Meiotic chromosome configurations in triploid progeny from reciprocal crosses between wild-type diploid and natural tetraploid loach *Misgurnus anguillicaudatus* in China

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Abstract

Here, we showed meiotic chromosome configurations prepared from oocyte germinal vesicles and spermatocytes of triploid loaches produced from reciprocal crosses between wild-type diploids (2n = 50) obtained from Dalian, Liaoning Province, China and natural tetraploids (4n = 100) from Chibi, Hubei Province, China. Major meiotic cells in triploids comprised 25 bivalents and 25 univalents, but cells with one to five trivalents were also observed. When three homologous chromosomes bearing nucleolar organizing regions (NOR) were identified with the detection of signals or positive sites by silver staining, chromomycin A_3 staining and fluorescence in situ hybridization with a 5.8S + 28S rDNA probe, two third of selected triploid cells gave a configuration including one bivalent with two NORs (association of two homologous chromosomes) and one univalent with one NOR. However, other triploid cells showed three univalent each of which had one NOR, suggesting a failure of synapsis between homologous chromosomes. These results suggested that triploid female and male should produce aneuploid gametes with the theoretical mode at 1.5n (37 or 38 chromosomes).

Keywords bivalent・gamete・hybrid・polyploid・trivalent・univalent

Introduction

When diploid females exhibit fully mature ovary, most oocytes cannot enter into vitellogenesis in the ovary of artificially induced triploid fishes (Benfey 1999, 2011; Piferrer et al. 2009). Ovarian development is largely retarded in induced triploid females in several teleosts (Benfey 1999, 2011; Piferrer et al. 2009). On the contrary, induced triploid males often generate a small quantity of aneuploid spermatozoa because testis develops to some extent and secondary sex characters arise (Benfey 1999, 2011; Piferrer et al. 2009). These reproductive characteristics are also the cases in artificially induced triploid loach or Oriental weatherfish, *Misgurnus anguillicaudatus* (Suzuki et al.1985; Zhang and Arai 1999; Arai and Fujimoto 2013). However, reproductive performance of spontaneous triploid fishes, which infrequently occur in nature, is different from that of artificially induced triploid fishes and their reproductive capacities are much more diversified (Arai and Fujimoto 2013).

In northern area of Hokkaido, Japan, natural triploid loaches infrequently arise from unreduced diploid eggs, which are spawned by the gynogenetically reproducing clonal diploid population, by accidental incorporation of a sperm nucleus into the egg (Morishima et al., 2002, 2008a; Itono et al. 2007). Such clone-origin triploid females mainly produce haploid eggs by the mechanism of meiotic hybridogenesis together with a small number of unreduced triploid eggs, but males are sterile (Morishima et al. 2008b; Arai and Fujimoto 2013).

Simultaneous production of unreduced triploid eggs and post-meiotic haploid eggs was also reported in triploid females which occurred from reciprocal crosses between wild-type diploids from Japanese population and natural tetraploids, which distributed in central part of China and presumably appeared in Japanese market samples, because Chinese loaches including such natural tetraploids are commercially imported to Japan as foods and live bait for fishing (Matsubara et al.1995; Zhang and Arai 1996; Arai and Fujimoto 2013). It is generally accepted that unreduced triploid eggs are formed by the mechanism of premeiotic endomitosis (Arai and Mukaino, 1997; Zhang et al., 1998), while haploid eggs by the meiotic hybridogenesis-like mechanism (Arai and Mukaino 1998). However, triploid males from the crosses between wild-type diploid and natural tetraploid could not generate fertile spermatozoa (Matsubara et al. 1995; Zhang and Arai 1996).

In Japanese wild populations, no tetraploid loach has been detected (Arai and Fujimoto 2013), but diploid-tetraploid complex exists in Chang Jiang River system in central China (Li et al. 2008, 2010). Li et al. (2012, 2013) verified gonochorisms and sexual reproduction both in Chinese diploid and tetraploid loaches: they reproduce by sexual fertilization of post-meiotic gametes (eggs and spermatozoa) and reciprocal hybridization between diploid and tetraploid produces viable triploid progeny. Li et al. (2012) also found sympatric occurrence of natural triploids and reported their

reproductive performance. Spontaneous occurring triploid female produced fertile haploid eggs from which normal viable progeny appeared after fertilization with spermatozoa of diploid and tetraploid males (Li et al. 2012). On the other hand, a triploid male produced abnormal progeny with about 2.5n chromosomes, suggesting the generation of aneuploid spermatozoa by meiosis of natural triploid males (Li et al. 2012).

Configuration of meiotic chromosomes was an essential information to consider the cytogenetic mechanism involved in the generation of gametes in triploid organisms. In the present study, we observed meiotic configurations of chromosomes in both the oocyte germinal vesicles (GVs) and the spermatocytes of the triploid progeny from the reciprocal crosses between wild-type diploids and natural tetraploids in 2010 (Li et al., 2012). In our study, the pairing behavior of homologous chromosomes (univalent, bivalents or other unusual multivalents) was carefully observed by the use of conventional Giemsa staining, silver staining of nucleolar organizing regions (Ag-NORs), chromomycin A_3 (CMA_3) differential staining and fluorescence in situ hybridization (FISH) using a 5.8S + 28S rDNA probe (Li et al. 2010, 2011, 2013), in order to infer reproductive performance of triploids from the reciprocal crosses between wild-type diploid and natural tetraploid loaches.

Materials and methods

Natural tetraploid loaches (4n = 100) were collected in Chibi, Hubei Province, China. Diploid loaches (2n =50) were collected in the fish market, Dalian, Liaoning Province, China. Ploidy status of each sample was determined by erythrocyte measurements and cellular DNA content flow cytometry (Li et al. 2008), in advance. All the samples were kept in the aquarium of the Dalian Ocean University (22 ± 1°C). In 2010, crosses diploid female × tetraploid male (2n x 4n) and tetraploid female x diploid male (4n x 2n) were performed. Fertilized eggs from each cross and larvae were incubated at a temperature of 25 ± 1°C. Water was changed daily, air was also provided and died larvae were removed.

In early summer spawning season of 2012, cytogenetic analyses of oocyte germinal vesicles (GVs) were made on four females from 2n x 4n crosses and five females from 4n x 2n crosses following the procedure of Itono et al. (2006) and Li et al. (2011). Briefly, fragments including full grown immature oocytes with sizes similar to mature eggs were removed from the ovaries and incubated in goldfish saline containing 17α-20β dihidroxy-4-pregnene-3-one (Sigma) at room temperature (Zhang et al. 1998). During germinal vesicle migration (GVM) but before germinal vesicle break down (GVBD), oocytes were fixed with Carnoy's fixative and GVs were isolated (Itono et al. 2006; Li et al. 2011). GVs were air-dried on a clean slide and then were observed after DAPI (4', 6-diamidino-2-phenylindole, Sigma) staining under a fluorescence microscope (Itono et al.

2006; Li et al. 2011).

In 2012, five males from 2n x 4n and five from 4n x 2n were injected twice with phytohemagglutinin-A as described in Li et al. (2011). The testes were removed and treated with a hypotonic solution (0.8% tri-sodium citrate). Then, the testes were fixed with Carnoy's fixative. A cell suspension was made from fixed testes of each male and then one droplet was pipetted on a slide glass. The slides were air-dried and stained with Giemsa (Merck) diluted with phosphate buffer.

Detection of homologous chromosomes bearing nuclear organizing regions (NORs) was made by silver nitrate staining (Ag-NOR) according to the method described in Li et al. (2013). The fluorochrome CMA_3 (chromomycin A_3, Wako) /DA (distamycin A, Sigma) staining was also performed to detect NORs according to Li et al. (2010, 2011, 2013). FISH with human 5.8S + 28S rDNA as a probe was also conducted to detect NORs as described in Li et al. (2010, 2011, 2013). Signals were detected under a Leica DM2000, and the image was captured with DF 450C CCD camera and processed with the software package Adobe Photoshop (ver. 7.0).

Results

In four triploid females developed from the crosses 2n x 4n, 15 out of 51 GVs gave a configuration including 25 bivalents (II) and 25 univalents (I) (Fig. 1a) and 16 out of 51 GVs showed another configuration including 24II, 24I and 1 trivalent (III) (Figs. 1b, 2, Table S1). Typical trivalent chromosomes often exhibited long rod-like shape and triangular or ring-like configuration (Fig. 1bc). Other triploid GVs exhibited 23I+23II+2III, 22I+22II+3III and 21I+21II+4III. Near triploid cells with extra chromosome numbers (CN) of 77-82 and those with insufficient CN of 67-74 also appeared (Fig. 2). In five 4n x 2n triploid females, 16 out of 25 triploid GVs showed clear mode with 25I and 25II (Fig. 2, Table S1). Other triploid cells had 24I+24II+1III and 23I+23II+2III. A few GVs with CN of 64-71 and those with CN of 81 and 90 also occurred.

In five triploid males from 2n x 4n cross, 48 out of 163 spermatocytes gave the mode with 25I + 25II (Fig. 1d), but other triploid meiotic cells includes trivalents with long rod-like shape (Fig. 1ef): 18 cells with 24I + 24II + 1III, 11 cells with 23I + 23II + 2III, 4 cells with 22I + 22II + 3III, one cell with 21I + 21 II + 4III, and one cell with 20I + 20II+ 5III (Fig. 3, Table S2). In spermatocytes, relatively large numbers of cells with polyploid range with CN of 81 to 157 appeared together with cells with near triploid CN of 70-74 and 76-80 (Fig. 3, Table S2).

Similar configurations were observed in spermatocytes of five triploid males from 4n x 2n cross (Fig. 3, Table S3). The modal triploid cells gave 25I + 25II configuration (53 out of 264 cells). Other triploid spermatocytes were 24I + 24II + 1III (6 cells), 23I + 23II +2III (6 cells) and 23I + 26II (2 cells). In testes of triploid (4n x 2n) males, cells with tetraploid range CN (84-116), pentaploid range CN (127-133) and hexaploid range CN (152) appeared together with near triploid CN (70-74,

76-82) with different meiotic configurations (Fig. 3, Table S3).

Triploid spermatocytes were screened by silver staining, differential CMA_3 staining or FISH profiles to detect the presence of three homologous chromosomes bearing NORs in meiotic spread, because diploid and tetraploid loaches have two and four homologous chromosomes with NORs, respectively (Li et al. 2010, 2011). Three Ag-NOR, CMA_3-positeive sites or FISH signals were detected in 34 out of 91 meiotic cells examined. Other 57 cells demonstrated one or two NORs or rDNA loci. Among 34 putative triploid spermatocytes, 19 cells demonstrated two signals in one bivalent with tail-to-tail association of long arm of two homologous chromosomes and one signal in one univalent were dominant (Table 1, Fig. 4ab). Rarely, three cells showed one Ag-NOR staining or one CMA_3-positive site at just intermediate position of one bivalent and one signal in one univalent (Table 1, Fig. 4c). The signal in bivalent presumably included two NORs or rDNA loci formed by head-to-head association of short arm of two homologous chromosomes (Fig. 4c). On the contrary, in other 12 meiotic cells, three independent chromosomes (presumably univalents) gave Ag-NOR, CMA_3-positive sites or FISH signals of rDNA loci (Table 1, Fig. 4d). In these cells, two homologous chromosomes could not be paired, indicating the failure of synapsis in these triploid spermatocytes.

Discussion

Both in triploid GVs and spermatocytes (3n = 75), the most frequent meiotic configuration comprised equal number of 25 bivalents and 25 univalents (25II + 25I), which was explained by the pairing between two homologous chromosomes from 50 chromosomes to form 25II and the failure of synapsis of other 25 chromosomes to form 25I. Differential staining and FISH analyses also supported that two homologous chromosomes paired to one bivalents and additional one chromosome became univalent in typical triploid spermatocytes. From such GVs and spermatocytes, gametes with the theoretical mode at 1.5n (chromosome number 37 or 38) can be generated by equal segregation of 25 bivalents and random segregation of 25 univalents. Thus, aneuploid progeny with the theoretical chromosome mode at 2n=62 or 63 will be produced when the triploid fish with aneuploid gametes is back-crossed to wild-type diploid (2n = 50). The same configuration with 25II + 25I was previously reported in triploid loaches produced by artificial inhibition of the second polar body after fertilization of wild-type diploids (Zhang and Arai 1999) and those arisen from the cross between gynogenetic diploid female induced from diploid eggs of spontaneously occurred tetraploid female and natural tetraploid male (Zhang et al. 2002). However, homologous chromosomes were not always synapsed in the course of meiosis and each of three homologous chromosomes behaved as univalent. These results suggest that miss-pairing might occur between homologous chromosomes in triploid meiotic cells, which results in the formation of unusual gametes by the involvement of many univalent in meiotic divisions.

In this study, presence of one, two, three or several trivalents (III) was observed in significant number of triploid meiotic cells. Trivalent chromosomes were detected by unusually long-rod like shape and triangular or ring-like shape probably due to the synapses among three homologous chromosomes. Thus, the appearance of trivalents may relate to the presence of three sets of homologous chromosomes in triploid animals. No such trivalents have been seen in the meiosis of wild-type diploid loaches (Zhang et al., 1998; Yoshikawa et al. 2009; Li et al.2011). However, all the triploids did not exhibit the configuration including trivalents: artificially induced triploids and previous triploids from diploid x tetraploid hybridization, they did not exhibit trivalents in the process of meiosis (Zhang and Arai 1999; Zhang et al. 2002).

As multivalent configuration, a few or several quadrivalents (IV) were detected together with many bivalents in meiotic cells of natural tetraploid loaches with four sets of chromosomes, but meiotic cells only with 25IV (100 chromosomes) have never appeared in tetraploid loaches (Li et al. 2011). These previous results suggest that the formation of bivalents should take precedence of all other configurations like quadrivalents and trivalents even in polyploid germ cells. Relatively low frequencies of quadrivalents and predominance of bivalents were reported in meiosis of salmonid species which were considered as evolutionary tetraploidy (Nygren et al. 1972; Davidson et al. 1973; Lee and Wright 1981).

Presence of trivalents presumably makes the results of chromosome segregation more complicated, because each of two homologous chromosomes will be distributed to a different gamete in the case of bivalent, but distribution of homologous chromosome is presumably irregular in a trivalent: two chromosomes to one gamete and one chromosome to another or three chromosomes to one gamete and no chromosome to another.

In addition, many near triploid cells with extra and insufficient chromosome numbers were detected together with unusual polyploid (tetraploid to hexaploid range) cells. The mechanism responsible for the occurrence of aneuploid cells is unknown at present. Here, many polyploid cells were detected in spermatocytes of natural tetraploids. Such polyploid cells were considered to have resulted from spontaneous endomitosis (endoreduplication) (Li et al. 2011). Similar polyploid cells were also noticed in spermatocytes of salmonids (Nygren et al. 1972). Such unusual polyploids presumably due to endomitosis have been observed in the unreduced egg production process of triploid (Zhang et al. 1998) and clonal diploid (Itono et al. 2006), and in the unreduced sperm formation of sex-reversed clonal diploid (Yoshikawa et al. 2009) in the loach. Other possible mechanism responsible for the presence of polyploid spermatocytes may be the meiotic arrest after chromosome replication, followed by formation of spermatozoa or speramatozoon-like cells with unusual proceeding of spermiogenesis. Such kind of unusual spermatozoa or spermatozoon-like cells with duplicated DNA contents were reported in medaka hybrid male (Shimizu et al. 1997) and hyper-triploid loach (Zhao et al. 2014).

It was reported that artificially induced triploid loach males produced aneuploid spermatozoa presumably due to the meiotic configuration with 25II + 25I, but induced triploid females could not form fertile eggs (Zhang and Arai 1999). However, it was reported that induced triploid koi (ornamental carp) females spawned fertile eggs and then produced viable aneuploid (mean value 2.6n, range 2.3n to 2.9n) progeny after crossing with diploid males (Gomelsky et al. 2015). Unexpected well-developed ovaries were also reported in common carp (Wu 1990; Cherfas et al. 1994). These previous studies suggest possible gonadal development and subsequent production of fertile gametes (eggs and sperm) even in triploid females from crosses between diploid and tetraploid Chinese loach, because meiotic configuration of 25II + 25I giving rise to the theoretical mode of 1.5n gametes, which was reported in fertile induced triploid males (Zhang and Arai 1999), was predominant in both oocytes and spermatocytes in the present triploid loaches. Thus, further experimental studies are required to examine reproductive capacity of gametes generated by these triploid loach.

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Legends of figures

Fig. 1. Meiotic configurations in oocyte germinal vesicles of triploid females (a-c) and those in spermatocytes of triploid males (d-f) in loach *Misgurnus anguillicaudatus*. DAPI-stained germinal vesicles comprising 25 univalents (I) and 25 bivalents (II) (a), 24I + 24II + one trivalent (III) (b), and

23I + 23II + 2 III (c) prepared from triploid females. Conventional Giemsa-stained spermatocytes comprising 25 I and 25 II (d), 24I + 24II + 1 III (e), and 23I + 23II + 2 III (f). White, yellow and red arrows denote univalents, bivalents and trivalents, respectively. Scales denote 10μm.

Fig. 2. Number of univalents (I), bivalents (II) and trivalents (III), and chromosome numbers in 51 oocyte germinal vesicles (GVs) from four triploid (diploid female x tetraploid male) females (top) and those in 25 GVs from five triploid (tetraploid female x diploid male) females (bottom) in loach *Misgurnus anguillicaudatus*.

Fig. 3. Number of univalents (I), bivalents (II) and trivalents (III), and chromosome numbers in 163 spermatocytes from five triploid (diploid female x tetraploid male) males (top) and those in 264 spermatocytes from five triploid (tetraploid female x diploid male) males (bottom) in loach *Misgurnus anguillicaudatus*.

Fig. 4. Triploid spermatocytes showing one Ag-NOR site in one univalent and two Ag-NOR sites in one bivalent with tail-to-tail association of homologous chromosomes (a), one CMA_3-positive site in one univalent and two CMA_3-positive sites in one bivalent with tail-to-tail association of homologous chromosomes (b), one CMA_3-positive site in one univalent and one signal fused by two CMA_3-positive sites in one bivalent with head-to-head association of homologous chromosomes (c), and one FISH signal probed by 5.8S+28S rDNA sequences in each of three univalents (d). NORs or rDNA loci shown by signals are indicated by arrows. NOR shown in the site just intermediate of one bivalent formed by head-to-head association of two homologous chromosomes is indicated by bold arrow. Scales denote 10μm.

Table 1. Meiotic configurations of homologous chromosomes bearing nucleolar organizing regions (NORs) identified by silver staiing, CMA_3–positive site and FISH probed by rDNA sequence in spermatocytes of triploid males

	2 in 1II (tail–to–tail) 1 in 1 I	2 in 1 II (head–to–head) 1 in 1I	3 in 3I	Sum
Ag–NOR	6	2	5	13
CMA_3–positive	12	1	3	16
FISH	1	0	4	5
Sum	19	3	12	34

Table S1. Number of univalents, bivalents and trivalents in the germinal vesicle of oocytes from triploid loaches from reciprocal crosses between diploid and tetraploid (2n x 4n, 4n x 2n). I, II and III mean univalent,bivalent, and trivalent, respectively. Number in parenthesis denotes chromosome number of meiotic cell.

Sample	Female no.	24I+20II (64)	25I+21II (67)	26I+22II (70)	21I+25II (71)	23I+23II+1III (72)	24I+25II (74)	21I+21II+4III (75)	22I+22II+3III (75)	23I+23II+2III (75)	24I+24II+1III (75)	25I+25II (75)	23I+27II (77)	27I+25II (77)	25I+25II+2III (81)	23I+28II+1III (82)	26I+29II+2III (90)	Total
2n × 4n	1	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	4
	2	0	0	0	0	1	0	0	0	3	7	4	0	0	0	0	0	15
	3	0	1	0	0	0	1	1	1	3	4	6	0	0	0	1	0	18
	4	0	0	0	0	0	0	0	2	3	3	3	2	1	0	0	0	14
	Total	0	1	0	0	1	1	1	3	9	16	15	2	1	0	1	0	51
4n × 2n	1	0	0	1	0	0	0	0	0	0	0	3	0	0	0	0	0	4
	2	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	4
	3	0	0	0	0	0	0	0	0	1	0	7	0	0	0	0	0	8
	4	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	4
	5	1	0	0	1	0	0	0	0	0	2	1	0	0	0	0	0	5
	Total	1	0	1	1	0	0	0	0	1	3	16	0	0	1	0	1	25

Table S2. Number of univerlents, bivalents and trivalents in spermatocytes from triploid loach males from the cross between diploid female and tetraploid male (2n x 4n). I, II and III indicate univalent, bivalent, and trivalent chromosomes. Number in parenthesis means chromosome number of spermatocyte.

Male (2n x 4n) no.	Hyper-diploid to hypo-triploid cells											
	16I+19II (54)	15I+20II (55)	13I+20II+3III (62)	18I+22II (62)	14I+23II+1III (63)	23I+20II (63)	12I+20II+5III (67)	20I+22II+1III (67)	15I+26II (67)	17I+25II (67)	21I+22II+1III (68)	22I+23II (68)
1#	0	0	1	0	0	0	0	1	0	0	1	0
2#	0	1	0	0	1	0	1	0	1	1	0	0
3#	1	0	0	1	0	1	0	0	0	0	0	2
4#	0	0	0	0	0	0	0	0	0	0	0	1
5#	0	0	0	0	0	0	0	0	0	0	0	0
Total	1	1	1	1	1	1	1	1	1	1	1	3

Male (2n x 4n) no.	Hyper-diploid to hypo-triploid cells											
	19I+25II (69)	23I+23II (69)	24I+23II (70)	18I+22II+3III (71)	25I+23II (71)	21I+24II+1III (72)	23I+23II+1III (72)	24I+24II (72)	20I+22II+3III (73)	23I+25II (73)	24I+22II+2III (74)	24I+25II (74)
1#	0	0	0	0	0	1	1	0	1	0	1	0
2#	1	0	0	1	1	0	0	1	0	0	0	1
3#	0	0	0	0	0	0	1	0	0	0	0	0
4#	0	1	1	0	1	0	0	0	0	0	0	0
5#	0	0	0	0	0	0	0	0	0	1	0	0
Total	1	1	1	1	2	1	2	1	1	1	1	1

Male (2n x 4n) no.	Triploid cells						Hyper-triploid to hypo-tetraploid cells				
	20I+20II+5III (75)	21I+21II+4III (75)	22I+22II+3III (75)	23I+23II+2III (75)	24I+24II+1III (75)	25I+25II (75)	26I+25II (76)	24I+22II+3III (77)	25I+26II (77)	27I+25II (77)	26I+26II (78)
1#	0	1	3	5	1	4	0	0	1	0	0
2#	1	0	0	5	1	7	1	1	2	0	1
3#	0	0	0	0	4	16	1	0	1	1	0
4#	0	0	1	1	7	8	0	0	1	0	0
5#	0	0	0	0	5	13	0	0	1	0	0
Total	1	1	4	11	18	48	2	1	6	1	1

Male (2n x 4n) no.	Hyper-triploid to hypo-tetraploid cells										
	24I+26II+1III (79)	29I+22II+2III (79)	25I+27II (79)	29I+25II (79)	24I+25II+2III (80)	24I+28II (80)	26I+27II (80)	28I+26II (80)	25I+28II (81)	29I+26II (81)	30I+26II (82)	29I+27II (83)
1#	0	1	0	0	0	0	0	0	0	0	0	0
2#	1	0	0	0	1	0	1	0	1	0	0	0
3#	0	0	1	1	0	1	0	0	0	0	0	0
4#	0	0	1	0	0	0	0	1	2	1	0	0

5#	0	0	2	0	0	0	0	1	1	0	1	1
Total	1	1	4	1	1	1	1	2	4	1	1	1

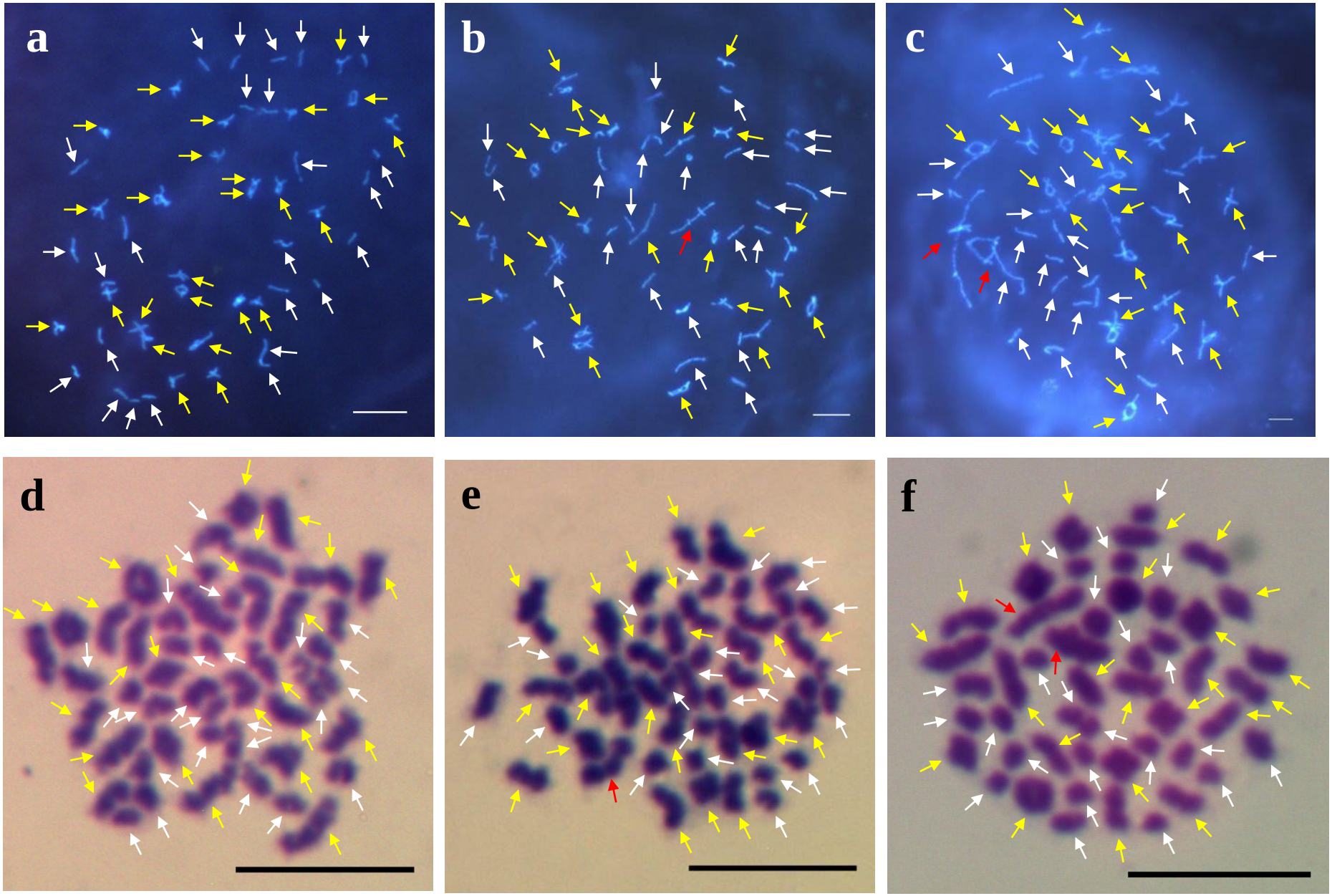
Male (2n x 4n) no.	Hyper-triploid l to hypo-tetraploid cells											
	31I+26II (83)	28I+25II+2III (84)	28I+28II (84)	27I+29II (85)	29I+28II (85)	26I+27II+2III (86)	31I+26II+1III (86)	24I+31II (86)	27I+30II (87)	26I+31II (88)	31I+29II (89)	25I+33II (91)
1#	0	0	0	0	0	0	0	0	0	0	0	1
2#	0	1	0	0	0	1	0	0	0	0	0	0
3#	0	0	0	0	0	0	0	1	0	0	0	0
4#	0	0	1	0	0	0	0	0	0	0	1	0
5#	1	0	0	1	1	0	1	0	1	1	0	0
Total	1	1	1	1	1	1	1	1	1	1	1	1

Male (2n x 4n) no.	Hyper-triploid to hypo-tetraploid cells	Hyper-tetraploid to hyper-hexaploid cells								Total n
	37I+28II (93)	21I+39II+1III (102)	22I+41II (104)	41I+32II (105)	36I+36II (108)	28I+41II (110)	48I+35II (118)	32I+49II (130)	41I+53II (147)	45I+56II (157)	
1#	1	1	1	0	1	0	1	1	0	0	30
2#	0	0	0	0	0	1	0	0	1	1	38
3#	0	0	0	0	0	0	0	0	0	0	33
4#	0	0	0	1	0	0	0	0	0	0	30
5#	0	0	0	0	0	0	0	0	0	0	32
Total	1	1	1	1	1	1	1	1	1	1	163

Table S3. Number of univerlents, bivalents and trivalents in spermatocytes from triploid loach males from the cross between tetraploid female and diploid male (4n x 2n)..
I, II and III indicate univalent, bivalent and trivalent chromosomes, respectively. Number in parenthesis means chromosome number of spermatocyte .

male (4n x 2n) no.	Diploid cell	Hyper-diploid to hyper-triploid cells												
	20I+12II+2III	25I+13II	14I+19II	18I+18II	14I+20II	18I+15II+2III	13I+21II	14I+21II	20I+18II	19I+19II	18I+20II	20I+19II	17I+21II	19I+20II
	(50)	(51)	(52)	(54)	(54)	(54)	(55)	(56)	(56)	(57)	(58)	(58)	(59)	(59)
1#	0	0	0	0	0	0	0	0	0	0	0	0	1	0
2#	1	1	1	1	0	0	1	1	1	0	1	1	1	2
3#	0	0	0	0	1	0	0	0	0	1	0	0	1	0
4#	0	0	0	0	0	1	0	0	0	0	0	0	0	0
5#	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	1	1	1	1	1	1	1	1	1	1	1	1	3	2
male (4n x 2n) no.	Hyper-diploid to hyper-triploid cells													
	23I+18II	16I+21II+1III	11I+25II	14I+24II	19I+22II	21I+21II	17I+22II+1III	14I+25II	16I+24II	18I+23II	26I+19II	23I+18II+2III	19I+23II	21I+22II
	(59)	(61)	(61)	(62)	(63)	(63)	(64)	(64)	(64)	(64)	(64)	(65)	(65)	(65)
1#	0	0	0	0	1	0	0	0	0	0	0	0	1	0
2#	1	1	1	0	0	0	0	1	1	1	0	1	0	1
3#	0	0	0	1	0	1	1	0	0	0	1	0	0	0
4#	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5#	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	1	1	1	1	1	1	1	1	1	1	1	1	1	1
male (4n x 2n) no.	Hyper-diploid to hypo-triploid cells													
	23I+21II	24I+21II	26I+20II	15I+23II+2III	20I+22II+1III	19I+24II	21I+23II	18I+25II	20I+24II	22I+23II	19I+25II	21I+24II	23I+23II	25I+22II
	(65)	(66)	(66)	(67)	(67)	(67)	(67)	(68)	(68)	(68)	(69)	(69)	(69)	(69)
1#	0	0	1	1	1	0	0	0	0	0	0	0	1	0
2#	1	0	0	0	0	0	2	1	2	1	0	1	0	1
3#	0	0	0	0	0	1	0	0	2	1	0	0	0	0
4#	0	1	0	0	0	0	0	0	0	0	1	0	0	0
5#	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	1	1	1	1	1	1	2	1	4	2	1	1	1	1
male (4n x 2n) no.	Hyper-diploid to hypo-triploid cells													Triploid cells
	22I+24II	19I+26II	21I+25II	23I+23II+1III	20I+26II	22I+25II	24I+24II	20I+25II+1III	22I+24II+1III	23I+25II	25I+24II	24I+25II		23I+23II+2III
	(70)	(71)	(71)	(72)	(72)	(72)	(72)	(73)	(73)	(73)	(73)	(74)		(75)
1#	1	0	0	0	0	1	0	1	0	1	0	1		1
2#	1	1	2	0	2	1	0	0	0	0	0	3		1
3#	1	0	0	1	0	1	2	0	1	0	0	0		0
4#	0	0	0	0	0	0	0	0	0	0	0	2		1
5#	0	0	0	0	0	0	1	0	0	1	1	1		3
Total	3	1	2	1	2	3	3	1	1	2	1	7		6

male (4n x 2n) no.	Triploid cells				Hyper-triploid to hypo-tetraploid cells									
	24I+24II+1III	25I+25II	23I+26II		25I+24II+1III	26I+25II	25I+26II	23I+23II+3III	24I+27II	26I+26II	28I+25II	24I+23II+3III	25I+27II	27I+26II
	(75)	(75)	(75)		(76)	(76)	(77)	(78)	(78)	(78)	(78)	(79)	(79)	(79)
1#	1	8	1		0	0	1	0	0	0	0	0	0	0
2#	2	12	0		0	1	1	0	0	0	0	0	0	0
3#	0	8	0		0	0	1	0	0	0	0	1	1	0
4#	2	17	0		0	4	6	1	1	6	0	0	5	1
5#	1	8	1		1	1	2	0	0	5	1	0	6	3
Total	6	53	2		1	6	11	1	1	11	1	1	12	4
male (4n x 2n) no.	Hyper-triploid to hypo-tetraploid cells													
	25I+26II+1III	26I+27II	28I+26II	32I+24II	25I+25II+2III	26I+26II+1III	27I+24II+2III	25I+28II	27I+27II	24I+29II	25I+27II+1III	26I+28II	28I+27II	25I+29II
	(80)	(80)	(80)	(80)	(81)	(81)	(81)	(81)	(81)	(82)	(82)	(82)	(82)	(83)
1#	0	1	0	0	0	0	0	1	0	0	1	0	0	0
2#	0	0	0	1	0	0	0	1	0	0	0	0	0	0
3#	0	0	0	0	0	0	0	0	1	0	0	1	0	0
4#	1	3	1	0	0	2	0	3	0	3	0	4	1	3
5#	0	3	0	0	1	0	1	3	1	0	0	2	2	0
Total	1	7	1	1	1	2	1	8	2	3	1	7	3	3
male (4n x 2n) no.	Hyper-triploid to hypo-tetraploid cell													
	27I+28II	31I+26II	25I+28II+1III	26I+29II	28I+28II	30I+27II	25I+30II	27I+29II	35I+25II	26I+30II	28I+28II+1III	29I+29II	33I+27II	26I+31II
	(83)	(83)	(84)	(84)	(84)	(84)	(85)	(85)	(85)	(86)	(87)	(87)	(87)	(88)
1#	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2#	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3#	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4#	3	0	0	1	1	0	1	1	0	1	1	1	1	2
5#	1	1	1	1	0	1	0	0	1	0	0	0	0	0
Total	4	1	1	2	1	1	1	1	1	1	1	1	1	2
male (4n x 2n) no.	Hyper-triploid to hypo-tetraploid cells				Tetraploid cells		Hyper-tetraploid to hyper-hexaploid cells							Total n
	32I+28II	30I+31II	26I+36II		30I+32II+2III		30I+37II	32I+36II+1III	44I+36II	41I+43II	45I+41II	37I+48II	49I+50II+1III	
	(88)	(92)	(98)		(100)		(104)	(107)	(116)	(127)	(127)	(133)	(152)	
1#	0	0	1		0		0	0	0	1	1	0	0	30
2#	1	0	0		0		0	0	0	0	0	1	0	60
3#	0	0	0		0		1	1	0	0	0	0	1	33
4#	0	1	0		1		0	0	0	0	0	0	0	85
5#	0	0	0		0		0	0	1	0	0	0	0	56
Total	1	1	1		1		1	1	1	1	1	1	1	264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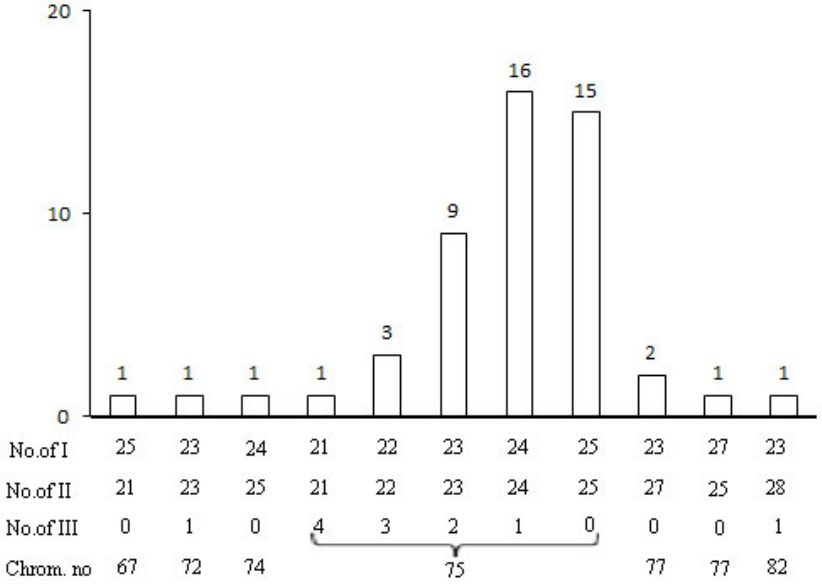
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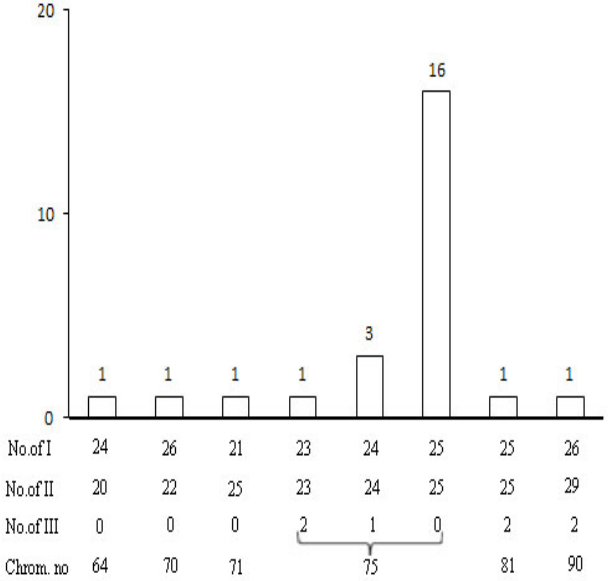
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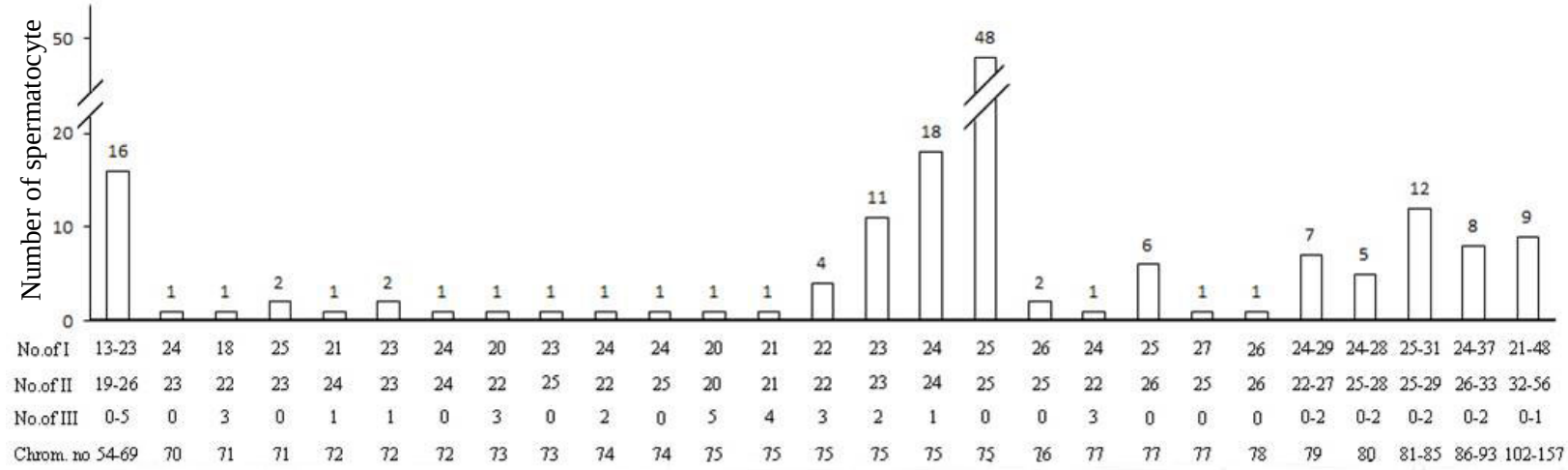
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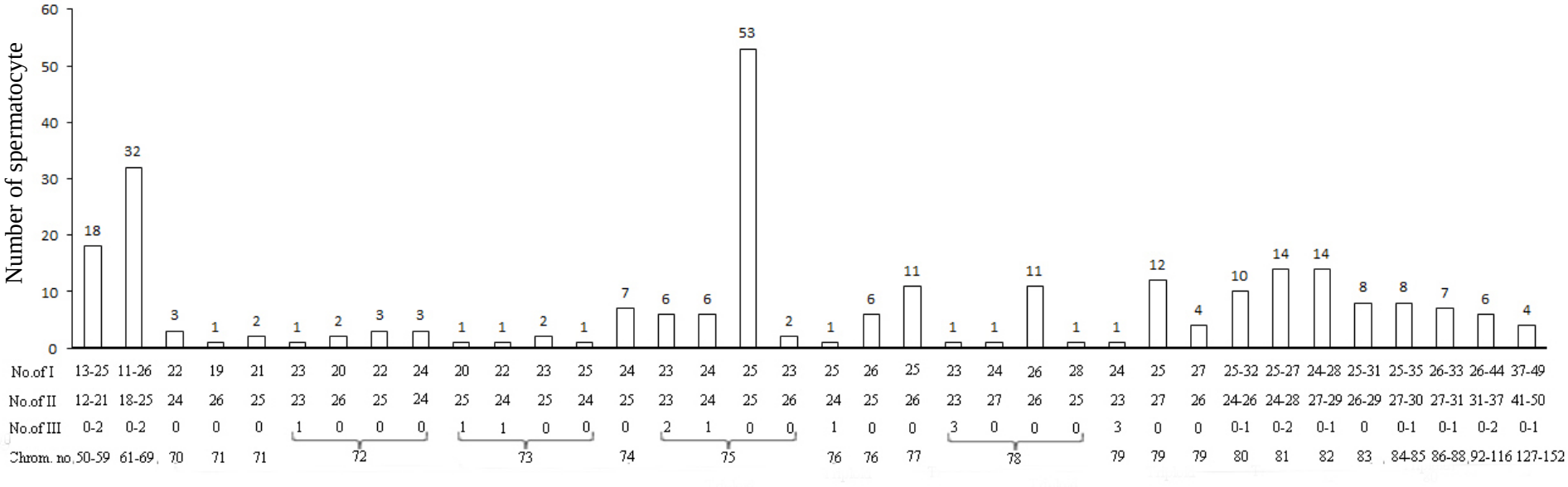
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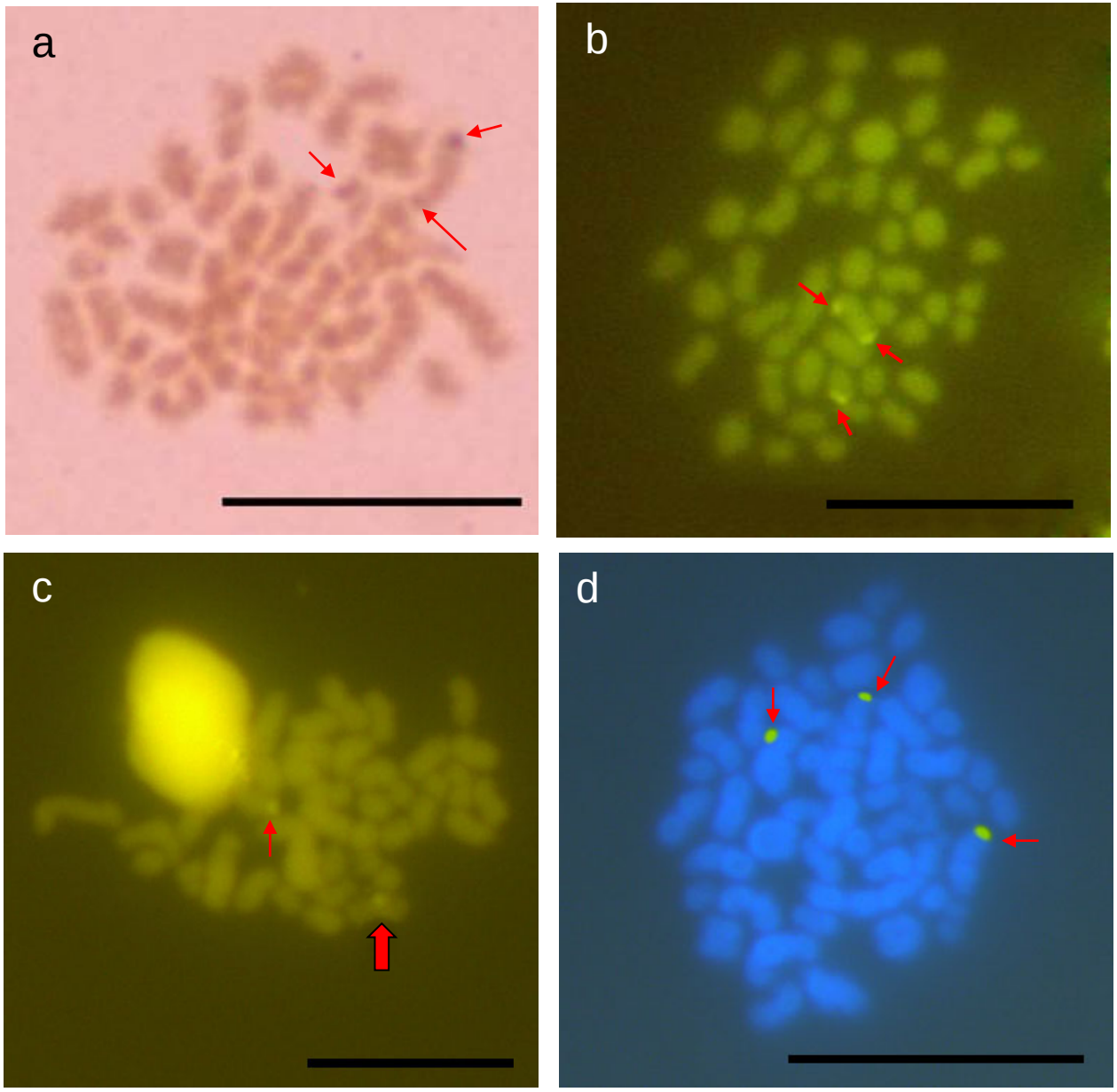
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