Aneuploid progenies of triploid hybrids between diploid and tetraploid loach *Misgurnus anguillicaudatus* in China

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Abstract

Triploid Chinese loach, *Misgurnus anguillicaudatus*, hybrids between tetraploids from Hubei Province and diploids from Liaoning Province were mated with either diploid wild-type or triploid hybrids to analyze viability and ploidy of the resultant progenies. Both triploid males and females generated fertile gametes, but progenies from the crosses 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 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 triploid hybrid females and diploid males gave a modal ploidy level at approximately 2.5n 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 approximately 3.0n (chromosome numbers 74, 75, 76) was most frequent. The cytogenetic results of the progenies suggest the production of aneuploid gametes with a modal ploidy level at approximately 1.5n in triploid hybrids. However, a shift to higher chromosome numbers in gametes was observed in certain cases, suggesting the involvement of mortality selection of gametes and/or zygotes with lower chromosome numbers.

Key-words: Bivalent・Gamete・Meiosis・Polyploid・Trivalent・Univalent

Abbreviation

Ag-NORs; silver staining nucleolus organizer regions

CMA_3; chromomycin A_3,

CN; chromosome number,

DA; distamycin A,

DAPI; 4’6-diamidino-2-phenylindole

FISH; fluorescence in situ hybridization

Introduction

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 inhibiting the second polar body release just after fertilization, gametes with a ploidy 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 al. 2015). Among them, however, meiotic configurations have not been well investigated except for induced triploid loach *Misgurnus anguillicaudatus*, in which about 25 bivalents (IIs hereafter) and 25 univalent (Is hereafter) were observed (Zhang 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.

Materials and methods

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 × 4n, 4n × 2n, 4n × 4n, 2n × 3n, 3n × 2n, and 3n × 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 × 2n, 2n × 3n, 3n × 2n and 3n × 3n progenies in 2014.

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

Chromosome preparation was individually conducted on each optic vesicle stage embryo after manually removing the yolk in physiological saline under a stereoscopic microscope. Chromosome preparation procedures were the same as those described in Li et al. (2013). Chromosome counting was made on conventional Giemsa-stained metaphases on a slide directly under a microscope and/or on their photographed images. In the progenies, the modal chromosome number was determined in each embryo. Karyotyping was conducted according to Levan et al. (1964). Differential staining with CMA_3/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 et al. (2010). FISH using human 5.8S + 28S rDNA sequences as a probe was applied according to Li et al. (2010).

Results

Survival potential of triploid hybrid progenies

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

Triploid hybrid egg sizes

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

Triploid hybrid chromosomes

Here, we confirmed the genomic constitution of triploid hybrids based on chromosome numbers, karyotypes, and NOR numbers detected by differential staining and FISH. In 20 embryos from each of the 2n × 2n, 2n × 4n, 4n × 2n and 4n × 4n crosses, chromosomes were individually counted (Table S1). Out of 543 metaphases from 2n × 2n embryos (n=20), 266 cells had 50 chromosomes. Out of 594 metaphases from 2n × 4n embryos (n=20), 272 cells had 75 chromosomes. Out of 529 metaphases from 4n × 2n embryos (n=20), 267 cells had 75 chromosomes. Out of 463 metaphases from 4n × 4n embryos (n=20), 249 cells had 100 chromosomes. Thus, 2n x 2n, 2n x 4n, 4n x 2n and 4n x 4n crosses generated diploid (2n = 50), triploid (3n = 75), triploid (3n = 75) and tetraploid (4n = 100) progenies, respectively.

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

(SM), and 18 telocentric (T) triplet chromosomes clearly indicated triploidy with three sets 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 were detected on the short arms of the three largest M chromosomes (Fig. 2d, e). CMA_3 positive sites were detected on the short arms of the three largest M chromosomes (Fig. 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 FISH, indicated that triploid hybrids had three sets of homologous chromosomes, one set from diploid wild-type and two sets from tetraploid loaches.

Flow cytometry of testicular cells

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

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

Chromosomes were individually counted in progenies from the 2n × 3n-1 (Table S2), 3n × 2n-1 (Table S3) and 3n × 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 × 3n-1, 3n × 2n-1, and 3n × 3n-1, respectively (Table S2–4). The mean chromosome numbers calculated from the cells of 2n × 3n-1, 3n × 2n-1, and 3n × 3n-1 progenies were 58.76 (2.4n), 67.18 (2.7n) and 73.49 (2.9n), respectively (Fig. 4).

In the 2n × 3n-1 cross (Fig. 4a), 2.2n (CN 54, 55, 56: 12.4%), 2.3n (CN 57, 58: 13.7%), 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, 68: 5.4%), 2.8n (CN69, 70, 71: 1.3%) and 3.0n (CN74: 0.1%) occurred in lower frequencies. In the 3n × 2n-1 cross (Fig. 4b), embryos with 2.1n (CN 52, 53) to 2.5n (CN 62, 63) cells appeared at a total rate of 19.7%, while 2.6n (CN 64, 65, 66) to 2.9n (CN 72, 73) aneuploid cells occurred at higher rates (total 52.1%) (Fig. 4b). Triploids (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 × 3n-1 cross (Fig. 4c), 2.8n (CN 69, 70, 71) to 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.

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

Chromosomes were individually counted in the progenies of the 2n × 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 × 3n-3, 3n × 2n-3, and 3n × 3n-3, respectively (Table S5–7). The mean chromosome number calculated from the cells of the 2n × 3n-3, 3n × 2n-3, and 3n × 3n-3 progenies were 61.63 (2.5n), 61.11(2.4n), and 73.00 (2.9n), respectively (Fig. 5).

In the 2n × 3n-3 cross (Fig. 5a), aneuploid cells with 2.5n (CN 62, 63: 34.7%) appeared most frequently, followed by those with 2.4n (CN 59, 60, 61: 32.1%) and 2.6n (CN 64, 65, 66: 19.2%). The occurrence rates of other 2.1n, 2.2n, 2.3n, 2.7n and 2.8n cells were low. In the 3n × 2n-3 cross (Fig. 5b), aneuploid cells with 2.4n (CN59, 60, 61: 37.3%) appeared most frequently, followed by those with 2.5n (CN62, 63: 28.9%) and 2.6n (CN64, 65, 66: 17.3%). The occurrence rates of other 2.1n to 2.3n cells and 2.7n to 2.8n cells were low. In the 3n × 3n-3 cross (Fig. 5c), no cells with < 2.5n ploidy occurred. 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: 18.7%). The occurrence rates of other cells were low.

Discussion

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 × 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.

Although the appearance of 2.5n and 3.0n was also predicted in the 2n × 3n-1 (and 3n × 2n-1) and 3n × 3n-1progenies, respectively, wide ranges of aneuploid cells < 2.3n occurred in the 2n × 3n-1 cross. In the 3n × 2n-1 cross, frequencies of cells ranging from 2.6n to 3.0n were much higher than the expectation. In the 3n × 3n-1 cross, frequencies of cells < 2.8n ploidy and > 3.1n were higher than those in 3n × 3n-3 cross. Such deviations from the predicted distribution based on the modal meiotic configuration might be related to the formation of diversified gametes with various chromosomes from non-typical meiotic configurations including various numbers of chromosomes, such as 24Is + 24IIs +1III (trivalent), 23Is + 23IIs + 2IIIs, 22Is + 22IIs + 3IIIs 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 materials was also predicted (Li et al. 2015).

Fankhauser and Humphrey (1950, 1954) observed a shift toward the lower chromosome numbers in diploid × triploid axolotl progenies and suggested that this was caused by the elimination of some lagging chromosomes and the resultant failure of gametogenesis in gametes with higher numbers of extra chromosomes. A shift to lower chromosome numbers was also described in a cross between triploid and diploid rice plants (Fukui and Tsujimoto 2010). However, in the present study, a shift toward the higher chromosome numbers was found in triploid hybrid progeny 3n x 2n-1 and 3n x 3n-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 contaminated by unpredicted triploid embryos that arose from the fertilization of diploid eggs with haploid sperm. Oshima et al. (2005) reported the spawning of a few diploid eggs in natural triploid loaches. A similar shift of the modal ploidy level toward 2.6n to 2.7n was recently reported in the progenies of fertile induced triploid ornamental carp (Gomelsky et al. 2015). This is presumably explained by selective mortality against eggs during oogenesis and the resultant zygotes (embryos) < 2.5n. The other explanation for the shift to higher chromosome numbers may be the failure of gamete formation 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 × 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.

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

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

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Legends of figures

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

Fig. 2 Conventional Giemsa-stained metaphase (a); karyotype (b) including five metacentric (M), two submetacentric (SM), and 18 telocentric (T) triplet chromosomes; presence 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 metacentric chromosomes (e); CMA_3 stained metaphase (f); its partial karyotype showing CMA_3 positive site on short arms of the largest metacentric chromosomes (g); FISH metaphase probed by rDNA sequences (h); its partial karyotype showing rDNA loci with FISH signals on short arms of the largest metacentric chromosomes (i). Scale bar = 10 μm.

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.

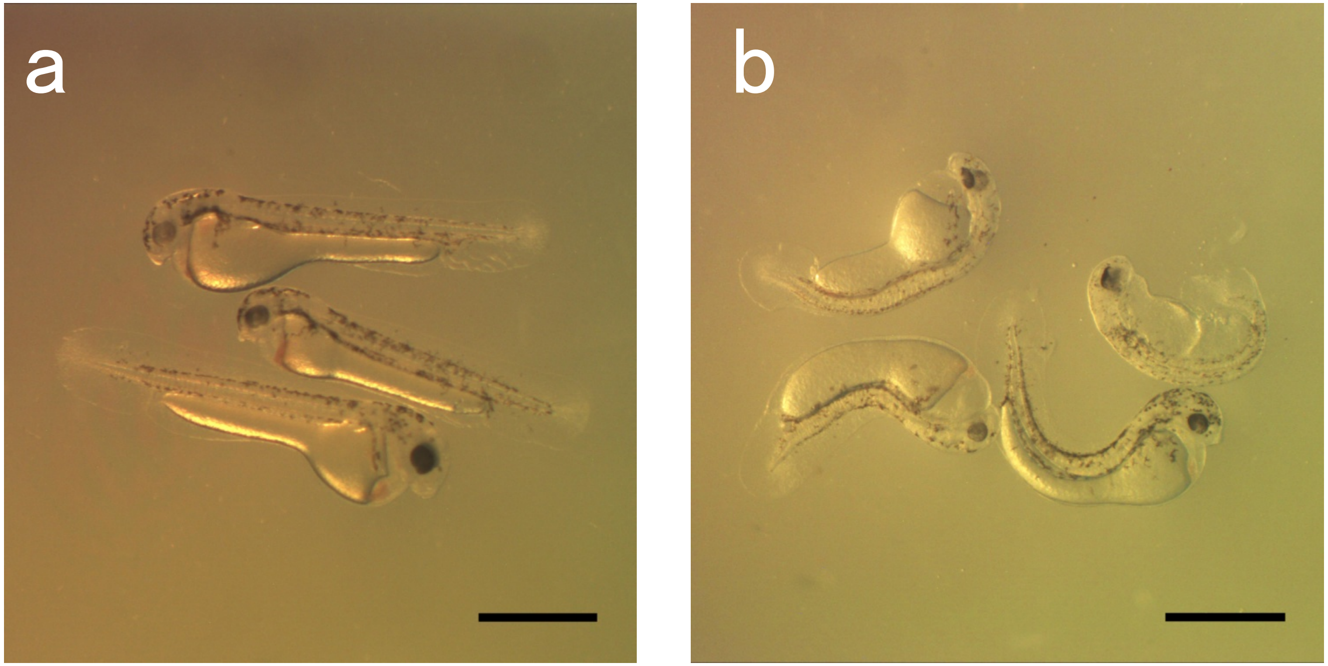
Fig. 4 Chromosome distributions in progenies from 2n × 3n-1 (a), 3n × 2n-1 (b) and 3n × 3n-1 (c) crosses using the triploid hybrids (tetraploid female × diploid male). Y-axis denotes cell numbers and X-axis denotes chromosome numbers and ploidy levels in each graph. Numbers in parenthesis under ploidy level indicate percentage of cells.

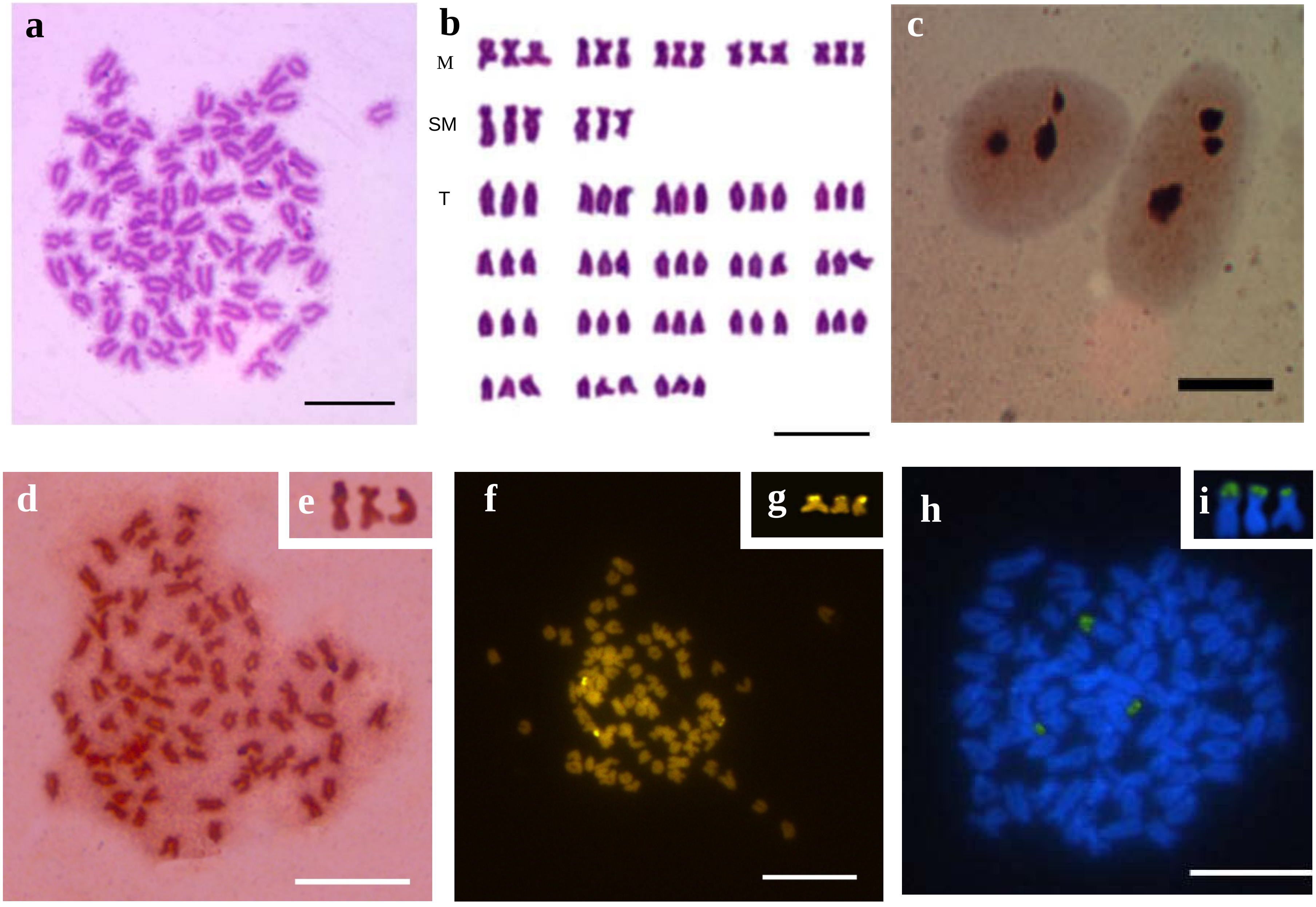
Fig. 5 Chromosome distributions in progenies from 2n × 3n-3 (a), 3n × 2n-3 (b) and 3n × 3n-3 (c) crosses using the triploid hybrids (diploid female × tetraploid male). Y-axis denotes cell numbers and X-axis denotes chromosome numbers and ploidy levels in each graph. Numbers in parenthesis under ploidy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

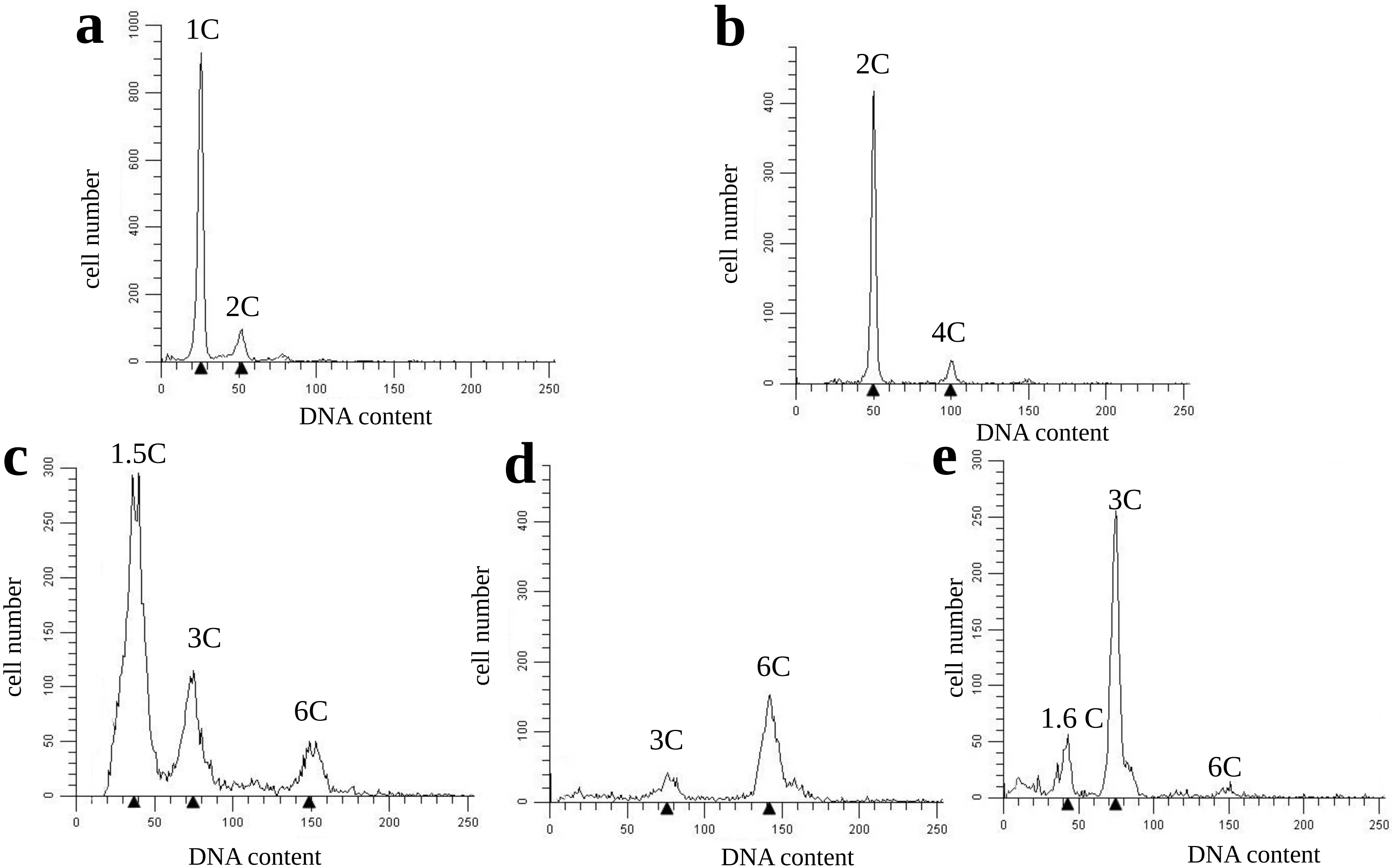
Table 1. Number of eggs, fertilization rate, hatching rate, normal rate and survival rate at 7 days after hatching in the different crosses using diploid, tetraploid and triploid (diploid female × tetraploid male) loaches in 2012 and 2014.

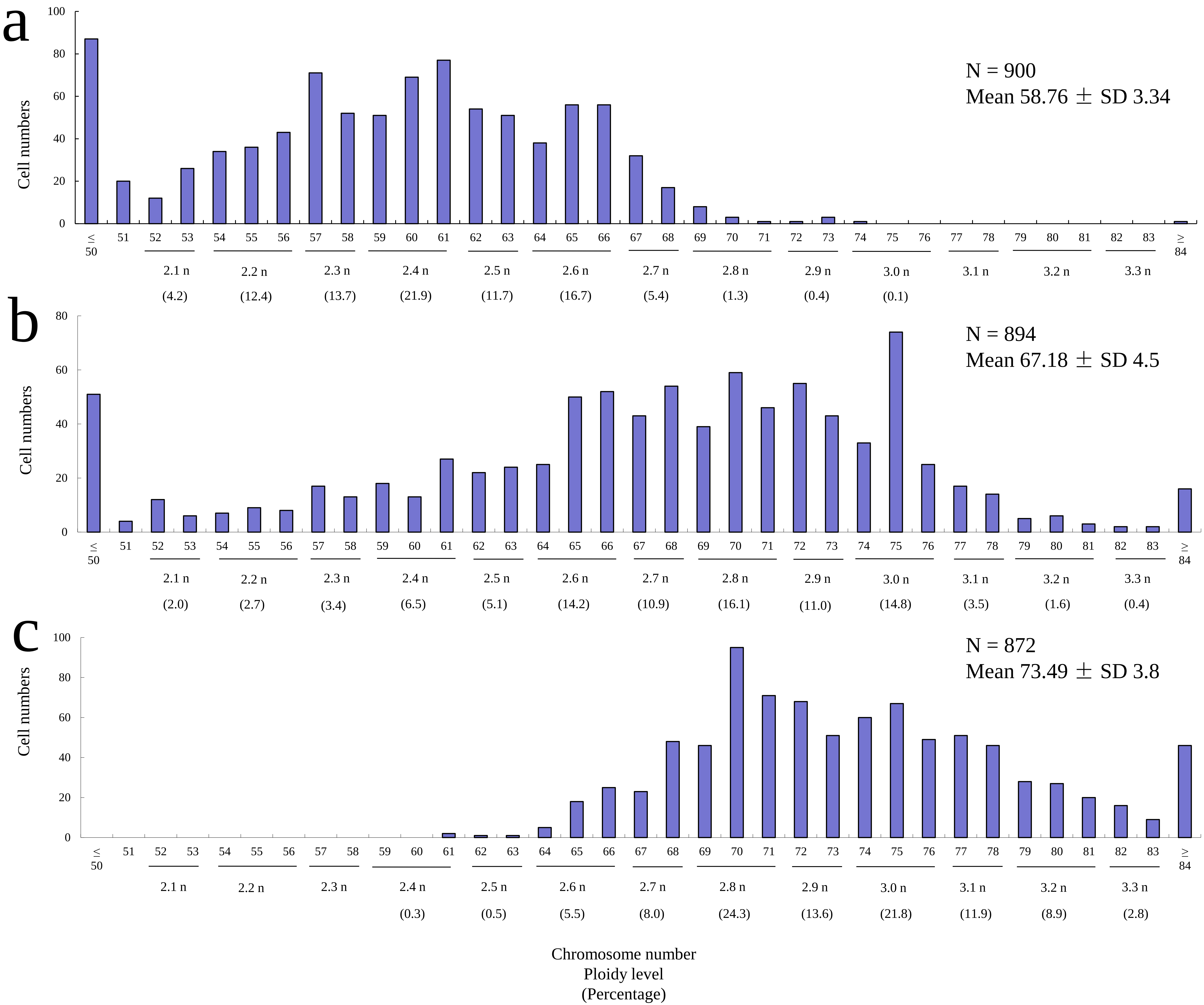
Year	Cross	No. of eggs	Fertilization rate (%)	Hatching rate (%)	Normal rate (%)	Survival rate at 7 days after hathcing (%)
2012	2n × 2n-1	1562	87.00	82.10	94.30	83.60
	2n × 2n-2	1663	87.90	80.60	91.70	83.50
	Mean ± SD		87.45 ± 0.64[a]	81.35 ± 1.06[b]	93.00 ± 1.84[a]	83.55 ± 0.07[d]
	2n × 4n-1	1509	80.73	89.25	96.22	92.31
	2n × 4n-2	770	82.21	86.21	96.65	91.55
	Mean ± SD		81.47 ± 1.05[a]	87.73 ± 2.15[ab]	96.44 ± 0.30[a]	91.93 ± 0.54[b]
	4n × 2n-1	1521	81.22	91.14	91.82	87.98
	4n × 2n-2	1021	80.13	89.11	92.21	87.33
	Mean ± SD		80.68 ± 0.77[a]	90.13 ± 1.43[a]	92.02 ± 0.28[a]	87.66 ± 0.46[c]
	4n × 4n-1	640	83.60	80.60	96.80	98.37
	4n × 4n-2	804	82.70	86.60	96.70	96.86
	Mean ± SD		83.15 ± 0.64[a]	83.60 ± 4.24[b]	96.75 ± 0.07[a]	97.62 ± 1.07[a]
	2n × 3n-1	566	57.10	32.00	56.00	31.90
	2n × 3n-2	1066	70.50	37.80	53.60	34.20
	Mean±SD		63.8±9.48[b]	34.9±4.10[c]	54.8±1.70[b]	33.05±1.63[e]
	3n × 2n-1	399	33.80	16.30	48.10	7.41
	3n × 2n-2	421	31.60	16.20	51.10	9.02
	Mean±SD		32.7±1.56[c]	16.25±0.07[d]	49.6±2.12[b]	8.22±1.14[f]
	3n × 3n-1	331	34.70	16.00	45.20	7.82
	3n × 3n-2	644	32.90	12.40	37.70	7.07
	Mean±SD		33.8±1.27[c]	14.2±2.55[d]	41.45±5.30[c]	7.45±0.53[f]
2014	2n × 2n-3	1329	87.13	88.17	91.77	82.56
	2n × 2n-4	1560	88.91	90.12	94.24	85.36
	Mean±SD		88.02±1.26[a]	89.15±1.38[a]	93.01±1.75[a]	83.96±1.98[a]
	2n × 3n-3	1458	58.30	55.29	56.6	3.41
	2n × 3n-4	1617	64.56	59.39	53.87	3.07
	Mean±SD		61.43±4.43[c]	57.34±2.90[c]	55.24±1.93[c]	3.24±0.24[b]
	3n × 2n-3	589	74.87	65.53	81.31	4.08
	3n × 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±1.47[b]	3.88±0.29[b]
	3n × 3n-3	667	55.62	52.02	47.67	3.50
	3n × 3n-4	656	58.69	57.66	53.15	4.42
	Mean±SD		57.16±2.17[c]	54.84±3.99[c]	50.41±3.87[c]	3.96±0.65[b]

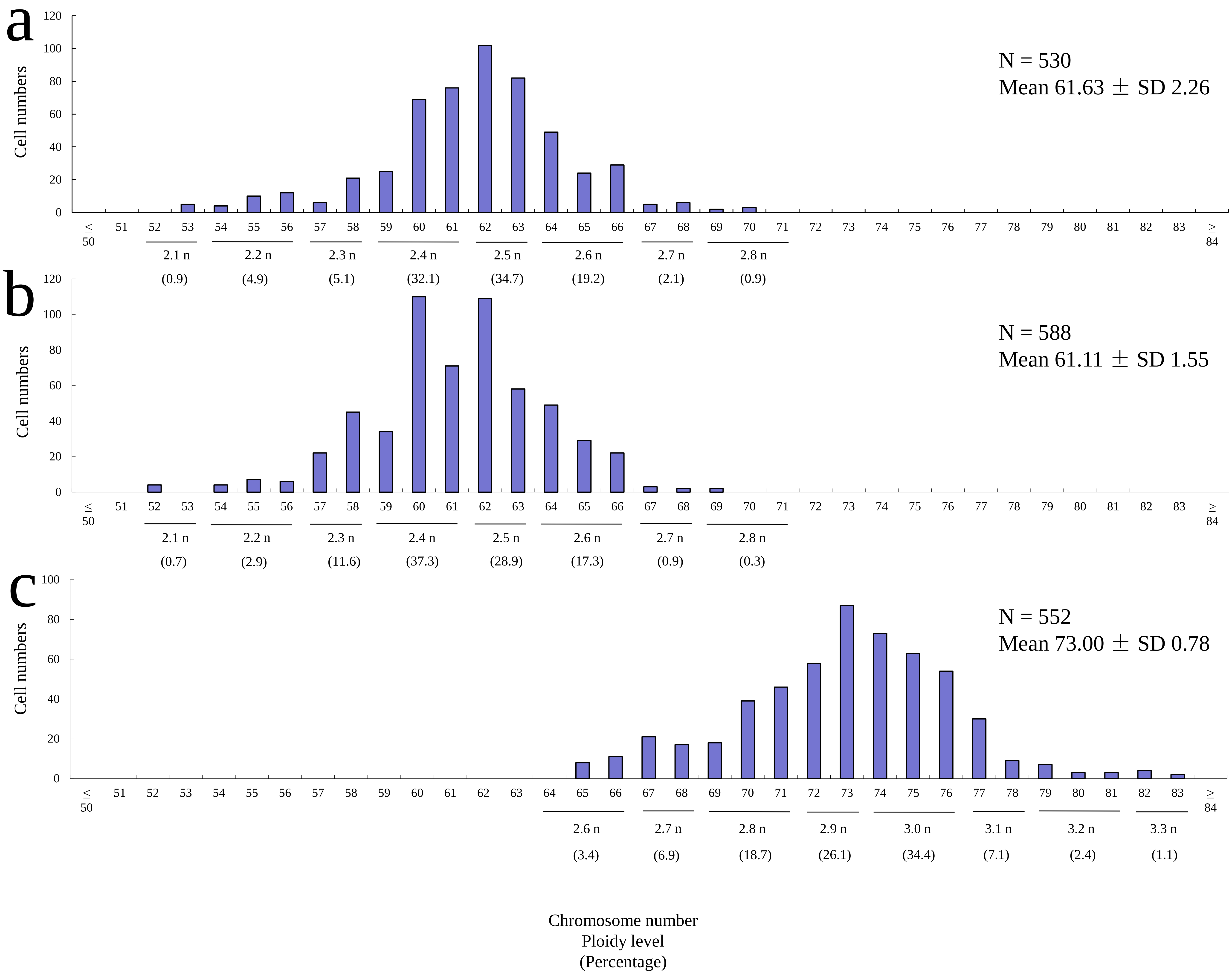
Note: Different lowercase letters in the same row indicated significant differences (P <0.05) among different crosses; and same letters indicated no significance (P >0.05).


a
b


a
b
M
SM
T
c
d
e
f
g
h
i


a
1C
2C
cell number
0
200
400
600
800
1000
0
50
100
150
200
250
DNA content
b
2C
4C
cell number
0
100
200
300
400
0
50
100
150
200
250
DNA content
c
1.5C
3C
6C
cell number
0
50
100
150
200
250
300
0
50
100
150
200
250
DNA content
d
3C
6C
cell number
0
100
200
300
400
0
50
100
150
200
250
DNA content
e
3C
1.6 C
6C
cell number
0
50
100
150
200
250
300
0
50
100
150
200
250
DNA content


a
N = 900
Mean 58.76 ± SD 3.34
2.1 n (4.2)
2.2 n (12.4)
2.3 n (13.7)
2.4 n (21.9)
2.5 n (11.7)
2.6 n (16.7)
2.7 n (5.4)
2.8 n (1.3)
2.9 n (0.4)
3.0 n (0.1)
3.1 n
3.2 n
3.3 n
b
N = 894
Mean 67.18 ± SD 4.5
2.1 n (2.0)
2.2 n (2.7)
2.3 n (3.4)
2.4 n (6.5)
2.5 n (5.1)
2.6 n (14.2)
2.7 n (10.9)
2.8 n (16.1)
2.9 n (11.0)
3.0 n (14.8)
3.1 n (3.5)
3.2 n (1.6)
3.3 n (0.4)
c
N = 872
Mean 73.49 ± SD 3.8
2.1 n
2.2 n
2.3 n
2.4 n (0.3)
2.5 n (0.5)
2.6 n (5.5)
2.7 n (8.0)
2.8 n (24.3)
2.9 n (13.6)
3.0 n (21.8)
3.1 n (11.9)
3.2 n (8.9)
3.3 n (2.8)
Cell numbers
Chromosome number
Ploidy level
(Percentage)


a
Cell numbers
0
20
40
60
80
100
120
N = 530
Mean 61.63 ± SD 2.26
≤ 50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
≥ 84
2.1 n
2.2 n
2.3 n
2.4 n
2.5 n
2.6 n
2.7 n
2.8 n
(0.9)
(4.9)
(5.1)
(32.1)
(34.7)
(19.2)
(2.1)
(0.9)
b
Cell numbers
0
20
40
60
80
100
120
N = 588
Mean 61.11 ± SD 1.55
≤ 50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
≥ 84
2.1 n
2.2 n
2.3 n
2.4 n
2.5 n
2.6 n
2.7 n
2.8 n
(0.7)
(2.9)
(11.6)
(37.3)
(28.9)
(17.3)
(0.9)
(0.3)
c
Cell numbers
0
20
40
60
80
100
N = 552
Mean 73.00 ± SD 0.78
≤ 50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
≥ 84
2.6 n
2.7 n
2.8 n
2.9 n
3.0 n
3.1 n
3.2 n
3.3 n
(3.4)
(6.9)
(18.7)
(26.1)
(34.4)
(7.1)
(2.4)
(1.1)
Chromosome number
Ploidy level
(Percentage)

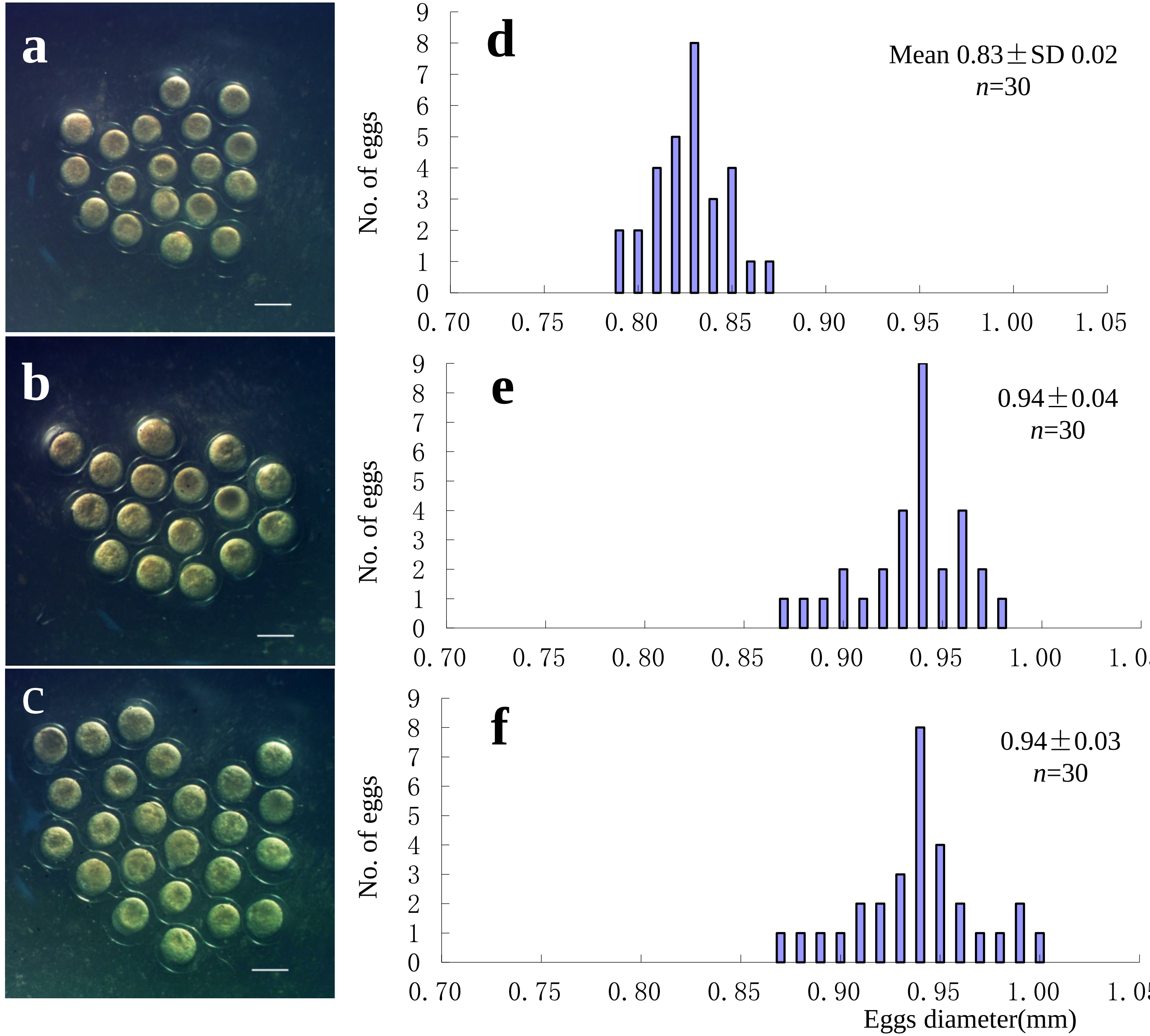


Fig. S1 External appearance (a-c) and diameters (d-f) of eggs from three crosses of loaches (a, d:2n female × 2n male-1, b, e: 3n × 2n-1, and c, f: 3n × 3n-1) at 30 min after fertilization. Scales denote 1 mm.

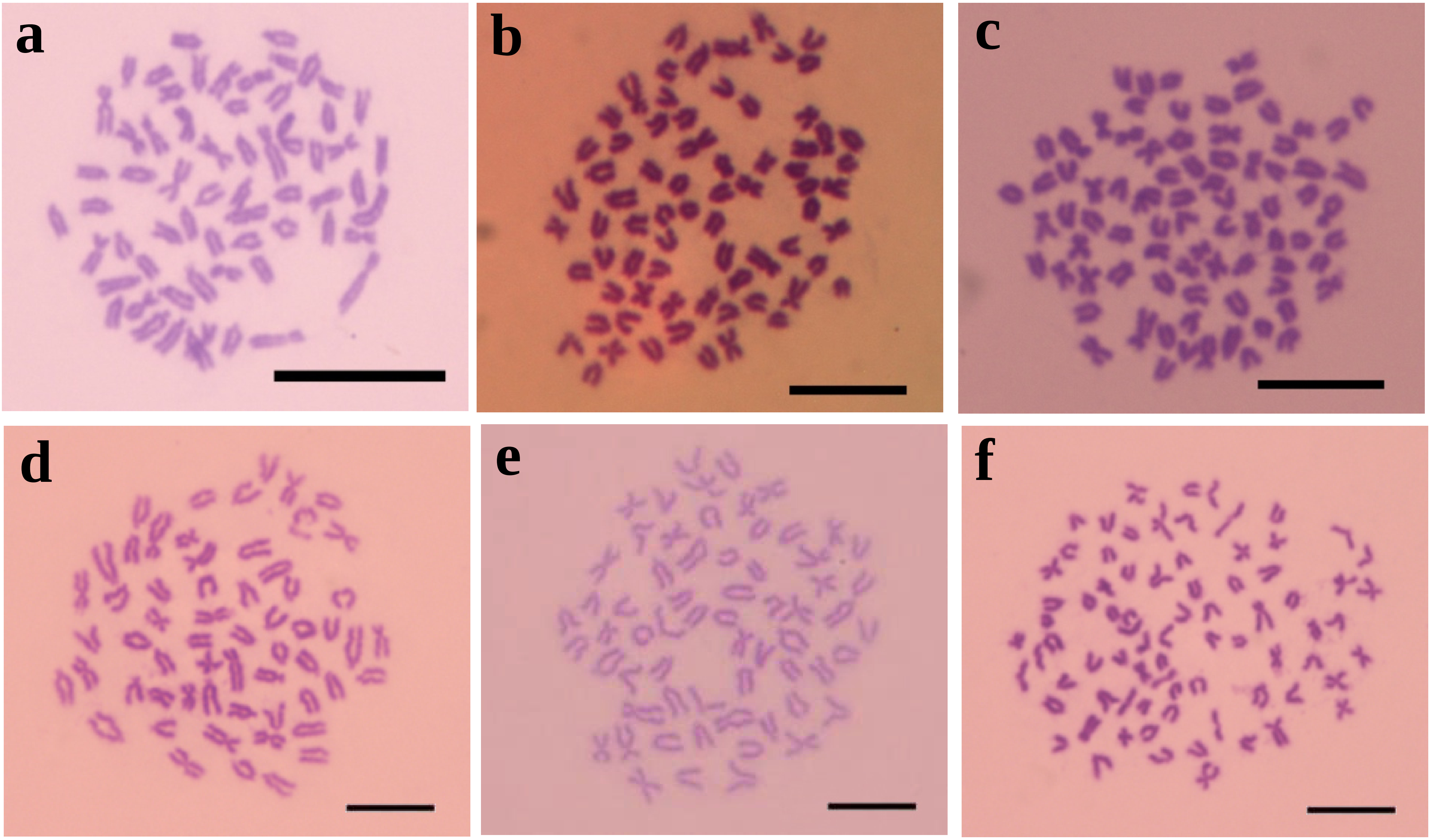


Fig. S2 Metaphase spreads observed in embryos from 2n × 3n (a, d), 3n × 2n (b, e) and 3n × 3n crosses (c, f). (a) Chromosome number (CN) = 62, 7m (metacentric chromosomes) + 12sm (submetacentric chromo.) + 43 t (telocentric chromo.) from 2n × 3n-1 cross; (b) CN = 71, 13m + 6 sm + 52t from 3n × 2n-1 cross; (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 × 3n-3 cross. Scales denote 10 μm.

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

cross	Chromosome no.	Embryo 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 × 2n	47	2	2	1	1	4	3	3	2	2	2	0	4	1	1	4	1	0	3	2	2	40
	48	4	5	7	4	6	2	3	6	4	4	7	5	2	0	3	5	6	2	5	8	88
	49	6	2	4	8	4	3	2	9	4	5	5	5	2	1	5	8	2	2	2	2	81
	50	6	6	11	11	5	17	21	15	17	14	18	14	19	20	13	13	12	18	2	14	266
	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 × 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	0	0	0	0	2	1	1	0	2	0	0	0	2	8
	total	27	30	30	30	30	30	30	30	30	28	29	30	30	30	30	30	30	30	30	30	594
	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 × 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
	94	0	0	0	0	0	3	1	2	0	1	0	0	0	2	1	0	1	2	0	1	14
	95	2	1	1	1	1	0	1	1	0	1	2	0	1	2	3	2	0	0	2	3	24
	96	1	0	0	4	1	2	5	2	0	1	2	1	2	1	1	1	0	1	0	0	25
4n × 4n	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
	101	0	0	1	1	1	0	0	0	2	1	1	1	0	2	1	1	1	1	1	0	15
	102	0	0	0	2	1	1	0	0	1	1	0	1	0	1	1	0	1	1	1	0	12
	total	22	21	20	26	22	29	20	23	20	22	30	21	20	28	23	23	22	25	24	22	463

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

Ploidy level	Chromosome number	Embryo number																															Sum (%)
		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	
1.3n	33															1																1	1(0.1)
	34																															0	
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	15(1.7)
	40		1												1								1									3	
	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				7	
1.8n	44			1						1				1	1			1					1									6	19(2.1)
	45																							2		1						3	
	46		1							1	1						1		1	1		1		1		1	1					10	
1.9n	47		1	1							1							1		1												5	19(2.1)
	48																						1		1	3	1					6	
	49										1	1			1		1		1				1				1			1		8	
2.0n	50				1				1		1	1				1	1					1					1				1	9	29(3.2)
	51								1	1	1			1		1			2	1	1	1	2			2		1	1	4		20	
2.1n	52										1	1						1	1			2				3	1	1		1		12	38(4.2)
	53		1			2			2			1			2	1	1			2	2	2	2	2	1		1		2		2	26	
2.2n	54						1		3	1	2			1		1	1	1		1	2	4	3	4		1	3	1	3	1		34	112(12.4)
	55			1	1	2			1	1	2	3			1		2	2	1				6	2		3	2		3	3		36	
	56			1		1	2		3	1					1	1	3	6	1	1	3	6	3	2		4	1		2	1		43	
2.3n	57		2	3	1	1	2		19	2	1				4	1	1	2	1	3	3	4	3	1		5	2	2	2	3	3	71	123(13.7)
	58	1		2		3	2	3		1	4		1	3	1	1	3	3	2		3	2	2	3		3	2	1		4	2	52	
2.4n	59	1		2	2		3	5		2	3		1		4			3	3	1		1		1	2	1	1		5	5	5	51	197(21.9)
	60	1		1	1	6		11			5		4			1	2	1	5	3	1	3		5	5	1	4		1	3	5	69	
	61	1			1	6	2	6		1	1	1		9	5	1	2	5	3	2	5	1		2	9		2	2	5	1	4	77	
2.5n	62	1		3	1	7		4		1	1		4	1			4	2	2	1	3	1		3	4		2	2	3	1	3	54	105(11.7)
	63	7	6		1	1	5			1	2	1	1	3	3	2		1	1	4	4			1	2		1	1			3	51	
2.6n	64	6	4	3	1		4			2		2	4		1	3	1		1		1				4			1				38	150(16.7)
	65	1	4	2	3		3			3	1	4	12	5	1	1	2		2	2	1	1			2		1	5				56	
	66	8	8		3		2	1		4		8	2	3	1	7				2	1							6				56	
2.7n	67	2	1	2	3		4			4	1	3	1	2		4				1								1	2		1	32	49(5.4)
	68	1		1	6					2		2				3	1											1				17	
2.8n	69			1	2					1		1		1														2				8	12(1.3)
	70			2	1																											3	
	71																											1				1	
2.9n	72																														1	1	4(0.4)
	73			3																												3	
3.0n	74			1																												1	1(0.1)
	75																															0	
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 S3 Distribution of chromosome numbers and ploidy levels in embrys from 3n x 2n-1 cross

Ploidy level	Chromosome number	Embryo number																														Total	Sum(%)
		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		
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	4(0.4)
	40						1							1																		2	
	41		1																													1	
1.7n	42					1	1			1	1			1																		5	9(1.0)
	43						1				1			1												1						4	
1.8n	44						1						1																			2	10(1.1)
	45				1					1															1							3	
	46										1							1		1			1			1						5	
1.9n	47	1				1				1								2		1					1							7	17(1.9)
	48				1						1		1													1						4	
	49					1	2	1																		2						6	
2.0n	50						1	1			1			1										1								5	9(1.0)
	51						1				1				1														1			4	
2.1n	52							1	1	1		1	3	1					1	1						1			1			12	18(2.0)
	53		1	1				1				1		1	1																	6	
2.2n	54		1						1		1	1										1			2							7	24(2.7)
	55				2	1											2	1		1			2									9	
	56	1					1				1	1			1					1				1		1						8	
2.3n	57	2					1	3		1					1						1		1	1		1	1		2		2	17	30(3.4)
	58				1		1				1						3			1		1				2			2		1	13	
2.4n	59		1	1	1		1	1		2	2		1		2		1						1		1	2			1			18	58(6.5)
	60						1			1	1	2	1		2		1	1											1		2	13	
	61		1		2		2	2			3	1			1			3	1	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	22	46(5.1)
	63				1			2	1		1		1		1	1	1	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	127(14.2)
	65	1		3	1	1	3	5			2	2			3	1	3	4		2	2	1	4			4	1	1	3		3	50	
	66	1		1		2		1	2	3	2	1	2		1	1	5	1		2	1	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	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	144(16.1)
	70	2	4	2	1		1	1		3	2	3	2	2	5	2	1	1	3	4		1			5	2	1	9		1	1	59	
	71		4		2	3	1		2	2		1		3	1		1		5	4	2	1		1	4		5	4				46	
2.9n	72	4		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				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	132(14.8)
	75	2	2	3	9	5			6			1		6		3		1	7		8	3		6			6			6		74	
	76	3		3	4				2			3				3			1			3					1			2		25	
3.1n	77	1		1	1									1		2	1		1			4								5		17	31(3.5)
	78	4		1	1				1				1			1						1	1						1	2		14	
3.2n	79			1					2							1						1										5	14(1.6)
	80			2		1																1	1				1					6	
	81															1						1		1								3	
3.3n	82																1										1					2	4(0.4)
	83			1												1																2	
3.4n	84															1											1					2	7(0.7)
	85												1									1										2	
	86																			1							1			1		3	
3.5n	87			1																		1										2	3(0.3)
	88																		1													1	
3.6n	89	1																														1	2(0.2)
	90																													1		1	
3.7n	93																							1								1	1(0.1)
3.8n	95														1																	1	3(0.3)
	96	1																						1								2	
	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	28	30	30	26	30	894	

Table S4. Distribution of chromosome numbers and ploidy level in embryos from the 3n x 3n cross

Ploidy level	Chromosome 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	Sum(%)
		Embryo number																															
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	48(5.5)
	65		3		3	3															2				3				4			18	
	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	
2.8n	69		2	5	2	1		5	2								2			10	1		8		3				3	1		45	212(24.3)
	70	4	1	6	2	3		7	10	1			4			1	3	6	2	7	3	8	8		5			1	1	12	1	96	
	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	119(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	
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	190(21.8)
	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	
	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	
3.2n	79	1	2			1	1			1	1	1		1	2	7								4		2	3			1	1	29	78(8.9)
	80		1								3	3	1	3	3	4						1		3		3	2		1		1	29	
	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	
3.4n	84											2	1																			3	8(0.9)
	85		1													1																2	
	86	2																							1							3	
3.5n	87	1										2	1																			4	6(0.7)
	88															1											1					2	
3.6n	89								1										1													2	4(0.5)
	90																										1		1			2	
	91																															0	
3.7n	92																															0	1(0.1)
	93														1																	1	
3.8n	94																															0	1(0.1)
	95								1																							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	

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

Ploidy level	Chromosome number	Embryo number																					Sum(%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Tota	
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	
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	
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	
2.8n	69											1				1						2	5(0.9)
	70			1							1										1	3	
	Total	30	22	28	22	27	25	25	23	30	28	30	25	22	30	30	27	26	27	23	30	##	

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

Ploidy level	Chromosome number	Embryo number																					Sum(%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total	
2.0n	50																					0	0(0)
	51																					0	
2.1n	52			2					1					1								4	4(0.7)
	53																					0	
2.2n	54								2					1				1				4	17(2.9)
	55			2	2				1				1	1								7	
	56				1	1	1		2	1												6	
2.3n	57	1		1	5	1			2	6		2					1	1			2	22	67(11.6)
	58			6	10	1	2	1	3	7		1	1	4			1	3			5	45	
2.4n	59	5		7	2	1	1		1	2		2	2	5	1		1		1	1	2	34	215(37.3)
	60	11	4	7	5	21	7	1	5	7	6	2	3	4		2	6	7	4	2	6	110	
	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	167(28.9)
	63		9				2	4	1		1		5	1	3	7	5	2	6	11	1	58	
2.6n	64		2			1	2	8		2	3		5	3	12	2	1	1	4	1	2	49	100(17.3)
	65		1				1	8				1			6	5	2		3	2		29	
	66							6		1	2	1	2	1	5	2	2					22	
2.7n	67							2						1								3	5(0.9)
	68														1				1			2	
2.8n	69																		1		1	2	2(0.3)
	70																					0	
	Total	22	30	30	30	30	30	30	25	30	30	30	29	28	30	30	30	27	30	30	26	577	

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

Ploidy level	Chromosome number	Embryo number																					Sum (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Tota	
2.6n	65			2			2	1	1					1						1		8	19(3.4)
	66	2		1			1						1	1	2	1			1		1	11	
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	
2.8n	69	1		2		3		2	1		1	1			1	4	1			1		18	103(18.7)
	70	3	2	6	1	1	1	3	2	2			2	4		3	1		3	3	2	39	
	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	
3.0n	74	6	6		4		4	2	4	10	1	6	3	5	5	2	2	3	3	2	5	73	190(34.4)
	75	1	2			5	2	3	7	3	13	5	2		2		4	7	4		3	63	
	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	
3.2n	79	1				1			1		1		1		1			1				7	13(2.4)
	80						1						1								1	3	
	81											1			1				1			3	
3.3n	82						2									1		1				4	6(1.1)
	83										1		1									2	
	Total	27	23	26	20	25	29	30	26	24	27	30	26	30	30	30	29	30	30	30	30	##	