

# Synaptonemal complex analysis of interspecific hybrids of *Poecilia* (Teleostei, Poeciliidae)

Marina I. Rodionova, Sergei V. Nikitin and Pavel M. Borodin

## ABSTRACT

The pattern of chromosome pairing at meiotic prophase in males of the guppy (*Poecilia reticulatus*), black molly (*P. sphenops*), marble molly (*P. velifera*) and hybrids between these species was examined by means of electron microscopy of surface spread synaptonemal complexes. Meiotic chromosomes of the pure species and *P. sphenops* x *P. velifera* hybrid showed complete pairing at pachytene. No indication of heteromorphism for any chromosomes was found. The vast majority of pachytene cells of the *P. velifera* x *P. reticulatus* hybrid demonstrated various signs of pairing failure: univalents, interlocks, multiple non-homologous pairing and end-to-end associations. However, a few cells were found to contain all completely paired homomorphic bivalents. These findings imply that: (1) genetic divergence between these species was not accompanied by gross chromosomal rearrangements; (2) pairing failure in the *P. velifera* x *P. reticulatus* hybrid is apparently determined by a difference in the species-specific mechanism controlling meiotic prophase, rather than chromosome divergence.

## INTRODUCTION

The comparative analysis of the karyotypes of closely related species has helped in understanding of the mechanisms of chromosomal evolution and phylogenetic relationships in animals, especially in mammals. Study of the chromosome evolution of fish is inherently difficult because their chromosomes are usually not accessible for G-band staining (Sola *et al.*, 1981). Similarity in morphology of routinely stained chromosomes does not necessarily reflect true homology. The resolution of classical cytogenetic methods is insufficient to detect chromosome rearrangements other than those changing chromosome number and/or arm ratio. An alternative way to estimate the degree of homology between the chromosomes of closely related species is to observe their pairing in the meiotic prophase of inter-species hybrids (Liming and Pathak, 1981; Poorman, 1982; Derr *et al.*, 1991; Dollin *et al.*, 1991; Hale *et al.*, 1993). A wide variety

of chromosome rearrangements may be detected by means of electron microscopic analysis of the surface spread silver stained synaptonemal complexes (SC). Heterozygosity for the rearrangements (as well as heteromorphism for the sex chromosomes) is displayed at the level of SC as heteromorphic synaptic configurations (Moses, 1980).

Karyotypes of the guppy *P. reticulatus* (Yosida and Hayashi, 1970; Scheel, 1972, Nanda *et al.*, 1990), the black molly *P. sphenops* (Prehn and Rasch, 1969, Rishi and Gaur 1976, Haaf and Schmid, 1984), and the marble molly *P. velifera* (Post, 1965; Prehn and Rasch, 1969) have been described. All of them contain 23 pairs of acrocentric chromosomes. Data (Post, 1965) suggesting  $n = 24$  for *P. sphenops* have not been confirmed.

Genetic data indicate that *P. reticulatus* has an XX/XY system of sex determination (see Kirpichnikov, 1981 for review). Nanda *et al.* (1990, 1992) reported a difference in the C-band patterns between the telomeres of the homologous No. 1 chromosomes in male. One of the homologues (Y) contained more telomeric heterochromatin than the other (X). Nanda *et al.* (1990, 1992)

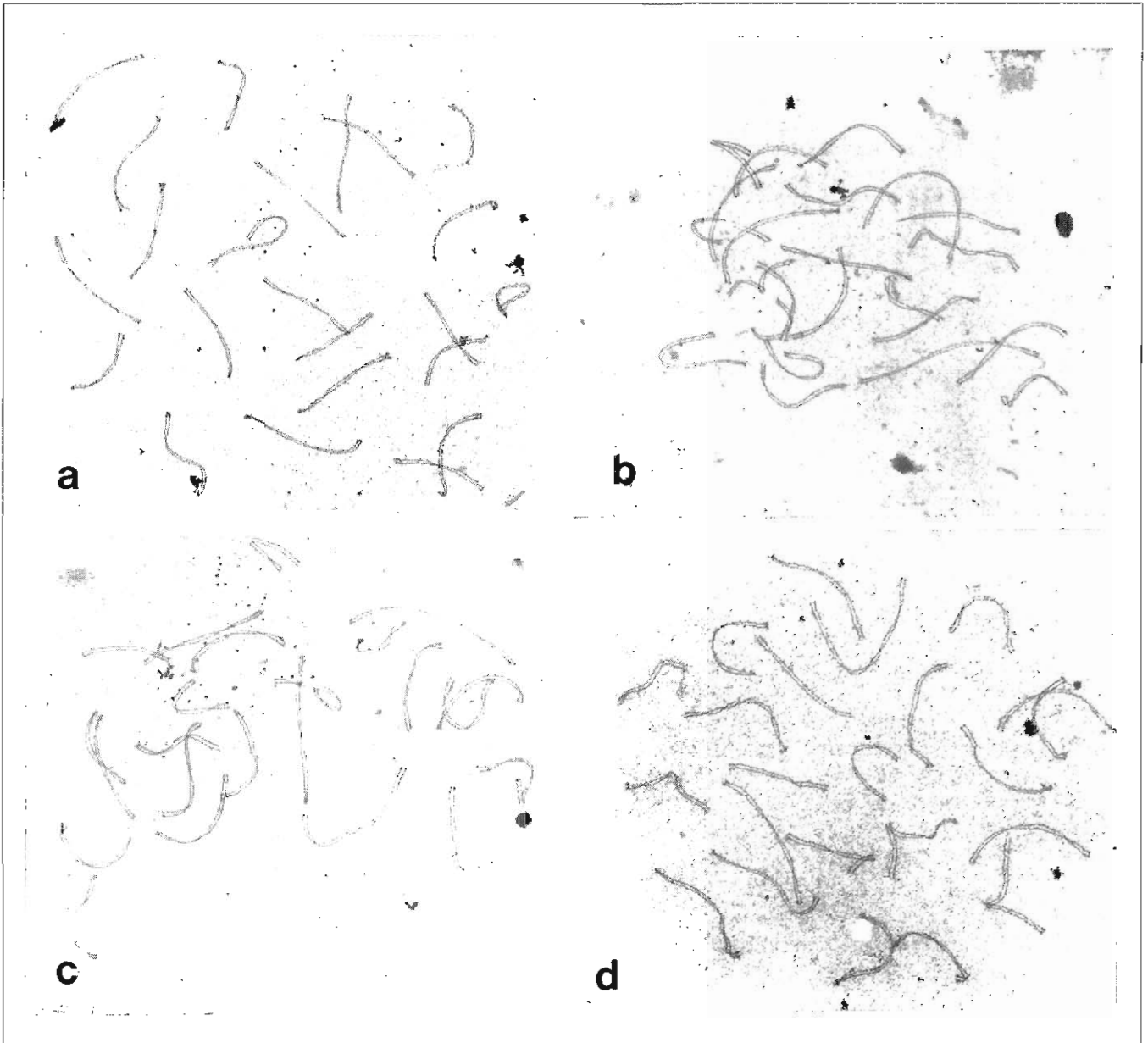


Figure 1 - SC complement of *Poecilia reticulatus* (a), *P. sphenops* (b), *P. velifera* (c), and *P. sphenops* x *P. velifera* (d) hybrids.

demonstrated that this difference was determined by accumulation of simple repeats on the telomeric heterochromatin of the Y chromosome. A distinct C-heteromorphism between two homologous chromosome 1 was observed in *P. sphenops* females (Haaf and Schmid, 1984), and interpreted as an indication of the WZ/ZZ system of sex determination. Such heteromorphism has not been found in *P. velifera*, indicating that the Z and W are still structurally equal (Nanda *et al.*, 1992).

## MATERIAL AND METHODS

Three adult males of each of the three species: *P. reticulatus*, *P. sphenops*, *P. velifera*, six of *P. sphenops* x

*P. velifera* and two of *P. velifera* x *P. reticulatus* hybrids were used in this study. Female *P. sphenops* were crossed to male *P. velifera* and female *P. velifera* to male *P. reticulatus*, to generate interspecific hybrids.

The technique for obtaining microspread spermatocytes for analysis of the whole cell complements of SC was essentially the same as that of Speed (1982). Testes were removed and placed in Hanks medium in watchglasses. Then they were transferred onto a separate slide with three drops of 0.15 M sucrose. The testes were torn apart to free the germ cells. The cell suspension was transferred onto a slide precoated with 0.5% Optilux plastic in chloroform. The slides were dried and fixed with 4% paraformaldehyde in 0.1 M sucrose for 10 min, washed, dried and stained with

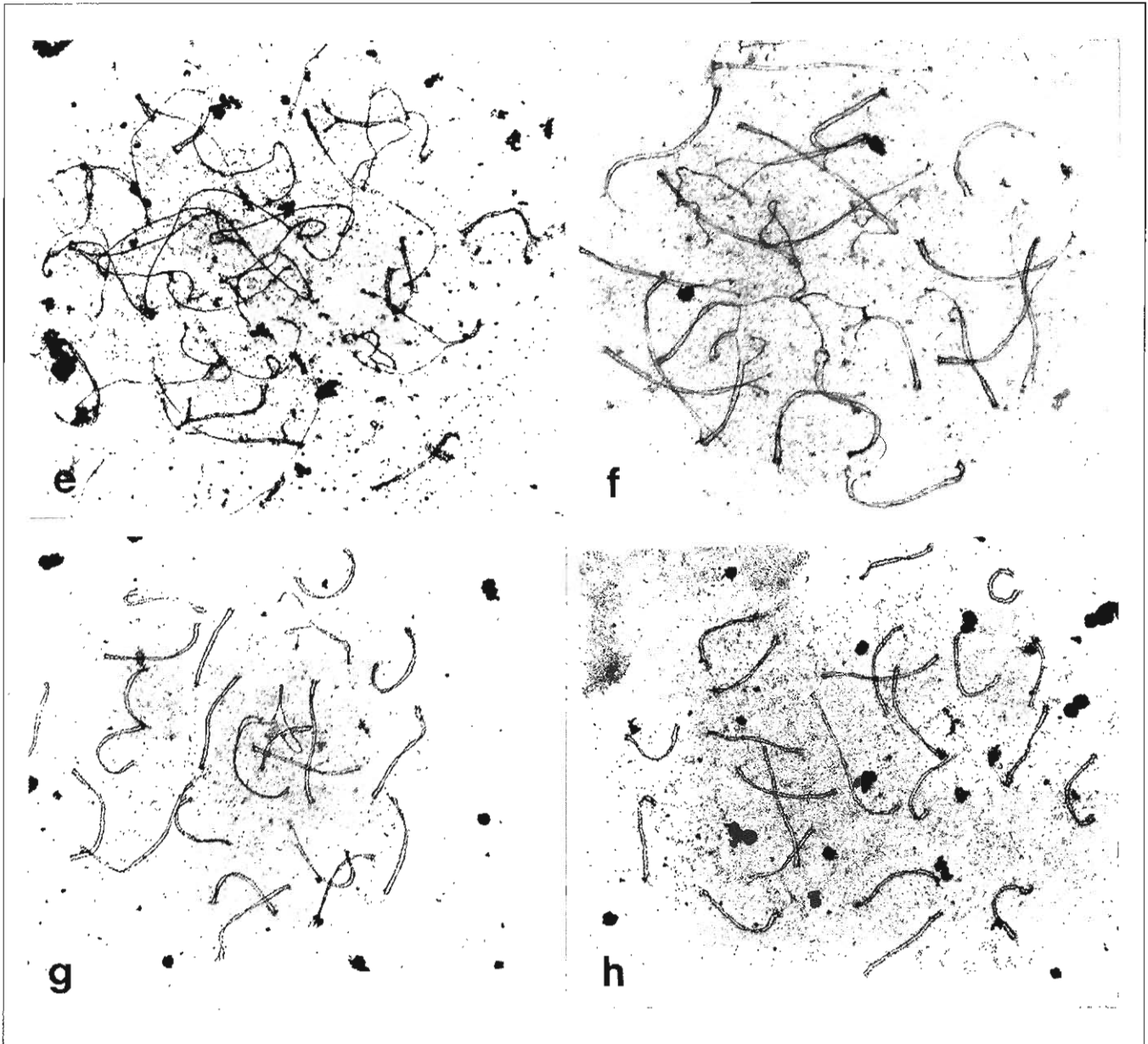


Figure 1 - (Continued). SC complement of *Poecilia velifera* x *P. reticulatus* (e, f, g, h) hybrids.

silver nitrate. The spreads of good quality after light microscopic examination were transferred to specimen grids, and examined and photographed with an electron microscope JEM-100 (JEOL, Japan) at 80 kV.

Approximately 100 spermatocytes per specimen were analyzed in *P. reticulatus*, *P. sphenops*, *P. velifera* and *P. sphenops* x *P. velifera*, and 869 cells in *P. velifera* x *P. reticulatus* hybrids.

## RESULTS AND DISCUSSION

### Pure species

In general, meiotic prophase in spermatocytes of *P. reticulatus*, *P. sphenops* and *P. velifera* had a similar

appearance and was similar to that described for other fish (Lin and Yu, 1991). The formation of SC started at zygotene and was completed at pachytene. A very small proportion of zygotene cells was found. This indicates that zygotene is a rather short stage in these species. Pairing was mainly initiated at the chromosome ends, but some interstitial sites of synaptic initiation were also detected.

At pachytene, 23 completely paired bivalents were found in the males of all three species studied (Figure 1a,b,c). It was impossible to identify individual SCs in any species as they formed a continuous series when arranged in decreasing size.

Taking into account the data of Nanda *et al.* (1990, 1992) about the C-band heteromorphism in

chromosome 1 in males of *P. reticulatus*, we paid special attention to the longest bivalent in the SC complement of male guppies (Figure 1a). We found that its lateral elements had equal length, were uniformly stained and completely paired with each other in the majority of pachytene cells. No bivalent had axes of unequal length. Some bivalents displayed a temporary asynapsis of the terminal segments at early and late pachytene, but usually there were several of these.

This means that the terminal regions of the X and Y chromosomes of the guppy pair non-homologously with each other. Non-homologous synapsis in structural heterozygotes has been found in many species. It has been shown that in male mammals the Y chromosome undergoes extensive non-homologous pairing with the differentiating part of the X chromosome (Tres, 1977; Chandley *et al.*, 1984; Ashley, 1987; Pack *et al.*, 1993). The lateral elements of Z and W chromosomes in birds demonstrate an ability to equalize their size in heteromorphic bivalents. (Solari, 1992; Solari and Pigozzi, 1993).

Genetic data show that crossing over between differentiated parts of the X and Y chromosomes is suppressed (Kirpichnikov, 1981). Non-homologous synapsis of the telomeric regions of the sex chromosomes, detected in our study, may be a mechanism of the suppression of crossing over.

### *P. sphenops* x *P. velifera* hybrid

All stages of spermatogenesis (including mature sperm of normal morphology) were represented in testes of the male hybrids between these two species. The SC complement consisted of 23 bivalents, as expected (Figure 1d). All bivalents demonstrated complete pairing at pachytene. No indication of heteromorphism for any chromosome was found. This implies that the divergence between *P. sphenops* and *P. velifera* was not accompanied by chromosomal rearrangements. The genic divergence did not lead to incompatibility of the species-specific genetic systems controlling meiotic progression and spermatogenesis.

### *P. velifera* x *P. reticulatus* hybrid

One of two hybrid males was completely sterile: neither sperm, nor prophase spermatocytes were found in the spreads of its testes. A little sperm was found in the other male hybrid. Pachytene cells were abundant, but many of them showed definite

signs of degeneration, such as complete asynapsis of the axial elements and accumulation of large globules of electron dense material in the nuclei. A majority of pachytene cells demonstrated various pairing failures: univalents, interlocks, multiple non-homologous pairing and end-to-end associations (Figure 1e,f,g). The number of chromosomes which failed to pair varied from cell to cell. In some cells, almost all chromosomes were unpaired (Figure 1e). In the other cells, some chromosomes were non-homologously paired with each other, forming chains, some displayed partial or complete asynapsis and some formed normal bivalents (Figure 1f). Figure 1g shows a cell in which all but three bivalents are completely normal. A few cells (three of 869) were found to contain only completely paired homomorphic bivalents (Figure 1h).

The variable appearance of pairing disturbances and the finding of normal spermatocytes (although extremely rare) imply that pairing failure in the majority of pachytene cells of the *P. velifera* x *P. reticulatus* hybrid, and arrest of spermatogenesis are determined by genic incompatibility of the species-specific mechanisms controlling meiotic prophase in the parental species, rather than loss of homology between their chromosomes. The finding of mature sperm (though a very small number) in the second hybrid male demonstrates that meiotic arrest in the hybrid is not complete, and some cells are able to surmount it.

Thus, we can conclude that divergence between *P. reticulatus* and *P. velifera* as well as between *P. velifera* and *P. sphenops* was not accompanied by gross chromosomal rearrangements. All of them have similar SC karyotypes. All of their chromosomes are able to form homomorphic bivalents in meiosis in the hybrid males. This confirms the conclusions about karyotypic similarity between these species inferred from comparative analysis of routinely stained karyotypes. However, the degree of genic divergence between these three species is substantially different. While no postzygotic reproductive barriers were formed between *P. velifera* and *P. sphenops*, the genetic system controlling meiotic prophase of the guppy has undergone significant divergence, and has become incompatible with those of mollies. In the latter case we are observing the final steps of formation of a postzygotic mechanism of isolation.

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## RESUMO

O padrão de pareamento de cromossomos na prófase meiótica em indivíduos machos das espécies de peixes *Poecilia reticulatus*, *P. sphenops* e *P. velifera* e em híbridos entre estas espécies foi examinado através de microscopia eletrônica do complexo sinaptonêmico. Os cromossomos meióticos das espécies puras e do híbrido *P. sphenops* x *P. velifera* mostraram pareamento completo em paquitene. Em nenhum cromossomo foi encontrado indício de heteromorfismo. A grande maioria das células paquitênicas do híbrido *P. velifera* x *P. reticulatus* demonstraram vários sinais de falha de pareamento: univalentes, "interlocks", pareamento múltiplo não homólogo e associações término-términos. Contudo, umas poucas células continham todos os bivalentes homomórficos completamente pareados. Estes achados significam que: 1) a divergência genética entre estas espécies não foi acompanhada por rearranjos cromossômicos grosseiros; 2) a falha de pareamento no híbrido *P. velifera* x *P. reticulatus* é aparentemente determinada por diferença gênica do mecanismo espécie-específico que controla a prófase meiótica, em vez de por divergência cromossômica.

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