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Authors
Avise, JC
Gold, JR

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CYTOGENETIC STUDIES IN NORTH AMERICAN MINNOWS (CYPRINIDAE). IV. SOMATIC POLYPLOIDY IN *GILA BICOLOR*

JOHN C. AVISE
Department of Zoology, University of Georgia, Athens, Georgia 30602

AND

JOHN R. GOLD
Genetics Section, Texas A. & M. University, College Station, Texas 77843

The kidney tissue of a single individual of the California minnow *Gila bicolor* (Girard) contained polyploid cells in about 1.7% frequency. Chromosome spreads of triploid, tetraploid, hexaploid, octaploid, and dodecaploid cells were observed and may have arisen through endoreduplication of ancestral diploid and triploid cells. The cytological mechanism producing the triploid cells is unknown. Diplochromosomes were not present. The distribution of ploidy in cells of this individual is not random. In particular, cells having undergone one round of chromosomal increase appear increasingly susceptible to additional rounds of chromosomal gain.

On a trouvé dans le tissu du rein d'un seul spécimen de vairon de Californie, *Gila bicolor* (Girard) des cellules polyploïdes à une fréquence d'environ 1.7%. Des préparations broyées de chromosomes ont révélé des cellules triploïdes, tétraploïdes, hexaploïdes, octaploïdes et dodécaploïdes. Ces dernières sont peut-être le résultat d'une endomitose des cellules ancestrales diploïdes et triploïdes. Le mécanisme cytologique qui provoque la formation des cellules triploïdes n'est pas connu. On n'a pas vu de diplochromosomes. Chez ce spécimen la distribution des cellules ploïdales n'est pas aléatoire. En particulier, les cellules qui ont déjà subi un taux d'accroissement en nombre de chromosomes semblent susceptibles à un autre accroissement. [Traduit par le journal]

Introduction

Scattered polyploid cells in predominantly diploid plant tissues were first reported early in the century (Stomps, 1910; Husted, 1932; Larter, 1932; Lorz, 1937; Ervin, 1941). Somatic polyploidy is of wide-spread if not universal occurrence in plants, particularly Angiospermae (Lorz, 1947; D'Amato, 1964). Many cases have also been reported in animals, including man (Holt, 1917; Schwarzacher and Schnedl, 1965; Kelly and Almy, 1969). Polyploid cells most often occur in strongly differentiated tissue. Typically, such cells contain $(2n)2^x$ chromosomes, where $2n$ is the diploid number and x is a small integer. This suggests that the extra chromosomes arise through duplications of entire chromosome sets without intervening mitotic divisions. The adaptive significance, if any, of somatic polyploidy is not known, although it has been suggested that the additional chromosome sets increase and economize the synthesizing capacity of a cell or tissue (Nagl, 1976a; see also Nagl, 1976b).

In this paper, we report the finding of a number of polyploid cells in the kidney of the California minnow *Gila bicolor* (Girard). In tissue from a single individual, we have observed diploid, triploid, tetraploid, hexaploid, octaploid, and dodecaploid cells. The distribution of ploidy within this individual, and among other minnows examined, does not appear random. Furthermore, the appearance of $3x$, $6x$, and $12x$ cells cannot be explained simply by the repeated doubling of whole chromosome sets in a diploid ancestral cell.

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It is appropriate here to briefly review some of the terminology applied to somatic polyploid cells, since the terms are confusing and have not been used consistently. As originally used by Geitler (1939), "endomitosis" referred to the appearance of additional chromosome-like structures during an abortive mitosis. With the recognition that chromosome replication and mitosis are separate events, "endomitosis" has largely been discarded and "endoreduplication" now refers to chromosomal replication in the absence of mitosis leading to polyploidy (Mittwoch *et al.*, 1965). "Endopolyploidy" results from "endoreduplication," as opposed to other possible mechanisms of generating polyploidy such as nuclear fusion. Since endoreduplication may not be the only process leading to polyploid *Gila* cells, we will use interchangeably the more general terms "polysomaty" or "somatic polyploidy."

Materials and Methods

The specimen of *Gila bicolor* exhibiting polysomaty was found during a routine karyological study of Cyprinidae inhibiting California (Gold and Avise, 1977a). A total of four *Gila bicolor*

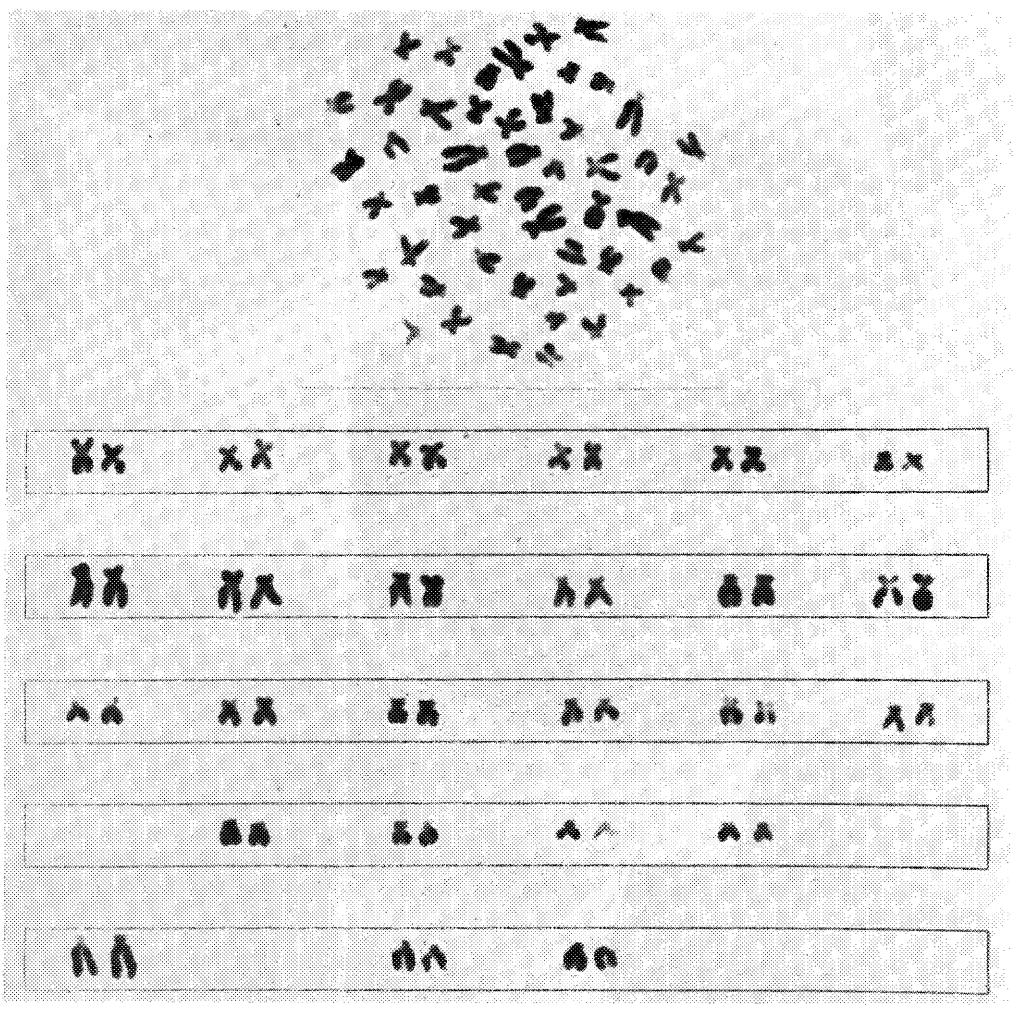

Fig. 1. Diploid metaphase karyotype ($2n = 50$) from kidney cell of *Gila bicolor*.

were collected by seine near Crowley Lake, Mono County, California, and returned live to the laboratory for karyotyping. Chromosome preparation and analysis was carried out using the technique of Gold (1974), employing kidney as the source tissue and colchicine as the mitotic inhibitor. Colchicine (0.5% in 0.85% sterile saline solution) was injected into the dorsal musculature three hours prior to dissection of the kidney.

Results

The diploid karyotype of *Gila bicolor* consists of 44 chromosomes with median or submedian centromeres, and 6 chromosomes with subterminal or terminal centromeres (Fig. 1). This karyotype differs relatively little in gross configuration from those of eight other California minnow species, or from many of the nearly 250 members of North American Cyprinidae (Avise and Gold 1977). The diploid number was determined from actual counts of 145 well-spread cells, and more cursory examination of an additional 477 cells, taken from four specimens of *Gila bicolor* (Table I).

An examination of 43 cells in three *Gila* individuals revealed no evidence of polysomaty. In cells from a fourth individual, however, we observed apparently intact nuclei containing approximately integer multiples of the haploid chromosome number (Table I; Fig. 2). About 1.7% of the chromosome spreads of kidney cells were polyploid (one dodecaploid, one octaploid, one hexaploid, four tetraploid, and three triploid cells were observed). The polyploid karyotypes did not exhibit exactly the expected numbers of chromosomes. For example, we count about 291 chromosomes in the presumed dodecaploid cell; the additional chromosomes were no doubt obscured by overlap, or perhaps lost during cell preparation.

With our sample sizes, the frequency of occurrence of polyploid cells in this individual *Gila* is not demonstrably different from that of the other *Gila* examined. However, we have also examined a total of 696 cells in 33 diploid individuals belonging to nine cyprinid species, without evidence of any polysomaty.

TABLE I

Frequency of cells observed with various chromosome numbers in the California minnows

Sample	Number of individuals	Approximate chromosome number					
		50	75	100	150	200	300
Gila bicolor	1	569	3	4	1	1	1
Other *Gila bicolor*	3	43	0	0	0	0	0
Other cyprinid species	30	653	0	0	0	0	0

TABLE II

Observed numbers of cells having undergone a given minimum number of chromosome increasing episodes in the kidney tissue of an individual *Gila bicolor*. Expected frequencies under the relevant Poisson distribution are also given*

Number of cells	Minimum no. of chromosome increasing episodes				Total
	0	1	2	3	
Observed	569	7	2	1	579
Expected	(565.154)	(13.677)	(0.165)	(0.001)	579.0
			3		
			(0.166)		

*$\chi^2_{[1]} = 51.67$, $P < 0.01$; (since the frequency in one block used to calculate χ^2 is < 5, results should be considered indicative rather than conclusive).

We may also ask whether the chromosome replicating episodes occur independently of one another in the kidney cells of the fish exhibiting polysomaty. In order to do this we must make certain assumptions about the mode of generation of new chromosome complements. We assume that many new chromosomal sets are not generated spontaneously, in saltatory fashion, but rather arise in a sequential manner. We also assume that at least one of the ancestral cells of a cell now polyploid was diploid. For example, the minimum number of chromosome increasing events needed to generate a hexaploid cell is two ($2n \rightarrow 4x$, + $2n \rightarrow 6x$; or $2n \rightarrow 3x \rightarrow 6x$); the minimum number for an octaploid cell is also two ($2n \rightarrow 4x \rightarrow 8x$); and the minimum number for a dodecaploid cell is three (i.e., $2n \rightarrow 3x \rightarrow 6x \rightarrow 12x$). This is true whether new chromosome sets are generated through endopolyploidy of whole or partial chromosome complements, or whether nuclear fusions are involved.

The observed numbers of kidney cells having undergone a given minimum number of chromosome increasing episodes are listed in Table II, and compared to frequencies

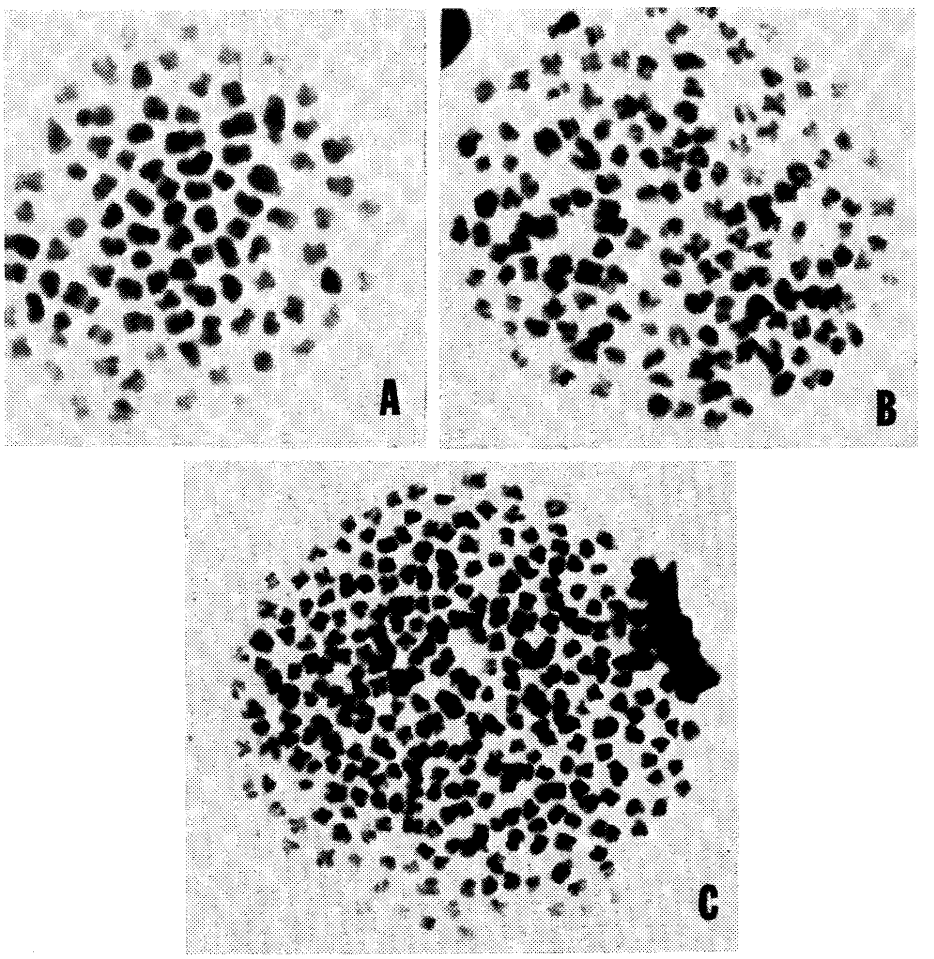

Fig. 2. Polyploid karyotypes in kidney cell of an individual *Gila bicolor:* A, tetraploid; B, hexaploid; C, dodecaploid.

expected under the relevant Poisson distribution. The discrepancy is highly significant. The excess numbers of cells exhibiting higher ploidy levels (dodecaploid, octaploid, hexaploid), and the deficiency of cells with low ploidy levels (tetraploids, triploids), suggest that the occurrence of one chromosome increasing event enhances the probability of second and later such events in the same cell line.

Discussion

In the first mitosis of nuclei following two series of replications, chromosomes characteristically occur in closely associated pairs in many organisms (Berger, 1941; Kelly and Almy, 1969; Levan, 1939; Levan and Hauschka, 1953). These pairs are called diplochromosomes, and provided the earliest and perhaps strongest evidence that endoreduplication rather than nuclear fusion is responsible for polysomaty (De Litardière, 1923; see Lorz, 1947). However, in subsequent mitoses the duplicated chromosomes become dispersed by movements of cell division, and diplochromosomes may be rare in a polysomatic cell population.

The chromosomes in all of the polyploid *Gila* cells appear unpaired, and hence we cannot convincingly argue from our data that endoreduplication was the cause of polysomaty. Endoreduplication of entire chromosome sets could readily account for the appearance of tetraploid and octaploid cells from a diploid ancestor, and hexaploid and dodecaploid cells from a triploid ancestor. In most organisms, endoreduplication involves all the chromosomes of a somatic cell. Hence the appearance of triploid cells and their multiples in *Gila* is most surprising. For the present, we can offer no empirical rationale for the origin of a triploid somatic cell.

The colchicine treatment probably did not cause the polysomaty in *Gila*. Using the same technique, we have now examined numerous karyotypes of more than 500 fish without observing any other instances of higher ploidy levels. Colchicine is widely used as a polyploid inducing agent in plants; its success in inducing polyploidy in animals has been much more limited (Dermen, 1940). It seems unlikely that if colchicine were the causal agent in *Gila* its effect would be so unevenly distributed among cells. Furthermore, only three hours elapsed between injection and sacrifice, probably too little time for the appearance of colchicine-induced ploidy at the higher levels observed. Finally, polysomaty is probably not the result of artificial treatment of any kind in many other organisms (Berger, 1941).

Whatever the cause of polysomaty in *Gila*, the data suggest that cells which contain more than two genomes are increasingly susceptible to additional rounds of chromosome increase. This finding is potentially important, since any mutationally derived genetic differences between cells, or any environmental parameters to which cells are differentially exposed (e.g., position effects or viruses), might produce this result. This observation in *Gila* needs to be verified by additional study as an important first step in determining the genetic and environmental factors responsible for polysomaty.

A few cases of polysomaty in fishes have previously been noted (Nygren *et al.*, 1968, 1971, 1975). One of us (J.R.G.) has also observed tetraploid cells in about 10-12 percent of kidney cells in *Salmo aguabonita* (unpublished data). Lorz (1947) writes, "It is significant that in most cases polysomaty is not manifest in cells which undergo meiotic reduction into spores or gametes. Otherwise, naturally occurring polyploid forms would be much more frequent than they are." However, we have recently reported the discovery of a single triploid individual of *Hesperoleucus symmetricus*, a minnow not too distantly related to *Gila* (Gold and Avise, 1977b). A likely origin of the triploid *Hesperoleucus* was fusion of a haploid sperm with a diploid ovum. Thus in fishes, the occurrence of cells containing multiples of the normal haploid or diploid numbers may occasionally be of some significance during sexual reproduction as well.

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