Where they have been summerized, teleost chromosome numbers presently range from 2n = 18 to 2n = 120. The former value is found in two central African rivulins (Cyprinodontidae), Aphyosemion christyi and Nothobranchius rachovi (Scheel, 1972), the latter in Corydoras aeneus, a South American smooth armored catfish (Heingarden and Rosen, 1972). Additionally, chromosome numbers of 2n = 104 are found in the carp (Cyprinus carpio) and goldfish (Crassius auratus) (Ohno et al., 1967). For other teleost groups, recent summeries of chromosome numbers can be found in Gyldenholm and Scheel (1971) for temperate and tropical freshwater species and in Denton (1973) for most of the teleosts examined. Summaries for DNA values in fishes can be found in Hinegardner and Rosen (1972).

In the two rivuline species mentioned above, the karyotypes are made up entirely of metacentrics or nearly so. However, other closely related rivuline species vary widely in their chromosome configurations and element numbers (Scheel, 1972). No DNA values are known specifically for any of the rivuline forms. Likewise, the karyotypes of the carp and goldfish are very similar with one-third of the elements metacentric, the remainder being terminal or sub-terminal. However, in this case, the DNA values for both

species approximate 50 % that of mammalian values (Ohno et al., 1967). These two species are considered by those authors to be in the tetraploid state in relation to other members of the Cyprinidae where diploid numbers are most frequently near 50-52 (Post, 1965; Lieppman and Hubbs, 1969) and DNA values 20-22 % that of mammalian values. Additionally, the barb (Barbus barbus) has 2n = 100, with a DNA value of 50 % that of placental mammals and is also considered a tetraploid species (Ohno, 1970). The ploidy condition of Corydoras is not known due to lack of comparative material.

Cases of polyploidy are suspected in other fish groups; most notably the salmonids, where may species have 60-80 chromosomes and DNA values of 80-90 % that of mammals. These species are considered to be in the tetra- or octoploid state in relation to other more primitive clupeiform species. For example, several of the presumably more primitive salmonoids (e.g., smelts in the family Osmeridae) have chromosome numbers around 50 and with low arm numbers (48-60) (Ohno et al., 1969a). These values are also found among some herring species (genus Clupea) giving some support to the idea of polyploidy if salmonids are derived from primitive clupeids. In addition, the putative tetraploid salmonids possess arm numbers up to 104, and while some have reduced diploid numbers, they retain high DNA amounts. These factors seem to suggest a series of inversions and/or Robertsonian fusions following polyploidization (Ohno et al., 1969a). These latter phenomena are thought to be part of the process of diploidization of the karyotype as discussed by Ohno (1970). Polyploidy is also believed to have occurred in the evolution of the suckers (Catostomidae) from ancestral cyprinids (Uyeno and Smith, 1971). These authors, in surveying 14 species of eight genera, found a near uniformity of 2n = 100 chromosomes. When measuring

DNA values, they found nearly a doubling of that present in most cyprinids. These data suggest that the ancestral cyprinid which gave rise to the suckers may have been in the direct evolutionary line of the modern day carp and goldfish.

The cases of polyploidization in fishes mentioned above, plus those for several kinds of frogs (Becak et al., 1966b; Wasserman, 1970) are the only known examples of such phenomena in naturally occuring bisexaul vertebrates (Schultz, 1969). These particular species of fishes and frogs lack well differentiated sex chromosomes and this seems to be a prerequisite for the occurance of polyploidy (Muller, 1925; White, 1946).

Aside from the presumed polyploid groups, the modal number of 2n = 48 is apparent and equally prominent in both the soft and spiny-rayed groups of teleosts. However, variability is dramatically increased in the Cyprinodontidae, especially among the Rivulinae (Scheel, 1972). Variation in DNA values has been discussed only superficially in cyprinodontids, compared to other fish taxa, and reflects the lack of material examined in this group. However, in the few species observed, Heingardner and Rosen (1972) demonstrated a two-fold increase in DNA in the family Cyprinodontidae as a whole when compared to the poeciliids and many perciform groups, but with equivalent values to most tropical cyprinids, characins and atherinids.

With regard to the extant variability in chromosome numbers and DNA values in most fish groups, Muramota et al. (1968) have proposed three mechanisms that may have contributed to the diversification in DNA content and karyotypes in fishes: 1) Increase in DNA by regional duplication of chromosome segments without changing the original karyotype (tandem duplication). This process seems evident when comparing the green sunfish (Lepomis cyanellus) of the order Perciformes, the anchovy (Engraulis mordax)

of the Clupeiformes and the hagfish (Eptatretus stouti) which all have a diploid complement of 48 acrocentrics but have DNA values of 30 %, 40 % and 80 % that of mammals, respectively; 2) Karyotypic changes with or without an increase in DNA content by tandem duplication. This phenomenon seems to be occuring most frequently in teleost karyotypes as cited previously; 3) Polyploidization, as described above.

The phenomenon of tandem duplication seems to have been the cause behind the enormous increase in lungfish DNA values as the diploid number of chromosomes in this species is rather low (Ohno, 1970). But tandem duplication is not considered to be important in the creation of dynamic genomes that produce novel phenotypes. It rather seems to lead to increased genome stability, as only a few segments are extensively duplicated, and also to an evolutionary dead end. Both phenomena are evidenced by the lack of phenotypic change in the lungfish since the Devonian (Ohno, 1970). Polyploidization, on the other hand, duplicates the whole genome and allows previously forbidden mutations to occur, creating new gene combinations that could be adaptive. Ohno (1970) has greatly elaborated on these processes and their importance in vertebrate evolution.

Several recent articles have reviewed the variability in DNA values found within and between various higher taxa of fishes. Hinegardner (1968) demonstrated that highly specialized fishes (those sharing fewer characteristics with other members of their taxa) tend to have less DNA per cell than do more generalized or less evolved fishes of the same phyletic grouping. It was assumed, in a more elaborate paper by Hinegardner and Rosen (1972), that evolution is moving toward specialization and is concomitant with a loss of parts in fishes (e.g., reductions in separate hyoid elements, gill arches, vertebrae, skull bones, fin rays, etc.). This process of

reduction has been described as a general pattern toward specialization in the evolutionary history of most animals by Rensch (1959). Also, there appears a decline in DNA content per cell from primitive to advanced fishes (Hinegardner and Rosen, 1972). This is in contrast to other living organisms where usually Dna values have increased toward the higher forms (e.g., fish to mammals). However, in observing variability, as related to the mode of specialization or habitat, ther cannot be clearly demonstrated an overall DNA loss or gain between higher fish taxa. But rather, there appears a great deal of variability within those taxa.

An excellent example of the above was observed by Ebeling et al. (1971). These authors compared DNA values between two marine fish groups containing shallow water, mesopelagic and bathypelagic species. They considered the first of those environments to be the more diverse and productive while the latter was thought to be more stable but less productive. DNA amounts quadrupled going from shallow-marine to deep ocean species within the primitive order Paracanthopterygia. Here, this phenomenon was proposed to have occurred via "saltatory replications" (large and rapid tandem duplications) in the large two-armed chromosomes found in the karyotypes of several of the deep-water forms. These large chromosomes in turn became repositories of large linkage groups (supergenes) that possibly contained replicate sets of genes securing evolutionarily stable specializations that would be inherited en bloc. However, in the second group of more advanced fishes, those inhabiting the bathypelagic zone had smaller amounts of DNA when compared to their shallow water relatives, but all had more DNA than the shallow water, more primitive paracanthoptergians. The advanced fish contained no large chromosome elements but rather had generalized karyotypes. These data suggested, to those authors, that the

amount of DNA in fishes may more closely reflect the age of the phyletic line and/or the stability of the environment. Also, there might be a natural tendency to continuously increase DNA in all organisms that is countered by selective pressures, which in this case may possibly be less in the deep sea or other very stable environments.

CHAPTER IV

RESULTS

Among the eleven species of Cyprinodon that I studied, the chromosome number was consistently 2n = 48 with a chromosome arm number (NF) of 50. The latter figure being the case as I maintain only the median centromere elements to have two arms. These same values were also found in seven additional Cyprinodon species from preparations done by T. Uyeno and provided my by R. R. Miller. An idiogram representing the basic pupfish karyotype is presented in Figure 1 of the Appendix with the karyograms for each species examined in this study following thereafter (Fig. 2-23). The nomenclature used in describing these karyotypes follows the abbreviated system of Levan et al. (1964) and is also presented in Figure 1 along with the morphometric calculations used to define the chromosome element categories.

The generalized pupfish karyotype can be defined as consisting of a single pair of median chromosomes, a graded series of 16 pairs of subterminal chromosomes and seven pairs of marginally submedian chromosomes. There seem to be no strictly terminal centromere elements (acrocentrics) in pupfishes where chromosome resolution is adequate (see Appendix Fig. 7). Secondary constrictions and satellites are lacking throughout the genus. Additionally, intraspecific differences were absent among three isolated stream populations of C. rubrofluviatilis and between the three nominal subspecies of C. variegatus. These findings are commensurate with the data of Uyeno (R. R. Miller, pers. comm.). No karyotypic differences were

demonstrable between any of the 18 nominal and undescribed species or species populations of Cyprinodon examined. Therefore, the pupfish karyotype is considered extremely conservative when compared to other cyprinodontids examined, such as Fundulus (Chen, 1970) or Rivulinae (Scheel, 1972).

The presence of heteromorphic sex elements in Cyprinodon was first suspected by Uyeno and Miller (pers. comm.) in the Armagosa pupfish C. n. nevadensis. These authors found the female of this species to have only one distinctly metacentric chromosome, its homolog being either a submedian or subterminal element. This condition does not exist in the populations I examined. Also, no univalents nor multivalents are evident and pairing among the 24 bivalents appears normal in all examined material (Appendix Fig. 19). If there are sex chromosomes in this karyotype, they are covert and have not differentiated to any appreciable degree. This holds for all other pupfish karyotypes examined.

To further elucidate chromsome morphology, C and G arm banding methods were employed. The results of those studies are demonstrated in Fig. 24 and 25 of the Appendix. Figure 24 represents a metaphase spread from an ovarian culture of C. bovinus in which C-banding was attempted. The dark staining areas are suspected to be regions of constituitive heterochromatin composed of highly repeated base sequences. It is also assumed that those areas are genetically inert (Arrighi and Hsu, 1971); and for this species of Cyprinodon, they appear to be mainly centromeric. These data have dual significance. First, heterochromatin appears to be sparse and localized in these fishes. Second, the short arms beyond the centromere are euchromatic and presumably carry functional genes.

Figure 25 represents an attempt to G-band chromosomes from cultured ovarian cells of C. bovinus. The lower spreads demonstrate the appearance

of some bands across the chromatid arms, while the assembled karyogram is an attempt to pair homologs (i.e., those with identical banding patterns).

CHAPTER V

DISCUSSION

The identical nature of the karyotypes observed among the species of Cyprinodon analysed in this study is unique among the killifish forms thus far examined. For example, Chen's (1971) study of twenty species of Fundulus revealed rather elaborate karyotypic differences between presumed closely related species.

Within that genus, diploid chromosome numbers ranged from 36 to 48 although arm numbers remained fairly constant at 48-52. However, those species with $2n < 48$ always had a pair or several pairs of large metacentric elements that were presumably derived from Robertsonian rearrangements of available subterminal elements. Thus, no drastic reorganization of genetic material had taken place. The nature of those changes led Chen to conclude that recent species of Fundulus comprise a natural karyotypic group. He also described differences between species in the length of the short-arm in acrocentrics. The longer condition was presumably derived from the shorter via pericentric inversions. Therefore, he envisioned a continual reduction of chromosome numbers in Fundulus, derived through centric fussions, from a primitive karyotype of $2n = 48$ terminal elements (as found in F. kansae). At the same time, pericentric inversions produced differences between species in the number of acrocentrics with long or short short-arms.

On the other hand, most of the Fundulus species had combinations of both primitive and derived chromosome types with enough similarity to

produce five karyotypic groups. This resulting classification of Chen's resembled more that of Brown (1957), which is based on gross morphology, than those of other authors (Miller, 1955; Farris, 1968). The karyotypic trends he described indicated a prolific species specific variability and a considerable potential for the adpative radiation of Fundulus karyotypes. Those differences presently observed may, in fact, account for the large number of superficially similar species inhabiting a variety of habitats found in that genus.

The second group of cyprinodontids that has been comparatively studied is the African Rivulinae (Scheel, 1972). These forms are also at variance with the karyotypic conservancy of pupfishes, especially within the genera Aphyosemion and Aplocheilus. Unfortunately, there is lack of taxonomic stability within and between species of these two genera, and in fact in most other rivuline groups as well. Meristic and morphometric characters are not especially diagnostic in rivulins while color patterns and habitat affinities seem to be. However, a great deal of color polymorphism and sibling species formation occur along with allopatric isolation between many of the annual species. Scheel (1972) found a complex situation of several color "phenotypes" that were similar with very different karyotypes and vice versa. With a rather complex set of karyotypic characters, Scheel did determine species or population specific distinctness using element and arm number, the ratio of long to short arms and "markers" such as large two-armed elements, oversized terminally centric and small "symmetrical" (metacentric) elements. However, he apparently found it difficult to generalize on these data to determine relationships, but rather chose to hypothesize a basic rivuline karyotype and subsequent evolution of complements from it. He had to presume a great deal of parallelism in some species and divergence in others. Scheel also inferred that centric

fusion and pericentric inversion were the principle methods of karyotypic change in rivulins but he proposed mechanisms of those changes opposite to those of Chen (1971) for the Fundulus material. That is, Scheel presumed, citing no evidence, that a pericentric inversion becomes irreversable if the centromere is moved terminally (i.e., it cannot be moved to a more median condition again). He further postulated that the karyotype with the maximum number of arms (all with median or sumbedian centromeres and n = 25-27) was the basic rivuline condition and designated it to be "symmetrical" in composition. From this basic type of many biarmed elements, he envisioned several "generations" of karyotypes where pericentric inversions would produce chromosomes with terminal centromeres. These later would fuse into large metacentrics that in turn could go through the centromeric shift and become overly large acrocentrics producing "asymmetrical" karyotypes. He found all levels of these "karyotypic generations" in his rivuline material.

The basis for much of Scheel's discussion comes from the ideas of Stebbins (1958), who discussed the evolution from symmetrical to asymmetical karyotypes as a method of increasing gene linkage. Stebbins proposed three types of karyotypic change leading to that condition: 1) reduction of the haploid number via fusion; 2) change from a median to a subterminal centromere position via pericentric inversion; 3) increase the size difference between members of the same complement. All these phenomena in turn would reduce crossing over and thus recombination. These changes would also convert a symmetrical karyotype, with the chromosomes all metacentric and about the same size, into an asymmetrical one with the chromosomes having terminal and subterminal centromeres and a wide range in element size. The end result would be to enhance adaptability to ex-

tremes of habitat by achieving temporary genetic constancy. The underlying assumption of this process is that if organisms find themselves in new and unstable conditions, certain gene combinations in the heterozygous condition may have a high selective value for adaptive adjustment. If structural changes occur that bring these genes closer together (increasing linkage) the proportion of gametes and zygotes containing these combinations will increase. Once this gene combination has become established, further adjustment could come about more easily through modification of this combination rather than building new ones on different chromosomes. In this way, progressively increasing asymmetry is explainable. Self-fertilization and apomixis are means to the same end and are found in fishes as well as plants (Atz, 1964; Harrington, 1971).

Scheel (1972) did not use the evidence of correlating asymmetry to habitat as he apparently did not have access to Stebbins' entire paper and thus those particular ideas. I believe he could have found such a correlation with annualism and isolation, two overt phenomena in rivulins.

The key to the acceptance of this particular scheme of karyotypic events, as proposed by Stebbins and Scheel, lies in determining the mechanism of the pericentric inversion. White (1973, p. 215) argues that "in the case of truly acrocentric chromosomes with very minute short arms, breaks through the latter will necessarily be very rare and pericentric inversions correspondingly infrequent". White also does not subscribe to the existence of true terminal centromeres. Acceptance of that notion would lend greater weight to the idea of halting further pericentric inversions at that stage. In telecentrics, the centromere itself would become a telomere and the "raw end" created in the longer arm would, in a subsequent break, remain as such having no short-arm break to reattach

to. If truly terminal centromeres exist, those data would give support to the irreversibility of the telocentric through pericentric inversion. That in turn strengthens the hypothesis of increasing asymmetry in karyotypes, or at least lessens the alternatives in moving toward that condition. However, in dealing with the present situation, do these novel hypothesis of karyotypic evolution relate to the problem of cyprinodont relationships?

Sethi (1960, p. 208), based on an extensive osteological study of cyprinodontids, concluded "that the aplochelids (rivulins of Scheel) or a similar group seemed to represent the ancestral stock from which all other major groups of both oviparus and viviparous cyprinodont fishes originated... Aplocheilus (in Africa and Asia) and Rivulus (in Central and South America) appear to be the most generalized aplocheilids. Except for the cyprinodontids (subfamily Cyprinodontinae), which probably originated from fundulids (Fundulinae), all other groups of oviparous cyprinodonts have evolved from the aplocheilids or a stock similar to them". In examining karyotypes of several aplocheilids, Scheel (1972) observed that many were similar in element and arm number with differences found in centromere position only. However, other species in the subfamily and within the same genus varied widely in number and size of elements. On the other hand, these differences could be easily derived from his generalized karyotype with n = 24-25 with a maximum number of arms; a configuration that occurs in many rivulins and other primitive atheriniforms. The cycle of pericentric inversion and fusion previously described could be responsible for the observed modifications in the karyotypes. This seems plausible in describing the rather widely divergent complement patterns found in the Rivulinae, if not most of the Atheriniformes. The contribution to the

speciation process by these events, however, may or may not be causal.

On the other hand, Chen (1971) assumed 2n = 48 acrocentrics (NF = 48) to be the primitive state in Fundulus, which by Scheel's definition is an asymmetrical (advanced) karyotype to begin with. In Fundulus, any increase in the NF would be derived, which is toward the symmetrical (primitive) type of Scheel. There seems to be no doubt that the enlarged biarmed elements in Fundulus have been derived through Robertsonian changes from terminal or subterminal chromosomes which is in accord with the Scheel-Stebbins model. This process would produce and/or amplify differences between large and small chromosomes and thus increase linkage within the large biarmed elements. Following Scheel, however, the short short-armed chromosomes would have to be assumed to be derived from the long short-arm ones via pericentric inversions if the problems encountered with the terminal centromere are real. Chen assumed the reverse. How much chromatin is actually needed at the terminal end to allow an inversion and how much is really there in a karyotype such as that found in Fundulus kansae are the obvious questions to be resolved.

That fundulins have undergone a rather prolific sequence of karyotypic changes in their evolution, however, is not disputed. Although, some of the salient arm length differences noted by Chen may be more apparent than real. Species in this genus are found in many different habitat types (e.g., lentic and lotic fresh water, brackish intertidal areas) and karyotypic differentiation may account for this.

This leads to the situation found in Cyprinodon. Here, there is also a proliferation of morphodemes that now live in a variety of environments. At the same time, they are extremely conservative in their karyotypes. However, the level of diversity seems important in comparing groups. The

fundulins have been subdivided into several subgenera by Brown (1957) based on external morphology. This has not been done in Cyprinodon. Furthermore, Drewry (1967) placed the genus Adenia into Fundulus but removed the sibling species Fundulus notatus and F. olivaceus into the genus Zygonectes. Thus pointing out that close genetic affinities between many of the species in Fundulus are not assumed. Unfortunately, the actual state of affairs in the rivulins, as mentioned above, is largely undetermined, but that they are multifarious is not disputed. On the other hand, Cyprinodon shows considerable cohesiveness in morphological, behavioral and genetic characters and the chromosome data presented herein adds karyotypic characters to that assembledge. Moreover, within the pupfishes, deviants from the narrow phenotype that distinguishes Cyprinodon (i.e., usually two mandibular pores, dark terminal bar on the caudal fin in males, enlarged scapular scale) have been placed into separate monotypic genera (Jordanella, Garmanella, Megupsilon and Cualac). Thus, the level of divergence used in classifying the pupfishes appears different from that used in Fundulus. In the latter, differences are viewed conservatively in retaining all forms within a single genus, while in the former they are accentuated to describe the distinct morphodemes as species in the first place and to erect new genera. Also, the frequency of allopatry between the nominal forms of Cyprinodon is of major concern, while it is not so extreme in Fundulus, making species boundaries even more arbitrary in the former than the latter.

Still, the fact that pupfishes (considering only the genus Cyprinodon) are comparatively conservative in their genetic and morphological constitutions is problematic. Base on his analysis of morphological characters, Miller (1950) proposed that the western pupfishes have undergone rapid and

extensive evolution since the Pleistocene. Liu's (1966) behavioral data also separated the existing taxa but chiefly in delimiting C. macularius from the other "western forms" and placing it close to the "eastern forms". His hybridization data, however, implied close relationships.

Most of the morphological data on Cyprinodon suggest a genetic divergence commensurate with the ecological differences among the habitats encountered. These, in turn, would imply different selective forces in operation. Also, stochastic processess usually associated with small and/or markedly fluctuating populations sizes, as are often found in pupfishes, should have enhanced this genetic divergence. However, the electrophoretic data presented by Turner (1974) do not support that prediction in the western forms he analysed.

It has been noted that morphological data presented from most fishes have an ecophenetic component that may be of some magnitude in those species of pupfishes examined by previous workers (Turner, 1973). However, electrophoretic data are biased also when utilizing enzyme loci that are considered to be involved exclusiely in intermediary metabolism. While realizing this, Turner (1974) proposed that there were two groups of genetic factors evolving independently in pupfishes. One group of genes seems to have been responsive to environmental and/or stochastic selective factors (e.g., the genes encoding morphological and behavioral traits) while the other group (metabolic control genes) have not. Further, Turner felt that coadaptation among the enzyme controlling genes may be important to physiological pathways and that conservancy may be demanded in adapting to large and frequent fluctuations in critical environmental parameters. He also postulated that this coadapted "metabolic core" of genes may have arisen in an ancestor originally adapted to such a fluctuating environment.

Additionally, when stable habitats were encountered, there may have been minimal selective pressure to substitute new allels. This idea is supported by Brown and Feldmeth (1971) who found that pupfish from stable environments (e.g., C. diabolis) and those from variable ones (C. salinus) had essentially the same broad thermal tolerance and ability to acclimate. This was true despite their separation since immediately post-Pleistocene.

The chromosomal data presented herein can be interpreted similarly to the genetic factors of Turner (1974) as karyotypes seem to be invariable between species. A karyotype presumably could have developed in some ancestral Cyprinodon stock (e.g., C. macularius or a close relative as suggested by Miller, 1948 and Turner, 1974) and, in fact, may well have determined the genetic conservancy. That is, certain gene combinations may be arranged en bloc through the various arm configurations that allowed survival in a fluctuating environment wherein karyotypic novelty would have been selected against. It is then also assumed that chromosome change has not been particularly selected for even when more stable habitats became available. That karyotype is hypothesized to be extant in modern pupfishes.

An additional factor, with regard to habitat, is a phenomenon occuring in stream dwelling Cyprinodon, where other fish species are present. Here, pupfishes tend to become peripherally located or disjunct from the other resident piscene populations. The former often thrive in the most harsh habitats available while the remaining fish species are presumably less adapted to these areas and do not successfully invade them. Echelle et al. (1972) have described this phenomenon in the Red River pupfish, C. rubrofluviatilis. This species seems adapted to a rather "generalized" ecological niche allowing it to exist in habitats of large thermal and

salinity fluctuations. On the other hand, in more equable environments, it appears competitively inferior to other fresh water forms that are more "specialized" or better adapted to exist in richer faunas. These ideas seem probable as Martin (1972) found that water quality parameters were not limiting the distribution of the Cyprinodon species he examined. Furthermore, he felt that their exclusion from fresh water was through unsuccessful competition for some resource(s) with primary fresh water fish, especially centrarchids. Additionally, predation did not seem to be a factor in excluding C. variegatus from fresh water as this species coexists with predatory marine species. From these data then, there appears an inverse relationship with regard to genes versus the environment. That is, the fixation of certain allels and a presumed lack of karyotypic change in maintaining those gene combinations is required in those species that are broadly adapted to extreme environments. Pupfish seem to be in the most extreme habitats occupied by fishes (Echelle et al., 1972). These adaptations in turn often remove those endowed species from direct competition with other forms.

Inferences from these data suggest that possibly the ancestral species of Cyprinodon did live or in fact evolved in harsh, fluctuating habitats. Therefore, the notion of karyotypic asymmetry being adaptable to rather unstable environments (Stebbins, 1958) might also apply here. There are but two metacentric (symmetrical) chromosomes present in the pupfish karyotype while the remaining elements are a graded series from the subterminal to the submedian condition. In that regard, the observed karyotype in pupfishes is not completely asymmetrical but does tend toward that direction.

The origin of the pupfish karyotype is unknown. The closely related forms placed in monotypic genera have similar if not identical karyotypes

to Cyprinodon except for the presence of the presumed sex elements. Their evolution is obviously from a Cyprinodon-like ancestor. There are also several species of Fundulus with similar karyotypes to pupfishes; the most similar being F. olivaceus and F. diaphanus, two forms not particularly closely related between themselves and certainly not to pupfishes directly. The point here is that there exists chromosome parallelism throughout the killifishes and the ancestral versus derived condition between higher taxa is obscure. Within Cyprinodon, single or small groups of mutations, rather than gross karyotypic changes, seem to be the differentiating factors and those presumably are outside the "metabolic core" gene segments.

CHAPTER VI

CONCLUSIONS

The pupfish genus Cyprinodon is one of the few North American groups of fishes that have most of its species allopatrically distributed. While the described forms are morphologically distinct, the level of differentiation between species is often somewhat arbitrarily defined.

In considering the described and un-named pupfish populations east of the Continental Divide, one is struck first with the many variations on a single morphological theme and secondly by the lack of comparative taxonomic treatments between these geographically placed species. However, by using Liu's (1968) behavioral groups, one can organize these several Cyprinodon forms into similar morphological and behavioral units that are coincidental with their distributional patterns. The purpose of this study was to determine if there was a common evolutionary history among those groupings that could be further elucidated by cytological data.

Liu (1968) attempted to deal with genetic aspects of relationship in his hybridization studies but found that all forms tested would hybridize to produce F_1's. However, F_1 fertility was not ascertained in most cases. Drewry (1967) found reduced fertility in laboratory produced hybrids between Cyprinodon elegans and an undescribed species refered to as C. bovinus while Stevenson and Buchanan (1972) found male sterility in a natural hybridization system between C. elegans and C. variegatus. However, data were minimal in both cases. In summary, these studies seem to reveal a

high level of interfertility between species that is, in turn, coupled with a low degree of species behavioral descrimination resulting in assumed close genetic relationships. In addition, Turner's (1974) electrophoretic analysis of proteins suggested very little genetic divergence between the various species of Death Valley pupfishes. His results demonstrated the highest degree of allelic homogeneity found in any vertebrate group thus far examined. However, those results are based chiefly on digestive enzymes, which are conservative in most organisms. Nevertheless, the data seem to demonstrate a paradox between the morphological data, which indicate differentiation between species and the hybridization and enzyme data that suggest little genetic divergence.

The karyological data presented herein reflect the situation found by Turner (1974) as the karyotypes at the level examined here show no variation within and between species. The cytological incongruencies suggested by Stevenson and Buchanan (1972) do not seem to exist. Thus, the reduction in fertility where observed between species of Cyprinodon is genetic, not cytological. And it only appears to occur between forms that are widely divergent in habitat and morphology. Therefore, it seems reasonable to conclude that members of the genus Cyprinodon are very similar genetically and that an identical karyotype has become established among the various species which may be the basis of the genetic conservativeness.

In examining the habitats of the various pupfish forms, two contrasting situations are evident. Some species live in rather constant environments of water quality conditions and temperature while others occur in areas of highly fluctuating environmental factors. Both types of environs have been described and documented extensively (e.g., Brown and Feldmeth, 1971

Echelle et al., 1972). Based on Turner's (1974) hypothesis of dichotomous selection of morphological versus coadapted metabolic control genes, the fluctuating habitats are the more primitive or ancestral for pupfishes. Furthermore, even in pluvial times, one could suppose Cyprinodon-types to be peripheral species (in the sense of Echelle et al., 1972) in those habitats. It seems possible that at that time, the modern karyotype and subsequent coadapted genome became established in Cyprinodon which confered increased adaptability in unstable, fluctuating environments. Those adaptive features apparantly have been retained even in those species that are restricted in distribution to non-fluctuating habitats (Brown and Feldmeth, 1971). If coadapted genomes are a reality, and it is advantageous for them to occur in close linkage groups (Stebbins, 1953; White, 1973), then the similar and rather asymmetrical karyotype observed within all Cyprinodon species may be the end result. Any change in gene arrangement via chromosomal alteration would be disadvantageous under fluctuating environmental regimes and not particularly selected for in more stable regimes.

An obvious question is, can more sophisticated techniques resolve relationships in this group of genetically conservative and cytologically identical fishes? Regarding the latter, better methods of karyotypic resolution will be necessary along with the application of the several chromosome banding methods available. The purpose being to determine individual chromosome identity in order to insure the reality of the suggested karyotypic conservancy. At present, karyological data are of little utility in Cyprinodon systematics. Electrophoretic data (Turner, 1974) suffer a similar dilemma of non-variance. Some morphological, behavioral and hybridization data have been produced, but more comparative work is needed in the first and last categories. Until then, Liu's (1968) super-

species-semispecies concept of Cyprinodon relationships seems the most parsimonious.

ACKNOWLEDGEMENT

Sincere appreciation is extended to Dr. Loren G. Hill, my major advisor, for encouragement, advice and above all patience throughout the course of this research. Additionally, his constructive criticism of this manuscript is also appreciated. In that regard, I wish to thank Drs. Gerald R. Braver, Charles C. Carpenter and James R. Estes, other members of my committee, for their critical review of this work and additional advice and assistance in that regard.

Financial support for this work was provided by a graduate assistantship in zoology at the University of Oklahoma, a teaching assistantship and N.S.F. grant-in-aid from the University of Oklahoma Biological Station, an N.S.F. pre-dissertation grant and a research assistantship from the Oklahoma Biological Survey.

Special thanks are due Dr. Robert R. Miller, who first suggested this problem, loaned unpublished material on California and New Mexico pupfish karyotypes, provided support for a collecting trip to Yucatan, Mexico and freely discussed, in light of his broad knowledge and experience with Cyprinodon, the results reported herein. Also, I am indebted to Dr. Anthony A. Echelle, another member of my committee, who provided field assistance, spiritual support and often room and board, along with tolerating lengthy lapses into pupfish lore. His knowledge of the latter was tapped frequently and his review of this work is especially appreciated.

I want also to thank A. Dean Stock, who originally stimulated my interest in karyology, plus aided in the perfecting of the chromosome

techniques for fishes used herein and in the interpretation of those results. In that regard, I must also thank Dr. T. C. Hsu, who graciously allowed the use of his laboratory facilities in Houston, Texas and gave of his time for discussing various aspects of this study.

More than acknowledgement is due Mr. Harry G. Chichester, who aided, more than his share, in the collecting of pupfishes, kept those activities organized and maintained coffee in the pot for late night enlightened conversation. I also wish to thank my many other friends and collegues at the University of Oklahoma and Tulane University who also provided much discussion, some of it relevant to this work. Additionally, appreciation is given to Drs. R. D. Suttkus and Leonard Thien, who provided materials and research space at Tulane to further this study.

And lastly, special appreciation is given to Nancy B. Stevenson, who started on this journey, but could not finish it, and to Gail Kathleen Nipper, who did see its finality. Without them both, this dissertation would not have been completed.

LITERATURE CITED

Alston, R. E. 1967. Cellular continuity and development. Scott-Foresman, Palo Alto, CA.

Arrighi, F. E. and T. C. Hsu. 1971. Localization of heterochromatin in human chromosomes. Cytogenetics, 10:81-86.

Atkin, N. B. and S. Ohno. 1967. DNA values of four primitive chordates. Chromosoma, 23:10-13.

Atz, J. W. 1964. Intersexuality in fishes. In: Armstrong, C. N. and A. J. Marshall (eds.), Intersexuality in Vertebrates Including Man. Academic Press, London:145-232.

Becak, W., M. L. Becak and S. Ohno. 1966a. Intraindividual chromosomal polymorphism in green sunfish (Lepomis cyanellus) as evidence of somatic segregation. Cytogenetics, 5:313-320.

_____, _____, and M. N. Rabello. 1966b. Cytological evidence of constant tetraploid in the bisexual South American frog Odontophrynus americanus. Chromosoma, 19:188-193.

Booke, H. E. 1968. Cytotaxonomic studies of the Corogoninae fishes of the Great Lakes, U.S.A.: DNA and karyotype analysis. J. Fish. Res. Brd. Canada, 24:1667-1687.

Boothroyd, E. R. 1959. Chromosome studies on three canadian populations of Atlantic salmon (Salmo salar). Canad. J. Genet. Cytol., 1:161-172.

Brown, J. H. and C. R. Feldmeth. 1971. Evolution in constant and fluctuating environments. Evolution, 25:390-398.

Brown, J. L. 1957. A key to the species and subspecies of the cyprinodont genus Fundulus in the United States and Canada east of the Continental Divide. J. Wash. Acad. Sci., 47:69-77.

Campos, H. H. and C. Hubbs. 1971. Cytomorphology of six species of gambusiine fishes. Copeia, 1971:566-569.

Chen, T. R. 1969. Karyological heterogamety of deep sea fishes. Postilla (Yale University), No. 130:29p.

_____. 1970. Fish chromosome preparations: air-dried displays of cultured ovarian cells in two killifishes (Fundulus). J. Fish. Res. Bd. Canada, 27:158-161.

_____. 1971. A comparative chromosome study of twenty killifish species of the genus Fundulus (Teleostei: Cyprinodontidae). Chromosoma, 32:436-453.

_____, and A. W. Ebeling. 1968. Karyological evidence of female heterogamety in the mosquito fish, Gambusia affinis. Copeia, 1968:70-75.

_____, and H. M. Reisman. 1970. A comparative chromosome study of the North American species of sticklebacks (Teleostei: Gasterosteidae). Cytogenetics, 9:321-332.

_____, and F. H. Ruddle. 1970. A chromosome study of four species and a hybrid of the killifish genus Fundulus (Cyprinodontidae). Chromosoma, 29:255-267.

Chernenko, Ye. V. 1969. Evolution and cytotaxonomy of the family Salmonidae. Problems in Ichthyology, 9:781-788.

Denton, T. E. 1973. Fish Chromosome Methodology. Charles C. Thomas, Publisher, Springfield, Illinois.

'Denver report'. 1960. Lancet, I:1063.

Drewery, G. E. 1967. Studies of relationships within the family Cyprinodontidae. Ph. D. dissertation, University of Texas, Austin.

DuPraw, E. J. 1970. DNA and Chromosomes. Holt, Rienhardt and Winston, New York.

Ebeling, A. W., N. B. Atkin and P. Y. Setzer. 1971. Genome sizes of teleostean fishes: increases in some deep-sea species. Amer. Natur., 105:549-561.

_____, and T. R. Chen. 1970. Heterogamety in teleostean fishes. Trans. Am. Fish. Soc., 99:131-138.

Echelle, A. A., A. F. Echelle and L. G. Hill. 1972. Interspecific interactions and limiting factors of abundance and distribution in the Red River pupfish, Cyprinodon rubrofluviatilis. Am. Midl. Nat., 88:109-130.

_____, and R. R. Miller. 1974. Rediscovery and redescription of the Leon Springs pupfish, Cyprinodon bovinus, from Pecos County, Texas. Southwest. Nat., 19:179-190.

Farris, J. S. 1968. The evolutionary relationships between the species of the killifish genera Fundulus and Profundulus (Teleostei: Cyprinodontidae). Ph. D. dissertation, University of Michigan.

Gordon, M. 1954. Two opposing sex-determining mechanisms, one XX-XY, the other WY-YY, in different natural populations of the platyfish, Xiphophorus maculatus. Caryologia, Supplemento IX, Congresso da Internazionale Genetica, 2:960-964.

Gyldenholm, A. O. and J. J. Scheel. 1971. Chromosome numbers of fishes. I. J. Fish Biol., 1971:479-486.

Harrington, R. W., Jr. 1971. How ecological and genetic factors interact to determine when self-fertilizing hermaphrodites of Rivulus marmoratus change into functional secondary males, with a reappraisal of the modes of intersexuality in fishes. Copeia, 1971:389-432.

Hinegardner, R. T. 1968. Evolution of cellular DNA in teleost fishes. Amer. Natur., 102:517-523.

_____, and D. E. Rosen. 1972. Cellular DNA content and the evolution of teleostean fishes. Amer. Natur., 106:621-644.

John, B. and K. R. Lewis. 1968. The chromosome complement. Protoplasmalogia, 6:1-206.

Kallman, K. D. 1965. Genetics and geography of sex determination in the poeciliid fish, Xiphophorus maculatus. Zoologica, 50:151-190.

Klinger, H. P. and D. O. Hammond. 1971. Rapid chromosome and sex-chromatin staining with pinacyanol. Stain Technol., 46:43-47.

Levan, A., K. Fredga and A. A. Sandberg. 1965. Nomenclature for centromeric position of chromosomes. Hereditas, 52:201-220.

Levin, C. B. and N. R. Foster. 1972. Cytotaxonomic studies in Cyprinodontidae: multiple sex chromosomes in Garmanella pulchra. Notulae Naturae, Acad. Nat. Sci. Philadelphia, No. 446:4p, 6 figs.

Lieppman, M. and C. Hubbs. 1969. A karyological analysis of two cyprinid fishes, Notemigonus crysoleucas and Notropis lutrensis. Texas Rep. Biol. Med., 27:427-435.

Liu, R. K. 1969. The comparative behavior of allopatric species (Teleostei-Cyprinodontidae: Cyprinodon). Ph.D. dissertation, University of California, Los Angeles.

Martin, F. D. 1972. Factors influencing local distribution of Cyprinodon variegatus (Pisces; Cyprinodontidae). Trans. Amer. Fish. Soc., 101:89-93.

Matthey, R. 1964. Evolution chromosomique et speciation ches les Mus du sous genre Leggada Gray 1837. Experientia, 20:657-665.

_____. 1965. Un type novvear de chromsomes sexuels multiples chez une souris africaine du groupe Mus (Leggada) minutoides (Mammalis-Rodentia). Male: X_1X_2Y. Femelle: X_1X_2/X_1X_2. Chromosoma, 16: 351-364.

McPhail, J. D. and R. L. Jones. 1966. A simple technique for obtaining chromosomes from teleost fishes. J. Fish. Res. Bd. Canada, 23:767-769.

Miller, R. R. 1948. The cyprinodont fishes of the Death Valley system of eastern California and southwestern Nevada. Misc. Publ. Mus. Zool. Univ. Michigan, No. 68:155p.

_____. 1950. Speciation in fishes of the genera Cyprinodon and Empetrichthys inhabiting the Death Valley region. Evolution, 4:55-63.

_____. 1955. An annotated list of the american cyprinodontid fishes of the genus Fundulus, with the description of Fundulus persimilis from Yucatan. Occ. Pap. Mus. Zool. Univ. Michigan, No. 568:25p.

_____. 1968. Two new fishes of the genus Cyprinodon from the Cuatro Cienegas Basin, Coahuila, Mexico. Occ. Pap. Mus. Zool. Univ. Michigan, No. 659:15p.

_____, and A. A. Echelle. 1975. Cyprinodon tularosa, a new cyprinodontid fish from the Tularosa Basin, New Mexico. Southwest. Nat., 19:365-377.

_____, and V. Walters. 1972. A new genus of cyprinodontid fish from Nuevo Leon, Mexico. Cont. Sci., Nat. Hist. Mus., Los Angeles Co., No. 233:13p.

Mittwock, U. 1967. Sex Chromosomes. Academic Press, New York.

Muller, H. J. 1925. Why polyploidy is rarer in animals than in plants. Amer. Natur., 59:346-353.

Muramoto, J., S. Ohno and N. B. Atkin. 1968. On the diploid state of the fish order Ostariophysi. Chromosoma, 24:59-66.

Navashin, M. 1932. The dislocation hypothesis of evolution of chromosome numbers. Z. indukt Abst. Verebl., 63:224-231.

Nogusa, S. 1960. A comparative study of the chromosomes in fishes with particular consideration on taxonomy and evolution. Mem Hyogo Univ. Agri. (Biol. Ser.), 3:1-62.

Ohno, S. 1967. Sex chromosomes and sex-linked genes. In: Labhart, A., T. Mann, L. T. Samuels and J. Zander (eds.), Monographs on Endocrinology 1. Springer-Verlag, New York.

_____. 1970. Evolution by Gene Duplication. Springer-Verlag, New York.

_____ and N. B. Atkin. 1966. Comparative DNA values and chromosome complements of eight species of fishes. Chromosoma, 18:455-466.

_____, J. Muramoto, L. Christian and N. B. Atkin. 1967. Diploid-tetraploid relationships among Old-world members of the fish family Cyprinidae. Chromosoma, 23:1-9

_____, _____, J. Klein and N. B. Atkin. 1969a. Diploid-tetraploid relationships in clupeoid and salmonoid fish. In: Darlington, C.D. and K.R. Lewis (eds.), Chromosomes Today, Vol. 2:139-147.

_____, _____, C. Stenius, L. Christian, W. A. Kettrell and N. B. Atkin. 1969b. Microchromosomes in holocephalian, chrondrostean and holostean fishes. Chromosoma, 26:35-40.

_____, C. Stenius, E. Faisst and M. T. Zenzes. 1965. Post-zygotic chromosomal rearrangements in rainbow trout (Salmo irideus). Cytogenetics, 4:117-129.

_____, U. Wolf, and N. B. Atkin. 1968. Evolution from fish to mammals by gene duplication. Hereditas, 59:169-187.

Pardue, M. L. and J. G. Gall. 1970. Chromosomal localization of mouse satellite DNA. Science, 168:1356-1358.

Post, A. 1965. Vergleichende untersuchungen der chromosomenzahlen bei susswasser-teleostern. Z. Syst. Evol. Forsch., 3:47-93.

Rensch, B. 1959. Evolution Above the Species Level. Columbia University Press, New York.

Roberts, F. L. 1964. A chromosome study of twenty species of Centrarchidae. J. Morph., 115:401-417.

_____. 1967. Chromosome cytology of the Osteichthys. Prog. Fish-Cult., 29:75-83.

Robertson, W. R. B. 1916. Chromosome studies. I. Taxonomic relationships shown in the chromosomes of Tettigidae and Acrididae. V-shaped chromosomes and their significance in Acrididae, Locustidae and Gryllidae: chromosomes and variation. J. Morph., 27:179-331.

Rosen, D. E. 1964. The relationships and taxonomic position of the halfbeaks, killifishes, silversides and their relative. Bull. Am. Mus. Nat. Hist., 127:219-267.

Ross, M. R. 1973. A chromosome study of five species of etheostomine fishes (Percidae). Copeia, 1973:163-165.

Scheel, J. J. 1972. Rivuline karyotypes and their evolution (Rivulinae, Cyprinodontidae, Pisces). Z. Syst. Evol. Forsch., 10:180-209.

Schultz, R. J. 1961. Reproductive mechanisms of unisexual and bisexual strains of the viviparous fish Poeciliopsis. Evolution, 15: 302-325.

_____. 1969. Hybridization, unisexuality and polyploidy in the teleost Poeciliopsis (Poeciliidae) and other vertebrates. Amer. Natur., 103:605-619.

Seabright, M. 1971. A rapid banding technique for human chromosomes. Lancet, II:971-972.

Sethi, K. P. 1960. Osteology and phylogeny of oviparous cyprinodont fishes (Order Cyprinodontiformes). Ph.S. dissertation, University of Florida, Gainsville.

Shiriaichi, Y. and T. H. Yosida. 1972. Banding pattern analysis of human chromosomes by use of a urea treatment technique. Chromosoma, 37:75-83.

Simon, R. C. 1963. Chromosome morphology and species evolution in the five North American species of Pacific salmon (Oncorhynchus). J. Morph., 112:77-95.

_____ and A. M. Dollar. 1963. Cytological aspects of speciation in two North American teleosts, Salmo gairdneri and Salmo clarki lewisi. Canad. J. Genet. Cytol., 5:43-49.

Stebbins, G. L. 1958. Longevity, habitat and release of genetic variability in the higher plants. Cold Apring Harbour Symp. Quant. Biol., 23: 365-378.

Stevenson, M. M. and T. M. Buchanan. 1973. An analysis of hybridization between the cyprinodont fishes Cyprinodon variegatus and C. elegans. Copeia, 1973:682-692.

Stewart, K. W. and C. B. Levin. 1968. A method of obtaining permanent dry mounted chromosome preparations from teleost fish. J. Fish. Res. Bd. Canada, 25:1091-1093.

Stock, A. D., D. B. Burnham and T. C. Hsu. 1972. Giemsa banding of meiotic chromosomes with description of a procedure for cytological preparations from solid tissue. Cytogenetics, 11:534-539.

Svardson, G. 1945. Chromosome studies on Salmonidae. Rep. Swed. Inst. Fresh Water., Drottningholm, No. 23.

Turner, B. J. 1973. Genetic divergence of Death Valley pupfish populations: species-specific esterases. Comp. Biochem. Physiol., 46B:53-70.

_____. 1974. Genetic divergence of Death Valley pupfish species: biochemical versus morphological evidence. Evolution, 24:281-294.

Uyeno, T. and R. R. Miller. 1971. Multiple sex chromosomes in a mexican cyprinodontid fish. Nature, 231:452-453.

_____ and G. R. Smith. 1972. Tetraploid origin of the karyotype of catostomid fishes. Science, 175:644-645.

Wasserman, A. O. 1970. Polyploidy in the common tree toad Hyla versicolor LeConte. Science, 167:385-386.

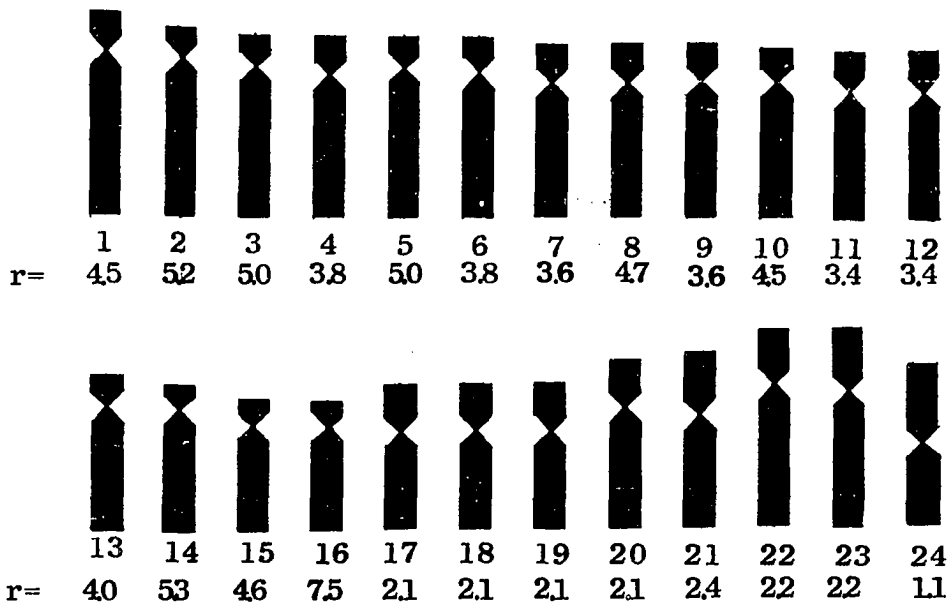
White, M. J. D. 1946. The evidence against polyploidy in sexually reproducing animals. Amer. Natur., 80:610-619.

_____. 1973. Animal Cytology and Evolution (3rd ed.). Cambridge at the University Press.

Yamamoto, T. O. 1969. Sex differentiation. In: Hoar, W.S. and R.J. Randall (eds.), Fish Physiology, Vol. e, Academic Press, NY:117-175.

APPENDIX

Figure 1. Idiogram of the Cyprinodon karyotype arranged by graded length and/or increasing medio-centrocy. Each element (1-24) represents a diploid pair. (r=l/s, where l is the length of the long arm, s the length of the short arm).



Pairs 1-16: subterminal (r= 3.4-7.5)

Pairs 17-23: submedian (r= 2.1-2.4)

Pair 24: median (r= 1.1)

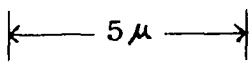


Figure 2. Karyogram of C. atrorus, female (gill epithelium).

Figure 3. Karyogram of C. atrorus, male (gill epithelium).

Figure 4. Karyogram of C. beltrani, female (gill epithelium).

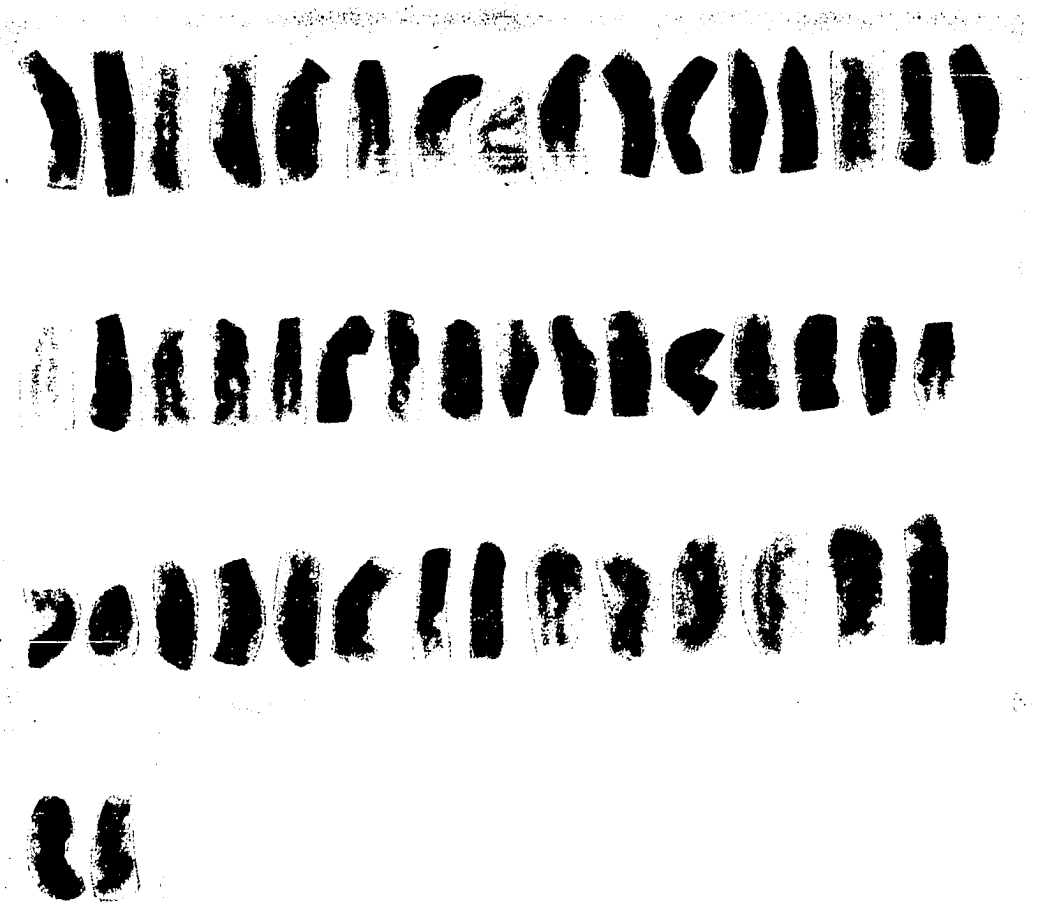


Figure 5. Karyogram of C. bifasciatus, female (gill epithelium).

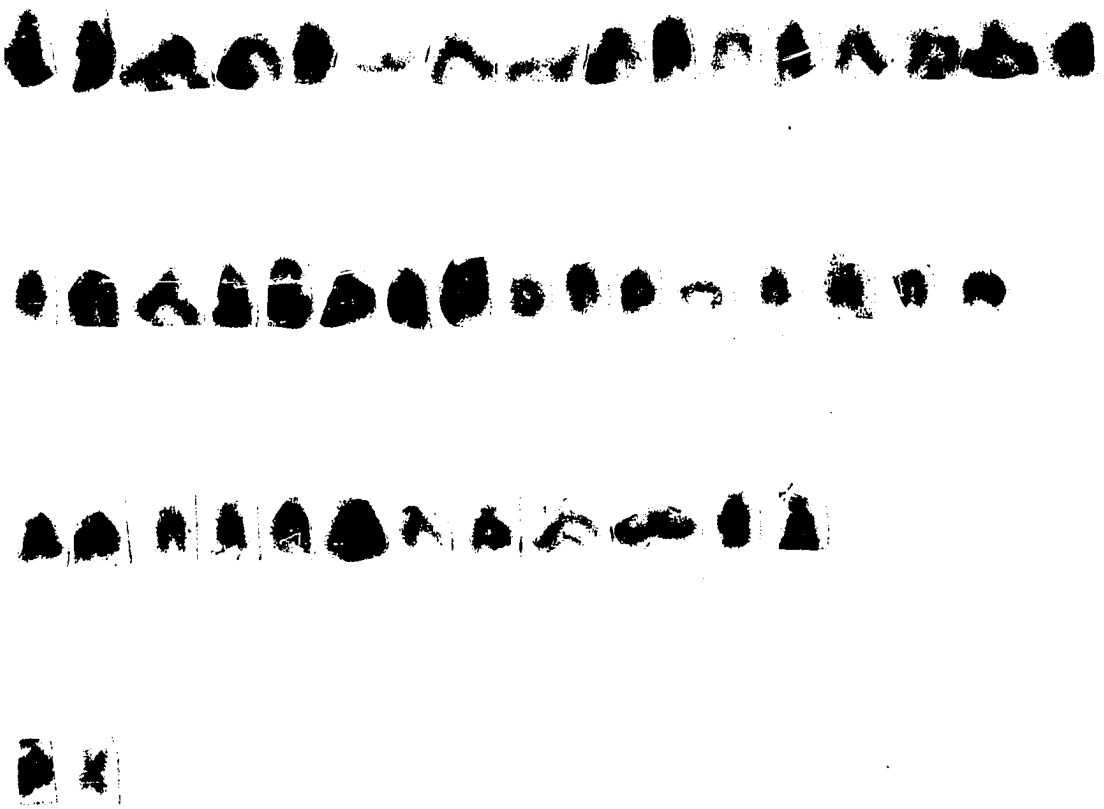


Figure 6. Karyogram of C. bifasciatus, male (gill epithelium).

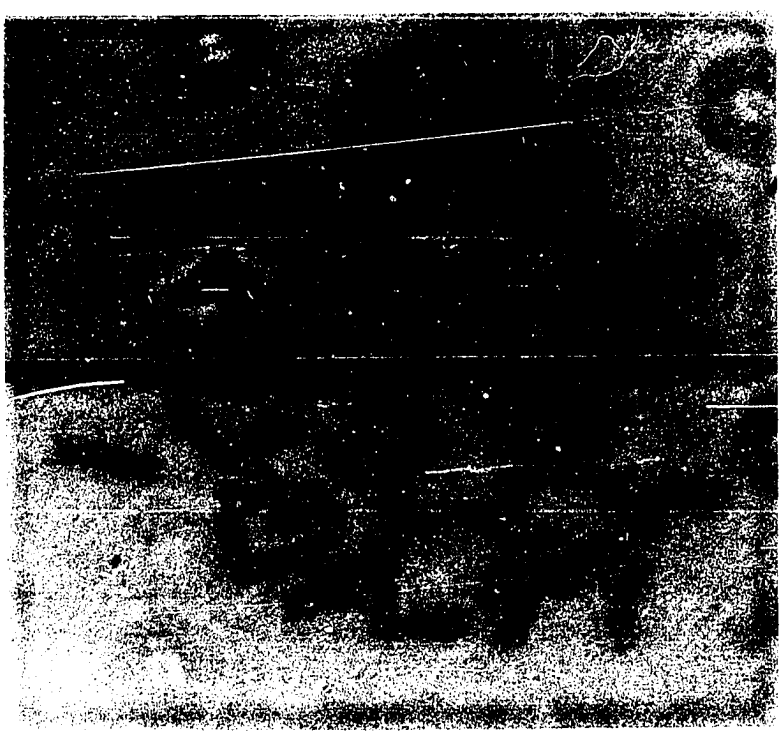


Figure 7. Karyogram of C. bovinus, female (ovarian culture).

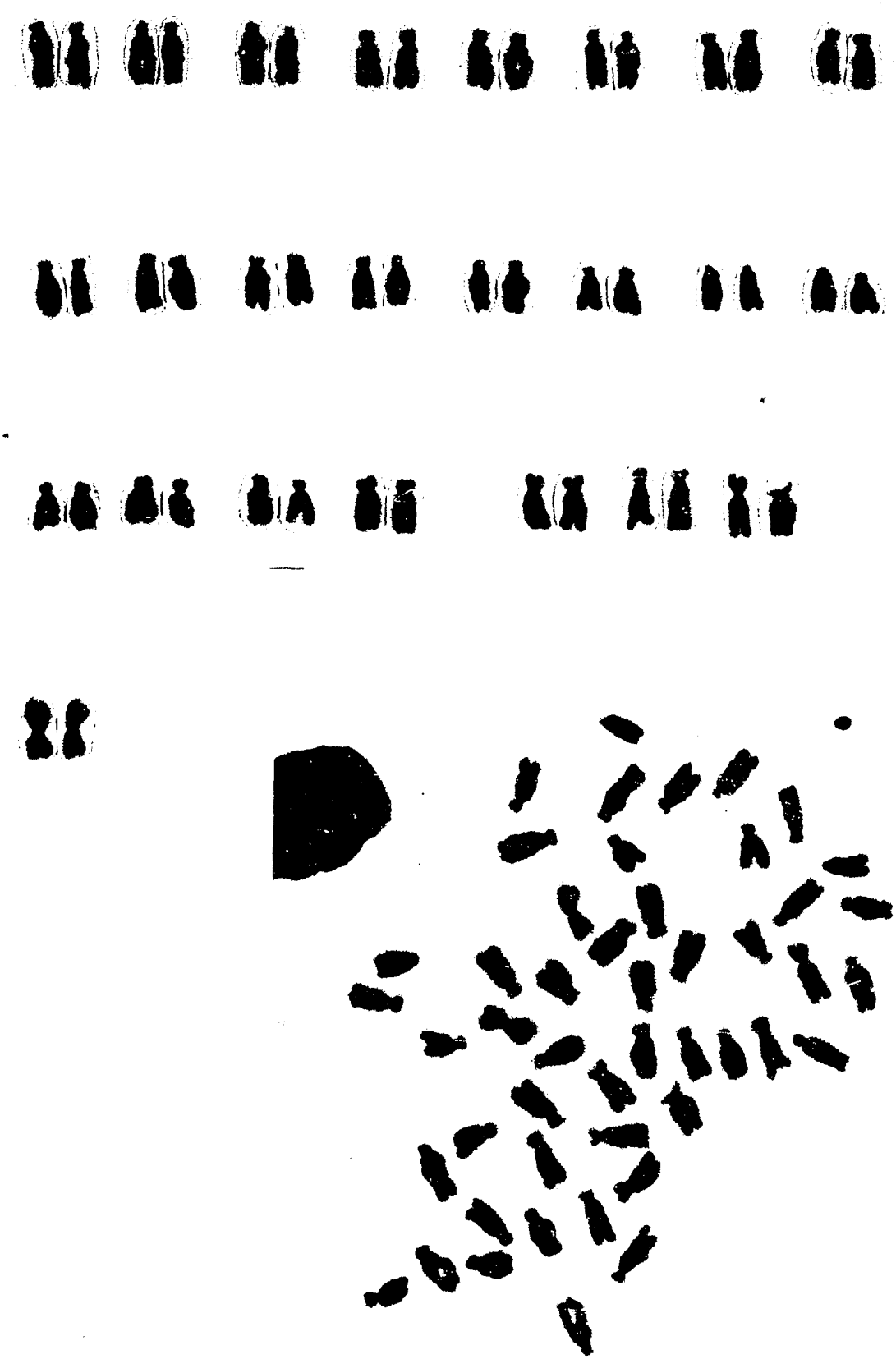


Figure 8. Karyogram of C. bovinus, male (gill cell culture).

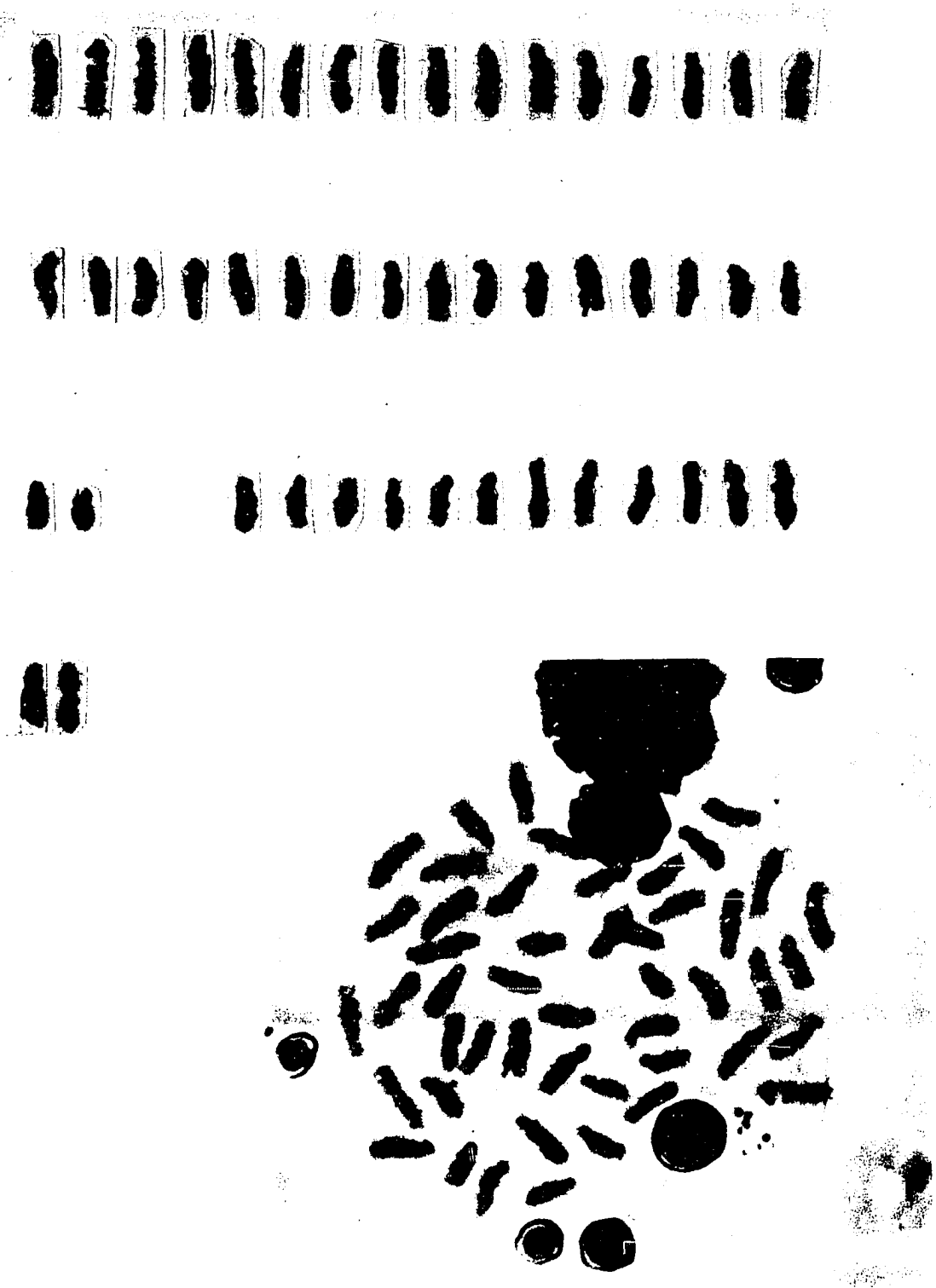
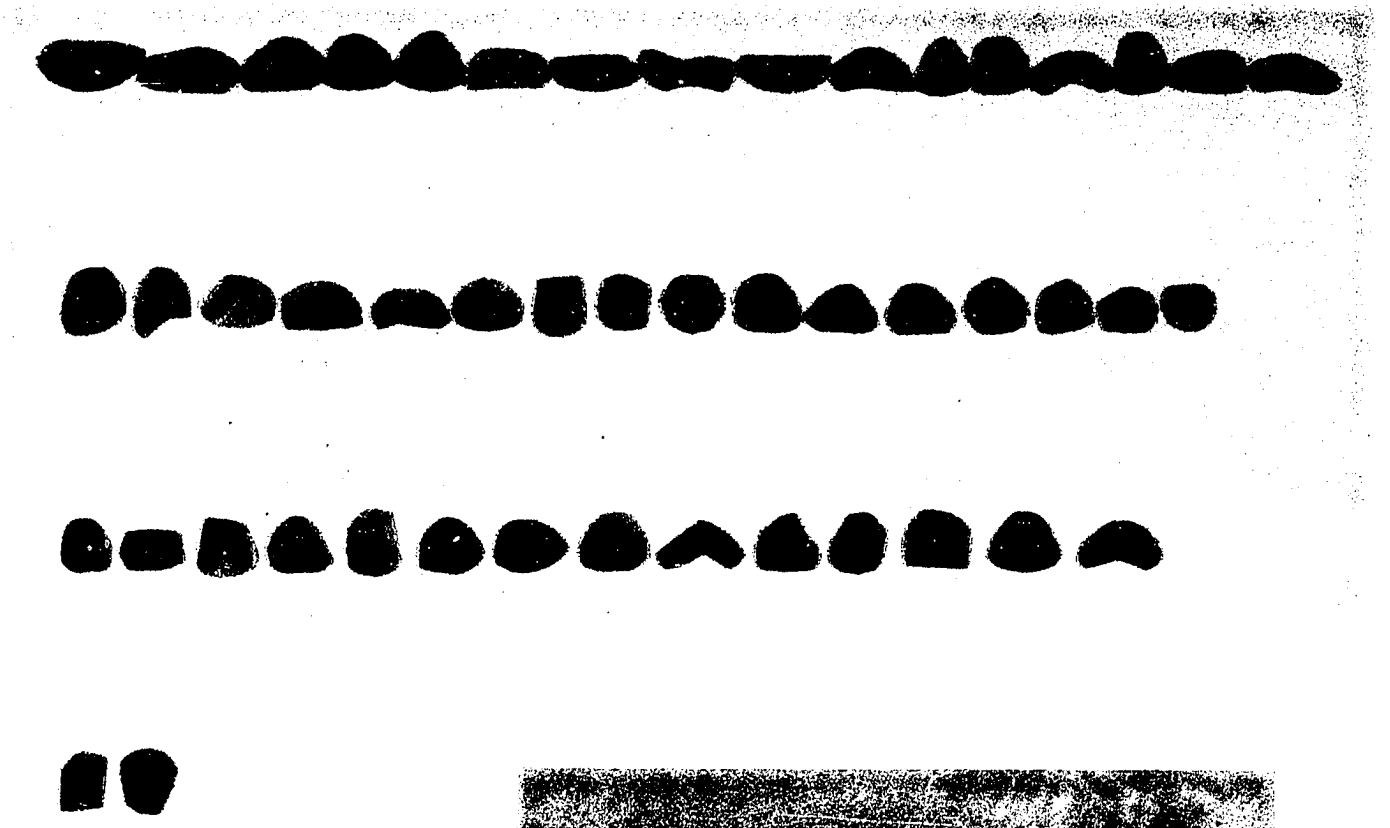


Figure 9. Karyogram of C. elegans, female (gill epithelium).



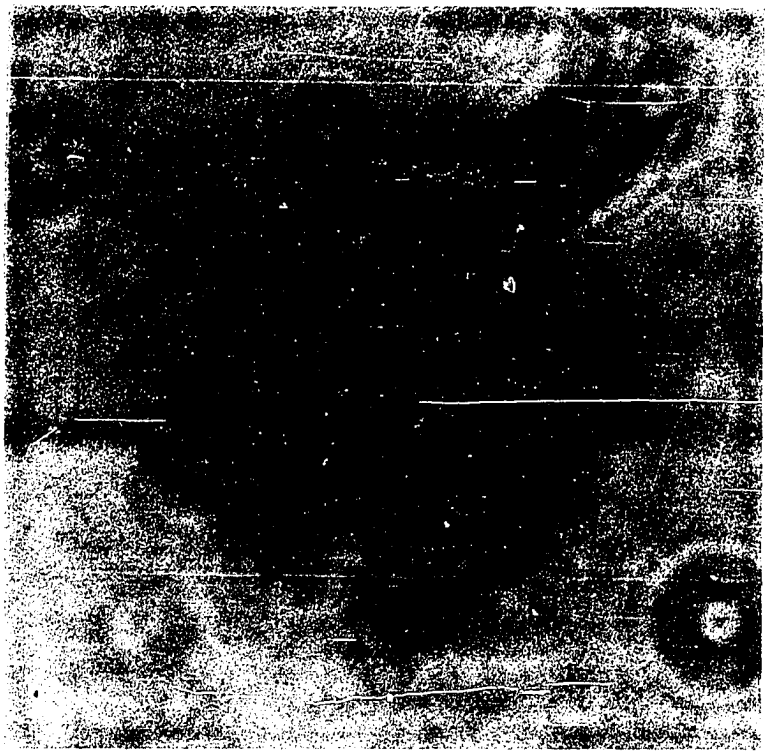


Figure 10. Karyogram of C. elegans, male (gill epithelium).

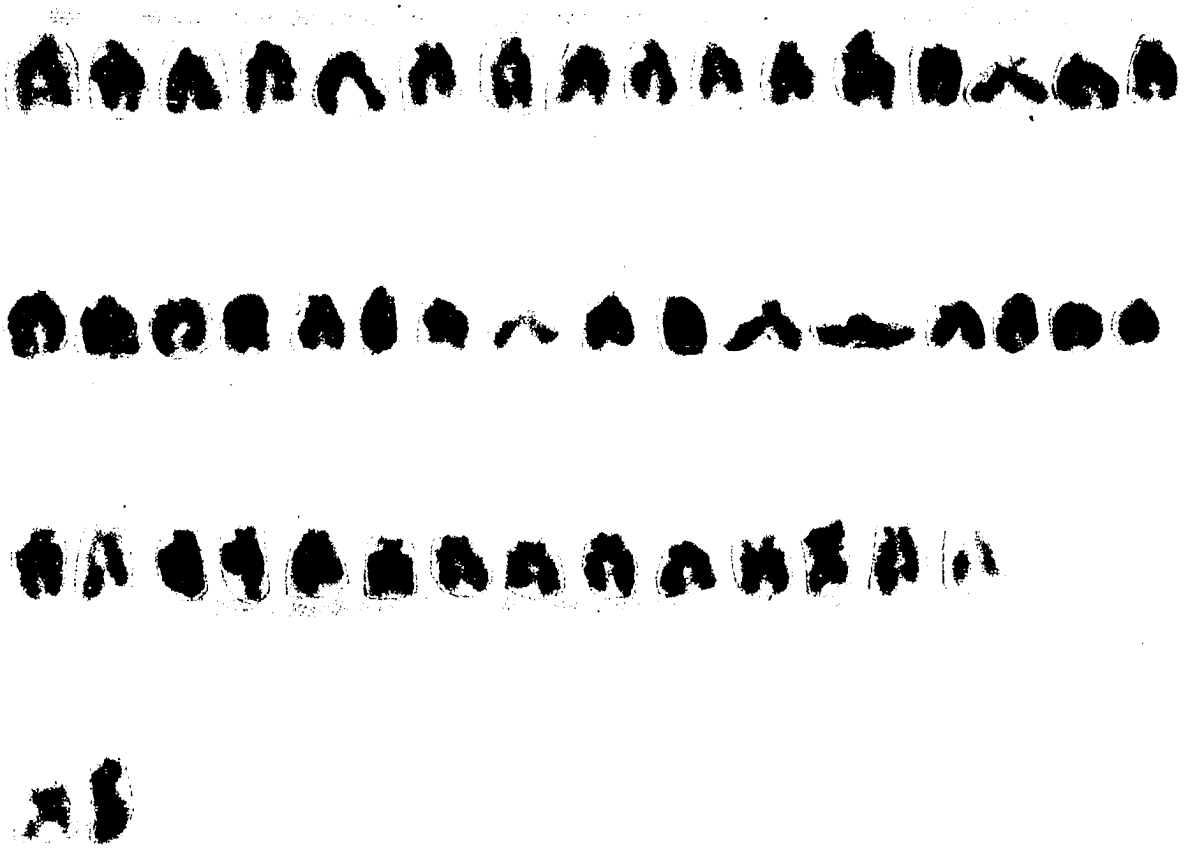


Figure 11. Karyogram of C. eximius, female (ovarian culture).

[redacted]

Figure 12. Karyogram of C. eximius, male (gill epithelium).

Figure 13. Karyogram of C. hubbsi, male (gill epithelium).

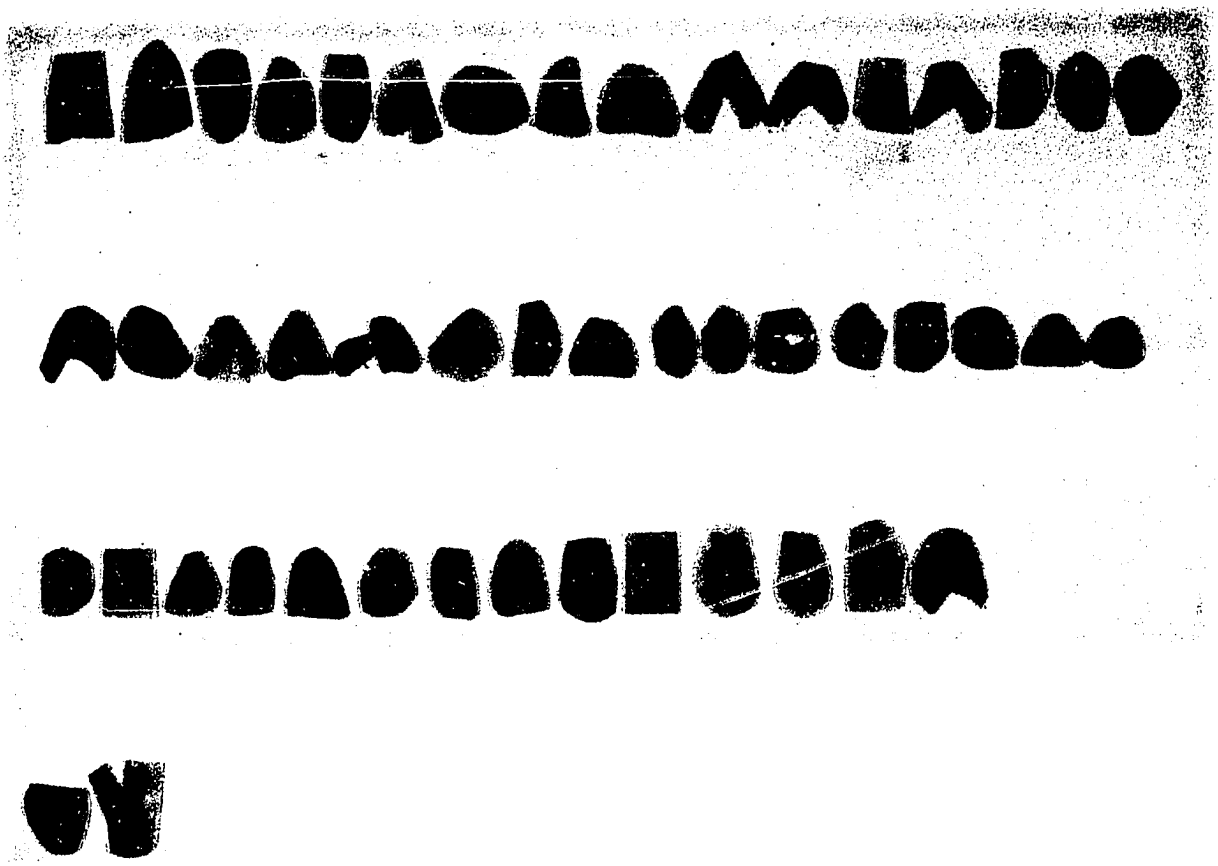


Figure 14. Karyogram of C. "pecosensis", female (gill epithelium).

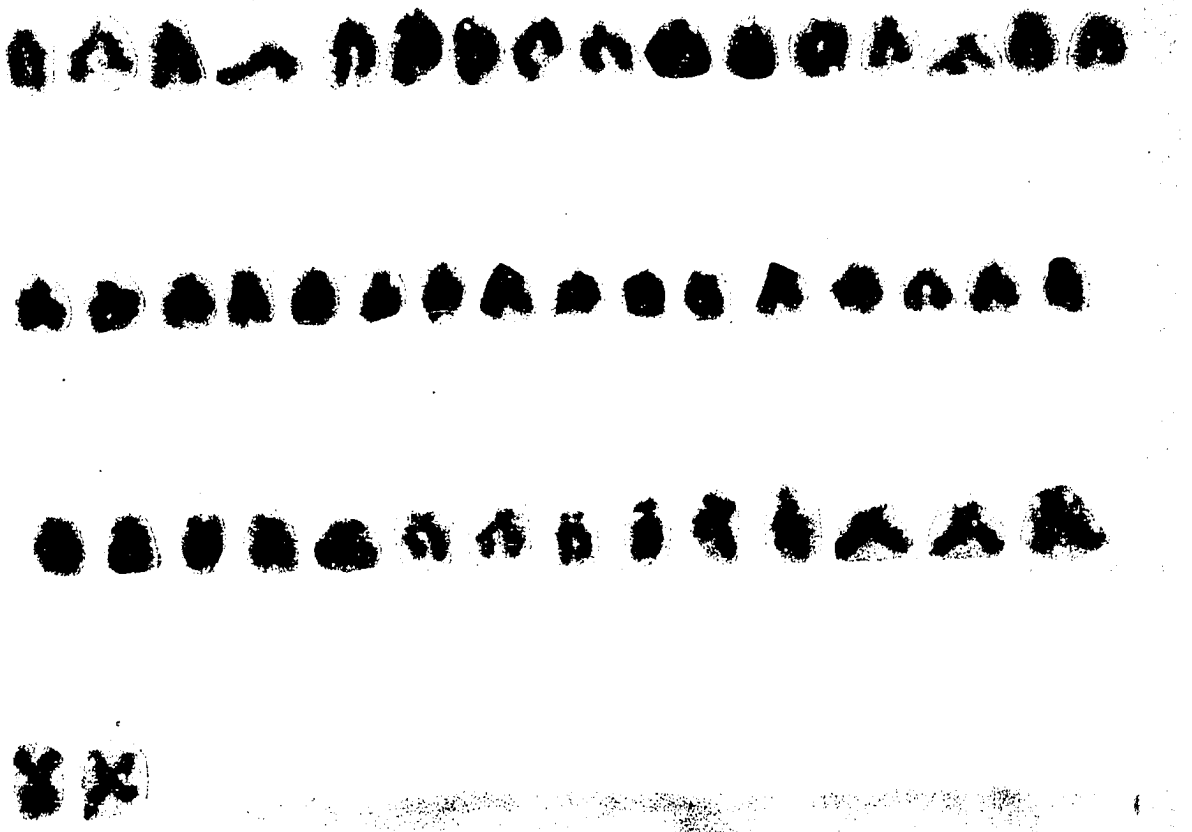


Figure 15. Karyogram of C. "pecosensis", male (gill epithelium).

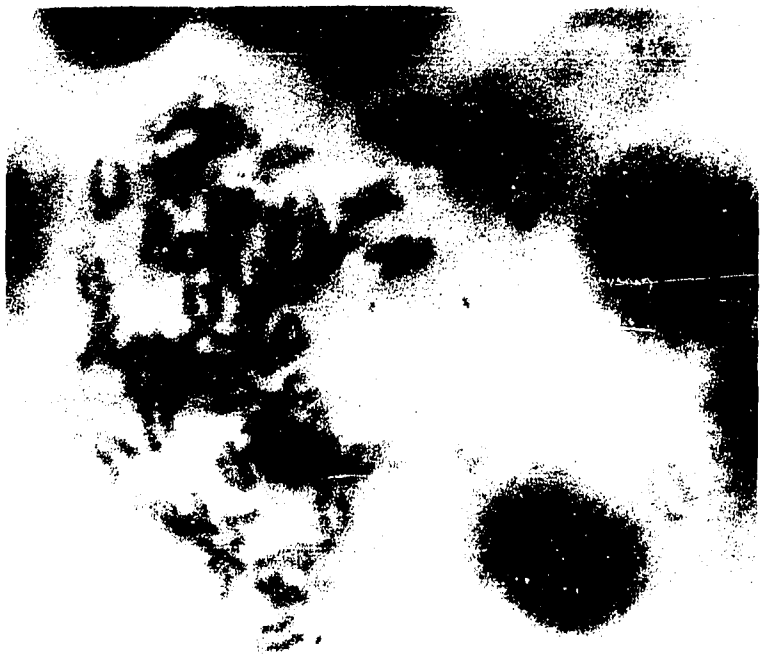


Figure 16. Karyogram of C. rubrofluviatilis, female (gill epithelium): Red River drainage.

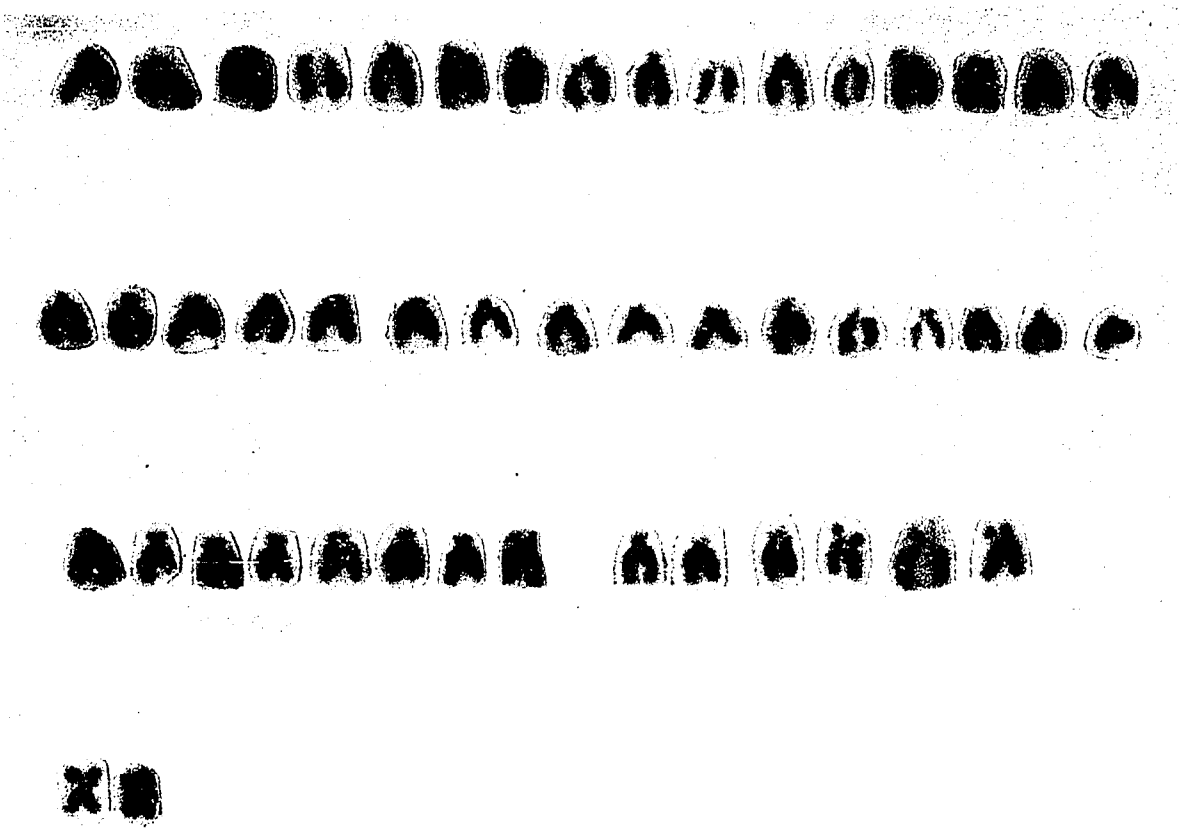


Figure 17. Karyogram of C. rubrofluviatilis, male (gill epithelium): Red River drainage.

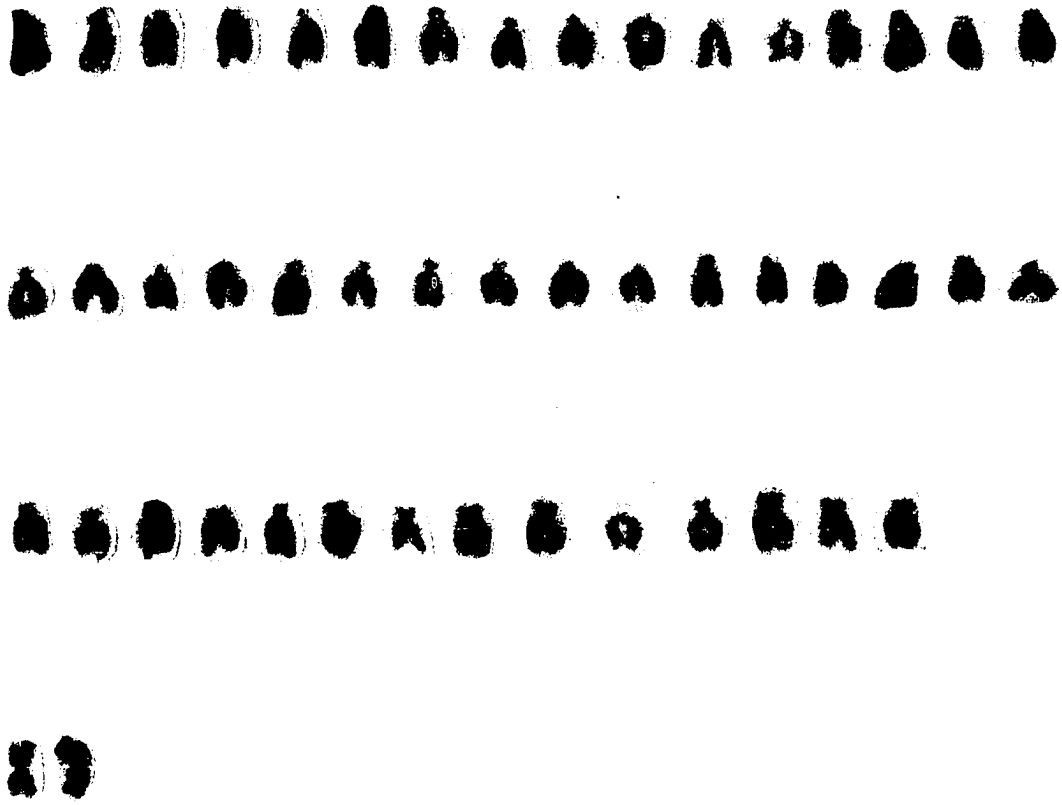


Figure 18. Karyogram of C. rubrofluviatilis, female (gill epithelium): Oscar Creek.

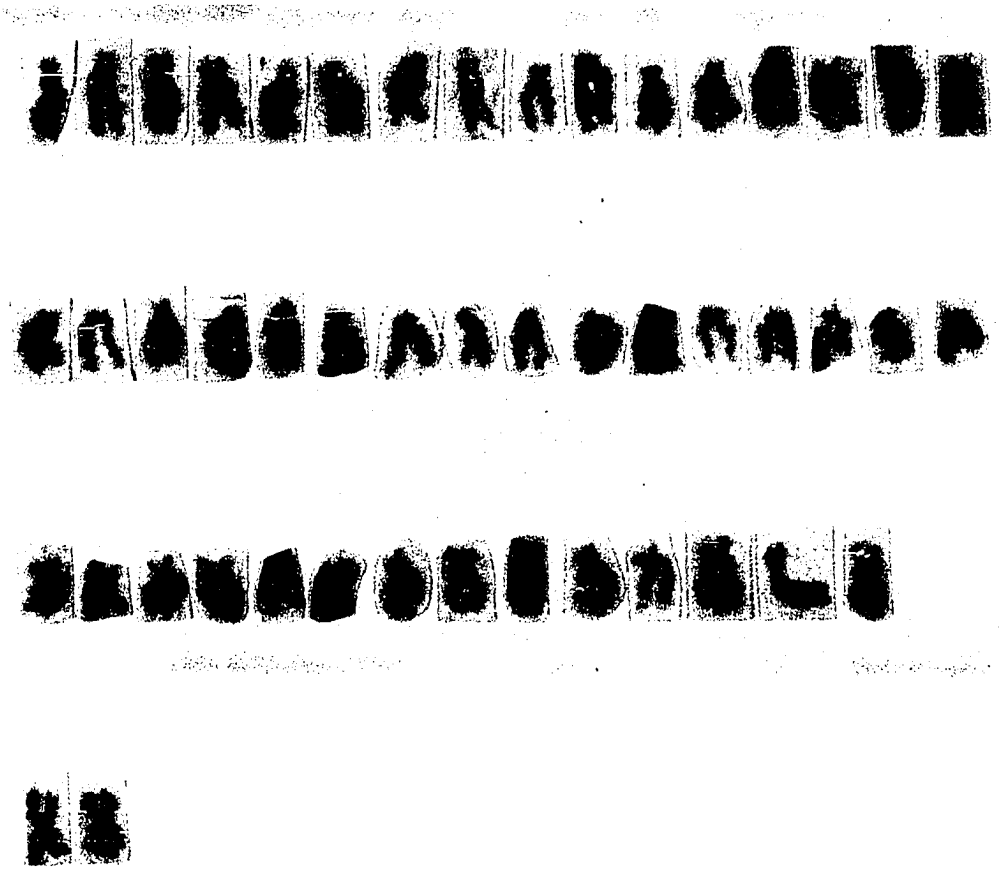


Figure 19. Diakinesis in testicular culture of C. rubrofluviatilis: Oscar Creek.

Figure 20. Karyogram of C. rubrofluviatilis, female (gill epithelium): Brazos River drainage.

Figure 21. Karyogram of C. rubrofluviatilis, male (gill epithelium): Brazos River drainage.

Figure 22. Karyogram of C. variegatus, female (ovarian culture).

Figure 23. Karyogram of C. variegatus, male (gill epithelium).

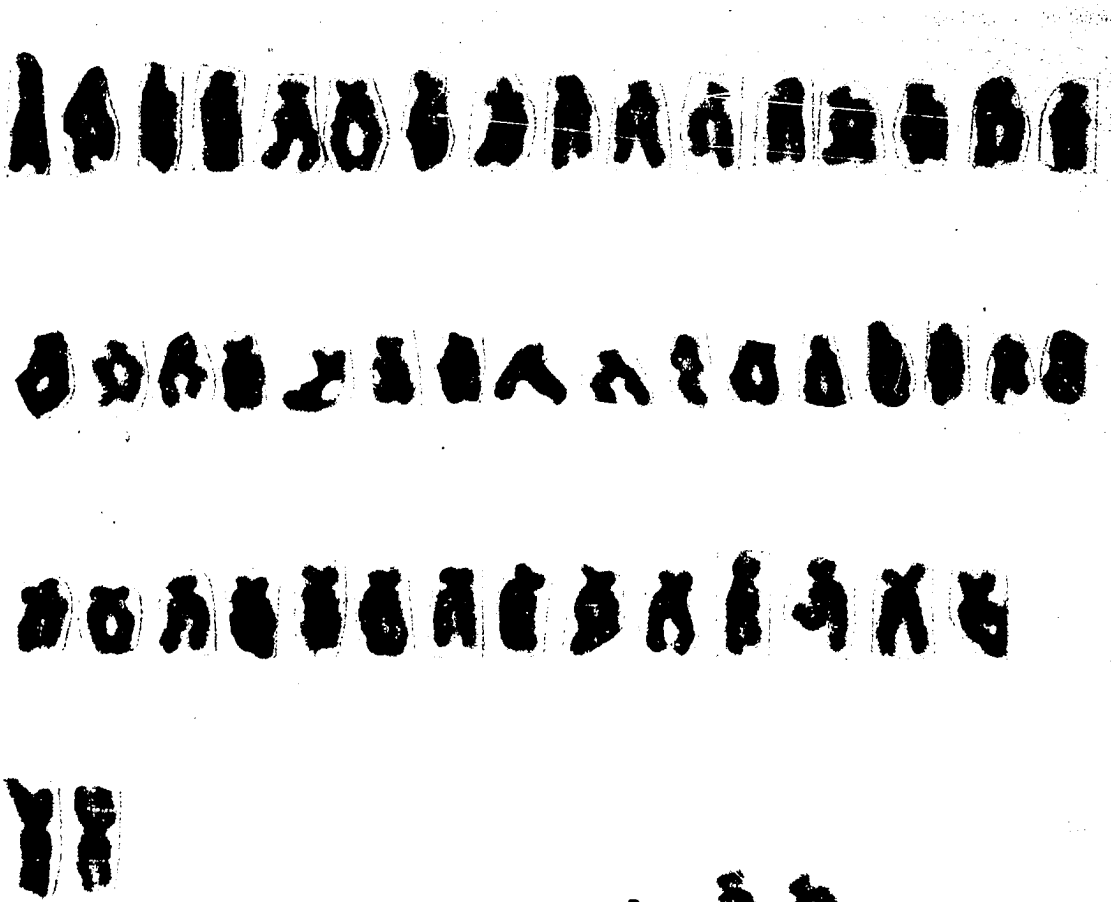


Figure 24. C-banding of ovarian culture metaphase of C. bovinus.

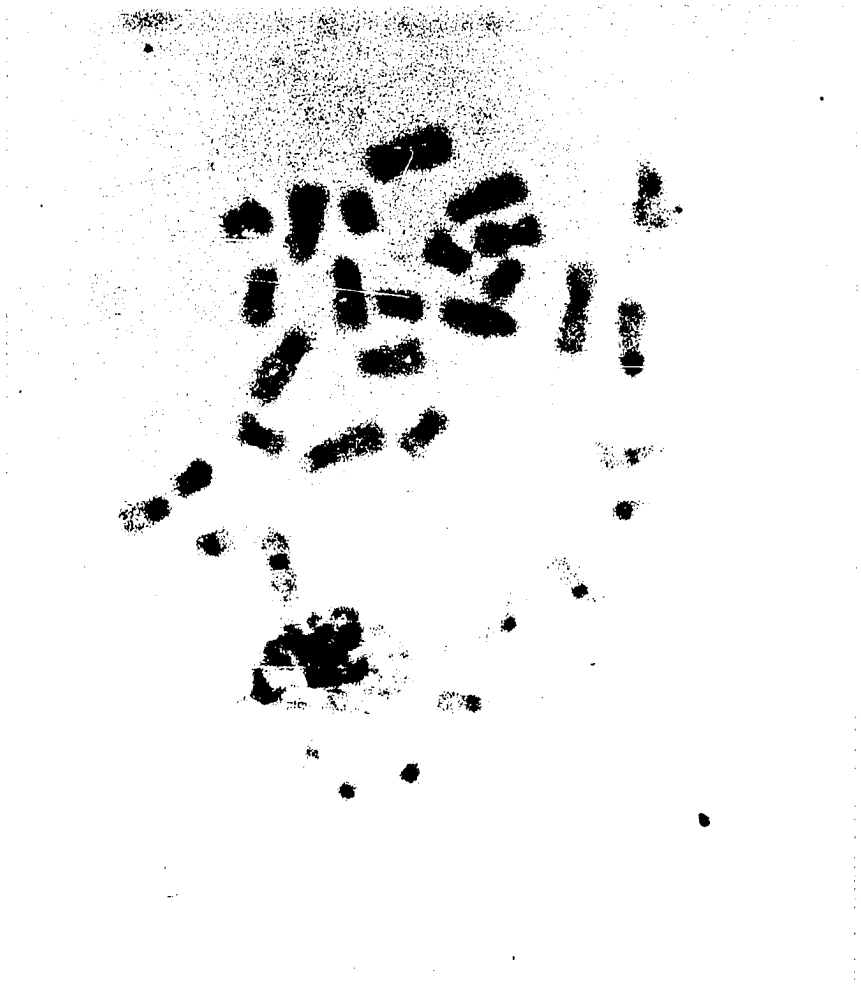


Figure 25. G-banding of ovarian culture metaphase of C. bovinus.