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37 Abstract

Triploid Chinese loach, Misgurnus anguillicaudatus, hybrids between tetraploids from 38 Hubei Province and diploids from Liaoning Province were mated with either diploid 39wild-type or triploid hybrids to analyze viability and ploidy of the resultant progenies. 40 41 Both triploid males and females generated fertile gametes, but progenies from the 42crosses using gametes of triploid hybrids did not survive beyond the larval stages. In crosses between wild-type diploid females and triploid hybrid males, embryos ranging 4344 from 2.2n to 2.6n were predominant with a mode of either 2.4n (chromosome numbers 59, 60, 61) or 2.5n (chromosome numbers 62, 63). Those from the crosses between 4546 triploid hybrid females and diploid males gave a modal ploidy level at approximately 472.5n in one case, but a shift to a higher ploidy level was observed in other embryos. In the progenies between triploid hybrid females and males, the ploidy level at 48approximately 3.0n (chromosome numbers 74, 75, 76) was most frequent. The 49cytogenetic results of the progenies suggest the production of aneuploid gametes with a 50modal ploidy level at approximately 1.5n in triploid hybrids. However, a shift to higher 51chromosome numbers in gametes was observed in certain cases, suggesting the 52involvement of mortality selection of gametes 53and/or zygotes with lower chromosome numbers. 5455Key-words: Bivalent · Gamete · Meiosis · Polyploid · Trivalent · Univalent 5657585960 61 62 63 64 Abbreviation Ag-NORs; silver staining nucleolus organizer regions 65

- $66 \qquad CMA_3; chromomycin A_{3,}$
- 67 CN; chromosome number,
- 68 DA; distamycin A,
- 69 DAPI; 4'6-diamidino-2-phenylindole
- 70 FISH; fluorescence in situ hybridization
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74 Introduction

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Meiotic chromosome configurations give insights into the pairing behavior of extra homologous chromosomes as well as the reproductive capacity of resultant gametes in polyploid animals. However, the relationship between meiotic configurations of chromosomes and gametogenesis has not well been clarified both in either natural or induced triploid teleosts.

In several auto- and allotriploid fishes, which were artificially induced by 81 82 inhibiting the second polar body release just after fertilization, gametes with a ploidy 83 level of approximately 1.5n have been reported (Allen et al. 1986; Benfey et al.1986; Ueda et al. 1987, 1991; Van Eenennaam et al. 1990; Zhang and Arai 1999; Gomelsky et 84 al. 2015). Among them, however, meiotic configurations have not been well 85 investigated except for induced triploid loach Misgurnus anguillicaudatus, in which 86 87 about 25 bivalents (IIs hereafter) and 25 univalent (Is hereafter) were observed (Zhang 88 and Arai 1999).

In China, bisexually reproducing diploid (2n = 50) - tetraploid (4n = 100) complex exists and thus triploid hybrids are easily produced by cross-breeding (Li et al. 2008, 2010, 2011, 2012, 2013). Since we observed that meiotic cells most frequently exhibited 25IIs and 25Is in triploid hybrids, we predicted the formation of gametes with a mode at 1.5n (37 or 38) chromosomes as a result of equal segregation of 25IIs and random segregation of 25Is (Li et al. 2015).

In the present study, we examined the chromosomes (ploidy level) and viability of progenies from inter-crosses between (1) diploid females and triploid males, (2) triploid females and diploid males, and (3) triploid females and triploid males, to elucidate the relationship between the complicated meiotic configurations and gametogenesis in triploid Chinese loach hybrids.

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101 Materials and methods

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Triploid hybrids were reciprocally produced between tetraploids from Hubei Province, China and diploids from Liaoning Province, as previously described in Li et al. (2012, 2013, 2015). Using two females and two males selected from each triploid (tetraploid females × diploid males) hybrid, diploid, and tetraploid brood-stock, we produced the two sets (no. 1 and 2) of following progenies in 2012: 2n (hereafter female first) × 2n (male), $2n \times 4n$, $4n \times 2n$, $4n \times 4n$, $2n \times 3n$, $3n \times 2n$, and $3n \times 3n$. Using two females and two males selected from each triploid (diploid females x tetraploid males) hybrid and diploid brood-stock, we produced two sets (no. 3 and 4) of $2n \times 2n$, $2n \times 3n$, $3n \times 2n$ and $3n \times 3n$ progenies in 2014.

112 At approximately 0.5h post fertilization, the diameter of fertilized eggs (n = 30) 113 from the 2n × 2n, 3n × 2n and 3n × 3n crosses was measured using a digital caliper on 114 photographed images in accordance with the procedures described in Li et al. (2012) 115 and then compared statistically (*t*-test).

Testicular cells were examined by flow-cytometry in 15 approximately 4-year-old triploid (tetraploid females x diploid males) hybrid males according to the methods described in Oshima et al. (2005) and Yoshikawa et al. (2007). As a control, one diploid and one tetraploid male of the same age were randomly taken to sample testicular cells.

Survival parameters were estimated as described in Li et al. (2013). Fertilization rate was calculated as the proportion of cleaved eggs relative to the initial number of eggs. Hatching rate was calculated as the proportion of hatched eggs relative to the initial number of eggs. Normal rate was calculated as the proportion of normal larvae relative to the number of hatched larvae. Survival rate at 7 days after hatching was calculated as the proportion of surviving larvae relative to the number of hatched larvae. Rearing water was changed daily after the larvae were first fed *Artemia*.

127Chromosome preparation was individually conducted on each optic vesicle stage 128embryo after manually removing the yolk in physiological saline under a stereoscopic 129microscope. Chromosome preparation procedures were the same as those described in 130 Li et al. (2013). Chromosome counting was made on conventional Giemsa-stained 131metaphases on a slide directly under a microscope and/or on their photographed images. 132In the progenies, the modal chromosome number was determined in each embryo. 133Karyotyping was conducted according to Levan et al. (1964). Differential staining with 134CMA₃/DA/DAPI (Schweizer 1976; Schweizer et al. 1978) and the Ag-NOR method (Howell and Black 1980) was applied to the chromosome slides in accordance with Li 135136et al. (2010). FISH using human 5.8S + 28S rDNA sequences as a probe was applied 137according to Li et al. (2010).

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139 **Results**

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141 Survival potential of triploid hybrid progenies

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Parameters for zygote survival capacity are shown in Table 1. Fertilization rates were >
80 % in all of the crosses using the eggs and sperm of wild-type diploid and natural

tetraploid loaches. In contrast, reduced fertilization rates were always observed in 145146crosses using triploid hybrid eggs and/or sperm. Hatching rates were > 80% in the $2n \times$ 2n, $2n \times 4n$, $4n \times 2n$ and $4n \times 4n$ crosses, but approximately 14 to 67% hatching rates 147were recorded in $2n \times 3n$, $3n \times 2n$ and $3n \times 3n$. Almost all (> 92%) of the hatched larvae 148 149were normal in the $2n \times 2n$, $2n \times 4n$, $4n \times 2n$ and $4n \times 4n$ crosses, but reduced normal 150rates (41 to 55%) were recorded in most of the crosses using triploid hybrid eggs and/or sperm except for $3n \times 2n$ crosses in 2014 (81 to 83%). Survival rates of 7-day-old larvae 151152after hatching were relatively high in the $2n \times 2n$, $2n \times 4n$, $4n \times 2n$ and $4n \times 4n$ crosses 153(about 84 to 98%), while reduced rates were recorded in the triploid hybrid progenies (3 154to 33%). The larvae from the $2n \times 2n$, $2n \times 4n$, $4n \times 2n$ and $4n \times 4n$ crosses survived 155beyond the beginning of feeding and most exhibited further growth. However, all the 156survivors from the $3n \times 2n$, $2n \times 3n$ and $3n \times 3n$ crosses exhibited external malformations including microcephaly, microphthalmia, edema, dwarfism, curved trunk 157and tail, and so on (Fig. 1) and no larvae survived for more than 10 days after hatching. 158

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- 160 Triploid hybrid egg sizes
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162 The mean egg diameters were 0.83 ± 0.02 mm (SD) in $2n \times 2n-1$, 0.94 ± 0.04 mm in 3n 163 \times 2n-1 and 0.94 ± 0.03 mm in $3n \times 3n-1$ crosses (Fig. S1). Both $3n \times 2n-1$ and $3n \times$ 164 3n-1 had significantly larger egg diameters than the control $2n \times 2n-1$ (p < 0.05). Thus, 165 eggs laid by triploid females were larger than those laid by wild-type diploids.

- 166
- 167 Triploid hybrid chromosomes
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169 Here, we confirmed the genomic constitution of triploid hybrids based on chromosome 170 numbers, karyotypes, and NOR numbers detected by differential staining and FISH. In 17120 embryos from each of the $2n \times 2n$, $2n \times 4n$, $4n \times 2n$ and $4n \times 4n$ crosses, 172chromosomes were individually counted (Table S1). Out of 543 metaphases from $2n \times$ 1732n embryos (n=20), 266 cells had 50 chromosomes. Out of 594 metaphases from $2n \times n$ 1744n embryos (n=20), 272 cells had 75 chromosomes. Out of 529 metaphases from $4n \times 10^{-10}$ 1752n embryos (n=20), 267 cells had 75 chromosomes. Out of 463 metaphases from $4n \times$ 176 4n embryos (n=20), 249 cells had 100 chromosomes. Thus, 2n x 2n, 2n x 4n, 4n x 2n and $4n \ge 4n$ crosses generated diploid (2n = 50), triploid (3n = 75), triploid (3n = 75) 177and tetraploid (4n = 100) progenies, respectively. 178

179 Based on a good quality conventional Giemsa-stained metaphase from triploid 180 hybrids (Fig. 2a), a karyotype comprising five metacentric (M), two submetacentric

(SM), and 18 telocentric (T) triplet chromosomes clearly indicated triploidy with three 181 182sets of homologous chromosomes (Fig. 2b). In static triploid hybrid somatic cells, maximum of three Ag-NORs were detected (Fig. 2c). In a triploid metaphase, Ag-NORs 183184 were detected on the short arms of the three largest M chromosomes (Fig. 2d, e). CMA₃ 185positive sites were detected on the short arms of the three largest M chromosomes (Fig. 186 2f, g). The rDNA loci FISH signals were also detected on the short arms of the three largest M chromosomes (Fig. 2h, i). All of these results from differential staining and 187 188 FISH, indicated that triploid hybrids had three sets of homologous chromosomes, one 189 set from diploid wild-type and two sets from tetraploid loaches.

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191 Flow cytometry of testicular cells

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193Flow cytometry was carried out on the testicular tissues taken from 15 triploid hybrid 194 males (tetraploid \times diploid). When control diploid and tetraploid males produced 195haploid (1C DNA content) and diploid (2C DNA content) sperm, respectively (Fig. 3a, 196 b), 12 out of 15 triploid males produced a major peak at approximately 1.5C DNA 197 content, with minor peaks at 3C and 6C DNA content (Fig. 3c). These histograms 198showed major production of 1.5n spermatozoa. While one of the 15 triploids produced 199 two major peaks at 3C and 6C DNA content and no peak corresponding to spermatozoa 200was detected (Fig. 3d). Two out of 15 triploids produced a major peak at 3C DNA 201content and minor peaks at approximately 1.6C and 6C DNA content (Fig. 3e). A minor 202peak at approximately 1.6C DNA content seemed to be cell populations of either 203spermatozoa or spermatids.

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Ploidy level and chromosomes of triploid (tetraploid female × diploid male) hybrid
 progenies

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Chromosomes were individually counted in progenies from the $2n \times 3n-1$ (Table S2), $3n \times 2n-1$ (Table S3) and $3n \times 3n-1$ (Table S4) crosses (Fig. S2a–c). Embryos with a mode at the ploidy level 2.4n (CN 59, 60, 61), 3.0n (CN 74, 75, 76) and 2.8n (CN 69, 70, 71) occurred most frequently in $2n \times 3n-1$, $3n \times 2n-1$, and $3n \times 3n-1$, respectively (Table S2–4). The mean chromosome numbers calculated from the cells of $2n \times 3n-1$, $3n \times$ 2n-1, and $3n \times 3n-1$ progenies were 58.76 (2.4n), 67.18 (2.7n) and 73.49 (2.9n), respectively (Fig. 4).

215In the 2n × 3n-1 cross (Fig. 4a), 2.2n (CN 54, 55, 56: 12.4%), 2.3n (CN 57, 58:21613.7%), 2.4n (CN 59, 60, 61: 21.9%), 2.5n (CN 62, 63: 11.7%), and 2.6n (CN 64, 65, 66:

16.7%) cells were predominant. Aneuploid cells with 2.1n (52, 53: 4.2%), 2.7n (CN 67, 21768: 5.4%), 2.8n (CN69, 70, 71: 1.3%) and 3.0n (CN74: 0.1%) occurred in lower 218219frequencies. In the $3n \times 2n-1$ cross (Fig. 4b), embryos with 2.1n (CN 52, 53) to 2.5n 220(CN 62, 63) cells appeared at a total rate of 19.7%, while 2.6n (CN 64, 65, 66) to 2.9n 221(CN 72, 73) aneuploid cells occurred at higher rates (total 52.1%) (Fig. 4b). Triploids 222(3.0n, CN74, 75, 76) appeared at the highest rate (14.8%) and hyper-triploid cells (3.1n-3.8n) appeared at the rate of 7.3%. In the $3n \times 3n-1$ cross (Fig. 4c), 2.8n (CN 69, 70, 71) 223224to 3.1n (CN 77, 78) aneuploid cells appeared most frequently (total 71.7%), but cells with less than 2.7n and more than 3.2n occurred at relatively lower rates. 225

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Ploidy level and chromosomes of triploid (diploid female × tetraploid male) hybrid
progenies

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Chromosomes were individually counted in the progenies of the $2n \times 3n-3$ (Table S5), 3n × 2n-3 (Table S6) and 3n × 3n-3 (Table S7) crosses (Fig. S2d–f). Embryos with a mode of the ploidy level 2.5n (CN 62, 63), 2.4n (CN 59, 60, 61), and 3.0n (CN74, 75, 76) occurred most frequently in $2n \times 3n-3$, $3n \times 2n-3$, and $3n \times 3n-3$, respectively (Table S5–7). The mean chromosome number calculated from the cells of the $2n \times 3n-3$, $3n \times$ 2n-3, and $3n \times 3n-3$ progenies were 61.63 (2.5n), 61.11(2.4n), and 73.00 (2.9n), respectively (Fig. 5).

237In the $2n \times 3n-3$ cross (Fig. 5a), an euploid cells with 2.5n (CN 62, 63: 34.7%) 238appeared most frequently, followed by those with 2.4n (CN 59, 60, 61: 32.1%) and 2.6n 239(CN 64, 65, 66: 19.2%). The occurrence rates of other 2.1n, 2.2n, 2.3n, 2.7n and 2.8n 240cells were low. In the $3n \times 2n-3$ cross (Fig. 5b), an euploid cells with 2.4n (CN59, 60, 24161: 37.3%) appeared most frequently, followed by those with 2.5n (CN62, 63: 28.9%) 242and 2.6n (CN64, 65, 66: 17.3%). The occurrence rates of other 2.1n to 2.3n cells and 2432.7n to 2.8n cells were low. In the $3n \times 3n-3$ cross (Fig. 5c), no cells with < 2.5n ploidy 244occurred. Aneuploid cells with 3.0n ploidy level (CN 74, 75, 76: 34.4%) appeared most frequently, followed by those with 2.9n (CN 72, 73: 26.1%) and 2.8n (CN 69, 70, 71: 24524618.7%). The occurrence rates of other cells were low.

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248 **Discussion**

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The most frequent occurrence of progenies with 2.4n to 2.5n in reciprocal crosses between diploid and triploid hybrids is easily explained by fertilization of haploid gametes (1n eggs, 1n sperm) from wild-type diploids with 1.4n to 1.5n gametes from triploid hybrids. The 1.5n gametes can be predicted by equal segregation of 25 IIs formed by 25 pairs of homologous chromosomes and random assortment of the extra 25 Is of 75 chromosomes in triploid, according to the binominal distribution previously reported by Zhang and Arai (1999) and Li et al. (2015). In the $3n \times 3n-3$ crosses, triploid to near-triploid progenies ranging from 2.6n to 3.3n with a modal ploidy level of about 3.0n appeared according to the expectation from the crosses between gametes with a modal ploidy level of approximately 1.5n.

260Although the appearance of 2.5n and 3.0n was also predicted in the $2n \times 3n-1$ (and 261 $3n \times 2n-1$) and $3n \times 3n-1$ progenies, respectively, wide ranges of an euploid cells < 2.3n 262occurred in the $2n \times 3n-1$ cross. In the $3n \times 2n-1$ cross, frequencies of cells ranging 263from 2.6n to 3.0n were much higher than the expectation. In the $3n \times 3n-1$ cross, frequencies of cells < 2.8n ploidy and > 3.1n were higher than those in $3n \times 3n$ -3 cross. 264265Such deviations from the predicted distribution based on the modal meiotic 266configuration might be related to the formation of diversified gametes with various 267chromosomes from non-typical meiotic configurations including various numbers of 268chromosomes, such as 24Is + 24IIs +1III (trivalent), 23Is + 23IIs + 2IIIs, 22Is + 22IIs + 2693IIIs and so on (Li et al. 2015). In our previous study, we also reported failure of synapsis between homologues and thus formation of gametes with unbalanced genetic 270271materials was also predicted (Li et al. 2015).

272Fankhauser and Humphrey (1950, 1954) observed a shift toward the lower 273chromosome numbers in diploid × triploid axolotl progenies and suggested that this was 274caused by the elimination of some lagging chromosomes and the resultant failure of 275gametogenesis in gametes with higher numbers of extra chromosomes. A shift to lower 276chromosome numbers was also described in a cross between triploid and diploid rice 277plants (Fukui and Tsujimoto 2010). However, in the present study, a shift toward the 278higher chromosome numbers was found in triploid hybrid progeny 3n x 2n-1 and 3n x 2793n-1 crosses. One explanation is the involvement of atypical diploid (2n) eggs generated by triploid hybrid females in such a cross-breeding. Thus, progenies might have been 280281contaminated by unpredicted triploid embryos that arose from the fertilization of diploid 282eggs with haploid sperm. Oshima et al. (2005) reported the spawning of a few diploid 283eggs in natural triploid loaches. A similar shift of the modal ploidy level toward 2.6n to 2842.7n was recently reported in the progenies of fertile induced triploid ornamental carp (Gomelsky et al. 2015). This is presumably explained by selective mortality against 285eggs during oogenesis and the resultant zygotes (embryos) < 2.5n. The other 286287explanation for the shift to higher chromosome numbers may be the failure of gamete 288formation with lower numbers of extra chromosomes (< 1.5n). However, it is difficult to

apply this assumption to the shift to lower ploidy, because the modal ploidy level in progenies from the $2n \times 3n-1$ cross was 2.5n. Flow cytometry indicated that the most frequent ploidy level of spermatozoa was approximately 1.5n in triploid hybrids, suggesting the production of ~2.5n progenies after the fertilization of eggs of wild-type female.

294In the present triploid hybrids, both males and females produced fertile aneuploid gametes and all progenies from the fertilization with these aneuploid gametes were 295296inviable, probably due to deficiency and/or excess of genetic materials. These results 297 differ from those previously reported in artificially induced triploids produced from 298wild-type diploids, in which triploid males generated unusual aneuploid spermatozoa, 299but triploid females were sterile (Zhang and Arai 1999). A part of progenies from fertilization of aneuploid spermatozoa of induced triploids were viable (Zhang and Arai 300 301 1999; Arai and Inamori 1999). In contrast, triploid hybrids between Japanese wild-type 302diploids and origin-unknown tetraploids from market samples were sterile in males, but 303 females laid both meiotic haploid and unreduced triploid eggs, which produced viable 304 progenies after fertilization with normal spermatozoa (Matsubara et al. 1995; Arai and 305 Mukaino 1997, 1998; Zhang et al. 1998). Clone-origin natural triploids were sterile in 306 males, but fertile haploid eggs were mainly formed in females by meiotic 307 hybridogenesis, followed by the appearance of normal diploid embryos after 308 fertilization with normal spermatozoa (Oshima et al. 2005; Morishima et al. 2008).

309 Such differences in gametogenesis and embryogenesis between present triploid 310 hybrids and previous induced triploids or other types of triploid hybrids are likely 311 related to the genomic constitution of resultant triploids. Genetic characteristics of 312 Chinese loach are poorly understood and the detailed mechanisms for explaining above 313 mentioned differences have not yet been elucidated. Thus, further genetic studies are 314 especially required on loaches in China.

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404 Legends of figures

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Fig. 1 External appearance of (a) normal larvae from crosses $(2n \times 2n, 2n \times 4n, 4n \times 2n)$ and $4n \times 4n$ using gametes of wild-type diploid and those of natural tetraploid loach and (b) abnormal larvae from crosses $(3n \times 2n, 2n \times 3n)$ and $3n \times 3n)$ using gametes of triploid hybrids and those of wild-type diploids. Scales denote $10\mu m$.

410

411 Fig. 2 Conventional Giemsa-stained metaphase (a); karyotype (b) including five 412metacentric (M), two submetacentric (SM), and 18 telocentric (T) triplet chromosomes; 413presence of three nucleoli shown by Ag-NOR in static cells (c); silver nitrate stained metaphase (d); its partial karyotype showing Ag-NOR on short arms of the largest 414 metacentric chromosomes (e); CMA₃ stained metaphase (f); its partial karyotype 415416 showing CMA₃ positive site on short arms of the largest metacentric chromosomes (g); 417 FISH metaphase probed by rDNA sequences (h); its partial karyotype showing rDNA 418 loci with FISH signals on short arms of the largest metacentric chromosomes (i). Scale 419 bar = $10 \mu m$.

420

Fig. 3 Flow cytometry histograms of testicular cells taken from diploid (a), tetraploid (b), and triploid hybrid males (c–e). Note the presence of a major peak of haploid (DNA content, 1C), diploid (2C), and 1.5n (1.5C) spermatozoa in diploid (a), tetraploid (b) and triploid hybrid (c) males. No spermatozoa peak (d) and presence of a minor peak of 1.6n (1.6C) spermatozoa (e) in triploid males. Y-axis denotes cell numbers and X-axis denotes channel numbers in each graph.

427

Fig. 4 Chromosome distributions in progenies from $2n \times 3n-1$ (a), $3n \times 2n-1$ (b) and $3n \times 2n-1$ (c) crosses using the triploid hybrids (tetraploid female × diploid male). Y-axis denotes cell numbers and X-axis denotes chromosome numbers and ploidy levels in

431 each graph. Numbers in parenthesis under ploidy level indicate percentage of cells.

432

- 433 Fig. 5 Chromosome distributions in progenies from $2n \times 3n-3$ (a), $3n \times 2n-3$ (b) and 3n
- $434 \times 3n-3$ (c) crosses using the triploid hybrids (diploid female × tetraploid male). Y-axis
- 435 denotes cell numbers and X-axis denotes chromosome numbers and ploidy levels in
- 436 each graph. Numbers in parenthesis under ploidy level indicate percentage of cells

Voor	Cross	No. of ages	Fertilization	Hatching	Normal	Survival rate at 7 days
i eai	CIOSS	No. of eggs	rate (%)	rate (%)	rate (%)	after hathcing (%)
	$2n \times 2n$ -1	1562	87.00	82.10	94.30	83.60
	$2n \times 2n$ -2	1663	87.90	80.60	91.70	83.50
	Mean \pm SD		87.45 ± 0.64^{a}	$81.35 \pm 1.06^{b} \\$	93.00 ± 1.84^a	$83.55 \pm 0.07^{d} \\$
	$2n \times 4n-1$	1509	80.73	89.25	96.22	92.31
	$2n \times 4n$ -2	770	82.21	86.21	96.65	91.55
	$Mean \pm SD$		81.47 ± 1.05^a	87.73 ± 2.15^{ab}	96.44 ± 0.30^a	91.93 ± 0.54^{bb}
	$4n \times 2n-1$	1521	81.22	91.14	91.82	87.98
	$4n \times 2n-2$	1021	80.13	89.11	92.21	87.33
	Mean \pm SD		$80.68 \pm 0.77^{\rm a}$	90.13 ± 1.43^{a}	92.02 ± 0.28^a	$87.66\pm0.46^{\rm c}$
	$4n \times 4n$ -1	640	83.60	80.60	96.80	98.37
2012	$4n \times 4n-2$	804	82.70	86.60	96.70	96.86
	$Mean \pm SD$		83.15 ± 0.64^a	83.60 ± 4.24^{b}	96.75 ± 0.07^a	97.62 ± 1.07^a
	$2n \times 3n-1$	566	57.10	32.00	56.00	31.90
	$2n \times 3n$ -2	1066	70.50	37.80	53.60	34.20
	Mean±SD		$63.8 {\pm} 9.48^{b}$	34.9±4.10 ^c	$54.8{\pm}1.70^{b}$	33.05±1.63 ^e
	$3n \times 2n-1$	399	33.80	16.30	48.10	7.41
	$3n \times 2n-2$	421	31.60	16.20	51.10	9.02
	Mean±SD		32.7±1.56 ^c	$16.25 {\pm} 0.07^{d}$	49.6±2.12 ^b	$8.22{\pm}1.14^{f}$
	$3n \times 3n$ -1	331	34.70	16.00	45.20	7.82
	$3n \times 3n$ -2	644	32.90	12.40	37.70	7.07
	Mean±SD		33.8±1.27 ^c	$14.2{\pm}2.55^{d}$	41.45±5.30°	7.45 ± 0.53^{f}
	$2n \times 2n$ -3	1329	87.13	88.17	91.77	82.56
	$2n \times 2n-4$	1560	88.91	90.12	94.24	85.36
	Mean±SD		$88.02{\pm}1.26^{a}$	$89.15{\pm}1.38^{a}$	93.01±1.75 ^a	83.96±1.98 ^a
	$2n \times 3n-3$	1458	58.30	55.29	56.6	3.41
	$2n \times 3n-4$	1617	64.56	59.39	53.87	3.07
2014	Mean±SD		61.43±4.43 ^c	$57.34{\pm}2.90^{\circ}$	$55.24{\pm}1.93^{c}$	3.24±0.24 ^b
2014	$3n \times 2n-3$	589	74.87	65.53	81.31	4.08
	$3n \times 2n-4$	632	73.26	67.60	83.39	3.67
	Mean±SD		74.07 ± 1.14^{b}	66.57±1.46 ^b	$82.35{\pm}1.47^{b}$	3.88±0.29 ^b
	$3n \times 3n$ -3	667	55.62	52.02	47.67	3.50
	$3n \times 3n$ -4	656	58.69	57.66	53.15	4.42
	Mean±SD		57.16±2.17°	54.84±3.99°	50.41±3.87°	3.96±0.65 ^b

Table 1. Number of eggs, fertilization rate, hatching rate, normal rate and survival rate at 7 days after hatching in the different crosses using diploid, tetraploid and triploid (diploid female × tetraploid male) loaches in 2012 and 2014.

Note: Different lowercase letters in the same row indicated significant differences (P < 0.05) among different crosses; and same letters indicated no significance (P > 0.05).







b м	FXL	121	323	\$X.X	223
SM	111	117			
Т	111	105			
		464			444
		-	-		









Chromosome number Ploidy level (Percentage)



Chromosome number Ploidy level (Percentage)



Fig. S1 External appearance (a-c) and diameters (d-f) of eggs from three crosses of loaches (a, d:2n female × 2n male-1, b, e: 3n × 2n-1, and c, f: 3n × 3n-1) at 30 min after fertilization. Scales denote 1 mm.



Metaphase spreads observed in embryos from 2n × 3n (a, d), 3n × 2n (b, e) and 3n × 3n crosses (c, f). (a) Fig. S2 Chromosome number (CN) = 62, 7m (metacentric chromosomes) + 12sm (submetacentric chromo.) + 43 t (telocentric chromo.) from 2n × 3n-1 cross; (b) CN = 71, 13m + 6 sm + 52t from 3n × 2n-1 cross; (c) CN = 80, 15m + 8sm + 57t from 3n x 3n-1 cross; (d) CN=58, 9m + 6sm + 45t from, 2n x 3n-3 cross; (e) CN = 62, 13m + 5sm + 44t from 3n × 2n-3 cross; (f) CN = 75, 12m + 6sm + 57t from $3n \times 3n-3$ cross. Scales denote 10 μ m.

cross	Chromosome									Eı	nbry	o no.										
01088	no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	total
	41	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	3
	42	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	43	0	0	0	2	1	1	0	2	0	0	0	0	0	0	0	0	1	0	0	0	7
	44	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	3
	45	0	4	0	0	0	0	1	2	2	0	1	1	0	0	0	1	0	0	0	2	14
	46	1	4	0	2	4	3	2	1	1	3	1	1	3	0	0	1	1	0	0	2	30
$2n \times 2n$	47	2	2	1	1	4	3	3	2	2	2	0	4	1	1	4	1	0	3	2	2	40
211 × 211	48	4	5	7	4	6	2	3	-	4	4	7	5	2	0	3	5	6	2	5	8	88
	40	-	2	,	•	4	2	2	0	4	-	, E	5	2	1	5	0	2	2	2	2	00
	49	0	2	4	0	4	3	2	9	4	3	10	5	2	1	12	0	2	2	2	2	200
	50	0	0			5	17	21	15	17	14	18	14	19	20	15	15	12	18	2	14	200
	51	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	1	0	4
	52	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	0	0	0	5
	total	22	23	23	29	24	30	32	38	30	29	32	30	29	26	28	29	22	25	12	30	543
	69	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	1	0	0	0	6
	70	1	2	1	0	1	1	2	1	2	2	1	1	1	1	1	1	0	1	1	0	21
	71	0	0	1	0	1	2	3	2	0	1	1	1	1	0	1	0	2	1	2	1	20
	72	3	1	1	1	2	0	0	1	3	0	2	1	1	3	0	2	1	0	2	1	25
	73	3	0	0	1	2	1	3	1	3	3	5	2	1	0	2	3	0		1	0	31
	74	1	2	1	3	3	1	3	3	3	3	0	1	4	2	3	4	2	1	2	1	43
$2n\times 4n$	75	8	9	15	19	18	13	12	15	16	11	11	13	15	16	11	12	16	14	16	12	272
	76	4	3	5	2	2	3	2	6	3	6	6	2	0	2	3	0	3	3	2	5	62
	77	3	7	3	1	1	4	0	0	0	0	1	3	3	2	4	2	2	4	2	1	43
	78	3	4	2	3	0	3	4	1	0	2	1	3	1	2	5	2	3	3	2	3	47
	79	0	2	1	0	0	2	1	0	0	0	0	1	1	0	0	1	0	3	0	4	16
	80	0	-		0	0	-		0	0	0	0	,		1	0		0	0	0	2	e e
	80	27	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	504
	total	21	50	30	30	30	30	30	30	30	20	29	50	30	30	30	50	30	30	30	30	394
	68	5	0	1	1	0	2	1	1	1	1	1	0	1	1	0	0	1	0	3	0	20
	69	0	0	0	0	0	1	1	1	1	1	1	1	0	1	1	0	0	0	2	0	11
	70	4	0	0	2	2	3	1	1	1	1	2	0	1	1	0	3	1	1	0	1	25
	71	0	3	0	3	0	0	2	0	1	2	1	0	2	1	3	0	1	0	1	4	24
	72	1	2	0	7	1	2	2	3	3	1	1	3	2	2	0	4	0	4	2	1	41
	73	3	3	4	4	4	7	0	1	2	6	1	1	0	1	3	3	4	5	5	7	64
4n imes 2n	74	0	2	1	2	2	1	3	1	0	2	1	3	2	1	1	2	3	1	3	1	32
	75	12	15	16	10	13	10	16	21	14	9	14	17	13	9	11	17	10	19	10	11	267
	76	2	2	0	0	0	0	2	0	1	2	2	0	4	1	1	0	1	0	0	0	18
	77	0	1	2	0	1	0	1	0	1	1	2	0	0	2	2	0	0	0	1	0	14
	78	1	0	1	1	0	0	0	0	1	2	0	1	1	1	0	0	1	0	0	0	10
	79	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3
	total	28	28	26	30	23	27	29	29	26	28	26	26	26	21	22	29	23	30	27	25	529
	90	1	0	1	0	2	1	2	1	0	3	1	0	0	0	0	0	0	0	0	0	12
	91	1	1	0	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0	1	0	8
	92	0	0	0	0	1	0	0	0	1	0	2	0	1	0	0	0	0	0	1	0	6
	93	2	0	1	0	0	0	0	1	0	2	2	0	0	1	0	1	1	2	1	1	15
	04	0	0		0	0	2	1	ว	0	-	2	0	0	2	1		1	2		1	14
	24	2	1			1	د م	1	4	0	1	2	0	1	2	1	0	1	4	0	1	14
	95	2	1	1	1	1	0	1	1	0	1	2		1	2	5	2	0		2	5	24
$4n\times 4n$	96	1	0	0	4	1	2	5	2	0	1	2	1	2	1	1	1	0	1	0	0	25
	97	1	0	0	2	2	4	1	0	1	1	3	3	0	3	1	2	2	4	0	1	31
	98	2	1	3	1	2	3	0	2	1	0	1	1	0	1	1	2	1	0	1	3	26
	99	1	2	2	1	0	6	0	0	2	0	0	1	1	1	2	1	1	2	2	1	26
	100	11	16	11	13	11	9	10	14	11	10	16	13	14	13	12	13	14	12	14	12	249
		0	0	1	1	1	0	0	0	2	1	1	1	0	2	1	1	1	1	1	0	15
	101	0	0	•																		
	101 102	0	0	0	2	1	1	0	0	1	1	0	1	0	1	1	0	1	1	1	0	12

Table S1. Chromosome numbers in the embryos from 2n (female) \times 2n (male), 2n \times 4n, 4n \times 2n and 4n \times 4n crosses.

Ploidy	Chromosome														Er	nbryo	num	iber															Sum (04)
level	number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Total	- Suii (%)
1.3n	33															1																1	1(0.1)
	34																															0	-(01-)
1.4n	35														1		1															2	6(0.7)
	36																2	1					1									4	
1.5n	37																						1			2	1		1			5	6(0.7)
	38																													1		1	
1.6n	39				1										1					1												3	
	40		1												1								1									3	15(1.7)
	41		1									1			1				1	1			1				2			1		9	
1.7n	42																		1				2	1				1				5	12(1.3)
	43				1	1					1						1		1	1								1					
1.8n	44			I						1				1	1			1					1	~								6	10(2.1)
	45																							2		1						3	19(2.1)
1.0	46		1							1	1						1		1	1		1		1		1	1					10	
1.9n	47		1	1							1							1		1			1		1	2	1					5	10(2,1)
	48										1	1			1		1		1				1		1	3	1			1		6	19(2.1)
2.0-	49								1		1	1			I		1		I				I									0	
2.0n	50				1				1	1	1	1		1		1	1		2	1	1	1	2			2	1	1	1	4	1	9	29(3.2)
2.1-	52								1	1	I	1		1		1		1	1	1	1		2			2	1	1	1	4		12	
2.1n	52		1			2			2		1	1			2	1	1	1	1	2	2	2	2	2	1	3	1	1	2	1	2	12	38(4.2)
2.2-	54						1		2	1		1		1		<u>1</u>	1	1		1	2		2		1	1	2	1	2	1		20	
2.2n	55			1	1	2	1		1	1	2	2		1	1	1	2	2	1	1	2	4	5	4		2	2	1	2	2		36	112(12.4)
	55			1	1	2	2		1	1	2	3			1	1	2	2	1	1	2	6	2	2		3	2		2	1		42	112(12.4)
2.2-	57		2	2	1	1	~2		10	2					1	<u>1</u>	1	2	1	2	2	4	2	1			2		2	2	2	43	
2.511	59	1	2	2	1	2	2	2	19	1	1		1	2	1	1	2	2	2	5	2	2	2	2		2	2	1	2	4	2	52	123(13.7)
2.4n	50	1		<u>-</u>	2	5	2	5		2			1		1	·····		2	2	1				1		1	1	1	5			51	
2.40	59	1		1	1	6	5	11		2	5		1		4	1	2	1	5	3	1	3		5	5	1	1		1	3	5	69	197(21.9)
	61	1		1	1	6	2	6		1	1	1	4	0	5	1	2	5	3	2	5	1		2	0	1	2	2	5	1	4	77	1)/(211))
2 5n	62	1		3	1	7	~	4		1	1	1	4	1	5	1	4	2	2	1	3	1		3	4		2	2	3	1	3	54	
2.511	63	7	6	5	1	1	5	4		1	2	1	1	3	3	2	4	1	1	4	4	1		1	2		1	1	5	1	3	51	105(11.7)
2 6n	64	<u> </u>	4	3	<u>-</u>	<u>-</u>	4			2		2	4		1	3	1	····	 1		1			···-	4		<u>-</u>	<u>-</u>				38	
2.01	65	1	4	2	3		3			3	1	4	12	5	1	1	2		2	2	1	1			2		1	5				56	150(16.7)
	66	8	8	2	3		2	1		4	1	8	2	3	1	7	2		2	2	1	1			2		1	6				56	
2 7n	67	2	1	2	3		4			4	1	3	1	2		4					·····							1	2		1	32	
2.71	68	1		1	6					2	•	2	•	-		3	1			•								1	-			17	49(5.4)
2.8n	69			1	2									1			····											2				8	
2.011	70			2	1					•		•		•														-				3	12(1.3)
	71			-	-																							1				1	. /
2.9n	72																														1	1	4/2 /2
	73			3																											-	3	4(0.4)
3.0p	74			1																												1	
	75																															0	1(0.1)
3.5n	87																			1												1	1(0.1)
	Total	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	900	. /

Table S2. Distribution of chromosome numbers and ploidy levels in embryos from 2n x 3n-1 cross

Ploidy	Chromosome												E	mbryc	num	ber														- Sum(%)
level	number	1	2 3	3 4	4 5	6	7	8	9	10	11 12	2 13	3 14	15	16	17	18	19	20	21 2	2 23	3 24	25	26	27	28	29 3	30	Total	
1.4n	35												2																2	4(0.4)
	36					1										1													2	
1.5n	37																		1				1						2	2(0.2)
	38																												0	=(*-=)
1.6n	39															1													1	
	40					1						1																	2	4(0.4)
	41		1																										1	
1.7n	42				1	1			1	1		1																	5	0(1.0)
	43					1				1		1											1						4)(1.0)
1.8n	44					1					1																		2	
	45			1	l				1													1							3	10(1.1)
	46									1						1		1		1			1						5	
1.9n	47	1			1				1							2		1				1							7	
	48			1	l					1	1												1						4	17(1.9)
	49				1	2	1																2						6	
2.0n	50					1	1			1		1									1								5	
	51					1				1			1													1			4	9(1.0)
2.1n	52						1	1	1		1 3	1					1	1					1			1			12	
2.111	53		1	1			1		•		1	1	1				•	•					•						6	18(2.0)
2 2n	54		1					1		1	1	[*]	<u>.</u>							1		2							7	
2.211	55			-	, 1										2	1		1		. ,		-							9	24(2.7)
	55	1		-	- 1	1				1	1		1		2	1		1		-	. 1		1						8	_ (()
2.25	57	2				1	3		1	1	1		<u>1</u>					1	1	1	1		1	1		2		2	17	
2.511	59	2		1		1	5		1	1			1		2			1	1	1	1		2	1		2		1	17	30(3.4)
2.4-	50		1 .	1 1		1	1				1									1		1						1	10	
2.4n	59		1	1 1		1	1		2	2	2 1		2		1					1		1	2			1		2	10	58(6.5)
	60					1	2		1	1	2 1		2		1	1							~			1		2	15	58(0.5)
	61		1				2			3	1		1			3	1			1	2	1	2			4			27	
2.5n	62		2		1	1	2	1	2	1	1		1	1	3			1		1			2		2			2	22	46(5.1)
	63						2	1			1		1	1	I	1			4	4			3			1		2	24	
2.6n	64		2	_		1	2		2	1		1	2		5	3			1	1 1					1	-		2	25	107(14.0)
	65	1	-	3 1		3	5			2	2		3	1	3	4		2	2	1 4			4	1	1	3		3	50	127(14.2)
	66	1		1	2		1	2	3	2	1 2		1	i	5	1		2	I	2 3		1	3	1	4	4		8	52	
2.7n	67	1		1 1			5	1	3	3	3			1		4	2	1		4	- 1	2	2		3	3		2	43	97(10.9)
	68	1	6 2	2	2	4	2		4	1	1 3		1	3		3		1	2	1 4	4	1	1		2	2		3	54	
2.8n	69		2	1	1	2		1	2	2	3	1	2		1	2			3	1	2	5		2	3	2		1	39	
	70	2	4 2	2 1	l	1	1		3	2	3 2	2	5	2	1	1	3	4		1		5	2	1	9		1	1	59	144(16.1)
	71		4	2	2 3	1		2	2		1	3	1		1		5	4	2	1	1	4		5	4				46	
2.9n	72	4	1	3	5	1		5	1		6 2	5					2	3	5	1	2	3		2		2	2	1	55	98(11.0)
	73	2	3 2	2	2			2			4	2		1	1		6	4		1	4	3		2			4		43	
3.0n	74	2		1	3			2			5	3	1	6						3	2			2	1		2		33	
	75	2	2 3	3 9	95			6			1	6		3		1	7		8	3	6			6			6		74	132(14.8)
	76	3		3 4	1			2			3			3			1			3				1			2		25	
3.1n	77	1		1 1	l							1		2	1		1			4							5		17	31(3.5)
	78	4		1 1	l			1			1			1						1 1						1	2		14	51(5.5)
3.2n	79			1				2						1						1									5	
	80		2	2	1															1 1				1					6	14(1.6)
	81													1						1	1								3	
3.3n	82														1									1					2	4(0.4)
	83			1										1															2	4(0.4)
3.4n	84													1										1					2	
	85										1									1									2	7(0.7)
	86																	1						1			1		3	
3.5n	87			1																1									2	2(0.2)
	88																1												1	3(0.3)
3.6n	89	1																											1	a (:
2.000	90																										1		1	2(0.2)
3 7n	93																				1								1	1(0.1)
3.8n	95												1								1								1	- (0.1)
5.00	96	1																			1								2	3(0.3)
	Total	30	30 2	20 2	0 20	20	30	30	30	30	30 20) 20) 20	20	20	30	30	30	30	30 2	0 20) 20	20	20	30	30	26 3	80	801	
	rotai	50	50 3	,5 3	0 50	50	50	50	50	50	20 20	ار ر	, 50	50	50	50	50	50	50	50 3	ט ט	, 50	50	20	50	50	20 3	,,,	074	

Table S3 Distribution of chromosome numbers and ploidy levels in embrys from 3n x 2n-1 cross

Table S4. Distribution of chromosome numbers and ploi	idy level in embryos from the 3n x 3n cross
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Ploidy	Chromosome														En	nbryo	num	ber															Sum(%)
level	number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Total	- Sun(70)
2.4n	61				2															1												3	3(0.3)
2.5n	62				1						1																					2	4(0.5)
	63				1																				1							2	
2.6n	64				3																1								1			5	
	65		3		3	3															2				3				4			18	48(5.5)
	66			4	4																2				4				10	1		25	
2.7n	67		2	2	6	2														1	2				4				2			21	70(8.0)
	68			4	2	1		2	4								3	1		10	2		8		4				6	2		49	70(0.0)
2.8n	69		2	5	2	1		5	2								2			10	1		8		3				3	1		45	
	70	4	1	6	2	3		7	10	1			4			1	3	6	2	7	3	8	8		5			1	1	12	1	96	212(24.3)
	71			4	1	4	1	5	5	1	1		6	1			8	8	1	1	4	4	1		1	2		6		4	2	71	
2.9n	72	1		4	1	1	1	6	2	2	2		6	1	2		6	4	3		2	10	1	1	1	1		3		1	4	66	110(13.6)
	73		2	1	1	1			3	1	3		1	4	2		4	5	1		2	1		3		2	4	6		2	4	53	11)(15.0)
3.0n	74	2	1		1	5	1	3		2	2	1	5	4	3	1	1	4	4		1	1	2	3			1	7		2	4	61	
	75	2				2	3	1	1	10	7	4	7	3	2	2	1		7		2	3		3	1	2	1	6		3	2	75	190(21.8)
	76	4	3			1	5	1	1	3	6			3	2		1	1	5			3		4	2	5	1				3	54	
3.1n	77	7	3				4			5	2	1		2	8	3	1		1		1		1	4		6	2				3	54	104(11.9)
	78	2	3			1	5			3	2	2		1	6	3		1	1		1		1	6		3	6	1			2	50	104(11.))
3.2n	79	1	2			1	1			1	1	1		1	2	7								4		2	3			1	1	29	
	80		1								3	3	1	3	3	4						1		3		3	2		1		1	29	78(8.9)
	81		1							2		6		1		4											5		1			20	
3.3n	82	3	1									3			1	3											3		1			15	24(2.8)
	83						1					5				1														1	1	9	24(2.8)
3.4n	84											2	1																			3	
	85		1													1																2	8(0.9)
	86	2																							1							3	
3.5n	87	1										2	1																			4	6(0.7)
	88															1											1					2	0(0.7)
3.6n	89								1										1													2	
	90																										1		1			2	4(0.5)
	91																															0	
3.7n	92																															0	1(0,1)
	93														1																	1	1(0.1)
3.8n	94																															0	1(0,1)
	95								1																							1	1(0.1)
	Total	29	26	30	30	26	22	30	30	31	30	30	32	24	32	31	30	30	26	30	26	31	30	31	30	26	30	30	31	30	28	872	

Ploidy	Chromosome									Em	bryo	nun	nber										$\mathbf{S}_{\text{rest}}(0/)$
level	number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Tota	Sum(%)
2.1n	53		1											1	1				1	1		5	5(0.9)
2.2n	54		1				2					1										4	
	55					1		2	1				1	3			1	1				10	26(4.9)
	56				2	3		1						2	1	1	1		1			12	
2.3n	57				2									4								6	27(5 1)
	58				5	1	2	1	1	1			1	2		2	4	1				21	27(3.1)
2.4n	59				3	2	3			1		1		8	1	2			4			25	
	60	4		2	8	1	5	6	4	1	2	2	3	2	6	4	4	1	9	4	1	69	170(32.1)
	61	4	1	7	1	3	7	9	6	3	1	5	4		6	4	1	4	4	2	4	76	
2.5n	62	7	3	6	1	4	3	3	3	9	5	6	3		12	8	12	2	6	3	6	##	184(34.7)
	63	7	5	5		6	1	3	7	4	2	6	4		1	3	2	10	1	11	4	82	104(34.7)
2.6n	64	4	2	1		1	2		1	8	3	7	2		1	2	1	6		2	6	49	
	65	1	2	2		4					5		6			1	1	1			1	24	102(19.2)
	66	2	4	2		1				2	9	1	1			2					5	29	
2.7n	67		3							1											1	5	$11(2 \ 1)$
	68	1		2											1				1		1	6	11(2.1)
2.8n	69											1				1						2	5(0.9)
	70			1							1										1	3	5(0.9)
	Total	30	22	28	22	27	25	25	23	30	28	30	25	22	30	30	27	26	27	23	30	##	

Table S5. Distribution of chromosome numbers and ploidy level in embryos from 2n x 3n-3 cross

Ploidy	Chromosome									En	ıbryo	num	ber										Sum(0/)
level	number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total	- Sull(%)
2.0n	50																					0	0(0)
	51																					0	0(0)
2.1n	52			2					1					1								4	4(0.7)
	53																					0	4(0.7)
2.2n	54								2					1				1				4	
	55			2	2				1				1	1								7	17(2.9)
	56				1	1	1		2	1												6	
2.3n	57	1		1	5	1			2	6		2					1	1			2	22	(7(11.6))
	58			6	10	1	2	1	3	7		1	1	4			1	3			5	45	0/(11.0)
2.4n	59	5		7	2	1	1		1	2		2	2	5	1		1		1	1	2	34	
	60	11	4	7	5	21	7	1	5	7	6	2	3	4		2	6	7	4	2	6	110	215(37.3)
	61	3	4	4	4	1	1		4	2	4	12	2	2		3	7	3	4	4	7	71	
2.5n	62	2	10	1	1	3	13		3	2	14	9	8	4	2	9	4	9	6	9		109	1(7(29.0))
	63		9				2	4	1		1		5	1	3	7	5	2	6	11	1	58	107(28.9)
2.6n	64		2			1	2	8		2	3		5	3	12	2	1	1	4	1	2	49	******
	65		1				1	8				1			6	5	2		3	2		29	100(17.3)
	66							6		1	2	1	2	1	5	2	2					22	
2.7n	67							2						1								3	5(0,0)
	68														1				1			2	5(0.9)
2.8n	69																		1		1	2	2(0,2)
	70																					0	2(0.3)
	Total	22	30	30	30	30	30	30	25	30	30	30	29	28	30	30	30	27	30	30	26	577	

Table S6. Distribution of chromosome numbers in embryos from 3n x 2n-3 cross

Ploidy	Chromosome									Em	bryo	nun	nber										$\mathbf{S}_{um}(0/0)$
level	number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Гota	Sum (%)
2.6n	65			2			2	1	1					1						1		8	19(3.4)
	66	2		1			1						1	1	2	1			1		1	11	17(3.4)
2.7n	67			3			2					2	2	3	3		4			2		21	38(6.9)
	68			2			1	1				3	1	2		1	4		1		1	17	50(0.7)
2.8n	69	1		2		3		2	1		1	1			1	4	1			1		18	
	70	3	2	6	1	1	1	3	2	2			2	4		3	1		3	3	2	39	103(18.7)
	71	1	2	6	2	2		6	1	1		2	2	1	1	3	4		3	6	3	46	
2.9n	72	3	7	3		2	2	4	2	3		3	2	5	1	7	1	1	3	6	3	58	144(26.1)
	73	7	2	1	1	5	5	4		5	3	4	1	7	4	7	6	5	4	8	7	86	144(20.1)
3.0n	74	6	6		4		4	2	4	10	1	6	3	5	5	2	2	3	3	2	5	73	
	75	1	2			5	2	3	7	3	13	5	2		2		4	7	4		3	63	190(34.4)
	76	2			5	4	3	3	4		4	3	2	1	5	1	1	10	3	1	2	54	
3.1n	77		2		7		3		3		2		4		4			2	2		1	30	39(7 1)
	78					2		1			1		1				1		2		1	9	57(7.1)
3.2n	79	1				1			1		1		1		1			1				7	
	80						1						1								1	3	13(2.4)
	81											1			1				1			3	
3.3n	82						2									1		1				4	6(1.1)
	83										1		1									2	0(1.1)
	Total	27	23	26	20	25	29	30	26	24	27	30	26	30	30	30	29	30	30	30	30	##	

Table S7. Distribution of chromosome numbers in embryos from 3n x 3n-3 cross