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CYTOGENETICS

J. R. GOLD

The chromosome structural arrangement is the umbilical cord of the species.
Verne Grant (1963)

I. INTRODUCTION: CHROMOSOMES AND FISHES

It is estimated that there are between 20,000 and 23,000 living species of fishes belonging to three diverse classes: *Agnatha* (lampreys and hagfishes); *Chondricthyes* (sharks, skates, and chimeras); and *Osteichthyes* (bony fishes). Of all these, the chromosome numbers of only 650–700 species have been reported; complete karyotypes are known for only about 500 species (ca. 2–3%). In contrast, over 30% of the living species of eutherian mammals have been studied cytologically, in some cases extensively.

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At the outset, it would be helpful to distinguish between "cytogenetics" and "cytotaxonomy." The former refers to the study of heredity through the study of chromosomes (the bearers of the genes), and the cytological mechanisms of inheritance. Cytotaxonomy, on the other hand, refers to the study of phenetic and/or phylogenetic relationships among species, based on comparisons of chromosome number and morphology.

In this review, emphasis is placed on cytogenetics of fishes. Readers interested more in cytotaxonomy of fishes are urged to consult the review by Ohno (1974), and the references contained in the checklists of fish chromosome numbers by Roberts (1967), Gyldenholm and Scheel (1971), Chiarelli and Capanna (1973), Denton (1973), and Park (1974).

II. TECHNIQUES AND METHODS OF KARYOTYPING FISHES

Obtaining consistent chromosome "spreads" of good quality is the limiting factor in the study of chromosome cytology in fishes. Early fish cytologists were handicapped by numerous technical difficulties, resulting in several reports of chromosome number and morphology now considered incorrect (Chiarelli and Capanna, 1973; Denton, 1973; Ohno, 1974). A possible exception was the sectioning technique utilized by Nogusa (1960), whose reports on chromosome numbers of several fish species are in agreement with later studies.

With the "revolution" in techniques of mammalian cytology during the last quarter century (German, 1973), several innovative procedures, including pretreatment with mitotic inhibitors and exposure of cells to hypotonic solution, have greatly simplified the preparation of fish chromosomes. Often the choice of method depends on the time and facilities available. Roberts (1967), Denton (1973), and Blaxhall (1975) have reviewed some of the literature on obtaining and preparing chromosomes from fish. A brief review of the sources from which fish chromosomes may be obtained is presented below.

A. Chromosomes Obtained from Live Fish or Embryos

Procedures involving preparations of mitotic chromosomes from actively dividing somatic tissues of live specimens or from embryos have been the most widely used among fish cytologists and have the dual advantages of being rapid and inexpensive. The soft organs (kidney, spleen, and liver) have proved to be good sources of chromo-

somes (Ohno *et al.*, 1965; Nygren *et al.*, 1968a,b, 1971a,b; Chen, 1969; Davisson *et al.*, 1972; Wilmot, 1974; Gold, 1974; Zenzes and Voiçulescu, 1975). Kidney probably gives the best results since in most fishes the renal intertubular tissue contains the hematopoietic organs (Catton, 1951), and thus provides numerous rapidly proliferating blood cells. Equally good sources are the epithelial cells from gills (McPhail and Jones, 1966; Lieppman and Hubbs, 1969), from fins or scales (Denton and Howell, 1969), and from cornea (Drewry, 1964). The use of epithelial cells instead of soft organ tissue has the advantage that the specimens may be kept alive. Swarup (1959a), Simon (1963), Booke (1968), and Endo and Ingalls (1968) developed techniques to obtain chromosomes from the blastula of early embryos. Several disadvantages to using embryonic material were summarized by Roberts (1967).

Testes are useful for meiotic as well as mitotic chromosome preparations (Roberts, 1964; Nygren *et al.*, 1968a,b; Chen, 1969), but usually can be used only during active spermatogonial division (Roberts, 1967; Blaxhall, 1975). Furthermore, the connective tissue stroma of testes often makes satisfactory spreading of cells difficult (Roberts, 1967). Meiotic chromosomes have also been obtained from ovaries (Ohno *et al.*, 1965; Davisson *et al.*, 1973).

Preparation of the chromosomes from any of the tissues noted above is relatively straightforward, and most of the various procedures are thoroughly outlined in Denton's (1973) book on fish chromosome methodology. Slight variations were suggested by Gold (1974) and Zenzes and Voiçulescu (1975) for soft organ tissues, and by Endo and Ingalls (1968) for embryos.

B. Chromosomes Obtained from Cell Culture

The overwhelming successes of mammalian cytologists in using short- or long-term cell cultures as sources of chromosomes have prompted several fish researchers to initiate studies along this line. Short-term cell cultures using soft organ tissue (Wolf *et al.*, 1960; Wolf and Quimby, 1969; Roberts, 1964; Barker, 1972; Yamamoto and Ojima, 1973; Abe and Muramoto, 1974; Wilmot, 1974), scale epithelium (Ojima *et al.*, 1972), fin explant (Regan *et al.*, 1968; Leuken and Foerster, 1969), gill (Chen and Ebeling, 1975), and gonad (Roberts, 1964, 1968, 1970; Chen, 1970), have all provided good sources of chromosomes from several different freshwater and marine species. The methods involved in initiating cell cultures, including digestion and centrifugation procedures, are well reviewed by Wolf

and Quimby (1969). Techniques for harvesting cells and preparing chromosomes are essentially the same as those from tissues of live specimens, and are outlined in Denton (1973).

Long-term cell cultures have also been used as sources of fish chromosomes (Clem *et al.*, 1961; Regan *et al.*, 1968; Wolf and Quimby, 1969; Rio *et al.*, 1973; Hayashi *et al.*, 1976), but are not recommended for general karyotyping (Chen and Ebeling, 1975). In attaining the potential for indefinite subculturing, several cell lines in both fishes (Wolf and Quimby, 1969) and mammals (Nelson-Rees *et al.*, 1967) have been found to be heteroploid. For example, Rio *et al.* (1973) examined the chromosomes of a cell line of the goldfish, *Carassius auratus,* which had been subcultured over 3 years, and found chromosome numbers ranging from 47 to 193, with an indistinct modal number of 94 (22%). The normal diploid number of *C. auratus* is 104 (Chiarelli *et al.*, 1969). In contrast, Regan *et al.* (1968) found the chromosome number of an 8-year cell line derived from fin tissue of the blue striped grunt, *Haemulon sciurus,* to be stable and $2n = 46$. Although the normal complement of *H. sciurus* contains $2n = 48$ chromosomes, the authors point out that their cell line could have been initiated from a population carrying a fixed polymorphism similar to that described by Roberts (1964) in the green sunfish, *Lepomis cyanellus.*

The most promising cell culture technique for obtaining chromosomes from fish is that of using leukocytes, since the tedious digestion and centrifugation procedures need not be employed. Leukocytes do not normally undergo cell division once in the circulating blood; however, Nowell (1960), Moorhead *et al.* (1967), and many others have shown that leukocytes in several eukaryotes may be stimulated to divide *in vitro* in the presence of certain mitogenic chemicals. Unfortunately, leukocyte culture has not enjoyed great success in fishes. Among the few reported successes are the following: Labat *et al.* (1967) and Ojima *et al.* (1970) in carp and goldfish; Heckman and Brubaker (1970) and Heckman *et al.* (1971) in goldfish and trout; Kang and Park (1975) in the Japanese eel, *Anguilla japonica;* Legendre (1975) in *Anguilla anguilla;* and Thorgaard (1976) in the rainbow trout. Heckman *et al.* (1971) noted that rainbow trout leukocytes grew only under increased oxygen tension, and suggested that perhaps alternate techniques might have to be developed for different groups of fishes. Barker (1972) devised a method for obtaining chromosomes from the few immature leukocytes in the circulating blood of the marine fish, *Pleuronectes platessa,* and indicated that the method was applied to other marine species as well.

C. Methods of Staining and Examining Chromosomes

Recently, staining techniques which result in differential banding of somatic metaphase chromosomes and permit the identification of individual chromosome pairs have been developed for a variety of organisms. Although there are few reports of successful "banding" of fish chromosomes (Abe and Muramoto, 1974; Zenzes and Voiçulescu, 1975; Thorgaard, 1976), there is little doubt with the successes in mammals that these techniques will also become widely used in studying fish chromosomes. The traditional methods of staining fish chromosomes using aceto-orecin or Giemsa (e.g., Fig. 1) are adequate for enumerating the chromosome complement of a species, but do not always permit the resolution necessary to ascertain possible chromosomal heteromorphy. Furthermore, there now are suggestions that the banding patterns observed on mammalian chromosomes not only reflect chromosomal phenotypes, but may also indicate functional genetic aspects of a given chromosome or chromosomal segment (Hoehn, 1975). A bibliography of the literature on banding techniques may be found in Nilsson (1973).

Another method which holds great promise for the study of fish chromosomes is the use of a scanning electron microscope. Webb (1974) was the first to use this technique in fishes, and his results are encouraging. The centromeres of each chromosome were readily visible (facilitating arm length determinations), and the three-dimensional surface structure of the chromosome was impressively revealed. According to Webb, the only difficulty seems to be obtaining sufficient numbers of spreads for analysis. This should be overcome by cell culture.

III. SEXUALITY, SEX CHROMOSOMES, AND SEX DETERMINATION

As a group, the fishes display an almost complete range of sexuality from hermaphroditism to unisexuality to bisexuality or gonochorism (Yamamoto, 1969). This diversity is unparalleled among the vertebrates. A few instances of unisexuality are known in the ambystomid salamanders and in a few lizard species (White, 1973a), but hermaphroditism is unknown elsewhere.

In the following, the various modes of sexuality found among fishes are briefly considered, with the emphasis placed on the genetic and/or cytogenetic mechanisms which influence sexuality. Readers more in-

terested in the physiology of sexuality, secondary sex characteristics, or sex differentiation are referred to the reviews by Gordon (1957) and Yamamoto (1969).

A. Hermaphroditism

Hermaphroditism is the normal and functional coexistence in an individual of both maleness and femaleness. In fishes, two basic types of hermaphroditism are recognized (Yamamoto, 1969). Synchronous or balanced hermaphrodites possess both male and female tissues which ripen together and function simultaneously. Histologically, the gonad of a synchronous form consists of an "ovotestes" divided into ovarian and testicular regions (D'Ancona, 1950). In theory, synchronous hermaphrodites have the capability for self-fertilization; this has been substantiated in the Florida serranid, *Serranus subligerus* (Clark, 1959, 1965), and in the oviparous cyprinodontid, *Rivulus marmoratus* (Harrington, 1961). Asynchronous or consecutive hermaphrodites are those that function as one sex when young and then transform to the other sex when aged. Protandrous forms function first as males, and then as females; protogynous forms are first females, and then transform to males. An important basic histological characteristic of asynchronous hermaphrodites, whether protandrous or protogynous, is that juveniles possess both ovarian and testicular tissue (Yamamoto, 1969).

Atz (1964) and Yamamoto (1969) listed the various hermaphroditic fish species which are found in thirteen families belonging to five orders; the majority belong to the order *Perciformes*. The synchronous hermaphrodites include species in several genera of the sea bass family *Serranidae*, the already mentioned cyprinodontid, *Rivulus marmoratus*, and a few species from four families of the order *Myctophiformes*. Atz (1964) also listed one Alaskan population of the stickleback, *Gasterosteus aculeatus*, of the order *Gasterosteiformes* as being a synchronous hermaphrodite.

The asynchronous type of hermaphroditism appears to be more prevalent, although this may reflect a paucity of information on hermaphroditic fishes. Protandrous forms (♂ to ♀) are encountered chiefly among the sea breams and porgies of the family *Sparidae;* a few others are cited in Atz (1964) and Yamamoto (1969). Protogynous forms (♀ to ♂) are found in at least four perciform families, including both the *Sparidae* and *Serranidae* (Yamamoto, 1969). The swamp eel, *Monopterus albus*, of the order *Synbranchiformes* is also a protogynous hermaphrodite (Liem, 1963; Chan, 1970). It should be pointed out that

many families contain both synchronous and asynchronous forms, and that in all families which contain hermaphrodites there are bisexual species.

The physiological and genetic bases of sexuality in the hermaphrodites are not well understood. Different histological patterns of gonadal development in protogynous forms have been described (Smith, 1959; Reinboth, 1967); essentially the process appears to be an orderly transition from one gonadal type to the other. There is evidence, however, that sex transformation in asynchronous forms may not always be completed (Larrañeta, 1964, cited in Yamamoto, 1969), and also that all individuals of a given species may not inverse sex in sequence (Smith, 1959; Reinboth, 1965). Nonetheless, in the asynchronous forms the transformation from one sex to the other may be viewed as ordinary histological differentiation; a decrease in the number of cells of one gonadal type is followed by an increase in the number of cells of the other type. An intervening "intersex" stage may prevail in some species (Yamamoto, 1969). Ohno (1974) has proposed that the switch may result from an antagonism between masculinizing inducers (androgenic steroids) and feminizing inducers (estrogenic steroids). In a protogynous species, the feminizing inducers produced by ovarian cells would suppress testicularization until a time—perhaps at ovulation—when the amount of feminizing inducer decreases and testicularization commences. Obviously, no such antagonism exists in synchronous hermaphrodites.

The above suggests that sexuality in a hermaphroditic species is a process of sex *differentiation*, rather than sex *determination*. Thus, one would not expect to find evidence of genetically or morphologically differentiated sex chromosomes (heterosomes) in either synchronous or asynchronous hermaphrodites. The chromosomes of ovarian and testicular tissue should differ to the same extent as they would between any other tissues of the same individual, for example, between liver and spleen. Vestigial heterosomes might possibly be found in a hermaphroditic species recently derived from a bisexual species which had possessed well-developed heterogamety, but even this seems unlikely. On the other hand, Ohno (1974) has pointed out the very puzzling fact that of all the bisexual species with well-differentiated (heteromorphic) heterosomes, several belong to orders which are known or thought to contain hermaphrodites (including the *Perciformes*, Nogusa, 1960; *Myctophiformes*, Chen, 1969; *Gasterosteiformes*, Chen and Reisman, 1970). One would expect those orders with hermaphroditic species to have maintained their chromosomes in the "least committed state" (Ohno, 1974). Since none of the hermaph-

rodites have yet been examined cytologically, no answer can be given for the paradox.

The experimental evidence of self-fertilization in *R. marmoratus* (Harrington, 1961) and *S. subligerus* (Clark, 1959) raises the question as to the extent of self-fertilization in natural populations of synchronous hermaphrodites. Genetically, complete selfing is the most intense form of inbreeding. Genetic variability within a lineage would be reduced by one-half every generation, and in the effective absence of genetic recombination the only new source of genetic variation would be mutation. On the other hand selfing could permit the selection for highly adapted genotypes, and prevent the breakup of co-adapted gene complexes which would normally occur with outcrossing. Theoretical aspects of this advantage are more thoroughly discussed by Crow and Kimura (1965). One also might expect that self-fertilization would be advantageous in colonizing a new habitat in areas of low population density, or in any situation where finding a mate is difficult. A single hermaphroditic individual capable of self-fertilization could successfully colonize an uninhabited area.

Few studies exist on the frequency of self-fertilization in natural populations of synchronous hermaphrodites. Clark (1959, 1965) found that isolated individuals of *S. subligerus* can fertilize their own eggs in captivity, but form spawning pairs when placed in proximity with other conspecifics. Harrington and Kallman (1968) demonstrated that the laboratory-reared offspring of wild-caught specimens of *R. marmoratus* were not only isogenic, but also homozygous for several histocompatibility loci. Whether the wild-caught individuals self-fertilized as a direct result of transplantation to the laboratory could not be determined. However, in a subsequent study, it was found that exposure to low temperature could transform both juvenile and adult *R. marmoratus* hermaphrodites into males (Harrington, 1971). Apparently, *R. marmoratus* has options for future changes in its mode of reproduction (Ohno, 1974).

B. Unisexuality

Unisexuality in fishes can be defined broadly as those modes of reproduction in which individual females produce female offspring exclusively. The term "thelytoky," defined by White (1973a) as unisexual reproduction by a process not involving fertilization (i.e., fusion of male and female pronuclei) is inadequate here. Some unisexual fishes undergo gametic fusion, and the paternal genome is expressed

phenotypically (*hybridogenesis*). Others merely use the sperm of closely related, usually sympatric, congeners to stimulate divisions of the egg nucleus; the male pronucleus degenerates and makes no genetic contribution to the developing embryo (*gynogenesis* or *pseudogamy*). In either case, the unisexual form is dependent on males of a bisexual species for sperm.

The first "all-female" species described was the Amazon molly, *Poecilia* (=*Mollienisia*) *formosa* (Hubbs and Hubbs, 1932), a small, viviparous toothcarp belonging to the family *Poeciliidae* (order *Atheriniformes*). Throughout its range from southern Texas to northeastern Mexico (Darnell and Abramoff, 1968), *P. formosa* is sympatric with one or both of the related species *Poecilia mexicana* and *Poecilia latipinna*. Hubbs and Hubbs (1932, 1946) initially suspected that *P. formosa* was an interspecific *P. mexicana* × *P. latipinna* hybrid which had lost its ability to produce bisexual offspring. Wild *P. formosa* females were phenotypically intermediate between *P. mexicana* and *P. latipinna*, and when crossed to males of either suspected parental species produced only female progeny of *formosa* phenotype. The hybrid origin of *P. formosa* was later substantiated beyond reasonable doubt by the genetic studies of Abramoff *et al.* (1968) using plasma protein markers, and by Prehn and Rasch (1969) using chromosomal markers. Further studies (Hubbs and Hubbs, 1946; Hubbs, 1955) revealed that matings of *P. formosa* with males from several different species invariably produced all-female progeny with strictly matroclinous inheritance; paternal characters were not apparently transmitted to the offspring (see also Meyer, 1938; Haskins *et al.*, 1960; Hubbs, 1964). Tissue transplant experiments gave similar results; female offspring were genetically identical to both their mothers and sisters (Kallman, 1962, 1970a). These findings led to the conclusion that unisexual reproduction in *P. formosa* was the result of gynogenesis.

Chromosome studies have shown that *P. formosa*, like its progenitors, *P. mexicana* and *P. latipinna*, is a diploid species with $2n = 46$ chromosomes (Drewry, 1964; Schultz and Kallman, 1968; Prehn and Rasch, 1969). A few triploid ($3n = 69$) individuals were identified among laboratory stocks of *P. formosa* perpetuated by crosses to males of *Poecilia sphenops* or *Poecilia vitatta* (Rasch *et al.*, 1965; Schultz and Kallman, 1968). Subsequently, Prehn and Rasch (1969) and Rasch *et al.* (1970) discovered *P. formosa*-like triploid "clones" in nature that bore a close phenotypic resemblance to *P. mexicana*, and which apparently had arisen from the addition of a functional haploid *P. mexicana* genome to the diploid one of *P. formosa* (Balsano *et al.*,

1972; Menzel and Darnell, 1973). Unlike the laboratory-produced triploids, the ones from nature were fertile and produced all-female triploid broods (Rasch and Balsano, 1973; Strommen *et al.*, 1975). Balsano *et al.* (1972) found that triploid clones comprise a significant, but variable fraction of several isolated *P. formosa* populations. Although not substantiated, the triploids are thought to reproduce by gynogenesis (Strommen *et al.*, 1975).

Production of diploid (or triploid) offspring by gynogenesis requires that the correct ploidy level be maintained in the developing embryo without the contribution of the paternal genome. This may occur cytologically by *apomixis*, where meiosis is abortive; or by *automixis*, where meiosis is normal, but correct ploidy is maintained by events occurring prior to or following the meiotic divisions (White, 1973a). In diploid or triploid *P. formosa* the exact cytological mechanisms of all-female production are not known. However, in another poeciliid genus, *Poeciliopsis*, found along the northwestern coast of Mexico (Moore *et al.*, 1970; Schultz, 1971), several unisexual forms have been discovered, and, in a few, the cytological features of all-female production have been verified.

Like *P. formosa*, the unisexuals of *Poeciliopsis* consist of both diploid and triploid forms, and apparently arose through interspecific hybridization (see review in Schultz, 1971). Initially a number of different *Poeciliopsis* unisexuals were identified. Some forms, known as *P. Cx* and *P. Cz*, were diploid with $2n = 48$ chromosomes; others, for example, *P. Cy*, were triploid with $3n = 72$ chromosomes (Miller and Schultz, 1959; Schultz, 1961, 1966, 1967, 1969, 1971). The *Poeciliopsis* diploid unisexuals maintain the all-female characteristic from generation to generation by a unique mechanism called hybridogenesis (Schultz, 1969), which prevents independent assortment of maternally and paternally derived chromosomes. Although both maternal and paternal genomes are expressed phenotypically, only the haploid female genome is transmitted to the ovum; diploidy is restored via fertilization with males from sympatric bisexual species. The cytological mechanism is essentially automictic, but not strictly so. Cimino (1972b) found that during the mitotic oogonial divisions preceding meiosis, a unipolar spindle is formed which attracts one set of chromosomes (maternal); the other set (paternal) is lost. The meiotic events, if any, are unknown but the ova produced are haploid and matroclinous (Schultz, 1973).

Additional important features of hybridogenesis were revealed by studies on the unisexual *P. Cx*. This form, which inhabits the Rio Mocorito, resembled another hybridogen, *Poeciliopsis monacha–*

lucida, in several morphological features, but differed slightly in attributes characteristic of the bisexual species, *Poeciliopsis viriosa* (Schultz, 1961, 1966). From biochemical and morphological studies (Schultz, 1969; Vrijenhoek, 1972), it was evident that *P. monacha–lucida* had arisen from interspecific hybridization between *Poeciliopsis monacha* and *Poeciliopsis lucida,* some 200 km to the north in the Rio Fuerte. Since *P. monacha* was unknown in the Rio Mocorito, and was closely related to *P. viriosa,* it was tentatively suggested that *P. Cx* arose in a similar fashion to *P. monacha–lucida,* but from interspecific hybridization between *P. viriosa* and *P. lucida.* In an extended series of studies, Vrijenhoek and Schultz (1974) demonstrated that *P. Cx* was in fact the *P. monacha–lucida* of the Rio Fuerte, but that *P. viriosa* genes had become introgressed into the *monacha* (maternal) genome. Laboratory crosses of genetically marked *P. monacha–lucida* × *P. viriosa* hybrids (which are chromosomally *monacha–viriosa* since the *monacha* genome is maternal in *P. monacha–lucida*) × *P. viriosa* males showed independent assortment both for the genetic markers used and for sex. Apparently, neither the cytological features of hybridogenesis nor the all-female character are irreversible, and when a hybridogen enters unfamiliar territory bisexuality and gene exchange with the endemic species may be favored by natural selection. Vrijenhoek and Shultz's (1974) study further revealed that it is the *monacha* genome which is invariably maternal and that "the *monacha–lucida* unisexuals have played a central role in the origin of other unisexual 'species' of *Poeciliopsis*" (p. 317). The dramatic spread of *P. monacha–lucida* via hybridogenetic combinations with the paternal genomes of *Poeciliopsis latidens* and *Poeciliopsis occidentalis* is now well documented (Schultz, 1961, 1966, 1969, 1971; Moore *et al.,* 1970; Vrijenhoek and Schultz, 1974). The adaptive advantages and evolutionary implications of hybridogenesis (and gynogenesis) are too lengthy to be considered here. Readers are referred to the reviews on the subject by Schultz (1971) and Maslin (1971).

The triploid *Poeciliopsis* unisexuals reproduce by gynogenesis. Schultz (1967) mated males from several different bisexual *Poeciliopsis* species to triploid *P. Cy,* and in each instance no evidence of paternally derived traits was found. The cytological mechanisms here are truly automictic. Prior to meiosis, the triploid oogonia undergo an endomitotic replication which raises the chromosome number to hexaploid (Cimino, 1972a). Meiosis then proceeds, and the ova produced are triploid with genetic complements identical to that of the mother. Chromosome segregation from the hexaploid meiocyte, however, is not random (Cimino and Schultz, 1970; Cimino, 1972a).

Other instances in natural populations of all-female unisexual fishes have been reported, based on observations of highly disproportionate sex ratios. Schultz (1971) cautioned, however, that skewed sex ratios could result from several causes (e.g., differential mortality) and should be viewed skeptically. Certain populations of the silver crucian carp, *Carassius auratus gibelio* (order *Cypriniformes*), are all-female producing, triploid with $3n = 141$* chromosomes, and gynogenetic (Cherfas, 1966, 1972). In nature, these triploid unisexuals can apparently utilize the sperm from related cyprinid species. Cherfas (1966, 1972) demonstrated that the cytological mechanism is apomictic. During late prophase and early metaphase of meiosis I, the chromosomes are unpaired (univalent). Multipolar figures appear which then culminate in a tripolar spindle that eventually disintegrates, resulting in an aborted reductional division. Although an equational division apparently occurs following ovulation, the ova are triploid and matroclinous. Most interestingly, Cherfas (1972) observed that the breakdown of the tripolar spindle first involved a transition to a bipolar spindle where, at least in some cells, the chromosomes were oriented in a 1:2 ratio. If, as suggested by Cherfas, this ratio corresponded to a haploid : diploid arrangement, then a similar mechanism might account for the rare diploid segregants found from *Poeciliopsis* triploids (Cimino and Schultz, 1970). Several *C. auratus* populations in Japan are also triploid, and probably gynogenetic (Kobayasi *et al.*, 1970; Kobayasi, 1971; Muramoto, 1975; Ojima *et al.*, 1975).

C. Bisexuality (Gonochorism)

The great majority of fishes reproduce bisexually, and have separate sexes which in nature are regularly encountered in an approximate 1:1 ratio. Because of this, it is frequently assumed that sex determination depends to a large extent on genes which reside on a single pair of "sex" chromosomes or *heterosomes*. In the highly evolved eutherian mammals, this is strictly the case. Males possess a pair of genetically nonhomologous heterosomes (X and Y) and produce both X- and Y-bearing sperm (*heterogamety*); females possess two X chromosomes and produce only X-bearing ova (*homogamety*). The two heterosomes, X and Y, are morphologically differentiated (*heteromorphic*) in size and shape and are easily identified cytologically. Insofar as sex determination in mammals is concerned, a single Y

* The high triploid chromosome number of *C. a. gibelio* reflects the apparent tetraploid origin of *Carassius auratus* [see Ohno and Atkin (1966) and Section IV,D of this review]. Reported diploid ($2n$) chromosome numbers of *C. auratus* range from 94 to 104 (see list in Chiarelli and Capanna, 1973).

chromosome is sufficient to determine maleness. Among the fishes, no such generalizations can be made.

1. HETEROGAMETY

Cytological evidence of heterogamety (heteromorphy) was claimed for several fishes by the early cytologists (for references, see Chen, 1969), but since has been questioned in view of the technical difficulties which then prevailed. Ebeling and Chen (1970) listed three criteria by which cytological heterogamety may be established: (1) the invariant occurrence in mitotic cells of a heteromorphic chromosome pair in one but not the other sex, (2) the atypical behavior—usually an end-to-end association—of a single bivalent at meiosis I, and (3) the presence of two different haploid karyotypes at meiosis II, each possessing one of the heteromorphic chromosome pairs. Although all three criteria are rarely fulfilled, relatively reliable evidence of sex chromosome heteromorphy has been reported for over twenty-five species of fish.

Chen (1969) described cytological heterogamety in twelve species from three orders of deep-sea fishes. In the mesopelagic deep-sea smelts of the family *Bathylagidae* (order *Salmoniformes*), four species, including *Bathylagus wesethi*, *B. stilbius*, *B. ochotensis*, and *B. milleri*, were classified as male heterogametic (XX : XY). In each species, the presumed X was the largest chromosome of the complement, and varied only slightly among the species in size (smaller in *B. ochotensis*) and in centromere position (submetacentric in *B. stilbius*, but metacentric in the others). The presumed Y also varied interspecifically, being the second largest chromosome in *B. stilbius* and *B. ochotensis*, and the smallest in *B. wesethi*. In *B. milleri*, the Y was indistinguishable from a number of very small chromosomes. Meiotic preparations were observed only in *B. wesethi*. At the first meiotic metaphase, a single "sex" bivalent was observed in an end-to-end configuration; at meiosis II, two morphotypes were found, one with the presumed X and the other without (see also Chen and Ebeling, 1966).

In another salmoniform, *Sternoptyx diaphana*, of the hatchetfish family *Sternoptychidae*, Chen (1969) found evidence of male heterogamety of the XX : XO type. This form of heterogamety is not at all rare in animal groups, and usually arises either from loss of the Y chromosome, or from fusion of the Y with an autosome or with the X (White, 1973a). Spermatogonial metaphases of *S. diaphana* contained $2n = 35$ chromosomes, the presumed X being the largest among five acrocentric chromosomes. In meiotic II metaphases, two morphotypes were observed, one with $n = 18$ and one with $n = 17$.

Of the remaining deep-sea fishes studied by Chen (1969), three lantern fish [one neoscopelid, *Scopelengys tristis*, and two myctophids, *Lampanyctus ritteri* and *Lampanyctus* (=*Parvilux*) *ingens*, of the order *Myctophiformes*] were apparently of the XX:XO type; while one myctophid, *Symbolophorus californiensis*, and three prepercoid melamphids, *Melamphaes parvus*, *Scopeloberyx robustus*, and *Scopelogadus mizolepis bispinosus* (order *Beryciformes*), were all of the XX:XY type. Full details of the cytological observations are given in Chen (1969) and Ebeling and Chen (1970). It should be noted, however, that females were available for analysis only in *L. ritteri*.

Among the shallow-water fishes, male heterogamety of the XX:XY type from cytological evidence is reported for the stickleback, *Gasterosteus wheatlandi* (Chen and Reisman, 1970; Ebeling and Chen, 1970); the gobiid, *Mogrunda obscura*, and the cottid, *Cottis pollux* (Nogusa, 1960); and two species of killifish, *Fundulus diaphanus* and *Fundulus parvipinnis* (Chen and Ruddle, 1970). In both fundulines, the heterosomes were identified as the fourth largest pair in the diploid complement. Interestingly, the presumed Y was metacentric in *F. diaphanus*, but acrocentric in *F. parvipinnis* (Ebeling and Chen, 1970; Chen and Ruddle, 1970). LeGrande (1975) found evidence of an XX:XO system in the flatfish, *Symphurus plagiusa* (*Pleuronectiformes*). Females of *S. plagiusa* contained $2n = 46$ chromosomes, whereas males had $2n = 45$. The missing element was a small metacentric.

Female heterogamety of the WZ:ZZ type (WZ = ♀, ZZ = ♂) also has been found cytologically. Chen and Ebeling (1968) discovered that karyotypes from several tissues of female mosquitofish, *Gambusia affinis*, invariably contained a large, unpaired metacentric chromosome (W) not found in males. The stickleback, *Apeltes quadracus*, is also female heterogametic (Chen and Reisman, 1970), but the presumed W is acrocentric.

Uyeno and Miller (1971) reported multiple sex chromosomes (X_1X_2Y) in an undescribed cyprinodontid killifish, related to the genus *Cyprinodon*. Mitotic karyotypes revealed that females ($2n = 48$) possessed five pairs of acrocentric chromosomes, whereas males ($2n = 47$) possessed only four pairs of acrocentrics, plus a single, outsized metacentric. In late spermatogonial prophase I, the long metacentric appeared in a trivalent configuration with two small acrocentrics. Based on similar cases described in other animals, Uyeno and Miller suggested that the large metacentric arose in the male karyotype through the fusion of a Y chromosome with an autosome. Since by definition the homologue of the fused autosome is considered a "sex"

chromosome (X_2), the species is said to possess multiple sex chromosomes, that is, ♀ = $X_1X_1X_2X_2$ and ♂ = X_1X_2Y where X_1 was the initial heterosome. Subsequently, Uyeno and Miller (1972) reported an apparently identical case of multiple sex chromosomes in an undescribed goodeid species.

Other fishes for which cytological heteromorphy has been described are the cyprinid, *Scardinus erythrophthalmus*, and the European eel, *Anguilla anguilla* (Chiarelli *et al.*, 1969). Since the sex of the individuals which were karyotyped was unspecified, it is not known whether the heteromorphic pair of chromosomes observed in both species were sex chromosomes.

In several species, heterogamety has been adduced from genetic rather than cytological evidence. In some instances, sex chromosomes have been identified by following the inheritance patterns of nonallelic sex-linked marker genes which affect morphological traits. Thus, male heterogamety of the XX:XY type has been demonstrated for several of the small, live-bearing poeciliid fishes, including two species in the genus *Poecilia* (Winge, 1922; Breider, 1935), and several species in the genus *Xiphophorus* (Kosswig, 1935, 1959; Bellamy, 1936; Gordon and Smith, 1938; Gordon, 1946, 1947; Kallman, 1965a), and for the cyprinodontid, *Oryzias latipes* (Aida, 1921). With one possible exception (see below), the heterosomes, X and Y, are not heteromorphic in these species (Winge, 1922; Iriki, 1932, cited in Denton, 1973; Friedman and Gordon, 1934; Wickbom, 1943), nor are they genetically nonhomologous throughout their length. Various authors, including Aida (1921), Winge and Ditlevsen (1947), Yamamoto (1961), and Kallman (1965b), have shown that genetic crossing over between the X and Y occurs at an appreciable frequency, although regions of nonhomology evidently exist since some genes behave as essentially X- or Y-linked. Genetic evidence of XX:XY male heterogamety also has been reported for the anabantid, *Betta splendens* (Kaiser and Schmidt, 1951), and for cultivated stocks of the cyprinid, *Carassius auratus* (Yamamoto and Kajishima, 1969).

The platyfish, *Xiphophorus maculatus*, is both male and female heterogametic. Initially, Bellamy (1922) and Gordon (1927) found that "domesticated" stocks of *X. maculatus* of unknown origin were female heterogametic and male homogametic (WZ:ZZ). When other xiphophorine species (e.g., *Xiphophorus variatus*) were then found to be XX:XY male heterogametic (Kosswig, 1935; Bellamy, 1936), it was questioned as to how or why two different modes of sex determination would arise in such closely related forms. This odd situation became confounded further by Gordon's (1946, 1947) discovery that *X. maculatus* populations in Mexico were male heterogametic. From a

series of crosses between "domesticated" ♀ heterogametic and "wild" ♂ heterogametic forms of *X. maculatus*, Gordon (1946, 1947) concluded that the Z of the ♀ heterogametic forms was equivalent to the Y of the ♂ heterogametic forms. Since he could find no evidence that W was equivalent to X, he suggested that the use of WY: YY (WY = ♀, YY = ♂) was more appropriate than WZ: ZZ. Later, Gordon (1951) found naturally occurring populations of ♀ heterogametic *X. maculatus* from the Belize River in the former British Honduras.

It now appears that Gordon's appreciation of the sex chromosomes in *X. maculatus* was correct, although at the time (Gordon, 1952) he believed that *X. maculatus* was separated into two major, isolated populations or races—one to the west in Mexico which was ♂ heterogametic (XX: XY), and one to the east in British Honduras which was ♀ heterogametic (WY: YY). Kallman (1965b, 1970b, 1973) sampled *X. maculatus* extensively throughout its native range from near Veracruz, Mexico, southeast to British Honduras, and found that the species is polymorphic for three sex chromosomes, W, X, and Y. Of the six possible zygotic combinations, four (WW, WX, WY, and XX) normally differentiate into females; the remaining two (XY and YY) normally differentiate into males. Since the Y is ubiquitous, the mode of heterogamety is dependent on the frequency of the W or X chromosome. For example, in the Belize River where Gordon (1951) first discovered ♀ heterogametic populations, the frequency of the X is low (ca. 0.045) and the dominant mode is ♀ heterogamety (WY: YY). To the northwest in the Rio Jampa, Mexico, the frequency of the W is apparently negligible, and the populations are ♂ heterogametic (XX: XY). A geographic cline, however, is not indicated. Both the W and X are widespread, and WX ♀ are not infrequent. In fact, the only trend noted by Kallman (1973) was that the X is possibly more prevalent in populations at the periphery of the platyfish distribution, suggesting that the W chromosome was a secondary modification of an already existant sex chromosome which arose in the center of the species' range. The existance of three different heterosomes leading to both ♀ and ♂ heterogamety is best documented in *X. maculatus*, but also may obtain in another poeciliid, *Poecilia sphenops* (Schröder, 1964, cited in Kallman, 1973), and in the cichlid, *Tilapia mossambica* (Hickling, 1960).

2. Sex Determination

As pointed out by Yamamoto (1969), sex in bisexual fishes is determined much in the same manner as demonstrated by Bridges

(1925) in *Drosophila*. That is, "a given property, the *sex included*, depends upon all the chromosomes, some of which pull in one direction and others in the other direction, some strongly and others faintly or not demonstrably at all" (Bridges, 1939).

The early genetic experiments by Winge (1922) on the guppy, *Poecilia reticulata*, indicated that the species was ♂ heterogametic (XX : XY). Occasionally, however, he found exceptional individuals which were heterosomally of one sex, but phenotypically and functionally of the other sex. These "exceptions" proved fertile in crosses to "normal" individuals of the same sex chromosome constitution, but of the opposite sex. Exceptional XX ♂ crossed to normal XX ♀ produced all XX (♀) progeny, and exceptional XY ♀ crossed to normal XY ♂ produced male (2XY, 1YY) and female (1XX) offspring in a 3 : 1 ratio. Winge (1934) and Winge and Ditlevsen (1947, 1948) interpreted these results as indicating that minor male (M) and female (F) determining genes were situated throughout the genome. Normally, these minor autosomal genes were hypostatic to the heterosomal sex-determining genes; the sex of an individual was a function of its sex chromosome constitution. However, through chance genetic or chromosomal recombinations, the sum of the autosomal male- or female-potency could override the usually epistatic sex chromosome genes, and thus produce the "exceptional" individuals.

Similar explanations have been proposed by several authors to account for the sporadic appearance in nature and in the laboratory of these so-called "sex reversals" among the heterogametic xiphophorines (Kallman, 1968), and for hormone-induced sex reversals of *O. latipes* (Yamamoto, 1963). In the latter species, Aida (1936) established an XX : XX bisexual strain by selective breedings of exceptional XX ♂, but suggested that the XX ♂ could have stemmed from a lowering of the female-potency of X chromosome genes. Although Aida's suggestion may have partial validity, the general consensus is that sex-determination (at least in a number of poeciliids and *O. latipes*) is polyfactorial, with epistatic sex genes located on the sex chromosomes. Kosswig (1964) has discussed this mode of sex determination in some detail, and Yamamoto (1969) has presented a simple, but useful, model based on three overlapping normal distribution curves.

The number, location, and mode of interaction of the autosomal M and F genes are unknown; the gene action, however, is not strictly additive. Kallman (1968) found evidence of specific sex transformer genes (♀ → ♂) in *X. maculatus*. In this instance, a fortuitous combination of autosomal genes, derived from crosses of two specific strains,

was apparently sufficient to override the strong female-potency of the W chromosome.

In several species, sex determination appears to be completely "polygenic," there being no genetic or cytological evidence of sex chromosome heterogamety. The most thoroughly studied example is the swordtail, *Xiphophorus helleri*. Over the years, Kosswig and his collaborators have found that sex ratios vary considerably among and within stocks of *X. helleri* and have proposed that "polygenes in their manifold recombinations decide about the sex of a specimen" (Kosswig, 1964, p. 195). A single pair of sex-indifferent autosomes, designated xx, are considered homologous to the sex chromosomes found in the heterogametic xiphophorines.

Interspecific hybridization studies between polygenic *X. helleri* and heterogametic *X. maculatus* have indicated that the M and F autosomal genes of *X. helleri* may, in certain combinations, be epistatic to sex-determining heterosomes (Kosswig, 1964). In the F_1 of crosses between *X. helleri* ♀♀ and *X. maculatus* ♂♂ (XY and YY), both male and female offspring were found among the chromosomal classes Xx and Yx. Sengün (1941, cited in Yamamoto, 1969), however, observed that Wx individuals from crosses of *X. helleri* ♂♂ to *X. maculatus* ♀♀ (WY) were all female, and that Xx individuals from crosses of *X. helleri* ♂♂ to *X. maculatus* ♀♀ (XX) were of both sexes. Presumably, this not only indicates that the W heterosome of *X. maculatus* has greater female-potency than the X, but also that the W itself has a very strong feminizing tendency—a fact substantiated by the somewhat infrequent occurrence of exceptional WY ♂♂ in natural populations of *X. maculatus* (Kallman, 1973). Based on his discovery that the sex transformer genes of *X. maculatus* may cause fluctuations in sex ratios, Kallman (1968) has suggested the interesting possibility that similar sex transformer genes may be prevalent in *X. helleri*.

Other species for which there is evidence of a polygenic mode of sex determination include two Caribbean poeciliids, *Poecilia caudofasciata* and *Poecilia vittata* (Breider, 1935, 1936), and possibly one anabantid, *Macropodus concolor* (references in Yamamoto, 1969).

3. Evolution of Sex Chromosomes

In those species for which there is genic evidence of heterogamety, the sex chromosomes do not appear morphologically differentiated.* Furthermore, the presumed sex chromosomes invariably show exten-

* A possible exception is one stock of *X. maculatus* which may have heteromorphic X and Y chromosomes (Anders *et al.*, 1969).

sive genetic homology. For example, in the guppy, *P. reticulata,* Winge (1934) and Winge and Ditlevsen (1947) have shown that during male meiosis a number of sex-linked genes cross over freely between the morphologically similar X and Y chromosomes. In the platyfish, *X. maculatus,* crossing over between the W and Y occurs about as frequently as between the X and Y (Gordon, 1937; Kallman, 1965b). These results suggest that the sex chromosomes in these species may be viewed simply as a homologous pair of autosomes which acquired one or more sex-determining genes. Further evidence that extensive genetic homology still exists between the sex chromosomes is demonstrated by the occurrence of viable WW (in *X. maculatus*) and YY individuals (many species) among laboratory or natural populations (Yamamoto, 1969; Kallman, 1973).

The number of sex-determining genes is unknown. Ohno (1974) has suggested that the key to sex determination in fishes may reside in the regulation of a single enzyme which converts androstenedione to testosterone. If the enzyme is present, testosterone is produced and testicular development is stimulated. In the functional absence of the enzyme, estrogens are produced and ovarian development results. Thus sex determination could depend upon allelic relationships at a single regulatory locus (Ohno, 1974). Such a hypothesis is not incompatible with the results from numerous studies on the physiology of sex determination in fishes (Yamamoto, 1969). Theoretically, crossing over between the sex chromosomes should have no consequence; an "X" of one generation would merely be the "Y" of the next. A single locus system determining sex, however, is suspect for several reasons, not the least of which is its susceptibility to mutation. It is reasonable to assume, then, that at least a few nonallelic, closely linked genes determine sex. The finding of essentially X- and Y-linked Mendelian genes in *P. reticulata* (Winge and Ditlevsen, 1947) indicates that stretches of nonhomology exist on the X and Y, and that crossovers within the region containing the sex determining loci are extremely rare (if they occur at all).

The position of the sex determining loci on the chromosome is of importance, since crossing over within the region containing the sex genes (or between this region and the centromere) might prove disruptive. Anders *et al.* (1973) have shown that the male and female determining regions of the *X. maculatus* and *X. variatus* sex chromosomes X and Y map adjacent to the centromere, and proximal to all known sex-linked Mendelian genes. Since the chromosomes of *X. maculatus* and *X. variatus* are acrocentric (Friedman and Gordon, 1934), this location is ideal. Chances of crossing over between the "sex" region

and the centromere would be small, and should be reduced further by an expected nonrandom distribution of crossovers along the length of the chromosome. In other experimentally tractable animals (e.g., *Drosophila*) there is ample evidence that crossing over itself is reduced in regions proximal to the centromere. Once situated, each sex-determining region might then undergo paracentric inversion(s) to further reduce any chance of recombination (Ohno, 1967).

Along these lines, Yamamoto's (1961) study on *O. latipes* is of interest. Using two alleles at a sex-linked locus for body color (*R* and *r*, where *R*– = orange red and *rr* = white), he constructed a stock in which the *R* allele was Y-linked, and the *r* allele X-linked. In normal ♂♂ (X^rY^R), crossing over between the *R* locus and the sex-determining loci occurred at a low frequency (ca. 0.2%). But when sex-reversed X^rY^R ♀♀ were examined, it was found that crossing over had increased more than fivefold. Apparently, there may also be physiological regulation of crossing over between the X and Y, which normally prevents the disruption of the specific "sex" genes.

The isolation of a "differential segment" containing the sex-determining genes in a position adjacent to the centromere may represent the initial stages leading to morphological differentiation or heteromorphy of the sex chromosomes. A subsequent pericentric inversion involving the sex region of one heterosome might further isolate the sex-determining genes, and result in detectable heteromorphy. In time, unequal crossing over, or perhaps nonreciprocal translocation, could bring about extreme heteromorphy with most if not all of the Mendelian genes eliminated from one heterosome. From studies on different families of snakes, Ohno (1967) has diagrammed this progression to heteromorphy, beginning with the cytologically detectable pericentric inversion.

Whether the chromosomal location of the sex-determiners in *X. maculatus* represents the incipient stages of heteromorphy is unknown. Anders *et al.* (1969) reported heteromorphic X and Y chromosomes in one stock of *X. maculatus*, which may be the result of unequal exchange or translocation (Anders *et al.*, 1973). Other *X. maculatus* populations, however, are apparently homomorphic, as are *O. latipes* and other closely related poeciliids (references in Denton, 1973; Chiarelli and Capanna, 1973). As an alternate hypothesis, Ebeling and Chen (1970) suggested that homomorphy may be adaptive since it could ensure a certain lability of sexual expression.

Among the deep-sea bathylagids studied by Chen (1969), the cytological progression to increasing heteromorphy (as outlined in Ohno, 1967) is apparently observed in the four species of the genus

Bathylagus which possess heteromorphic sex chromosomes (Ebeling and Chen, 1970). The same may also be true for the two fundulines, *F. diaphanus* and *F. parvipinnis*, even though the centromere position of the heteromorphic Y differs in these two species (Chen and Ruddle, 1970; Ebeling and Chen, 1970). However, in the remaining species where heteromorphic sex chromosomes have been reported, there is no indication as to whether they represent isolated exceptions or the initial stages of incipient heteromorphy. In short, the above examples notwithstanding, the sex chromosomes in most fishes have remained in a relatively undifferentiated state.

IV. CHROMOSOME NUMBERS, CHROMOSOME MORPHOLOGY, AND GENOME SIZES: THE EVOLUTION OF FISH KARYOTYPES

The karyotype, as far as its morphological features are concerned, is also part of the phenotype, as it is the result of an evolution whose course may have been varied.

Mario Benazzi (1973)

Considering the vast number of living fish species, their diversity in morphology, and the antiquity of the group as a whole, one might expect to find a corresponding wealth of karyotypic diversity. Surprisingly, this does not appear to be the case. Many orders are relatively uniform in karyotype, although they may differ in evolutionary age by tens of millions of years. For example, the haploid (n) karyotype of 24 acrocentric chromosomes is found throughout several diverse orders of the subclass *Teleostei* (class *Osteichthyes*) and appears to be the predominant karyotype in the recently evolved *Perciformes* (Roberts, 1964, 1967; Denton, 1973; Chiarelli and Capanna, 1973). This has led to the suggestion that the 24 acrocentric chromosome complement may be ancestral to all modern fishes, and perhaps was possessed by the primordial teleost (*Leptolepis*) over 100 million years ago (Ohno, 1974). An even more entertaining possibility is that this chromosome configuration may have been ancestral to all vertebrates (Ohno *et al.*, 1968; Ohno, 1974). Nogusa (1960) and Taylor (1967) found this karyotype in two hagfish species of the primitive order *Cyclostomata*. These hagfish species represent the primitive jawless *Agnatha* which separated from the main line of vertebrate evolution more than 300 million years ago. But other primitive species from the classes *Agnatha*, *Chondrichthyes*,

and *Osteichthyes*, do not have a 24 acrocentric karyotype (Denton, 1973; Chiarelli and Capanna, 1973).

A. Chromosome Numbers and Genome Sizes

Reported chromosome numbers in fishes range from a low of $n = 8$ in the cyprinodontid, *Notobranchius rachovii* (Post, 1965), and the anabantid, *Sphaerichthys osphromonoides* (Calton and Denton, 1974), to a high of $n = 84$ in the petromyzontiform lamprey, *Petromyzon marinus* (Potter and Rothwell, 1970). The wide range in chromosome number is misleading. About 35–40% of almost 500 assayed fish species from 76 families in 26 orders have $n = 24$ chromosomes (Denton, 1973; Chiarelli and Capanna, 1973). The distribution is strongly leptokurtic; almost 70% of species have chromosome numbers in the range $n = 22$–26, and ca. 80% of species fall in the range $n = 20$–28. Two minor peaks are found in the ranges $n = 40$–52 (ca. 5–6%) and $n = 82$–84 (ca. 0.8%). The former peak contains species from the teleost families *Salmonidae, Cyprinidae, Catostomidae,* and *Cobitidae,* a few petromyzontiform lampreys, one chondrostean, and a few skates of the order *Rajiformes*. The latter peak contains four species of petromyzontiform lampreys.

Chromosome numbers and variabilities in chromosome number distinguish certain major taxonomic groupings of fishes. For example, salmoniform species have higher chromosome numbers (median $\approx$ 36 haploid chromosomes) than cypriniform species (median $\approx$ 25 haploid chromosomes), and they also are more variable in chromosome number. Within the *Salmoniformes,* chromosome numbers are in the range $n = 11$–51 and are distributed platykurtically; within the *Cypriniformes,* chromosome numbers are in the range $n = 18$–52 but are distributed leptokurtically. Trends also are observed at lower taxonomic levels. Briefly, groups with chromosome numbers in the range $n = 22$–26 tend to be relatively invariant in chromosome number, whereas groups with higher or lower chromosome numbers tend to be more variable.

There are at least three cytological mechanisms which may bring about changes in chromosome number: (1) polyploidization, where the chromosome number is increased to an exact multiple of the basic chromosome set, (2) Robertsonian rearrangements, where centric fusion of two nonhomologous acrocentric chromosomes produces a single metacentric, or where centric dissociation of a single metacentric produces two nonhomologous acrocentrics (Robertson, 1916), and

(3) aneuploidy, where nondisjunction or endoreduplication results in gain or loss of individual chromosomes.

Genome size, or the amount of DNA per nucleus, also shows wide variation among fishes. Haploid DNA contents range from 0.4 pg (10^{-12} g) per nucleus in tetraodontiform puffers (Hinegardner and Rosen, 1972) to 124 pg per nucleus in the lungfish, *Lepidosiren paradoxa* (Markert, 1968). Bachmann *et al.* (1972), however, found that DNA contents of 195 fish species, including representatives from all three classes, exhibited a unimodal distribution, skewed towards higher DNA values. When log transformed, the distribution was normal around a strong mode at 1.7 pg (diploid amount). Among the teleost fishes, the distribution is similar but the estimated mode (haploid amount) is 1.0 pg (Hinegardner, 1968; Hinegardner and Rosen, 1972).

The evolutionary implications of changes in genome size in fishes have been studied by Hinegardner (1968), Hinegardner and Rosen (1972), Bachmann *et al.* (1972), and Ohno (1970, 1974), and only their broad conclusions are summarized here. There is usually homogeneity of DNA amounts within families and lower taxonomic categories, and genome sizes tend to be relatively stable despite changes in morphology and/or physiology. Notable exceptions occur in the families *Cyprinidae*, *Cyprinodontidae*, and *Callichthyidae*, where species may differ in DNA content by more than twofold. A greater variation in DNA amounts is observed among certain orders. For example, the average DNA content in 104 perciform species is 1.04 pg, whereas 32 siluriform species average 1.78 pg (Hinegardner and Rosen, 1972). The increased DNA content of the *Siluriformes* stems primarily from the high DNA contents of the families *Callichthyidae* and *Loricariidae* (averaging 2.68 pg per species).

Decreases in DNA content often are associated with increasing specialization in body form and design. More specialized species have less DNA per cell than do more generalized forms.* This inverse relationship between genome size and degree of specialization holds for fishes as a group, and also within certain taxa (Mirsky and Ris, 1951; Hinegardner, 1968; Hinegardner and Rosen, 1972). A rationale for this pattern of DNA loss has been developed by Ohno *et al.* (1968), Ohno (1970, 1974), and Bachmann *et al.* (1972). In their view, increases in genome size, particularly when provided by polyploidization, result

* The terms "specialized" and "generalized" as used here follow the definitions of Hinegardner and Rosen (1972). Specialized groups (or species) are those which share few features in common with related members of the same taxon; generalized species share many features in common with phylogenetic relatives.

in major adaptive shifts; following such a shift, loss of "excess" DNA accompanies specialization.

Within taxonomic families, a significant correlation exists between genome size and variation in genome size (Hinegardner and Rosen, 1972). Specialized families with small genome sizes tend to be less variable in genome size, and almost all families with very low average DNA contents per species (0.4–0.6 pg) have very little variation in DNA content among species.

Exceptions to the trend of decreasing genome size with increasing specialization are found among the catfishes of the order *Siluriformes*. Species in the specialized families *Callichthyidae* and *Loricariidae* have, on the average, two- to threefold more DNA per nucleus than does the average species in other siluriform families (Hinegardner and Rosen, 1972). Other exceptions include the specialized families *Scaridae* and *Gobiidae* of the order *Perciformes*, which have more DNA than 36 other perciform families (Hinegardner and Rosen, 1972). The biological significance of these exceptions is unknown.

The most poignant exceptions to the trend are the dipnoan lung fishes (order *Lepidosireniformes*) of the subclass *Crossopterygii*. These ancient, but specialized, fishes have genome sizes some 80–100 times as large as the average fish and at least 25–40 times as large as their closest living relative, *Latimeria chalumnae* (Mirsky and Ris, 1951; Ohno and Atkin, 1966; Cimino and Bahr, 1974). The enormous genome size of the dipnoans is a characteristic shared only with the Urodele *Amphibia* (Morescalchi, 1973).

Cytological mechanisms which could lead to increases in genome size include polyploidy (Ohno *et al.*, 1968; Ohno, 1970), "lateral increases" through differential polynemy (Rothfels *et al.*, 1966), "longitudinal increases" through accidental DNA doubling (Sparrow and Nauman, 1974), unequal crossing over (Spofford, 1972; Ohno, 1974), and regional disturbances in DNA replication (Keyl, 1965; Price, 1976). Mechanisms which might lead to decreases in genome size include unequal crossing over, regional disturbances in DNA replication, or misrepair of chromosome "breaks" (Bachmann *et al.*, 1972; Spofford, 1972; Sparrow *et al.*, 1972; Price, 1976).

Based on the log normal distribution of DNA content observed among fishes, Bachmann *et al.* (1972) suggested that changes in genome sizes were small, numerous, and cumulative, and most likely stemmed from successive duplications and/or deficiencies. Large changes such as implied by polyploidy were exceptional. Goin and Goin (1968) and Bachmann *et al.* (1972) view the decreases in genome size as due to loss of unnecessary and/or redundant DNA. Whether

these DNA losses occur subsequent to loss of gene function, or whether the losses themselves cause loss of gene function, is problematic (Hinegardner and Rosen, 1972).

Although there is little direct evidence to indicate the cytological processes responsible for the decreases in genome size in fishes, one line of reasoning suggests that much of the reduction in genome size occurs during chromosomal rearrangements which produce changes in chromosome number. Among diploid teleost fishes there is a highly significant, positive correlation between chromosome number and genome size (Hinegardner and Rosen, 1972). This correlation holds when species with probable polyploid ancestry (see Section IV,D) are excluded from the calculation. Species or species groups with higher chromosome numbers tend to have larger genome sizes. Although exceptions exist (Hinegardner and Rosen, 1972), the clear implication is that reduction in chromosome number is accompanied by reduction in genome size, and hence may be viewed as another process correlated with increasing specialization and advancement.

Even though the overall picture of fish karyotype evolution is as yet unclear, a few salient features are apparent. The trend in fish karyotype evolution is toward smaller genome size. This presumably is accomplished in part by chromosomal rearrangements which reduce chromosome number. DNA loss may in itself be adaptive by altering certain biophysical parameters related to genome size (Bennett, 1972; Price, 1976), and then too, reduction in chromosome number may be adaptive through tightening of linkage (Mather, 1953; Stebbins, 1958). Nikolsky (1976) has suggested that reduction in chromosome number (and also in genome size) in fishes may be associated with increasing habitat stability and effectiveness of food resource utilization.

One may further speculate that once a species or species group reaches a small genome size, chromosome structural changes which result in further DNA loss should no longer be easily tolerated. If this is true, highly specialized taxa should be relatively invariant in genome size and in karyotype. In general, this is the case. In the highly specialized order *Perciformes,* the average species (excluding those from the families *Scaridae* and *Gobiidae*) has ca. 0.97 pg of DNA per haploid nucleus (Hinegardner and Rosen, 1972). This estimate is relatively low when compared with most other teleostean orders. The *Perciformes* are also relatively homogeneous in genome size and chromosome number (Hinegardner and Rosen, 1972; Denton, 1973). In contrast, species from the less specialized order *Cypriniformes* [excluding two cyprinids, *Carassius auratus* and *Cyprinus carpio,* and

the family *Catostomidae*, which are apparently polyploid (see Section IV,D)] have on the average ca. 1.33 pg of DNA per haploid nucleus, and also are more variable in genome size and chromosome number (Hinegardner and Rosen, 1972; Denton, 1973). The same trend holds for comparisons within orders. Many species in the families *Gobiidae* (*Perciformes*), *Callichthyidae* and *Loricariidae* (*Siluriformes*) each have average DNA contents higher than the average species in their respective orders. They also appear to be more heterogeneous in genome size and chromosome number (Hinegardner and Rosen, 1972; Scheel *et al.*, 1972; Denton, 1973). The indication is that taxa with high DNA contents may have greater flexibility in terms of chromosomal rearrangement.

B. Chromosome Morphology and Polymorphism

1. Morphology

Among diploid species, each pair of homologous chromosomes is assumed to differ genetically from all other chromosome pairs in the same cell. Outward manifestations of some of these differences comprise the morphological "phenotype" or karyotype and include differences between chromosome pairs in relative size, shape, and centromere position. Karyotypic differences among species or taxa may be used to determine phenetic similarities and phylogenetic relationships.

The concept of "symmetrical" versus "asymmetrical" karyotypes has been developed by several authors (Stebbins, 1958, 1971; White, 1973a) to indicate the apparent degree of chromosomal heterogeneity within a karyotype. In a perfectly symmetrical karyotype all chromosomes are approximately the same size and shape and have medially located centromeres. The trout (family *Salmonidae*) karyotype (Fig. 1) is quite symmetric; the metacentrics essentially comprise one size group, and the acrocentrics a second. By contrast, the minnow (family *Cyprinidae*) karyotype (Fig. 2) is highly asymmetric; this is typical of most *Cyprinidae*. Not only are there apparent differences in chromosome size, there are also obvious differences in centromere position even within groups of chromosomes of approximate size.

Degrees of asymmetry in karyotype among the fishes are broadly taxon specific. As noted above, the *Cyprinidae* predominantly have highly asymmetrical karyotypes, the *Salmonidae* much less so. Examples also exist within orders, for example, the highly asymmetrical karyotypes of the salmoniform family *Bathylagidae* contrast sharply with the symmetry of the *Salmonidae* (Chen, 1969).

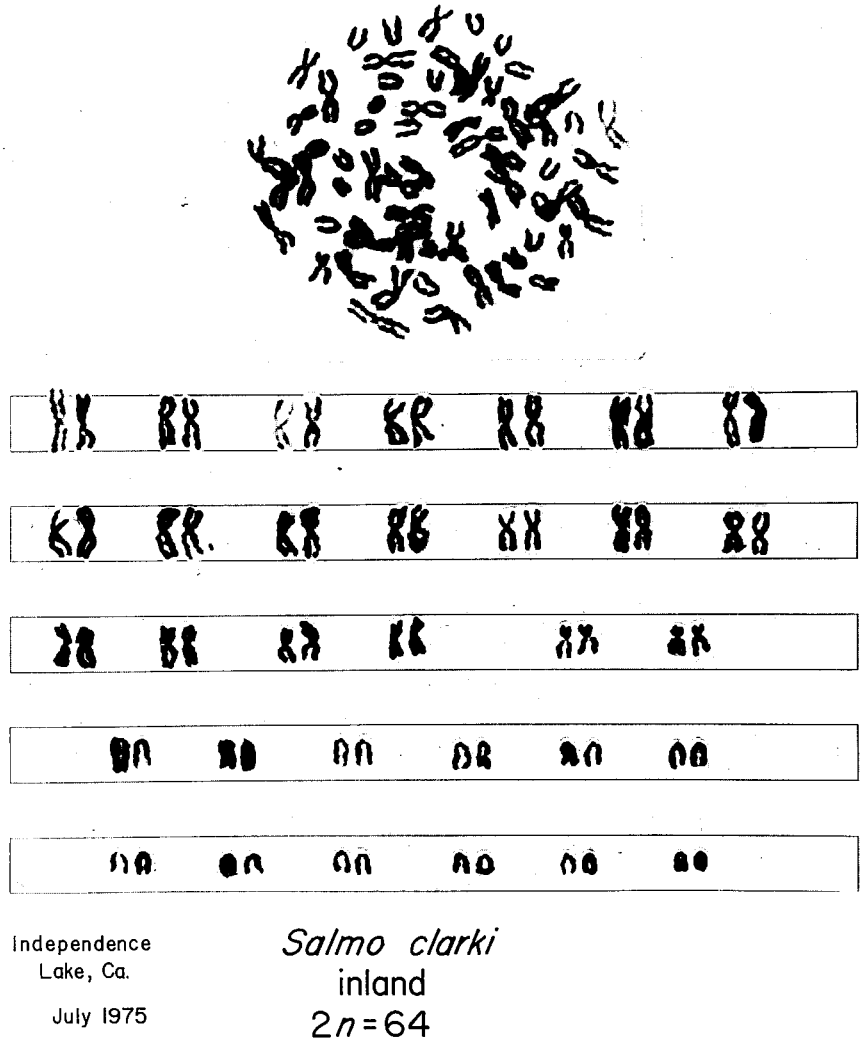

Fig. 1. Somatic metaphase karyotype from kidney cell of inland cutthroat trout, *Salmo clarki* (family *Salmonidae*), $2n = 64$.

Generally, fish chromosomes are smaller in size than chromosomes in most vertebrates. The length of the "average" fish chromosome is between 2 and 5 μm. Many species possess numerous small chromosomes of 2 μm or less, but which are nonetheless easily seen through the light microscope. Very large chromosomes of 15–30 μm in length, such as those found in the lungfish, *Lepidosiren paradoxa* (Ohno and

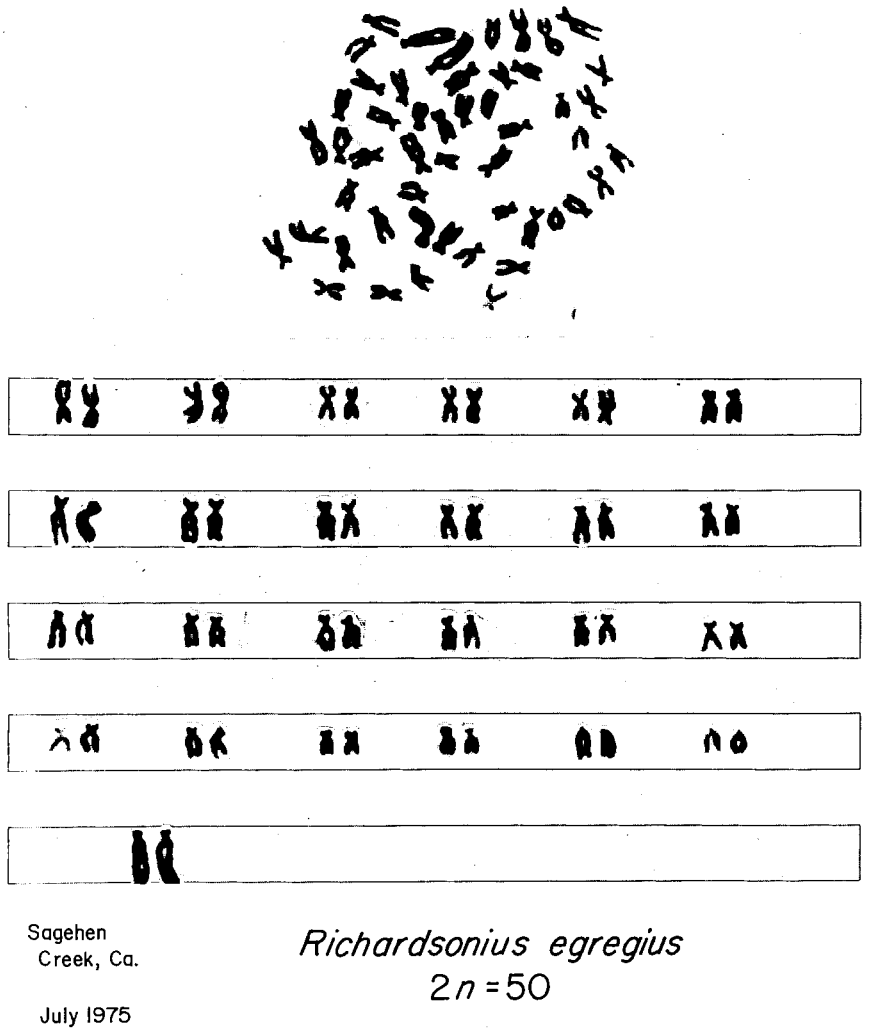

Fig. 2. Somatic metaphase karyotype from kidney cell of Lahontan redside, *Richardsonius ergregius* (family *Cyprinidae*), $2n = 50$.

Atkin, 1966), or the extremely bizzare SM (*supermacro*) chromosomes found by Post (1973) in two forms of the family *Diretmidae* (*Beryciformes*), are rare. In the past the overall small size of fish chromosomes often has precluded the accurate determination of centromere position, but improved cytological techniques now available should

remedy this situation. Most workers follow the standard nomenclature of Levan *et al.* (1964) in identifying particular chromosome shapes.

Extremely small chromosomes (microchromosomes) have been reported in a few species (Ohno and Atkin, 1966; Ohno *et al.*, 1969b; Chen, 1969). Ohno *et al.* (1969b) found between 26 and 48 microchromosomes in the karyotypes of three very primitive species, *Hydrolagus colliei* (ratfish), *Scaphirhynchus platorhynchus* (sturgeon), and *Lepisosteus productus* (gar). Elsewhere among the vertebrates microchromosomes are found in the birds (Ray-Chaudhuri, 1973) and in certain reptiles (Ohno, 1967; Gorman, 1973). Ohno *et al.* (1969b) pointed out that although microchromosomes could have arisen independently at the reptilian stage of vertebrate evolution, their presence in these relic fishes suggests that the evolution of terrestial vertebrates from the fishes was polyphyletic, and furthermore, that the fish ancestral to all birds may already have possessed microchromosomes.

2. POLYMORPHISM

A chromosomal rearrangement (inversion or translocation) arises in a single individual and may be passed on to its progeny. If adaptive, it may increase in frequency in the population. The "new" and "old" gene arrangements are inherited essentially as if they were Mendelian alleles at a single locus. Thus, when more than one chromosomal *morph* is present in a population, the population is said to be polymorphic and individuals are considered as homozygous or heterozygous in the traditional genetic sense.

The importance of chromosomal polymorphism to evolution is stated by White (1973a): "Where a parallelism exists . . . between intraspecific chromosomal rearrangements and interspecific ones we may legitimately conclude that balanced chromosomal polymorphism has given rise to cytotaxonomic differences between species through one chromosome sequence undergoing fixation in one incipient species while an alternative sequence undergoes fixation in a second incipient species" (p. 764). This correlation between fixation of alternate chromosomal sequences and incipient speciation is well documented in both plant and animal species, and has been considered as evidence that some chromosomal rearrangements are adaptive and strongly influenced by natural selection (Grant, 1963; Dobzhansky, 1951, 1970; White, 1973a,b). Occasionally, a chromosomal polymorphism may persist in a species in a "balanced" condition, presumably through a mode of selection which maintains heterozygosity. This too is well documented in both plants and animals (see above references).

In contrast to other animal species, there are few reports of chromosomal polymorphisms among the fishes, although they certainly must occur given the diversity of karyotypes observed in fishes. Why chromosomal polymorphism appears low in fishes is unknown. One possibility is that chromosomal rearrangements in fishes tend to be fixed rapidly [perhaps as a result of small effective population sizes (see Wright, 1943; Wilson *et al.*, 1975)]. A more plausible alternative is that the present techniques used in fish cytology provide insufficient resolution of chromosome structure to discern most polymorphisms. Further, the number of individuals examined per population usually is small, and existing polymorphisms may go unnoticed.

The most widely known instances of chromosomal polymorphism in fishes occur among certain genera of the family *Salmonidae*, notably in the genus *Salmo*. Thorgaard (1976) has recently presented convincing evidence of an intrapopulation chromosomal polymorphism in the rainbow trout (*Salmo gairdneri*). In the most thoroughly studied species, the Atlantic salmon, *Salmo salar*, reported chromosome numbers range from $2n = 54$ to $2n = 60$. European populations of *S. salar* range in chromosome number from $2n = 58$–60 (Prokofieva, 1934; Svärdson, 1945; Rees, 1964, 1967; Nygren *et al.*, 1968a, 1972), whereas North American populations range from $2n = 54$–57 (Boothroyd, 1959; Roberts, 1968, 1970).

Changes in chromosome number without change in the number of chromosome arms (*nombre fondamental*) constitute *primae facie* evidence for Robertsonian fusions or dissociations (Matthey, 1945, 1973). Arm number estimates in North American *S. salar* reveal that most of the polymorphism is Robertsonian. Boothroyd (1959) and Roberts (1968, 1970) found estimates of 72 arms in seven North American populations, despite variations in chromosome number from $2n = 54$–57. Among European *S. salar* there is evidence of variation in arm number as well as in chromosome number. Prokofieva (1934) and Svärdson (1945) reported karyotypes of *S. salar* consisting of $2n = 60$ chromosomes; in both, six chromosome pairs were metacentric giving an arm number estimate of 72. Rees (1964) also found a *S. salar* karyotype of $2n = 60$ from a hatchery population in Wales. After a 2-year study of the same population Rees (1967) acknowledged a karyotype of $2n = 58$ chromosomes containing 74 chromosome arms as the correct one. This same karyotype ($2n = 58$ with 74 arms) has been found among several Swedish *S. salar* populations (Nygren *et al.*, 1968a, 1972).

The karyotype data from *S. salar* (North American and European) suggest moderate chromosomal restructuring within the species in-

volving both Robertsonian and non-Robertsonian rearrangement. Centric fusions and/or dissociations could account for the observed variation in chromosome number, while uneven translocation or pericentric inversion could account for the discrepancies in arm number. The real situation is more complex. Several investigators have commented on the apparent absence in *S. salar* of a fixed karyotype *within* individuals; cells examined from the same or different tissue of a single specimen showed extensive intraindividual variation (or polymorphism) in chromosome number, sometimes by as much as 16 chromosomes (Nygren *et al.*, 1968a). Rees (1967) and Boothroyd (1959) felt that much of the observed variation was more apparent than real and was due to counting error and/or artifacts caused by chromosome preparation technique. It was also suggested (Svärdson, 1945; Boothroyd, 1959) that some of the variation could stem from genuine aneuploidy, without phenotypic alteration.

Ohno *et al.* (1965) observed similar intraindividual polymorphism in the rainbow trout, *Salmo gairdneri*, but found that much of the variation followed a distinct Robertsonian pattern. By counting both chromosome and chromosome arm numbers in several cells from various tissues of the same individual, they identified seven distinct karyotypes ranging from $2n = 58$–65, each of which possessed 104 chromosome arms. Since the modal karyotype (35% of counts) of $2n = 60$ with 104 arms was consistent with other published karyotypes of *S. gairdneri* (Wright, 1955; Bungenberg de Jong, 1955; Simon and Dollar, 1963), Ohno *et al.* (1965) concluded that they were "witnessing for the first time an example of Robertsonian polymorphism within single individuals" (p. 118). Similar patterns of intraindividual Robertsonian polymorphisms have subsequently been described in several salmonid species, including *S. salar* (Roberts, 1968, 1970; see also Ohno *et al.*, 1969a; Davisson *et al.*, 1973; Gold and Gall, 1975; Zenzes and Voiçulescu, 1975).

The cytological mechanisms that produce intraindividual polymorphism are not well understood. Beçak *et al.* (1966a) suggested two possible alternatives: (1) During zygotic development, certain chromosomes undergo Robertsonian exchange (fusion and dissociation) without apparent harm to cell viability, or (2) zygotes begin development structurally heterozygous for several Robertsonian rearrangements, then during cell division undergo a segregation pattern called *somatic segregation* which tends to restore structurally homozygous cell types. The first alternative predicts that all individuals, whether structurally heterozygous or homozygous for any number of Robertsonian rearrangements, should display the intrain-

dividual polymorphism pattern, at least to some extent; the second predicts that individuals should exist which as zygotes were structurally homozygous for all the Robertsonian rearrangements segregating in the population, and hence should display a fixed karyotype in all cells. Whether either or both of the two alternatives is operative in salmonid species which show this polymorphic pattern is yet unknown (Beçak *et al.*, 1966a; Ohno *et al.*, 1969a; Gold and Gall, 1975; Roberts, unpublished observations).

C. Chromosome Changes and Speciation

The idea that chromosomal rearrangement is involved in the process of speciation is an old one in evolutionary biology, and is thoroughly discussed in the literature (Dobzhansky, 1951, 1970; Mayr, 1973; White, 1973a,b). White (1968) notes that "it is a matter of empirical observation that . . . even the most closely related [higher animal] species are usually found to differ in karyotype. . . . The only sure exceptions to this generalization seem to be certain (*homosequential*) species complexes in the genus *Drosophila*" (p. 1065).

There are at least two reasons why chromosomal rearrangements may be important to speciation. The first is by providing a postmating reproductive isolation mechanism that renders F_1 hybrids partially or completely sterile (White, 1973a,b). Individuals heterozygous for one or more structural rearrangements would be expected to produce duplication-deficiency gametes due to chromosome pairing and segregation irregularities at meiosis.

The second reason is that chromosomal restructuring, rather than point mutation, may effect significant changes in the patterns of gene regulation (Wallace, 1963; Stebbins, 1969; Wilson *et al.*, 1974a,b). Insofar as speciation is concerned, it has been proposed that "genetic revolutions" may occur by changes in gene arrangement rather than by accumulated changes in structural genes. The evidence for this is that rates of organismal evolution (e.g., anatomy or way of life) in certain groups are correlated with rates of chromosomal evolution, but not with rates of protein evolution (Wilson *et al.*, 1974a,b, 1975; Prager and Wilson, 1975).

Among the fishes, several taxa show evidence of extensive chromosomal rearrangement, much of which presumably is associated with speciation. Several cyprinodontids in the genera *Aplocheilus*, *Aphyosemion*, *Epiplatys*, and *Fundulus* differ markedly in karyotype. These differences apparently stem from both Robertsonian rear-

rangement and uneven translocation or pericentric inversion (Post, 1965; Scheel, 1968, 1972; Chen, 1971; see also Gyldenholm and Scheel, 1971; Denton, 1973). Similar examples are found among species or taxa of certain pleuronectiforms (LeGrande, 1975), the perciform *Gobiidae* (Nogusa, 1960; Chen and Ebeling, 1971), and neotropical cichlids (Thompson, 1976).

Among the *Salmonidae,* several genera reflect rather extensive chromosomal rearrangement. Booke (1968, 1970, 1974) examined eleven North American species from *Prosopium* and *Coregonus* and found evidence of both Robertsonian rearrangement and uneven translocation or pericentric inversion; six species of *Prosopium* had the same arm number (100) but different chromosome numbers (from 64 to 82), and five species of *Coregonus* had the same chromosome number (80) but different arm numbers (from 98 to 106). European species of *Coregonus* may differ in chromosome number (references in Booke, 1968). Simon (1963) found that five species of *Oncorhynchus* ranged in chromosome number from $2n = 52–74$, and in arm number from 102 to 112. Perhaps the most karyotypically variable taxa is the genus *Salmo;* reported chromosome numbers (seven species) range from $2n = 54–80$, and arm numbers range from 72 to 106 (Svärdson, 1945; Wright, 1955; Boothroyd, 1959; Simon and Dollar, 1963; Rees, 1967; Roberts, 1967, 1968, 1970; Nygren *et al.,* 1968a, 1972; Miller, 1972; Gold and Gall, 1975).

It is well known, however, that many species and genera within the *Salmonidae* hybridize in culture and in nature (Hubbs, 1955; Buss and Wright, 1956; see list of references in Dangel, 1973). A case in point are the trouts (*Salmo*) endemic to the Pacific Northwest. The chromosome numbers (arm numbers) reported for these trouts are *S. clarki clarki* (coastal subspecies), $2n = 68$ (104); *S. clarki henshawi* (inland subspecies), $2n = 64$ (104); *S. gairdneri,* $2n = 60$ (104); *S. aguabonita,* $2n = 58$ (104); and *S. apache,* $2n = 56$ (106) (Wright, 1955; Ohno *et al.,* 1965; Miller, 1972; Wilmot, 1974; Gold and Gall, 1975; Gold *et al.,* 1977; Thorgaard, 1976). These species apparently comprise a Robertsonian series with the predominant rearrangement being centric fusion (Gold *et al.,* 1977). And yet, hybridization between several of these species occurs freely, and often at a very high frequency (Gould, 1966; Behnke, 1970, 1972; Dangel, 1973; Gold and Gall, 1975; Gold *et al.,* 1976). Moreover, in several cases the hybrids and their offspring are apparently fertile (Behnke, 1972). The overall indication is that among related species in the *Salmonidae,* partial or complete sterility due to structural heterozygosity may not contribute to reproductive isolation.

The same appears to be true among fish taxa which by present cytological resolution are extremely conservative karyotypically. Roberts (1964) examined twenty of the thirty extant species of the North American perciform family *Centrarchidae* and found that fifteen had virtually indistinguishable karyotypes of $n = 24$ acrocentric chromosomes. All fifteen are known to produce fertile hybrids (references in Avise and Gold, 1977). The implication is again that sterility due to structural heterozygosity may not contribute to reproductive isolation.

In Section IV,A, it was noted that fishes are remarkably conservative in karyotype. About 35–40% of all species karyotyped have $n = 24$ chromosomes, and about 70% fall in the range $n = 22$–26. Of these, many show little or no variation in arm number (Denton, 1973). This is more pronounced in certain taxa. In the order *Perciformes*, 70–75% of the species examined have $n = 24$ chromosomes, and with three exceptions, all have karyotypes in the range $n = 22$–26. Furthermore, of those perciforms (excluding the gobiids) for which arm number data are available (Denton, 1973), ca. 70% have arm numbers of 48. In view of the large number of extant fish species and the relatively high frequency of hybridization which generally characterizes fish (Hubbs, 1955), the overall conservative nature of fish karyotypes suggests that chromosomal restructuring in fishes may not be a prime contributor to reproductive isolation. It also suggests that some speciation events may not necessarily be accompanied by (observable) chromosomal changes.

Wilson *et al.* (1975) recently published estimates of rates of chromosomal change in fifteen vertebrate groups. Their estimate for teleost fishes (23 genera) was 1.5 changes in arm number and 1.1 changes in chromosome number per lineage per 100 million years. This may possibly be an overestimate. Of the 23 teleost genera (one-hundred eight species) sampled, 6 (forty-seven species) were from genera which are extremely variable in karyotype (G. L. Bush, personal communication). However, since Wilson *et al.* (1975) restricted their sample only to genera known to occur as fossils, the estimate for teleosts may be regarded as reasonable, although perhaps somewhat elevated. When compared to other vertebrates the rates of chromosomal evolution in teleosts were over threefold less than in placental mammals, about the same as in snakes and lizards, higher than in frogs, and much higher than in turtles, crocodiles, and salamanders (Wilson *et al.*, 1975). Since the number of extant fish species is easily double that of most other vertebrate groups, the indi-

cation again is that many speciations in fishes may not be accompanied by gross chromosomal change.

In a recent study, Avise and Gold (1977) compared the karyotypes of several North American cyprinids (*Leuciscinae*) with those of the North American sunfish genus *Lepomis* (see also Gold and Avise, 1977). The subfamily *Leuciscinae* is a highly speciose taxon (ca. two-hundred fifty species), whereas the genus *Lepomis* is species poor (eleven species); both are thought to be of approximate evolutionary age. At the level of gross chromosomal organization, we found little evidence of greater chromosomal evolution among the speciose *Leuciscinae* than among the species-poor *Lepomis*. This suggests that the rates of regulatory evolution, as reflected in gross chromosomal rearrangement, do not appear more rapid among the speciose *Leuciscinae*.

The foregoing discussion indicates that many speciations in fishes may occur in the absence of chromosomal rearrangement. The data, however, should only be treated as suggestive, since important chromosome structural changes could have occurred beyond the resolution of present cytological techniques. Certainly, the application of higher resolution methodology as discussed in Section II,C is to be encouraged.

D. Polyploidy and Aneuploidy

1. POLYPLOIDY

Incipient polyploidy among bisexual vertebrates is extremely rare, and has been verified only recently among certain *Amphibia* (Beçak *et al.*, 1966b, 1967, 1970). In contrast, polyploidy is common among higher plants, and apparently has played a major role in speciation and evolution (Stebbins, 1971). White (1973a) has listed a few reasons why polyploidy should be rare in bisexual species, including Muller's (1925) suggestion that heterogametic sex-determining mechanisms might be disturbed.

In fishes, the all-female triploid unisexuals (Section III,B) are the only substantiated instances in nature of polyploidy at the population level. There are reports of tetraploid ($4n$) individuals among Japanese populations of *Carassius auratus* (Kobayasi *et al.*, 1970), and moreover that some of these populations contain triploids of both sexes (Muramoto, 1975). However, since unisexuality occurs in *C. auratus* these populations may be gynogenetic (Kobayasi, 1971). Isolated and

very rare triploid individuals have been identified cytologically in the rainbow trout, *Salmo gairdneri* (Cuellar and Uyeno, 1972), and the western roach, *Hesperoleucus symmetricus* (Gold and Avise, 1976), but they are expected to be sterile.

There is, however, circumstantial evidence that a few extant groups or species of fish are ancestral polyploids. Ohno and his colleagues have published in depth on the subject. In their view these "polyploids" are likely the result of nature's experimentation with gene duplication (Ohno and Atkin, 1966; Atkin and Ohno, 1967; Muramoto *et al.*, 1968; Ohno *et al.*, 1967, 1968, 1969a; Ohno, 1970, 1974). White (1946, 1973a) and others have questioned whether polyploidy could have occurred at all in a bisexual species and have noted that the evidence "has never been of a conclusive kind."

Actually, there are only a few living fishes for which ancestral polyploidy of this sort has been suspected. These include the Northern Hemisphere genera of the lamprey family *Petromyzontidae* (Ohno *et al.*, 1968; Howell and Denton, 1969; Potter and Robinson, 1973; but see Robinson *et al.*, 1975), the cypriniform family *Catostomidae* (Uyeno and Smith, 1972), three species in the *Cyprinidae* (*C. auratus*, *Cyprinus carpio*, and *Barbus barbus*, Ohno and Atkin, 1966; Ohno *et al.*, 1967, 1968; Ohno, 1974), the loach, *Misgurnus fossilis* (Raicu and Taisescu, 1972), the family *Salmonidae* (see below), and one form of the beryciform genus *Diretmus* (Post, 1973). The usual evidence for ancestral polyploidy is that the species or groups in question have both genome sizes and chromosome numbers which are approximately twofold greater than those of closely related taxa.

Some caution is advised, however, before one considers a species as ancestrally polyploid since there are instances where either genome size or chromosome number (but not both) appear to have been increased substantially. For example, the clown loach, *Botia macracantha*, of the cypriniform family *Cobitidae* has $n = 49$ chromosomes as compared to the Khulli loach, *Ancanthophthalmus khulli*, which has $n = 25$. Both species have genome sizes of about 1.0 pg per haploid nucleus which is average for most cypriniforms (Muramoto *et al.*, 1968). A similar example is found in the salmoniform *Bathylagidae* (Ebeling *et al.*, 1971).

The reverse situation, substantial increase in genome size without apparent concomitant increase in chromosome number, also has been observed in genera of the siluriform *Callichthyidae* and *Loricariidae* (Muramoto *et al.*, 1968; Hinegardner and Rosen, 1972), the hagfish family *Eptatretidae* (Atkin and Ohno, 1967; Taylor, 1967), and the

Cyprinidae (references in Ohno, 1970, 1974). A most striking example occurs in the salmoniform suborder *Esocoidei* (Beamish *et al.*, 1971).

Much of the literature on polyploidy in fishes has centered on the family *Salmonidae.** Initially, Svärdson (1945) suggested the group comprised a polyploid series with a haploid set of 10 chromosomes. This hypothesis has since been questioned by several authors, and no longer seems tenable in view of Rees's (1964) critical study. There is accumulated evidence, however, which suggests that the *Salmonidae* are ancestral polyploids which arose from tetraploidization, rather than successive events as suggested by Svärdson. Ohno (1974) recently reviewed most of this evidence and observed that (1) with one possible exception (Ohno *et al.*, 1969a), the chromosome arm numbers and genome sizes in species examined from each salmonid subfamily are approximately double those of related taxa, (2) there are now a number of biochemical–genetic studies which indicate duplication for several nonlinked genes (see also Chapter 8), and (3) in a few salmonid species there are ring and rod multivalents observed during prophase of meiosis I. The latter is an important point since if the salmonids are autotetraploids (as seems to be indicated—see Ohno *et al.*, 1969a) a few tetravalents might be expected to occur.

Unfortunately, in those species where meiotic multivalents are regularly observed, for example, *Salmo salar, Salmo gairdneri,* and *Salmo aguabonita* (Ohno *et al.*, 1965; Nygren *et al.*, 1968a; Gold and Gall, 1975), there is ample evidence of extensive Robertsonian rearrangement at both the interspecific and intraindividual levels. This could in itself explain any number or type of multivalent by assuming a karyotype of more than two metacentric chromosomes with partial or heterobrachial homologies (Gropp *et al.*, 1972). Zenzes and Voiçulescu (1975) published a C-banded somatic karyotype of *Salmo trutta,* in which the $2n = 80$ chromosomes were arranged into sets of twos (metacentrics) and fours (acrocentrics). However, since specific identification of all individual chromosomes was not possible, whether the chromosomes in all sets of two or four were homologous could not be determined.

Somewhat different reasoning led Nygren *et al.* (1972) to the conclusion that "reciprocal translocations rather than autopolyploidy cause the occurrence of multivalents in the meiosis of (*S. salar*)," although at the time they were apparently considering Svärdson's model. Nevertheless, even if all the multivalents observed in some

* The family *Salmonidae* is considered here to comprise the three subfamilies *Salmoninae, Coregoninae,* and *Thymallinae* (after Norden, 1960).

species were the result of numerous Robertsonian rearrangement between nonhomologues, this could still reflect a process of "diploidization" from a tetraploid state (Ohno *et al.*, 1969a).

2. Aneuploidy

As noted previously, the only substantiated report of an aneuploid fish was a trisomic ($2n = 85$) Eastern brook trout, *Salvelinus fontinalis*, identified from both cytological and biochemical–genetic evidence by Davisson *et al.* (1972). It is not surprising perhaps that the aneuploid should be found in a salmonid species, or that the apparently nondisjoined chromosome was a metacentric, presumably derived from a Robertsonian fusion event. Among strictly diploid species, aneuploids are expected to be extremely rare because of their severe effect on developmental processes.

V. CYTOGENETICS AND FISH CULTURE

The discipline of "cytogenetics," along with its practical application, has yet to be used extensively in fish breeding or fish culture. In contrast, the manipulation of chromosomes and/or chromosome sets has proved a valuable technique in plant breeding, and general karyology has been useful to some extent in animal breeding. Many of these methods should be useful in fish breeding or in other phases of fish culture. A few potentialities for the practical application of cytogenetics to fish culture are briefly discussed below.

A. Manipulation of Chromosome Sets

A serious difficulty in fish breeding programs is the establishment and maintenance of inbred lines, since the amount of time, labor, and expense is very often prohibitive. Purdom and Lincoln (1973), however, have suggested that highly inbred strains could be produced in only a few generations by increasing the rate of inbreeding through artificial parthenogenesis, that is, sperm-stimulated development or gynogenesis of diploidized eggs.

Earlier, several Russian investigators working with carp (*Cyprinus carpio*), loach (*Misgurnus fossilis*), and sturgeon (*Acipenser ruthenus*) found that a low frequency of diploids was recovered from haploid eggs "fertilized" with radiation-inactivated sperm (references in Purdom and Lincoln, 1973). Most of the gynogenetic embryos were

monoploid, but the frequency of diploids could be increased by exposing the embryos to temperature shocks subsequent to fertilization. Purdom and Lincoln (1973) extended this work to the plaice, *Pleuronectes platessa,* and by carefully studying the duration of temperature shock they were able to recover substantially high frequencies of diploid "gynogenomes."

The reestablishment of diploidy following temperature shock in these gynogens probably stems from a failure of either the first or second meiotic divisions of the egg, or of the first mitotic division of the embryo. In *P. platessa* and *C. carpio,* the failure is apparently at the second meiotic division (Purdom and Lincoln, 1973; Cherfas, 1975). Regardless, the degree of inbreeding (homozygosity) should be at least 50% (failure of meiosis I), and could be as high as 100% (failure of first mitosis). Failure of meiosis II should result in inbreeding levels between 50 and 100%, depending on the amount of crossing-over occurring during meiosis I (Purdom and Lincoln, 1973). Thus, since each gynogenome is expected to be genetically unique, a different inbred line should result following a second generation.

The survival of the first generation diploid gynogens was low in both *P. platessa* and *C. carpio* (Purdom and Lincoln, 1973; Cherfas, 1975), but such an approach to inbreeding should be considered for other fish species.

The application of temperature shocks along with fertilization by *normal* sperm may also be useful to fish culture since polyploids should be produced. This has been accomplished in the stickleback, *Gasterosteus aculeatus* (Swarup, 1959a,b), in plaice and plaice × flounder (*Platichthys flesus*) hybrids (Purdom, 1973), and the blue tilapia, *Tilapia aurea* (Valenti, 1975). Usually, triploids are the result, although Swarup (1959a,b) recovered several heteroploid *G. aculeatus*, and Valenti (1975) may have recovered a few tetraploid *T. aurea.*

The potential value of the triploids is twofold. First, it is known in animals that nuclear and cell sizes increase in proportion to increases in chromosome number (Fankhauser, 1945; Swarup, 1959b). Thus, if cell number and division time are the same for both diploid and triploid individuals, the triploids should have increased growth rates. Swarup (1959b) found that growth rate and final size of *G. aculeatus* triploids were the same as diploid controls. Since the triploids possessed larger overall cell size, Swarup (1959b) concluded that size regulating mechanisms were operating in such a way that the increased cell size of the triploids was compensated for by a reduction in the number of cells per organ. Purdom (1973), however, found that plaice

× flounder triploids grew considerably faster than expected, and suggested that the effect was due to triploidy per se. The difference between the growth rates of diploid and triploid *G. aculeatus* and those of plaice × flounder hybrids may be due to the determinate growth pattern of *G. aculeatus* (Purdom, 1973); in plaice, growth is continuous throughout life and certain size regulating mechanisms may be absent. Valenti (1975) has obtained similar results with *T. aurea;* in all cases, polyploid fish were larger at 14 weeks of age than were diploid siblings.

A second practical use of triploid fish follows from the fact that they are expected to be sterile due to irregular segregation during meiosis. Sterile fish would be useful in stocking programs where the genetic integrity of wild populations may be threatened by hatchery introductions. Other possible advantages of stocking sterile fish were discussed by Purdom and Lincoln (1973).

B. Use of General Karyology

In several animal species, including man, many instances of embryonic rejection may be attributed to chromosomal anomalies, particularly those arising from nondisjunction and polyploidy (see reviews in Carr, 1966, 1970; Fechheimer, 1968, 1972; Bruere, 1974). The role of chromosomal anomalies in reproductive failure is not known, yet several abortuses are apparently either aneuploid, polyploid, or chromosomally mosaic. Possible causes of chromosomal anomalies in animals also are unknown, but may include ionizing radiation, delayed fertilization, or aging of ova. The anomalies are easily identified through general karyology or karyotyping.

Instances of reproductive failure among cultured fish species are common, but these usually are attributed to inadequate management practices which result in physiological stress on the adults, eggs, or embryos. It is also conceivable that chromosomal anomalies play a major role in fish reproductive failure. Adults with altered reproductive ability due to structural heterozygosity for chromosomal rearrangement might be identified prior to their use in breeding programs.

Bruere (1974) has reviewed the evidence for reduced fertility in animals due to structural heterozygosity for Robertsonian rearrangement. In some species such as the tobacco mouse, *Mus poschiavinus*, heterozygosity for Robertsonian rearrangement apparently leads to aneuploid gametes, and hence to reduced fertility (Gropp *et al.*, 1972; Tettenborn and Gropp, 1970). In other species (e.g., sheep) there is little evidence that either aneuploid gametes or aneuploid embryos

result from matings of individuals heterozygous for a Robertsonian rearrangement to structurally normal individuals (Bruere, 1974). In fishes, very little is known on this subject, although it is interesting that progeny survival of rainbow trout, a species with apparently numerous Robertsonian chromosomal polymorphisms, may be as high as 85–90% (Gold and Gall, unpublished observations).

A final possibility is that individual chromosomes could potentially serve to identify specific strains or hybrids, much in the same manner as biochemical–genetic markers (see Chapter 8). Cytological techniques presently used in fishes, however, would have to be improved appreciably. To date, only a few species hybrids have been identified cytologically (Prehn and Rasch, 1969; Setzer, 1970; Chen and Ebeling, 1975).

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REFERENCES

Abe, S., and Muramoto, J. (1974). Differential staining of chromosomes of two salmonoid species, *Salvelinus leucomaenis* (Pallas) and *Salvelinus malma* (Walbum). *Proc. Jpn. Acad.* **50**, 507–511.

Abramoff, P., Darnell, R. M., and Balsano, J. S. (1968). Electrophoretic demonstration of the hybrid origin of the gynogenetic teleost *Poecilia formosa*. *Am. Nat.* **102**, 555–558.

Aida, T. (1921). On the inheritance of color in a fresh-water fish *Aplocheilus latipes* Temminck and Schlegel, with special reference to the sex-linked inheritance. *Genetics* **6**, 554–573.

Aida, T. (1936). Sex reversal in *Aplocheilus latipes* and a new explanation of sex differentiation. *Genetics* **21**, 136–153.

Anders, A., Anders, F., and Rase, S. (1969). XY females caused by X-irradiation. *Experientia* **25**, 851.

Anders, A., Anders, F., and Klinke, K. (1973). Regulation of gene expression in the Gordon–Kosswig melanoma system. II. The arrangement of chromatophore determining loci and regulating elements in the sex chromosomes of xiphophorin fish, *Platypoecilus maculatus* and *Platypoecilus variatus*. *In* "Genetics and Mutagenesis of Fish" (J. H. Schröder, ed.), pp. 53–63. Springer-Verlag, Berlin and New York.

Atkin, N. B., and Ohno, S. (1967). DNA values of four primitive chordates. *Chromosoma* **23**, 10–13.

Atz, J. W. (1964). Intersexuality in fishes. *In* "Intersexuality in Vertebrates Including Man" (C. N. Armstrong and A. J. Marshall, eds.), pp. 145–232. Academic Press, New York.

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

Bachmann, K., Goin, O. B., and Goin, C. J. (1972). Nuclear DNA amounts in vertebrates. *Brookhaven Symp. Biol.* **23**, 419–450.

Balsano, J. S., Darnell, R. M., and Abramoff, P. (1972). Electrophoretic evidence of triploidy associated with populations of the gynogenetic teleost *Poecilia formosa*. *Copeia* No. 2, pp. 292–297.

Barker, C. J. (1972). A method for the display of chromosomes of plaice, *Pleuronectes platessa,* and other marine fishes. *Copeia* No. 2, pp. 365–368.

Beamish, R. J., Merrilees, M. J., and Crossman, E. J. (1971). Karyotypes and DNA values for members of the suborder *Esocoidei* (Osteichthyes: Salmoniformes). *Chromosoma* **34**, 436–447.

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

Beçak, M. L., Beçak, W., and Rabello, M. N. (1966b). Cytological evidence of constant tetraploidy in the bisexual South American frog *Odontophrynus americanus*. *Chromosoma* **19**, 188–193.

Beçak, M. L., Beçak, W., and Rabello, M. N. (1967). Further studies on polyploid amphibians (Ceratophrydidae). I. Mitotic and meiotic aspects. *Chromosoma* **22**, 192–201.

Beçak, M. L., Beçak, W., and Vizotto, L. D. (1970). A diploid population of the polyploid amphibian *Odontophrynus americanus* and an artificial intraspecific triploid hybrid. *Experientia* **26**, 545.

Behnke, R. J. (1970). The application of cytogenetic and biochemical systematics to phylogenetic problems in the family Salmonidae. *Trans. Am. Fish. Soc.* **1**, 237–248.

Behnke, R. J. (1972). The systematics of salmonid fishes of recently glaciated lakes. *J. Fish. Res. Board Can.* **29**, 639–671.

Bellamy, A. W. (1922). Sex-linked inheritance in the teleost *Platypoecilus maculatus*. *Anat. Rec.* **24**, 419–420.

Bellamy, A. W. (1936). Interspecific hybrids in *Platypoecilus:* One species ZZ–WZ; the other XY–XX. *Proc. Natl. Acad. Sci. U.S.A.* **22**, 531–535.

Benazzi, M. (1973). Cytotaxonomy and evolution: General remarks. *In* "Cytotaxonomy and Vertebrate Evolution" (A. B. Chiarelli and E. Capanna, eds.), pp. 3–14. Academic Press, New York.

Bennett, M. D. (1972). Nuclear DNA content and minimum generation time in herbaceous plants. *Proc. R. Soc., Ser. B* **181**, 109–135.

Blaxhall, P. C. (1975). Fish chromosome techniques—A review of selected literature. *J. Fish Biol.* 7, 315–320.

Booke, H. E. (1968). Cytotaxonomic studies of the coregonine fishes of the Great Lakes, USA: DNA and karyotype analysis. *J. Fish Res. Board Can.* **25**, 1667–1687.

Booke, H. E. (1970). Speciation parameters in coregonine fishes: I. Egg-size. II. Karyotype. *In* "Biology of Coregonid Fishes" (C. C. Lindsey and C. S. Woods, eds.), pp. 61–66. Univ. of Manitoba Press, Winnipeg.

Booke, H. E. (1974). A cytotaxonomic study of the round whitefishes, genus *Prosopium*. *Copeia* No. 1, pp. 115–119.

Boothroyd, E. R. (1959). Chromosome studies on three Canadian populations of Atlantic salmon, *Salmo salar* L. *Can. J. Genet. Cytol.* 1, 161–172.

Breider, H. (1935). Geschlechtsbestimmung und-differenzierung bei *Limia nigrofascida, Caudofasciata, vittata* und deren Artbastarden. *Z. Indukt. Abstamm.- Vererbungsl.* **68**, 265–299.

Breider, H. (1936). Weiteres über die Geschlechtsbestimmung und Geschlechts-differenzierung bei *Limia vittata* Poy. *Zool. Anz.* **114**, 113–119.

Bridges, C. B. (1925). Sex in relation to chromosomes and genes. *Am. Nat.* **59**, 127–137.

Bridges, C. B. (1939). Cytological and genetic basis of sex. *In* "Sex and Internal Secretions" (E. Allen, ed.), 2nd Ed., pp. 15–63. Williams & Wilkins, Baltimore, Maryland.

Bruere, A. N. (1974). The discovery and biological consequences of some important chromosome anomalies in populations of domestic animals. *World Congr. Genet. Appl. Livestock Prod., 1st* pp. 151–175.

Bungenberg de Jong, C. M. (1955). Cytological studies in *Salmo irideus*. *Genetics* **27**, 472–485.

Buss, K., and Wright, J. E. (1956). Results of species hybridization within the family Salmonidae. *Prog. Fish Cult.* **18**, 149–158.

Calton, M. S., and Denton, T. E. (1974). Chromosomes of the chocolate gourami: a cytogenetic anomaly. *Science* **185**, 618–619.

Carr, D. H. (1966). Cytogenetics and abortions. *In* "Comparative Aspects of Reproductive Failure" (K. Benirschke, ed.), pp. 96–117. Springer-Verlag, Berlin and New York.

Carr, D. H. (1970). Chromosome abnormalities and spontaneous abortions. *In* "Human Population Cytogenetics" (P. A. Jacobs, W. H. Price, and P. Law, eds.), Pfizer Medical Monographs, No. 5, pp. 103–118. Univ. of Edinburgh, Edinburgh.

Catton, W. T. (1951). Blood cell formation in certain teleost fishes. *Blood* **6**, 39–60.

Chan, S. T. H. (1970). Natural sex reversal in vertebrates. *Philos. Trans. R. Soc. London, Ser. B* **259**, 59–71.

Chen, T. R. (1969). Karyological heterogamety of deep-sea fishes. *Postilla* **130**, 1–29.

Chen, T. R. (1970). Fish chromosome preparation: Air-dried displays of cultured ovarian cells in two killifishes (*Fundulus*). *J. Fish. Res. Board Can.* **271**, 158–161.

Chen, T. R. (1971). A comparative study of twenty killifish species of the genus *Fundulus* (Teleostei: Cyprinodontidae). *Chromosoma* **32**, 436–453.

Chen, T. R., and Ebeling, A. W. (1966). Probable male heterogamety in the deep-sea fish *Bathylagus wesethi* (Teleosti: Bathylagidae). *Chromosoma* **18**, 88–96.

Chen, T. R., and Ebeling, A. W. (1968). Karyological evidence of female heterogamety in the mosquitofish *Gambusia affinis* (Baird and Girard). *Copeia* No. 1, pp. 70–75.

Chen, T. R., and Ebeling, A. W. (1971). Chromosomes of the Goby fishes in the genus *Gillichthys*. *Copeia* No. 1, pp. 171–174.

Chen, T. R., and Ebeling, A. W. (1975). Karyotypes from short- and long-term cultures of hybrid killifish and platyfish tissues. *Copeia* No. 1, pp. 178–181.

Chen, T. R., and Reisman, H. M. (1970). A comparative chromosome study of the North American species of sticklebacks (Teleostei: Gasterosteidae). *Cytogenetics* **9**, 321–332.

Chen, T. R., and Ruddle, F. H. (1970). A chromosome study of four species and a hybrid of the killifish genus *Fundulus* (Cyprinodontidae). *Chromosoma* **29**, 255–267.

Cherfas, N. B. (1966). Natural triploidy in females of the unisexual forms of the goldfish (*Carassius auratus gibelio* Bloch). *Sov. Genet.* **2**(5), 9–13.
Cherfas, N. B. (1972). Results of a cytological analysis of unisexual and bisexual forms of silver crucian carp. *In* "Genetics, Selection, and Hybridization of Fish," pp. 79–90. Isr. Program Sci. Transl., Jerusalem.
Cherfas, N. B. (1975). Investigation of radiation-induced diploid gynogenesis in the carp (*Cyprinus carpio* L.). *Genetika* **11**, 78–86.
Chiarelli, A. B., and Capanna, E. (1973). Checklist of fish chromosomes. *In* "Cytotaxonomy and Vertebrate Evolution" (A. B. Chiarelli and E. Capanna, eds.), pp. 206–232. Academic Press, New York.
Chiarelli, B., Ferrantelli, O., and Cucchi, C. (1969). The caryotype of some Teleostea fish obtained by tissue culture *in vitro*. *Experientia* **25**, 426–427.
Cimino, M. C. (1972a). Meiosis in triploid all-female fish (*Poeciliopsis*, Poeciliidae). *Science* **175**, 1484–1486.
Cimino, M. C. (1972b). Egg production, polyploidization and evolution in a diploid all-female fish of the genus *Poeciliopsis*. *Evolution* **26**, 294–306.
Cimino, M. C., and Bahr, G. F. (1974). The nuclear DNA content and chromatin ultrastructure of the coelacanth *Latimeria chalumnae*. *Exp. Cell Res.* **88**, 263–272.
Cimino, M. C., and Schultz, R. J. (1970). Production of a diploid male offspring by a gynogenetic triploid fish of the genus *Poeciliopsis*. *Copeia* No. 4, pp. 760–763.
Clark, E. (1959). Functional hermaphroditism in a serranid fish. *Science* **129**, 215–216.
Clark, E. (1965). Mating of groupers. *Nat. Hist.* **74**, 22–25.
Clem, W. L., Moewus, L., and Sigel, M. M. (1961). Studies with cells from marine fish in tissue culture. *Proc. Soc. Exp. Biol. Med.* **108**, 762–766.
Crow, J. F., and Kimura, M. (1965). Evolution in sexual and asexual populations. *Am. Nat.* **99**, 439–450.
Cuellar, O., and Uyeno, T. (1972). Triploidy in rainbow trout. *Cytogenetics* **11**, 508–515.
D'Ancona, U. (1950). Détermination e differenciation du sexe chez les poissions. *Arch. Anat. Microscop. Morphol. Exp.* **39**, 274–294.
Dangel, J. R. (1973). An annotated bibliography of interspecific hybridization of Salmonidae. *FAO (FAO UN) Fish. Circ.* No. 133, pp. 1–32.
Darnell, R. M., and Abramoff, P. (1968). Distribution of the gynogenetic fish, *Poecilia formosa*, with remarks on the evolution of the species. *Copeia* No. 2, pp. 354–361.
Davisson, M. T., Wright, J. E., and Atherton, L. M. (1972). Centric fusion and trisomy for the LDH-B locus in brook trout, *Salvelinus fontinalis*. *Science* **178**, 992–994.
Davisson, M. T, Wright, J. E., and Atherton, L. M. (1973). Cytogenetic analysis of pseudolinkage of LDH loci in the teleost genus *Salvelinus*. *Genetics* **73**, 645–658.
Denton, T. E. (1973). "Fish Chromosome Methodology." Thomas, Springfield, Illinois.
Denton, T. E., and Howell, W. M. (1969). A technique for obtaining chromosomes from the scale epithelium of teleost fishes. *Copeia* No. 2, pp. 392–393.
Dobzhansky, T. (1951). "Genetics and the Origin of Species," 3rd Ed. Columbia Univ. Press, New York.
Dobzhansky, T. (1970). "Genetics of the Evolutionary Process." Columbia Univ. Press, New York.
Drewry, G. (1964). Appendix I, Chromosome number. *Bull. Tex. Mem. Mus.* No. 8, pp. 5–72.
Ebeling, A. W., and Chen, T. R. (1970). Heterogamety in teleostean fishes. *Trans. Am. Fish. Soc.* **1**, 131–138.
Ebeling, A. W., Atkin, N. B., and Setzer, P. Y. (1971). Genome sizes of teleostean fishes: Increases in some deep-sea species. *Am. Nat.* **105**, 549–562.

Endo, A., and Ingalls, T. H. (1968). Chromosomes of the zebra fish. *J. Hered.* **59**, 382–384.

Fankhauser, G. (1945). The effect of change in chromosome numbers on amphibian development. *Q. Rev. Biol.* **20**, 20–78.

Fechheimer, N. S. (1968). Consequences of chromosomal aberrations in mammals. *J. Anim. Sci.* **27**, Suppl. 1, 27–50.

Fechheimer, N. S. (1972). Causal basis of chromosome abnormalities. *J. Reprod. Fertil., Suppl.* **15**, 79–98.

Friedman, B., and Gordon, M. (1934). Chromosome numbers in Xiphophorin fishes. *Am. Nat.* **68**, 446–455.

German, J. (1973). Studying human chromosomes today. *Am. Sci.* **58**, 1–20.

Goin, O. B., and Goin, C. J. (1968). DNA and the evolution of vertebrates. *Am. Midl. Nat.* **80**, 289–298.

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

Gold, J. R., and Avise, J. C. (1976). Spontaneous triploidy in the California roach, *Hesperoleucus symmetricus* (Pisces: Cyprinidae). *Cytogenet. Cell Genet.* **17**, 144–149.

Gold, J. R., and Avise, J. C. (1977). Cytogenetic studies in North American minnows (Cyprinidae). I. Karyology of nine California genera. *Copeia* No. 3, pp. 541–549.

Gold, J. R., and Gall, G. A. E. (1975). Chromosome cytology and polymorphism in the California High Sierra golden trout (*Salmo aguabonita*). *Can. J. Genet. Cytol.* **17**, 41–53.

Gold, J. R., Pipkin, R. E., and Gall, G. A. E. (1976). Artificial hybridization between rainbow (*Salmo gairdneri*) and golden trout (*Salmo aguabonita*). *Copeia* No. 3, pp. 597–598.

Gold, J. R., Avise, J. C., and Gall, G. A. E. (1977). Chromosome cytology in the cutthroat trout, *Salmo clarki* (Salmonidae). *Cytologia* **42**, 377–382.

Gordon, M. (1927). The genetics of a viviparous top-minnow *Platypoecilus;* the inheritance of two kinds of melanophores. *Genetics* **12**, 253–283.

Gordon, M. (1937). Genetics of *Platypoecilus*. III. Inheritance of sex and crossing over of the sex chromosomes in the platyfish. *Genetics* **22**, 376–392.

Gordon, M. (1946). Interchanging genetic mechanisms for sex determination. *J. Hered.* **37**, 307–320.

Gordon, M. (1947). Genetics of *Platypoecilus maculatus*. IV. The sex-determining mechanism in two wild populations of Mexican platyfish. *Genetics* **32**, 8–17.

Gordon, M. (1951). Genetics of *Platypoecilus maculatus*. V. Heterogametic sex-determining mechanisms in females of a domesticated stock originally from British Honduras. *Zoologica (N.Y.)* **36**, 127–134.

Gordon, M. (1952). Sex determination in *Xiphophorus* (*Platypoecilus*) *maculatus*. III. Differentiation of gonads in platyfish from broods having a sex ratio of the three females to one male. *Zoologica (N.Y.)* **37**, 91–100.

Gordon, M. (1957). Physiological genetics of fishes. *In* "The Physiology of Fishes" (M. E. Brown, ed.), Vol. 2, pp. 431–501. Academic Press, New York.

Gordon, M., and Smith, G. M. (1938). The production of a melanotic neoplastic disease in fishes by selective matings. IV. Genetics of geographical species hybrids. *Am. J. Cancer* **34**, 543–565.

Gorman, G. C. (1973). The chromosomes of the Reptilia, a cytotaxonomic interpretation. *In* "Cytotaxonomy and Vertebrate Evolution" (A. B. Chiarelli and E. Capanna, eds.), pp. 349–424. Academic Press, New York.

Gould, W. R. (1966). Cutthroat trout (*Salmo clarkii* Richardson) × golden trout (*Salmo aguabonita* Jordan) hybrids. *Copeia* No. 3, pp. 599–600.

Grant, V. (1963). "The Origin of Adaptations." Columbia Univ. Press, New York.

Gropp, A., Winking, H., Zech, I., and Müller, H. (1972). Robertsonian chromosomal variation and identification of metacentric chromosomes in feral mice. *Chromosoma* **39**, 265–288.

Gyldenholm, A. O., and Scheel, J. J. (1971). Chromosome numbers of fishes. I. *J. Fish Biol.* **3**, 479–486.

Harrington, R. W., Jr. (1961). Oviparous hermaphroditic fish with internal self-fertilization. *Science* **134**, 1749–1750.

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 among fishes. *Copeia* No. 3, pp. 389–432.

Harrington, R. W., Jr., and Kallman, K. D. (1968). The homozygosity of clones of the self-fertilizing hermaphroditic fish *Rivulus marmoratus* Poey (Cyprinodontidae, Atheriniformes). *Am. Nat.* **102**, 337–343.

Haskins, C. P., Haskins, E. F., and Hewitt, R. E. (1960). Pseudogamy as an evolutionary factor in the poeciliid fish *Mollienisia formosa*. *Evolution* **14**, 473–483.

Hayashi, M., Ojima, Y., and Asano, N. (1976). A cell line from teleost fish: Establishment and cytogenetical characterization of the cells. *Jpn. J. Genet.* **51**, 65–68.

Heckman, J. R., and Brubaker, P. E. (1970). Chromosome preparation from fish blood leukocytes. *Prog. Fish Cult.* **32**, 206–208.

Heckman, J. R., Allendorf, F. W., and Wright, J. E. (1971). Trout leukocytes: Growth in oxygenated cultures. *Science* **173**, 246–247.

Hickling, C. F. (1960). The Malacca *Tilapia* hybrids. *J. Genet.* **57**, 1–10.

Hinegardner, R. (1968). Evolution of cellular DNA content in teleost fishes. *Am. Nat.* **102**, 517–523.

Hinegardner, R., and Rosen, D. E. (1972). Cellular DNA content and the evolution of teleostean fishes. *Am. Nat.* **106**, 621–644.

Hoehn, H. (1975). Functional implications of differential chromosome banding. *Am. J. Hum. Genet.* **27**, 676–686.

Howell, W. M., and Denton, T. E. (1969). Chromosomes of ammocoetes of the Ohio brook lamprey, *Lampetra aepyptera*. *Copeia* No. 2, pp. 393–395.

Hubbs, C. (1964). Interactions between a bisexual fish species and its gynogenetic sexual parasite. *Bull. Tex. Mem. Mus.* No. 8, pp. 1–72.

Hubbs, C. L. (1955). Hybridization between fish species in nature. *Syst. Zool.* **4**, 1–20.

Hubbs, C. L., and Hubbs, L. C. (1932). Apparent parthenogenesis in nature, in a form of fish of hybrid origin. *Science* **76**, 628–630.

Hubbs, C. L., and Hubbs, L. C. (1946). Breeding experiments with the invariable female, strictly matroclinous fish, *Mollienisia formosa*. *Rec. Genet. Soc. Am.* **14**, 48.

Kaiser, P., and Schmidt, E. (1951). Vollkommene Geschlechtsumwandlung beim weiblichen siamesischen Kampffish, *Betta splendens*. *Zool. Anz.* **146**, 66–73.

Kallman, K. D. (1962). Gynogenesis in the teleost, *Mollienisia formosa* (Girard), with a discussion of the detection of parthenogenesis in vertebrates by tissue transplantation. *J. Genet.* **58**, 7–24.

Kallman, K. D. (1965a). Sex determination in the teleost *Xiphophorus milleri*. *Am. Zool.* **5**, 246–247.

Kallman, K. D. (1965b). Genetics and geography of sex determination in the poeciliid fish, *Xiphophorus maculatus*. *Zoologica (N.Y.)* **50**, 151–190.

Kallman, K. D. (1968). Evidence for the existance of transformer genes for sex in the teleost *Xiphophorus maculatus*. *Genetics* **60**, 811–828.

Kallman, K. D. (1970a). Genetics of tissue transplantation in Teleostei. *Transplant. Proc.* **2**, 263–271.

Kallman, K. D. (1970b). Sex determination and the restriction of sex-linked pigment patterns to the X and Y chromosomes in populations of a poeciliid fish, *Xiphophorus maculatus*, from the Belize and Sibun Rivers of British Honduras. *Zoologica (N.Y.)* **55**, 1–16.

Kallman, K. D. (1973). The sex-determining mechanism of the platyfish, *Xiphophorus maculatus*. *In* "Genetics and Mutagenesis of Fish" (J. H. Schröder, ed.), pp. 19–28. Springer-Verlag, Berlin and New York.

Kang, Y. S., and Park, E. H. (1975). Leukocyte culture of the eel without autologous serum. *Jpn. J. Genet.* **50**, 159–161.

Keyl, H. G. (1965). Demonstrable local and geometric increase in the chromosomal DNA of *Chironomus*. *Experientia* **21**, 191–193.

Kobayasi, H. (1971). A cytological study on gynogenesis of the triploid Ginbuna (*Carassius auratus langsdorfi*). *Dobutsugaku Zasshi* **80**, 316–322.

Kobayasi, H., Kawashima, Y., and Takeuchi, N. (1970). Comparative chromosome studies in the genus *Carassius*, especially with a finding of polyploidy in the Ginbuna (*C. auratus langsdorfi*). *Gyoruigaku Zasshi* 17, 153–160.

Kosswig, C. (1935). Genotypische und Phänotypische Geschlechts bestimmung bei Zahnkarpfen. V. Ein X(Z)-Chromosom als Y-Chromosom in fremden Erbgut. *Wilhelm Roux' Arch. Entwicklungsmech. Org.* **133**, 118–139.

Kosswig, C. (1959). Beiträge zur genetischen Analyse xiphophoriner Zahnkarpfen. *Biol. Zentralbl.* **78**, 711–718.

Kosswig, C. (1964). Polygenic sex determination. *Experientia* **20**, 190–199.

Labat, R., Larrouy, G., and Malaspina, L. (1967). Technique de culture des leucocytes de *Cyprinus carpio* L. *C. R. Acad. Sci., Ser. D* **264**, 2473–2475.

Legendre, P. (1975). A field-trip microtechnique for studying fish leukocyte chromosomes. *Can. J. Zool.* **53**, 1443–1446.

LeGrande, W. H. (1975). Karyology of six species of Louisiana flatfishes (Pleuronectiformes: Osteichthyes). *Copeia* No. 3, pp. 516–522.

Leuken, W., and Foerster, W. (1969). Chromosomenuntersuchungen bei Fischen mit einer vereinfachten Zellkulturtechnik. *Zool. Anz.* **138**, 168–76.

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

Liem, K. F. (1963). Sex reversal as a natural process in the *Synbranchiform* fish *Monopterus albus*. *Copeia* No. 2, pp. 303–312.

Lieppman, M., and Hubbs, C. (1969). A karyological analysis of two cyprinid fishes, *Notemigonus crysoleucas* and *Notropis lutrensis*. *Tex. Rep. Biol. Med.* **27**, 427–435.

McPhail, J. D., and Jones, R. L. (1966). A simple technique for obtaining chromosomes from teleost fishes. *J. Fish. Res. Board Can.* **23**, 767–768.

Markert, C. L. (1968). Panel discussion: Present status and perspectives in the study of cytodifferentiation at the molecular level. I. Initial remarks. *J. Cell. Physiol.* **72**, Suppl. 1, 213–219.

Maslin, T. P. (1971). Parthenogenesis in reptiles. *Am. Zool.* **11**, 361–380.

Mather, K. (1953). The genetical structure of populations. *Symp. Soc. Exp. Biol.* 7, 66–93.

Matthey, R. (1945). L'évolution de la formule chromosomiale chez les vertébrés. *Experientia* **1**, 50–56.

Matthey, R. (1973). The chromosome formulae of eutherian mammals. *In* "Cytotaxonomy and Vertebrate Evolution" (A. B. Chiarelli and E. Capanna, eds.), pp. 531–616. Academic Press, New York.

Mayr, E. (1973). "Animal Species and Evolution." Harvard Univ. Press, Cambridge, Massachusetts.

Menzel, B. W., and Darnell, R. M. (1973). Morphology of naturally-occurring triploid fish related to *Poecilia formosa*. *Copeia* No. 2, pp. 350–352.

Meyer, H. (1938). Investigations concerning the reproductive behavior of *Mollienisia "formosa."* *J. Genet.* **36**, 329–366.

Miller, R. R. (1972). Classification of the native trouts of Arizona with the description of a new species, *Salmo apache*. *Copeia* No. 3, pp. 401–422.

Miller, R. R., and Schultz, R. J. (1959). All-female strains of the teleost fishes of the genus *Poeciliopsis*. *Science* **130**, 1656–1657.

Mirsky, A. E., and Ris, H. (1951). The desoxyribonucleic acid content of animal cells and its evolutionary significance. *J. Gen. Physiol.* **34**, 451–462.

Moore, W. S., Miller, R. R., and Schultz, R. J. (1970). Distribution, adaptation and probable origin of an all-female form of *Poeciliopsis* (Pisces: Poeciliidae) in northwestern Mexico. *Evolution* **24**, 806–812.

Moorhead, J. F., Connolly, J. J., and McFarland, W. (1967). Factors affecting the reactivity of human lymphocytes *in vitro*. *J. Immunol.* **99**, 413–419.

Morescalchi, A. (1973). Amphibia. *In* "Cytotaxonomy and Vertebrate Evolution" (A. B. Chiarelli and E. Capanna, eds.), pp. 233–348. Academic Press, New York.

Muller, H. J. (1925). Why polyploidy is rarer in animals than in plants. *Am. Nat.* **59**, 346–353.

Muramoto, J. (1975). A note on triploidy of the Funa (Cyprinidae, Pisces). *Proc. Jpn. Acad.* **51**, 583–587.

Muramoto, J., Ohno, S., and Atkin, N. B. (1968). On the diploid state of the fish order *Ostariophysi*. *Chromosoma* **24**, 59–66.

Nelson-Rees, W. A., Kniazeff, A. J., and Darby, W. B., Jr. (1967). Debut and accumulation of centric fusion products: An index to age of certain cell lines. *Cytogenetics* **6**, 436–450.

Nikolsky, G. (1976). The interrelation between variability of characters, effectiveness of energy utilisation, and karyotype structure in fishes. *Evolution* **30**, 180–185.

Nilsson, B. (1973). A bibliography of literature concerning chromosome identification—with special reference to flourescence Giemsa staining techniques. *Hereditas* **73**, 259–270.

Nogusa, S. (1960). A comparative study of the chromosomes in fishes with particular considerations on taxonomy and evolution. *Mem. Hyogo Univ. Agric. (Hyogo Noka Daigaku Kiyo), Biol. Ser.* **3**(1), 1–62.

Norden, C. R. (1960). Comparative osteology of representative salmonid fishes, with particular reference to the grayling (*Thymallus articus*) and its phylogeny. *J. Fish. Res. Board Can.* **18**, 679–791.

Nowell, P. C. (1960). Phytohaemataggluti nin; an initiator of mitosis in cultures of normal leukocytes. *Cancer Res.* **20**, 462–466.

Nygren, A., Nilsson, B., and Jahnke, M. (1968a). Cytological studies in Atlantic salmon. *Ann. Acad. Regiae Sci. Ups.* **12**, 21–52.

Nygren, A., Edlund, P., Hirsch, U., and Ahsgren, L. (1968b). Cytological studies in perch (*Perca fluviatilis* L.), pike (*Esox lucius* L.), pike-perch (*Lucioperca lucioperca* L.), and ruff (*Acerina cernua* L.). *Hereditas* **59**, 518–524.

Nygren, A., Nilsson, B., and Jahnke, M. (1971a). Cytological studies in *Thymallus thymallus* and *Coregonus albula*. *Hereditas* **67**, 269–274.

Nygren, A., Nilsson, B., and Jahnke, M. (1971b). Cytological studies in Hypotremata and Pleurotremata (Pisces). *Hereditas* **67**, 275–282.

Nygren, A., Nilsson, B., and Jahnke, M. (1972). Cytological studies in Atlantic salmon from Canada, in hybrids between Atlantic salmon from Canada and Sweden and in hybrids between Atlantic salmon and sea trout. *Hereditas* **70**, 295–306.

Ohno, S. (1967). "Sex Chromosomes and Sex-linked Genes" (A. Labhart, T. Mann, L. T. Samuels, and J. Zander, eds.), Monographs on Endocrinology, Vol. 1. Springer-Verlag, Berlin and New York.

Ohno, S. (1970). "Evolution by Gene Duplication." Springer-Verlag, Berlin and New York.

Ohno, S. (1974). Protochordata, Cyclostomata and Pisces. *In* "Animal Cytogenetics" (B. John, ed.), Vol. 4, Chordata 1, pp. 1–91. Borntraeger, Berlin.

Ohno, S., and Atkin, N. B. (1966). Comparative DNA values and chromosome complements of eight species of fishes. *Chromosoma* **18**, 455–466.

Ohno, S., Stenius, C., Faisst, E., and Zenzes, M. T. (1965). Postzygotic chromosomal rearrangements in rainbow trout (*Salmo irideus* Gibbons). *Cytogenetics* **4**, 117–129.

Ohno, S., Muramoto, J., Christian, L., and Atkin, N. B. (1967). Diploid–tetraploid relationship among old-world members of the fish family *Cyprinidae*. *Chromosoma* **23**, 1–9.

Ohno, S., Wolf, U., and Atkin, N. B. (1968). Evolution from fish to mammals by gene duplication. *Hereditas* **59**, 169–187.

Ohno, S., Muramoto, J., Klein, J., and Atkin, N. B. (1969a). Diploid–tetraploid relationship in clupeoid and salmonoid fish. *In* "Chromosomes Today" (C. D. Darlington and K. R. Lewis, eds.), Vol. 2, pp. 139–147. Oliver & Boyd, Edinburgh.

Ohno, S., Muramoto, J., Stenius, C., Christian, L., Kittreel, W. A., and Atkin, N. B. (1969b). Microchromosomes in holocephalian, chondrostean and holostean fishes. *Chromosoma* **26**, 35–40.

Ojima, Y., Hitosumachi, S., and Hayashi, M. (1970). A blood culture method for fish chromosomes. *Jpn. J. Genet.* **45**, 161–162.

Ojima, Y., Takayama, S., and Yamamoto, K. (1972). Chromosome preparation from cultured scale epithelium of teleost fish. *Jpn. J. Genet.* 47, 445–446.

Ojima, Y., Hayashi, M., and Ueno, K. (1975). Triploidy appeared in the backcross offspring from Funa–carp crossing. *Proc. Jpn. Acad.* **51**, 702–706.

Park, E. H. (1974). A list of the chromosome numbers of fishes. *Rev. Coll. Liberal Arts Sci., Seoul Natl. Univ.* **20**, 346–372.

Post, A. (1965). Vergleichende Untersuchungen der Chromosomenzahlen bei Süsswasser-Teleosteern. *Z. Zool. Syst. Evolutionsforsch.* **3**, 47–93.

Post, A. (1973). Chromosomes of two fish species of the genus *Diretmus* (Osteichthyes, Beryciformes: Diretmidae). *In* "Genetics and Mutagenesis of Fish" (J. H. Schröder, ed.), pp. 103–111. Springer-Verlag, Berlin and New York.

Potter, I. C., and Robinson, E. S. (1973). The chromosomes of the cyclostomes. *In* "Cytotaxonomy and Vertebrate Evolution" (A. B. Chiarelli and E. Capanna, eds.), pp. 179–203. Academic Press, New York.

Potter, I. C., and Rothwell, B. (1970). The mitotic chromosomes of the lamprey, *Petromyzon marinus* L. *Experientia* **26**, 429–430.

Prager, E. M., and Wilson, A. C. (1975). Slow evolutionary loss of the potential for interspecific hybridization in birds: A manifestation of slow regulatory evolution. *Proc. Natl. Acad. Sci. U.S.A.* **72**, 200–204.

Prehn, L. M., and Rasch, E. M. (1969). Cytogenetic studies of *Poecilia* (Pisces). I. Chromosome numbers of naturally occurring poeciliid species and their hybrids from eastern Mexico. *Can. J. Genet. Cytol.* **11**, 880–895.

Price, H. J. (1976). Evolution of DNA content in higher plants. *Bot. Rev.* **42**, 27–52.
Prokofieva, A. (1934). On the chromosome morphology of certain pisces. *Cytologia* **5**, 498–506.
Purdom, C. E. (1973). Induced polyploidy in plaice (*Pleuronectes platessa*) and its hybrid with the flounder (*Platichthys flesus*). *Heredity* **29**, 11–24.
Purdom, C. E., and Lincoln, R. F. (1973). Chromosome manipulation in fish. *In* "Genetics and Mutagenesis of Fish" (J. H. Schröder, ed.), pp. 83–89. Springer-Verlag, Berlin and New York.
Raicu, P., and Taisescu, E. (1972). *Misgurnus fossilis*, a tetraploid fish species. *J. Hered.* **63**, 92–94.
Rasch, E. M., and Balsano, J. S. (1973). Cytogenetic studies of *Poecilia* (Pisces). III. Persistence of triploid genomes in the unisexual progeny of triploid females associated with *Poecilia formosa*. *Copeia* No. 4, pp. 810–813.
Rasch, E. M., Darnell, R. M., Kallman, K. D., and Abramoff, P. (1965). Cytophotometric evidence for triploidy in hybrids of the gynogenetic fish, *Poecilia formosa*. *J. Exp. Zool.* **160**, 155–170.
Rasch, E. M., Prehn, L. M., and Rasch, R. W. (1970). Cytogenetic studies of *Poecilia* (Pisces). II. Triploidy and DNA levels in naturally occurring populations associated with the gynogenetic teleost, *Poecilia formosa* (Girard). *Chromosoma* **31**, 18–40.
Ray-Chaudhuri, R. (1973). Cytotaxonomy and chromosome evolution in birds. *In* "Cytotaxonomy and Vertebrate Evolution" (A. B. Chiarelli and E. Capanna, eds.), pp. 425–483. Academic Press, New York.
Rees, H. (1964). The question of polyploidy in the Salmonidae. *Chromosoma* **15**, 275–279.
Rees, H. (1967). The chromosomes of *Salmo salar*. *Chromosoma* **21**, 472–474.
Regan, J. D., Sigel, M. M., Lee, W. H., Llamas, K. A., and Beasley, A. R. (1968). Chromosomal alterations in marine fish cells *in vitro*. *Can. J. Genet. Cytol.* **10**, 448–453.
Reinboth, R. (1965). Sex reversal in the black sea bass *Centropristes straitus*. *Anat. Rec.* **151**, 403.
Reinboth, R. (1967). Protogynie bei *Chelidoperca hirundinacea* (Cuv. et Val.) (Serranidae)—Ein Diskussionsbeitrag zur Stammesgeschichte amphisexueller Fische. *Annot. Zool. Jpn.* **40**, 181–193.
Rio, G. J., Magnavita, F. J., Rubin, J. A., and Beckert, W. H. (1973). Characteristics of an established goldfish cell line. *J. Fish Biol.* **5**, 315–321.
Roberts, F. L. (1964). A chromosome study of twenty species of Centrarchidae. *J. Morphol.* **115**, 401–418.
Roberts, F. L. (1967). Chromosome cytology of the Osteichthyes. *Prog. Fis Cult.* **29**, 75–83.
Roberts, F. L. (1968). Chromosomal polymorphism in North American landlocked *Salmo salar*. *Can. J. Genet. Cytol.* **10**, 865–875.
Roberts, F. L. (1970). Atlantic salmon (*Salmo salar*) chromosomes and speciation. *Trans. Am. Fish. Soc.* **99**, 105–111.
Robertson, W. R. B. (1916). Chromosome studies. 1. 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. Morphol.* **27**, 179–332.
Robinson, E. S., Potter, I. C., and Atkin, N. B. (1975). The nuclear DNA content of twenty lampreys. *Experientia* **31**, 912–913.
Rothfels, K., Sexsmith, E., Heimburger, M., and Krause, M. O. (1966). Chromosome size

and DNA content of species of *Anemone* L. and related genera (Ranunculaceae). *Chromosoma* **20**, 54–74.

Scheel, J. J. (1968). "Rivulins of the Old World." T. F. H. Publications, Jersey City, New Jersey.

Scheel, J. J. (1972). Rivulin karyotypes and their evolution (Rivulinae, Cyprinodontidae, Pisces). *Z. Zool. Syst. Evolutionsforsch.* **10**, 180–209.

Scheel, J. J., Simonsen, V., and Gyldenholm, A. O. (1972). The karyotype and some electrophoretic patterns of fourteen species of the genus *Corydoras*. *Z. Zool. Syst. Evolutionsforsch.* **10**, 144–152.

Schultz, R. J. (1961). Reproductive mechanism of unisexual and bisexual strains of the viviparous fish *Poeciliopsis*. *Evolution* **15**, 302–325.

Schultz, R. J. (1966). Hybridization experiments with an all-female fish of the genus *Poeciliopsis*. *Biol. Bull. (Woods Hole, Mass.)* **130**, 415–429.

Schultz, R. J. (1967). Gynogenesis and triploidy in the viviparous fish *Poeciliopsis*. *Science* **157**, 1564–1567.

Schultz, R. J. (1969). Hybridization, unisexuality, and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. *Am. Nat.* **103**, 605–619.

Schultz, R. J. (1971). Special adaptive problems associated with unisexual fishes. *Am. Zool.* **11**, 351–360.

Schultz, R. J. (1973). Origin and synthesis of a unisexual fish. *In* "Genetics and Mutagenesis of Fish" (J. H. Schröder, ed.), pp. 207–211. Springer-Verlag, Berlin and New York.

Schultz, R. J., and Kallman, K. D. (1968). Triploid hybrids between the all-female teleost *Poecilia formosa* and *Poecilia sphenops*. *Nature (London)* **219**, 280–282.

Setzer, P. Y. (1970). An analysis of a natural hybrid swarm by means of chromosome morphology. *Trans. Am. Fish. Soc.* **99**, 139–146.

Simon, R. C. (1963). Chromosome morphology and species evolution in the five North American species of Pacific salmon (*Oncorhynchus*). *J. Morphol.* **112**, 77–97.

Simon, R. C., and Dollar, A. M. (1963). Cytological aspects of speciation in two North American teleosts, *Salmo gairdneri* and *Salmo clarki lewisi*. *Can. J. Genet. Cytol.* **5**, 43–49.

Smith, C. L. (1959). Hermaphroditism in some serranid fishes from Bermuda. *Pap. Mich. Acad. Sci., Arts Lett.* **44**, 111–119.

Sparrow, A. H., and Nauman, A. F. (1974). Evolutionary change in genome and chromosome sizes and in DNA content in the grasses. *Brookhaven Symp. Biol.* **25**, 367–389.

Sparrow, A. H., Price, H. J., and Underbrink, A. G. (1972). A survey of DNA content per cell and per chromosome of prokaryotic and eukaryotic organisms: Some evolutionary considerations. *Brookhaven Symp. Biol.* **23**, 451–494.

Spofford, J. B. (1972). A heterotic model for the evolution of duplications. *Brookhaven Symp. Biol.* **23**, 121–143.

Stebbins, G. L. (1958). Longevity, habitat, and release of genetic variability in the higher plants. *Cold Spring Harbor Symp. Quant. Biol.* **23**, 365–378.

Stebbins, G. L. (1969). "The Basis of Progressive Evolution." Univ. of North Carolina Press, Chapel Hill.

Stebbins, G. L. (1971). "Chromosomal Evolution in Higher Plants." Addison-Wesley, Reading, Massachusetts.

Strommen, C. A., Rasch, E. M., and Balsano, J. S. (1975). Cytogenetic studies of *Poecilia* (Pisces). V. Cytophotometric evidence for the production of fertile offspring by triploids related to *Poecilia formosa*. *J. Fish Biol.* 7, 667–676.

Svärdson, G. (1945). Chromosome studies on Salmonidae. *Rep. Inst. Freshwater Res. Drottningholm* No. 23, pp. 1–151.

Swarup, H. (1959a). Production of triploidy in *Gasterosteus aculeatus*. *J. Genet.* **56**, 129–142.

Swarup, H. (1959b). Effect of triploidy on the body size, general organization and cellular structure in *Gasterosteus aculeatus* (L.). *J. Genet.* **56**, 143–155.

Taylor, K. M. (1967). The chromosomes of some lower chordates. *Chromosoma* **21**, 181–188.

Tettenborn, U., and Gropp, A. (1970). Meiotic nondisjunction in mice and mouse hybrids. *Cytogenetics* **9**, 272–283.

Thompson, K. W. (1976). Some aspects of chromosomal evolution of the Cichlidae (Teleostei: Perciformes) with emphasis on neotropical forms. Ph.D. Thesis, Univ. of Texas at Austin.

Thorgaard, G. H. (1976). Robertsonian polymorphism and constitutive heterochromatin distribution in chromosomes of the rainbow trout (*Salmo gairdneri*). *Cytogenet. Cell Genet.* **17**, 174–184.

Uyeno, T., and Miller, R. R. (1971). Multiple sex chromosomes in a Mexican cyprinodontid fish. *Nature (London)* **231**, 452–453.

Uyeno, T., and Miller, R. R. (1972). Second discovery of multiple sex chromosomes among fishes. *Experientia* **15**, 223–225.

Uyeno, T., and Smith, G. R. (1972). Tetraploid origin of the karyotype of Catostomid fishes. *Science* **175**, 644–646.

Valenti, R. J. (1975). Induced polyploidy in *Tilapia aurea* (Steindachner) by means of temperature shock treatment. *J. Fish Biol.* **7**, 519–528.

Vrijenhoek, R. C. (1972). Genetic relationships of unisexual hybrid fishes to their progenitors using lactate dehydrogenase isozymes as gene markers (*Poeciliopsis*, Poeciliidae). *Am. Nat.* **106**, 754–766.

Vrijenhoek, R. C., and Schultz, R. J. (1974). Evolution of a trihybrid unisexual fish (*Poeciliopsis*, Poeciliidae). *Evolution* **28**, 306–319.

Wallace, B. (1963). Genetic diversity, genetic uniformity, and heterosis. *Can. J. Genet. Cytol.* **5**, 239–253.

Webb, C. J. (1974). Fish chromosomes: A display by scanning electron microscopy. *J. Fish Biol.* **6**, 99–100.

White, M. J. D. (1946). The evidence against polyploidy in sexually reproducing animals. *Am. Nat.* **80**, 610–619.

White, M. J. D. (1968). Models of speciation. *Science* **159**, 1065–1070.

White, M. J. D. (1973a). "Animal Cytology and Evolution." Cambridge Univ. Press, London.

White, M. J. D. (1973b). Chromosomal rearrangements in mammalian population polymorphism and speciation. *In* "Cytotaxonomy and Vertebrate Evolution" (A. B. Chiarelli and E. Capanna, eds.), pp. 95–128. Academic Press, New York.

Wickbom, T. (1943). Cytological studies on the family Cyprinodontidae. *Hereditas* **29**, 1–24.

Wilmot, R. L. (1974). A genetic study of the red-banded trout. Ph.D. Thesis, Oregon State Univ., Corvallis.

Wilson, A. C., Maxson, L. R., and Sarich, V. M. (1974a). Two types of molecular evolution. Evidence from studies of interspecific hybridization. *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2843–2847.

Wilson, A. C., Sarich, V. M., and Maxson, L. R. (1974b). The importance of gene rearrangement in evolution: evidence from studies on rates of chromosomal, protein, and anatomical evolution. *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3028–3030.

Wilson, A. C., Bush, G. L., Case, S. M., and King, M. C. (1975). Social structuring of mammalian populations and rate of chromosomal evolution. *Proc. Natl. Acad. Sci. U.S.A.* **72,** 5061–5065.

Winge, Ö. (1922). One-sided masculine and sex-linked inheritance in *Lebistes reticulatus*. *J. Genet.* **12,** 145–162.

Winge, Ö. (1934). The experimental alteration of sex chromosomes into autosomes and vice versa, as illustrated by *Lebistes*. *C. R. Trav. Lab. Carlsberg, Ser. Physiol.* **21,** 1–49.

Winge, Ö., and Ditlevsen, E. (1947). Colour inheritance and sex determination in *Lebistes*. *Heredity* **1,** 65–83.

Winge, Ö., and Ditlevsen, E. (1948). Colour inheritance and sex determination in *Lebistes*. *C. R. Trav. Lab. Carlsberg, Ser. Physiol.* **24,** 227–248.

Wolf, K., and Quimby, M. C. (1969). Fish cell and tissue culture. *In* "Fish Physiology" (W. S. Hoar and D. J. Randall, eds.), Vol. 3, pp. 253–305. Academic Press, New York.

Wolf, K., Quimby, M. C., Pyle, E. A., and Dexter, R. P. (1960). Preparation of monolayer cell cultures from tissues of some lower vertebrates. *Science* **132,** 1890–1891.

Wright, J. E. (1955). Chromosome numbers in trout. *Prog. Fish Cult.* **17,** 172–176.

Wright, S. (1943). Breeding structure of populations in relation to speciation. *Am. Nat.* **74,** 232–248.

Yamamoto, K., and Ojima, Y. (1973). A PHA-culture method for cells from the renal tissue of teleosts. *Jpn. J. Genet.* **48,** 235–238.

Yamamoto, T. (1961). Progenies of induced sex-reversal males in the medaka, *Oryzias latipes*. *J. Exp. Zool.* **146,** 163–179.

Yamamoto, T. (1963). Induction of reversal in sex differentiation of YY zygotes in the medaka, *Oryzias latipes*. *Genetics* **48,** 293–306.

Yamamoto, T. (1969). Sex differentiation. *In* "Fish Physiology" (W. S. Hoar and D. J. Randall, eds.), Vol. 3, pp. 117–175. Academic Press, New York.

Yamamoto, T., and Kajishima, T. (1969). Sex-hormonic induction of reversal of sex differentiation in the goldfish and evidence for its male heterogamety. *J. Exp. Zool.* **168,** 215–222.

Zenzes, M. T., and Voiçulescu, I. (1975). C-banding patterns in *Salmo trutta*, a species of tetraploid origin. *Genetica (The Hague)* **45,** 531–536.