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2   The occurrence of hyper-tetraploid and other unusual polyploid loaches *Misgurnus anguillicaudatus*

3   among market specimens in Japan

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22 **Abstract**

23 Exotic animals may cause a genetic contamination of indigenous species if they escape and reproduce in

24 wild populations. Loach *Misgurnus anguillicaudatus* and its related species have been imported to Japan

25 for commercial uses. We collected live loach specimens from central wholesale market in Tokyo. Among

26 451 specimens, ploidy status was examined by DNA content flow cytometry and polyploid loaches with

27 triploid, tetraploid and other higher DNA content ranges were detected. Hyper-triploid and

28 hyper-tetraploid individuals could be easily detected by flow cytometry using a standard eudiploid,

29 eutriploid and eutetraploid controls and then reproductive capacity of these hyper-polyploid males was

30 examined. Sperm of hyper-triploid males did not exhibit active progressive motility and major

31 populations of spermatozoa or spermatozoon-like cells were detected in triploid and hexaploid ranges.

32 Motile haploid spermatozoa were very few in sperm from hyper-triploid males. Therefore, hyper-triploid

33 males were sterile, whereas hyper-tetraploid males produced fertile hyper-diploid spermatozoa with active

34 progressive motility after contact with ambient water. Viable progeny occurred in the cross between

35 normal wild-type diploid female and hyper-tetraploid males, but androgenotes induced by fertilization of

36 UV-irradiated eggs with sperm of hyper-tetraploid males were inviable hyper-diploid. Cytogenetic

37 analyses in such androgenotes indicated that hyper-tetraploid males should produce hyper-diploid

38 spermatozoa with 2n=54, i.e. presence of four supernumerary micro-chromosomes.

39 **Keywords**   Androgenesis・Aneuploidy・Conservation・Euploidy・Exotic species

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Introduction

Exotic live fishes, which are introduced for foods, live baits of fishing, aquarium animals and other purposes, often threaten biodiversity of indigenous ecosystems, when they escape into the wild. Genetic disturbances have been strongly suggested in Japanese wild populations of medaka [1] and bitterlings [2]. Most individuals of *M. anguillicaudatus* are sexually reproducing diploid organisms with 50 chromosomes (2n=50) in Japanese wild populations, but gynogenetically reproducing clonal diploid (2n=50) and clone-origin triploid (3n=75) individuals sympatrically occur in a few wild populations of northern Hokkaido and Noto Peninsula in Ishikawa Prefecture, Honsyu Island, Japan [3, 4]. In other localities, frequencies of natural triploid loach were generally low except for several places [5, 6]. Although tetraploid loaches with 100 chromosomes (4n=100) are distributed along Changjiang river system in central China together with diploid and triploid individuals [7-11], so far natural tetraploid individuals have not been found in Japanese wild populations [3-6]. Therefore, tetraploid individuals, which have been detected in Japanese market samples since 1970s [12, 13], are likely to be Chinese loaches imported to Japanese markets. Such an occurrence of tetraploid loaches in Japanese market samples suggests simultaneous import of sympatric diploid and triploid Chinese loaches to Japan.

Inferred from mitochondrial (mt) DNA sequences analyses, individuals of Japanese *M. anguillicaudatus* can be categorized into three different groups: A, B-1 and B-2 in Morishima et al. [4] or A, B and C in Koizumi et al. [14]. Arai [6] suggested that the group B-2 in [4] might be originated or strongly related to Chinese loach population. Koizumi [15] came to the same conclusion about the B group in [14] distributed in Japanese wild populations. Introduction of exotic individuals into natural waters in Ehime Prefecture, Shikoku Island was recently indicated by results of partial mtDNA analysis

64 [16]. These results suggest that genetic disturbance might occur by intra-specific hybrid event.

65 Inter-populational hybridization between genetically different groups in *M. anguillicaudatus* resulted in

66 disruption of regular meiosis and normal gametogenesis, causing formation of unreduced diploid and

67 other unusual aneuploid and polyploidy eggs in females [17] and sterility in males [18]. Molecular

68 genetic relationship between Japanese and Chinese loach populations is still fragmentary and

69 inconclusive, and it is impossible to distinguish them based on morphological characteristics. However,

70 polyploidy and other unusual cytogenetic characteristics are indicators of the cryptic invasion of exotic

71 loaches to find imported individuals among the market specimens.

72   In the present study, we obtained loach samples from the central wholesale market in Tokyo and

73 examined ploidy status by DNA content flow cytometry in order to find exotic loach individuals. We

74 found hyper-triploid, hyper-tetraploid and other unusual polyploids. Furthermore, the motility and ploidy

75 status of sperm from hyper-triploid and hyper-tetraploid males were examined. Normally-fertilized and

76 androgenetically induced progeny were then produced by using sperm of hyper-tetraploid males to

77 investigate their reproductive capacity.

78

79 **Materials and methods**

80 Fish specimens

81 A total of 451 loach individuals were obtained from the wholesale market in Tokyo from March to April,

82 2011. According to the fish dealers, these farmed animals were imported as food from Taiwan, but they

83 did not know their exact origin, and aquaculture conditions remain unknown. These specimens were

84 identified as *M. anguillicaudatus* on the basis of external appearance and kept in the Aquarium room of

85   the Environment Control Experiment Building, the Faculty and Graduate School of Fisheries Sciences,

86   Hokkaido University.

87

88   Flow cytometry and ploidy determination

89   The ploidy status was determined as relative value in each individual by measuring DNA content using a

90   flow-cytometer (Ploidy Analyzer, Partec GmbH, Münster, Germany) as described previously [3]. Briefly,

91   a fin clip of each specimen was suspended in 200 µl of solution A (Cystain DNA 2step, Nulei Extraction

92   solution, Partec GmbH) and incubated at room temperature for 20 min. The mixture was filtered with a

93   50-µm mesh (Cell Trics, Partec GmbH) and stained for 5 min with Solution B (Cystain DNA 2step, DAPI

94   (4'-6-diamidino-2-phenylindole) Staining solution, Partec GmbH). Ploidy status was determined by

95   relative DNA content of the target samples with the standard DNA content of eudiploid, eutriploid and

96   eutetraploid specimens. The normal wild-type eudiploid loach was taken from Kitamura, Iwamizawa City,

97   Hokkaido. The eutriploid sample was produced by fertilizing eggs of a normal wild-type female with

98   diploid sperm of a sex-reversed clonal loach [19]. Diploid sperm was considered to be formed by

99   premeiotic endomitosis (chromosome doubling in early spermatogonia and subsequent quasi-normal

100   meiotic divisions) in sex-reversed clonal diploid loach [20]. As standard control of eutetraploidy,

101   neo-tetraploid loach was induced by fertilizing eggs of normal wild-type female with diploid sperm of a

102   natural tetraploid male obtained in market samples, followed by inhibition of the second polar body

103   release shortly after fertilization [21].

104

105   Motility and ploidy of sperm

106 Based on DNA content flow cytometry of samples, four hyper-triploid males (hyper-3n-#1, hyper-3n-#2,

107 hyper-3n-#3 and hyper-3n-#4) and three hyper-tetraploid males (hyper-4n-#1, hyper-4n-#2 and

108 hyper-4n-#3) were sorted for further examination on reproductive capacity. Then, maturation was induced

109 by injecting hCG (human Chorionic Gonadotropin, Aska Pharmaceuticals, Tokyo) [22]. Fish were

110 maintained at 27℃ for 12 h, and then sperm was collected in hematocrit glass tube by gently squeezing

111 the abdomen. Ploidy status of sperm sample from each specimen was assayed by flow cytometry as

112 described in the previous study [23]. The sperm motility was analyzed with a video recorder (Sharp VHS

113 VC-HF920) according to procedures previously reported for loach [23].

114

115 Production of progeny

116 To verify the chromosome number of each specimen, we fertilized UV-inactivated eggs in order to

117 produce androgenetic progeny. Androgenetic individuals do not survive for long beyond first feeding

118 stage (3 to 4 days after hatching) due to so-called "haploid syndrome" although diploid individuals

119 develop normally into adults. To induce androgenetic progeny, mature eggs from the normal wild-type

120 diploid female were genetically inactivated by UV irradiation (150 mJ/cm^2) according to the procedures

121 previously optimized by Fujimoto et al. [24]. Irradiated eggs were then fertilized with the sperm from one

122 normal wild-type diploid male (eudiploid control producing 1n sperm), one neo-tetraploid male

123 (eutetraploid control producing 2n sperm), and three hyper-tetraploid males (hyper-4n-#1, hyper-4n-#2

124 and hyper-4n-#3). At the same time, putative triploid progeny were produced by fertilizing

125 non-inactivated eggs from the same female with sperm from the same neo-tetraploid and hyper-tetraploid

126 males. Fertilized eggs were reared in dechlorinated tap water at 20℃. Fertilization rate was percentage of

127   cleaved eggs at 8 to 64-cell stage in total eggs used for fertilization. Cleaved eggs were clearly observable

128   in 8 to 64-cell stages. Hatch rate was percentage of hatched larvae in fertilized eggs. Normal rate was

129   percentages of hatched larvae with normal external appearance.

130

131   Ploidy and chromosomes of progeny

132   The relative DNA contents of hatched larvae were measured by flow cytometry as described previously

133   [24]. Embryos at the 10-20 somite stage were randomly selected from different crosses for chromosome

134   preparation. Yolk sacs were mechanically removed from the embryos using fine forceps and the embryos

135   were then treated with 0.0025% colchicine in Ringer's solution for 30 min. The embryos were then placed

136   in 0.075M KCL for 25 min and fixed with Carnoy's solution (1 acetic acid : 3 methanol). Chromosome

137   preparations were made and chromosome numbers per metaphase plate were counted as described in

138   Inokuchi et al. [25]. Chromosomes were classified according to Levan et al. [26].

139

140   **Results**

141   By flow cytometry, loach specimens (total n=451) were categorized into diploid range (n=366, 81.2%),

142   triploid range (n=52, 11.5%),　tetraploid range (n=30, 6.7%), pentaploid range (n=1, 0.2%), hexaploid

143   range (n=1, 0.2%) and heptaploid range (n=1, 0.2%) (Fig. 1, Electronic Supplementary Material(ESM)

144   Table S1). Although eudiploid, eutriploid and eutetraploid had just twice, three times and four times DNA

145   content of normal haploid genome size, respectively, clear hyper-triploid (Fig. 2a) and hyper-tetraploid

146   (Fig 2b) were recognized when compared with control triploid or tetraploid (neo-tetraploid) sample.

147   Detection of hyper-diploid individuals and hypo-polyploid individuals has not been successfully

148   completed in the present study, because of the difficulty to distinguish them from variances of fluorescent

149   peak(s) in flow cytometrical histograms.

150   Although normal sperm exhibited only haploid cell population (Fig. 3a), haploid, triploid and

151   hexaploid-range cell populations were detected in sperm from hyper-triploid males (Fig. 3bc). Size of

152   each cell population was different, but hexaploid-range cell population was always dominant in any

153   hyper-triploid male (Fig. 3bc). Spermatozoa or spermatozoon-like cells with relatively bigger head sizes

154   did not exhibit active progressive motility after contact with ambient water, but the spermatozoa with the

155   smallest head sizes sometimes showed relatively active movement.

156   On the other hand, hyper-tetraploid males (hyper-4n-#1, hyper-4n-#2 and hyper-4n-#3)

157   demonstrated vigorous progressive motility of spermatozoa, as normally observed in haploid sperm of

158   wild-type diploid males after activation. The relative DNA content of spermatozoa from these

159   hyper-tetraploid males was half of their corresponding somatic cells and higher than that of normal

160   diploid spermatozoa from neo-tetraploid loach (Fig. 4), thus hyper-tetraploid males were apparent to

161   produce hyper-diploid spermatozoa.

162   When the eggs of a normal wild-type diploid female were fertilized with sperm from a normal

163   wild-type diploid male, 98.4% eggs were successfully cleaved and 90.6% hatched (Table 1). DNA content

164   flow cytometry indicated that the resultant progeny were diploids. In the cross between normal diploid

165   female and neo-tetraploid male, viable triploid progeny appeared (Fertility, 94.3%; Hatch, 92.2%; Normal,

166   94.5%; Table 1, Fig. 5a). In the cross between normal diploid female and hyper-tetraploid males, normal

167   larvae with triploid range DNA content appeared and viabilities were not different from the result used

168   neo-tetraploid male (Fertility, 90.6–95.8%; Hatch, 85.0–90.3%; Normal, 84.3–92.8%; Table 1, Fig. 5b).

169   When UV-irradiated eggs of normal diploid female were fertilized with normal sperm of wild-type male,

170   inviable haploid progeny appeared by induced androgenesis (Fertility, 90.3%; Hatch, 28.3%; Normal, 0%;

171   Figure not shown). Androgenetic progeny developed from the cross between UV-irradiated eggs of

172   normal diploid female and diploid sperm of neo-tetraploid males were viable diploid with high percentage

173   of normally hatched larvae exhibiting normal external appearance (Fertility, 95.1%; Hatch, 35.6%;

174   Normal, 90.4%; Table 1, Fig 5c). On the other hand, no viable progeny occurred in the crosses between

175   UV-irradiated eggs of normal diploid female and sperm of hyper-tetraploid males (Fertility, 80.2–90.7%;

176   Hatch, 25.2–34.7%; Normal, 0%; Table 1) and abnormal larvae exclusively occurred in androgenetically

177   activated eggs (Fig. 5d).

178   DNA content of viable progeny between normal diploid female and neo-tetraploid male was just

179   1.5 times higher of that of somatic diploid cells and thus showed eutriploidy in these progeny (Table 1,

180   Fig. 6a). Androgenetic progeny developing from sperm of neo-tetraploid male gave just eudiploid DNA

181   content (Fig. 6b). Eudiploidy of these androgenetic progeny was also verified by cytogenetic analysis:

182   modal chromosome number was 2n=50 in the UV-irradiated eggs of normal diploid female × sperm of

183   neo-tetraploid male cross (Table 2, Fig. 7ab). On the contrary, DNA content of viable progeny between

184   normal diploid female and hyper-tetraploid males was slightly larger than that of eudiploid progeny (Fig

185   6c). Similarly, slightly larger DNA content was also detected in progeny from UV-irradiated eggs of

186   normal diploid female × sperm of hyper-tetraploid males (Fig. 6d). These flow-cytometrical results

187   indicated that hyper-tetraploid males generated hyper-diploid spermatozoa. Hyper-diploidy of

188   spermatozoa of hyper-tetraploid males was also supported by modal chromosome number of 2n=54 found

189   in androgenetic progeny (Table 2). Most frequent diploid cell in the androgenetic progeny of

190 hyper-tetraploid males exhibited four supernumerary micro-chromosomes besides regular members of 50

191 chromosomes (Fig 7cd).

192

193 **Discussion**

194 In the present study, we detected various kinds of loaches with diploid, triploid, tetraploid, pentaploid,

195 hexaploid and heptaploid-range DNA content among Japanese market samples. The occurrence of

196 polyploid loaches is not surprising, because diploid, triploid and tetraploid loaches are sympatrically

197 distributed in wild populations along Changjiang river system in central China [7-11], and natural triploid

198 loaches have been detected among wild populations in Japan [3-6]. Recently, wild hexaploid loach with

199 6n=150 chromosomes was also discovered in China [27]. Viable hexaploid loaches, which were

200 experimentally induced from the cross-breeding between natural tetraploids followed by inhibition of the

201 second polar release after fertilization, exhibited viability as well as fertility, and tetraploid, pentaploid

202 and hexaploid progeny were produced by cross-breedings using fertile triploid gametes of the mature

203 hexaploid loaches[28]. All these facts indicate that loach species can tolerate an elevation of genome size

204 or ploidy status, in terms of surviving and reproductive capacity.

205 However, frequencies of euploid loaches were low in our present samples, and most individuals

206 were considered aneuploids. Viable hyper-diploid loaches (2.2n-2.5n) were successfully produced by

207 fertilization of eggs from wild-type diploids with aneuploid sperm from artificially induced triploids [29].

208 Adult-size hyper-diploid loaches with 2n=51, 52, 53 and 58, comprising additional telocentrics and

209 supernumerary micro-chromosomes, were found in families produced by the cross-breeding of wild-type

210 [30]. Hyper-triploid progeny (3.1n-3.8n) which were produced by cross-breeding between natural

211   tetraploid females and artificially induced triploid males were also viable [31]. These previous results

212   suggest that loach species can also tolerate hyperploidy comprising extra or supernumerary chromosomes.

213   The occurrence of such hyperploid loaches in market samples also suggests that hyperploid loaches

214   should have survival capacity, but it was difficult to distinguish hypoploid individuals from artifact in the

215   present study.

216   In our study, hyper-triploid males were shown to be sterile, because they produced no fertile

217   spermatozoa except for few cases. On the contrary, hyper-tetraploids produced motile hyper-diploid

218   spermatozoa, and crosses between diploid wild-type female and hyper-tetraploid males gave viable

219   progeny. Thus, hyper-tetraploid males are considered fertile.

220   When UV-irradiated eggs of wild-type were fertilized with sperm of hyper-tetraploid males,

221   androgenetic progeny were induced, and the resultant androgenetic hyper-diploid progeny exhibited four

222   supernumerary micro-chromosomes (2n=54) when compared with wild-type eudiploid (2n=50). Although

223   hyper-diploid loaches in previous studies were all viable [29, 30], these androgenetic hyper-diploid

224   progeny exhibited abnormal appearance and could not survive beyond feeding stage. At present, however,

225   we could not explain the genetic and cytogenetic reason why these androgenetic hyper-diploid progeny

226   were inviable and externally abnormal. Simple explanation may be deleterious influences of

227   supernumerary chromosomes on survival capacity of induced androgenetes.

228   In the present study, we reported sterility of hyper-triploid males and fertility of hyper-tetraploid

229   males found in Japanese market samples. Although reproductive capacity of such unusual aneuploid

230   females has not been investigated yet, it is noteworthy that fertile exotic hyper-tetraploid males may cause

231   genetic disorders on the wild populations when they escape to invade Japanese waters. Very small number

232   of higher-polyploids exhibiting DNA content corresponding to pentaploid, hexaploid and heptaploid

233   range also appeared in market samples, but reproductive capacity of these higher polyploids was not

234   examined herein. Further investigation on genetic and reproductive capacity of these higher polyploid

235   loaches is required in future to assess the risk of exotic species. The other important problem is the exact

236   origin of market samples. To identify the origin, mitochondrial DNA haplotypes and nuclear DNA

237   genotypes are also required to analyze in near future. The mechanism responsible for the occurrence of

238   aneuploids and higher polyploids are also enigmatic. Random mating among different fertile (hyper- or

239   hypo-) polyploid individuals should be imaginable.

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337 **Figure captions**

338 **Fig. 1** Histograms showing the number of specimens with different ploidy status inferred from relative

339 DNA content estimated by flow cytometry

340 **Fig. 2** Comparison of flow-cytometrical histograms between control eudiploid and hyper-triploid/

341 hyper-tetraploid in somatic cells. Fluorescent peak 1 of normal diploid loach indicates 2C DNA

342 content; fluorescent peak 2 of control triploid loach indicates 3C DNA content; fluorescent peak 3 of

343 hyper-triploid loaches indicates approximately 3.3C DNA content (a). Fluorescent peak 1' of normal

344 diploid loach indicates 2C DNA content; fluorescent peak 2' of neo-tetraploid loach indicates 4C

345 DNA content; fluorescent peak 3' of hyper-tetrapoid loaches indicates approximately 4.3C DNA

346 content (b)

347 **Fig. 3** Flow-cytometrical histograms of normal diploid and hyper-triploid in sperm. Fluorescent peak 1 of

348 haploid spermatozoa from normal wild-type diploid loach indicates 1C DNA content (a). Fluorescent

349 peak 1', 2' and 3' of spermatozoa or spermatozoa-like cells from sperm of hyper-triploid loach

350 indicate approximately 1.7C, 3.3C and 6.7C DNA content, respectively (b). Fluorescent peak 1" and

351 2" of spermatozoa or spermatozoa-like cells from sperm of hyper-triploid loach indicate

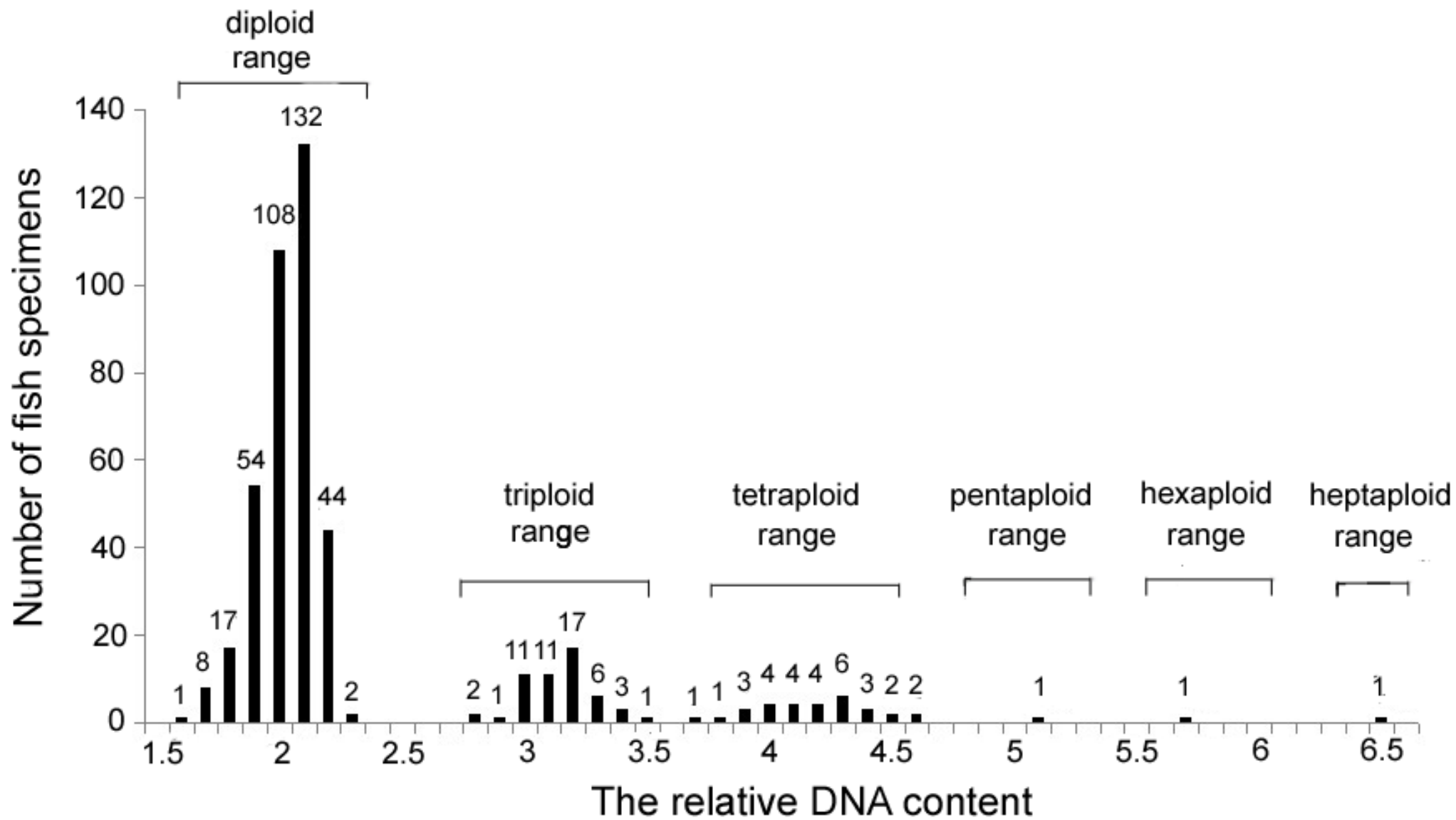352 approximately 3.3C and 6.6C DNA content, respectively (c)

353 **Fig. 4** Flow-cytometrical histograms of neo-tetraploid and hyper-tetraploid in sperm. Fluorescent peak 1

354 of haploid spermatozoa from normal diploid loach indicates 1C DNA content; fluorescent peak 2 of

355 diploid spermatozoa from neo-tetraploid loach indicates 2C DNA content; fluorescent peak 3 of

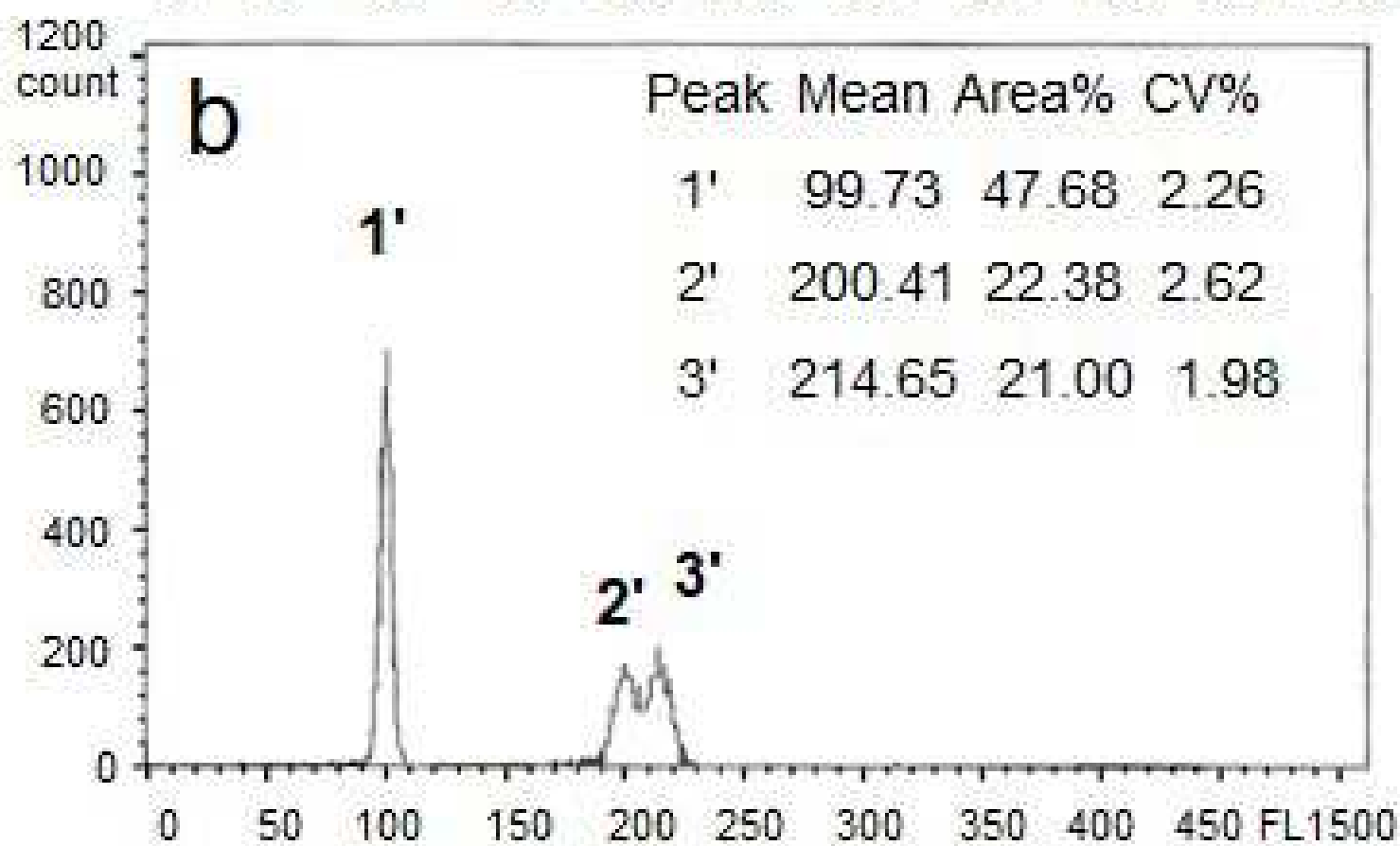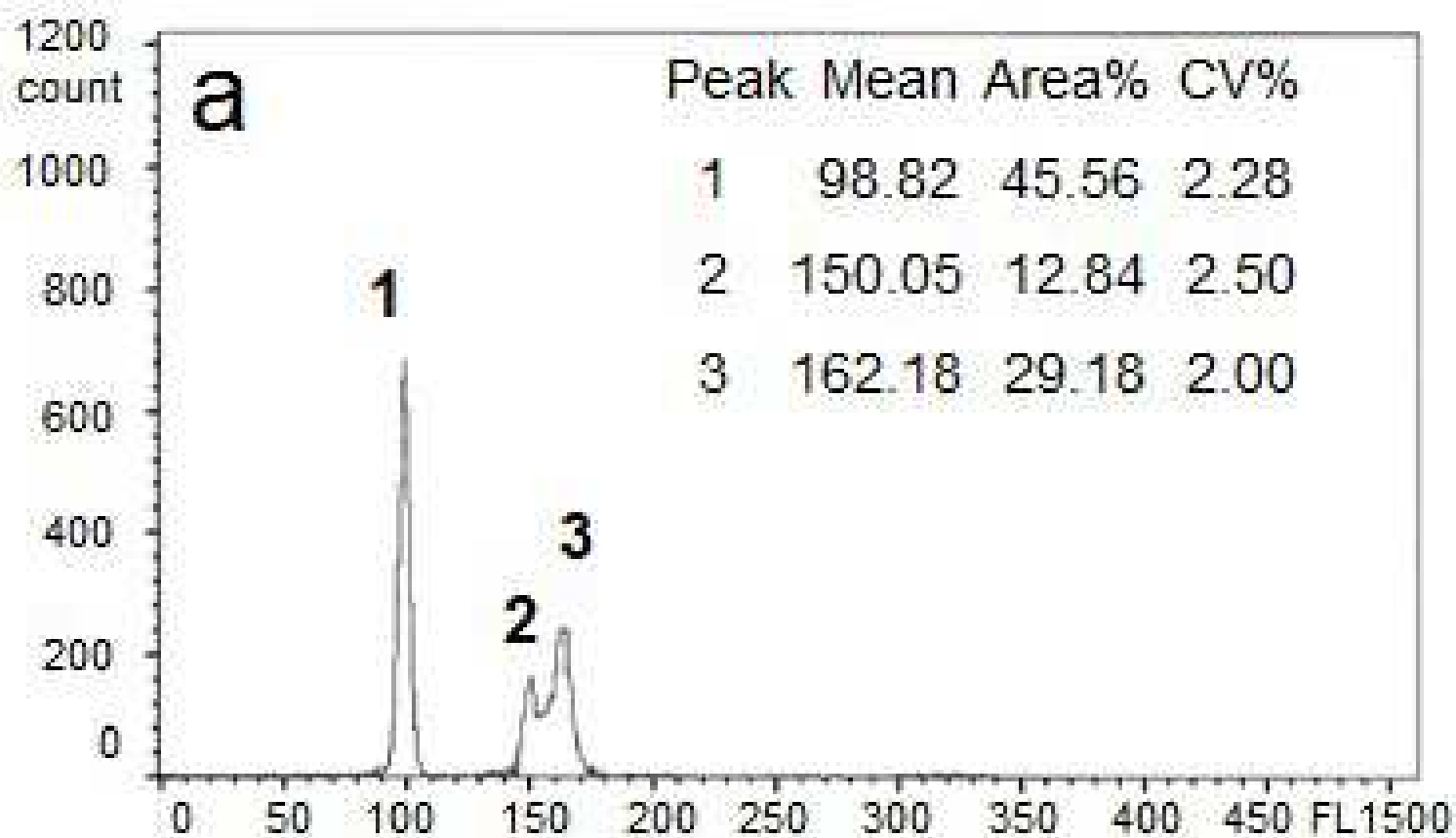356 spermatozoa from hyper-tetraploid loaches indicates approximately 2.3C DNA content

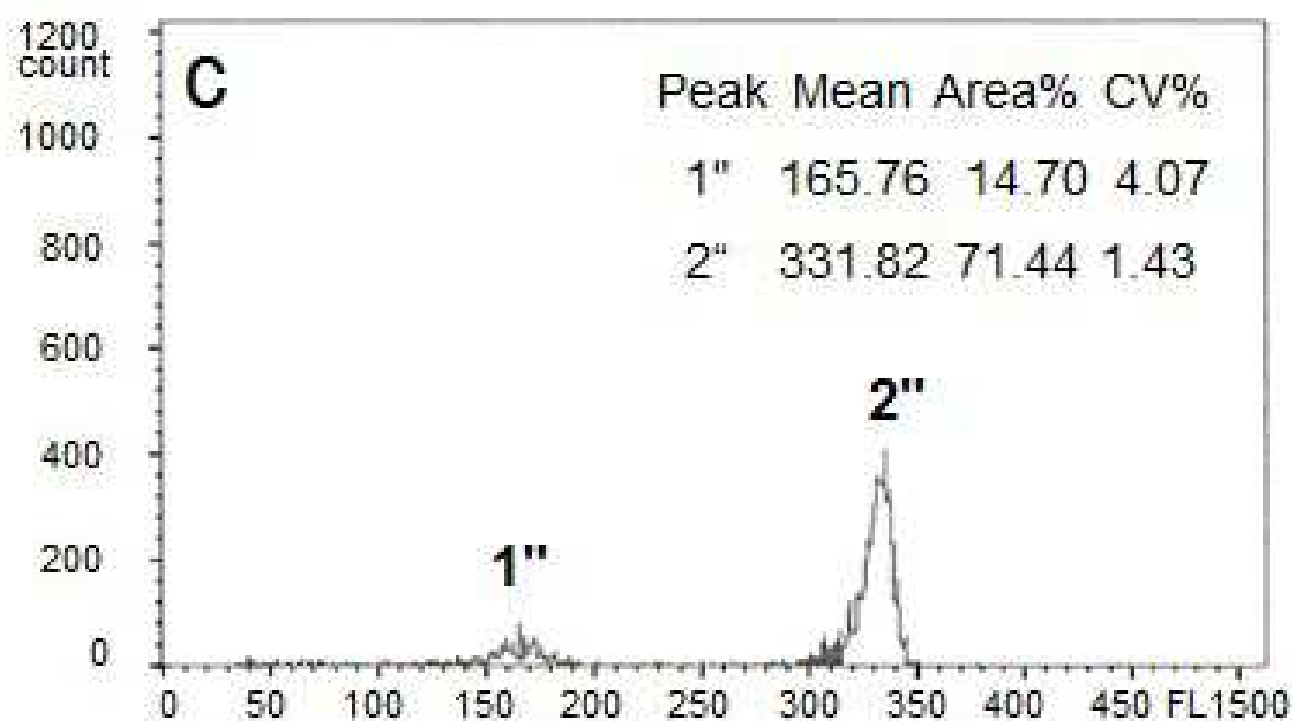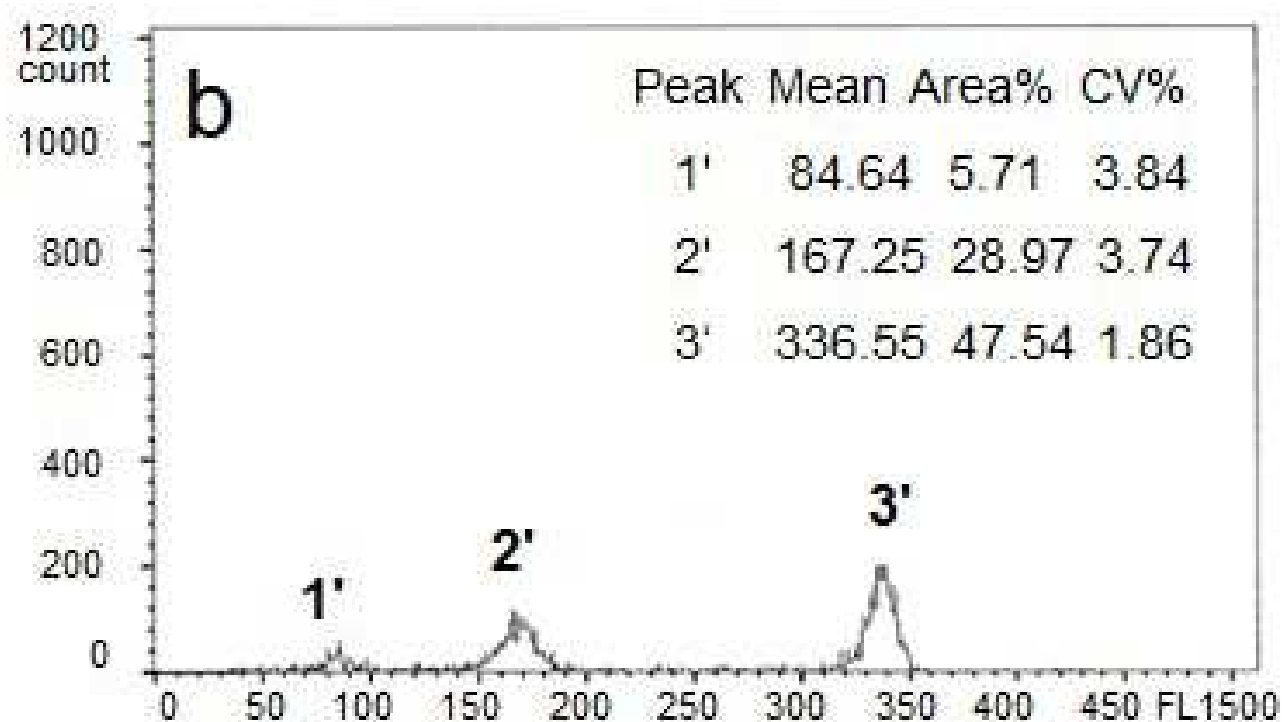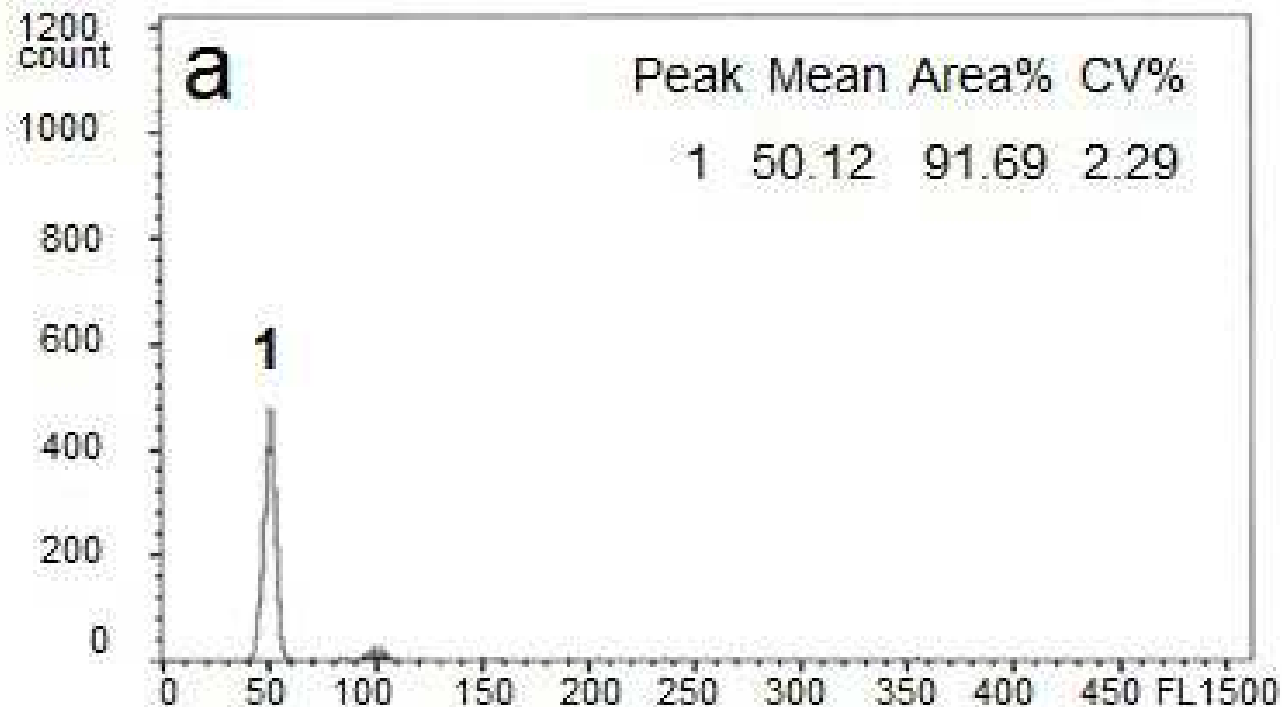357 **Fig. 5** External appearance of larvae. Normal larvae from diploid female × neo-tetraploid male (a).

358   Normal larvae from diploid female × hyper-tetraploid males (hyper-4n-#1, hyper-4n-#2 and

359   hyper-4n-#3) (b). Normal larvae produced by induced androgenesis, diploid female (UV-irradiated

360   eggs) × neo-tetraploid male (c). Abnormal larvae with a few or no melanophores produced by

361   induced androgenesis, diploid female (UV-irradiated eggs) × hyper-tetraploid males (d). Arrows

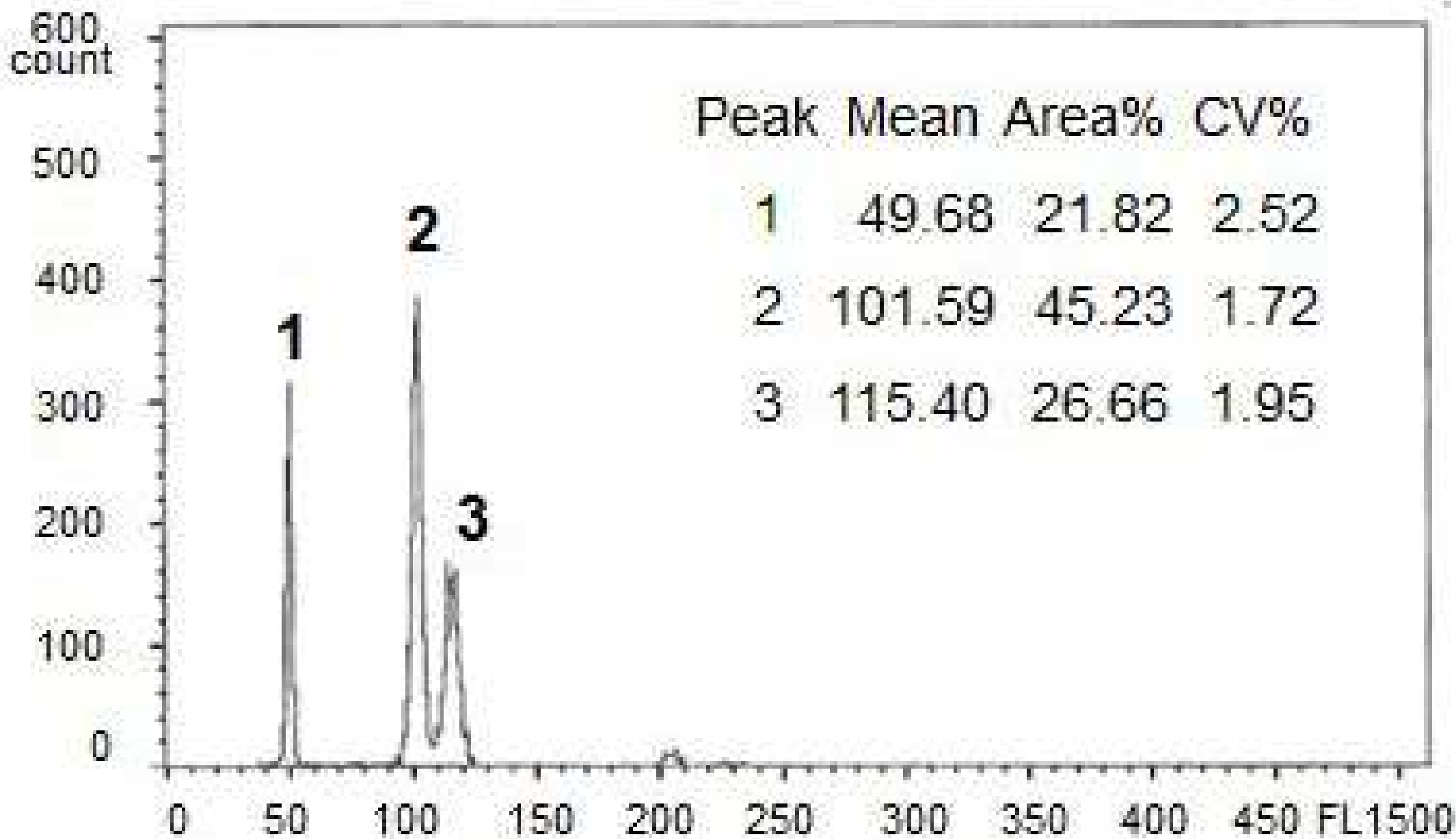362   indicate melanophores. A scale bar denotes 1mm

363   **Fig. 6** Relative DNA contents of larvae from different crosses measured by flow cytometry. Fluorescent

364   peak 1 of larvae from normal diploid female × normal diploid male cross indicates 2C DNA content.

365   Fluorescent peak 2 of embryos from diploid female × neo-tetraploid male indicates 3C DNA content

366   (a). Fluorescent peak 3 of larvae from diploid female (UV-irradiated eggs) × neo-tetraploid male

367   cross indicates 2C DNA content (b). Fluorescent peak 1' of larvae from normal diploid female ×

368   normal diploid male cross indicates 2C DNA content. Fluorescent peak 2' of Embryos from diploid

369   female × hyper-tetraploid males cross indicates approximately 3.3C DNA content (c). Fluorescent

370   peak 3' of larvae from diploid female (UV-irradiated eggs) × hyper-tetraploid males cross indicates

371   approximately 2.2C DNA content (d)

372   **Fig. 7** Metaphase chromosomes (a, c) and corresponding karyotypes (b, d) of the loach *Misgurnus*

373   *anguillicaudatus*.   Diploid female (UV-irradiated eggs) × neo-tetraploid male, 2n=50 (a, b). Diploid

374   female (UV-irradiated eggs) × hyper-tetraploid males, 2n=54 (c, d). Arrows indicate supernumerary
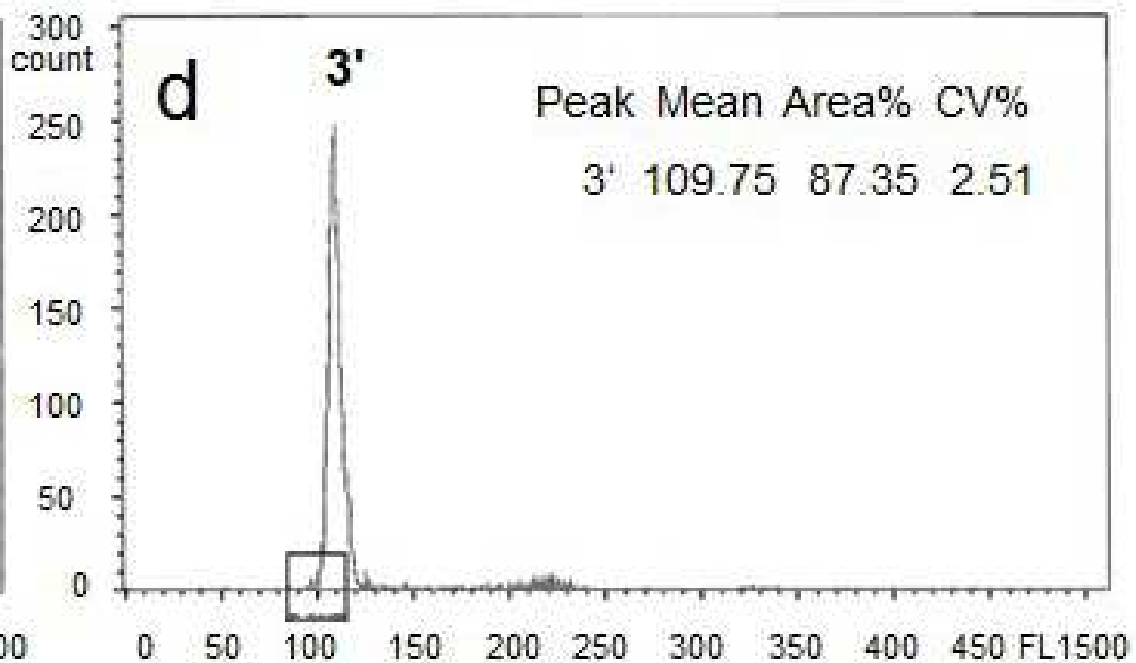
375   chromosomes. Scale bars denote 10 μm

a

Peak	Mean	Area%	CV%
1	98.82	45.56	2.28
2	150.05	12.84	2.50
3	162.18	29.18	2.00

b

Peak	Mean	Area%	CV%
1'	99.73	47.68	2.26
2'	200.41	22.38	2.62
3'	214.65	21.00	1.98

Peak	Mean	Area%	CV%
1	50.12	91.69	2.29

Peak	Mean	Area%	CV%
1'	84.64	5.71	3.84
2'	167.25	28.97	3.74
3'	336.55	47.54	1.86

Peak	Mean	Area%	CV%
1"	165.76	14.70	4.07
2"	331.82	71.44	1.43

a

Peak	Mean	Area%	CV%
1	97.45	12.20	2.31
2	149.30	79.49	1.84

b

Peak	Mean	Area%	CV%
3	99.16	88.12	2.77

c

Peak	Mean	Area%	CV%
1'	98.46	18.29	2.29
2'	160.97	70.49	2.02

d

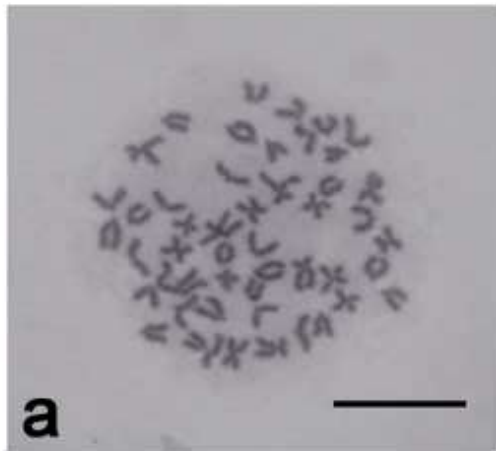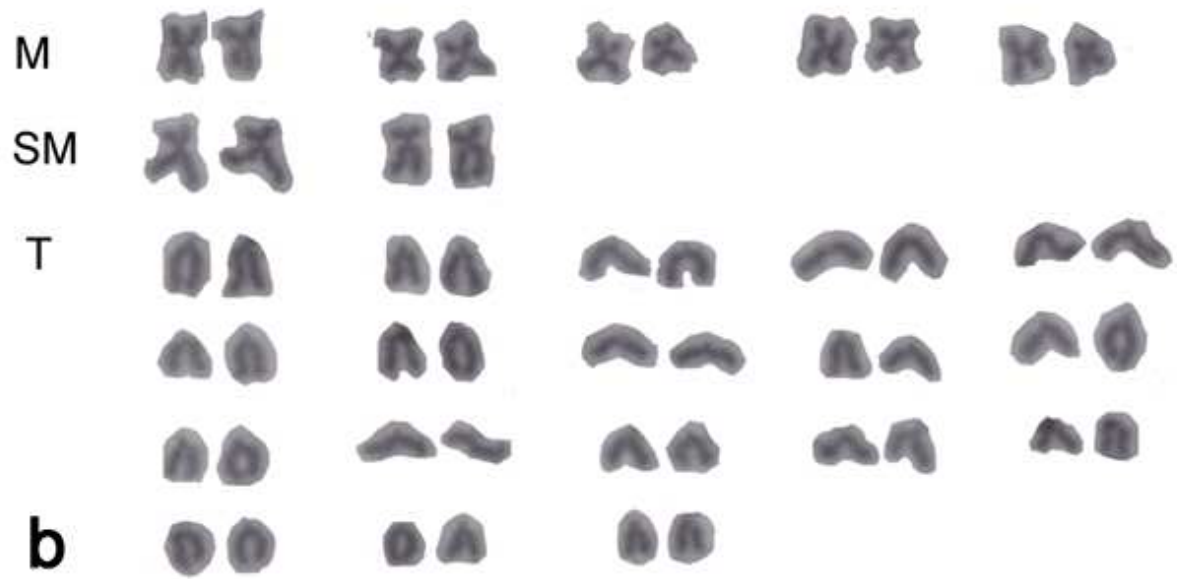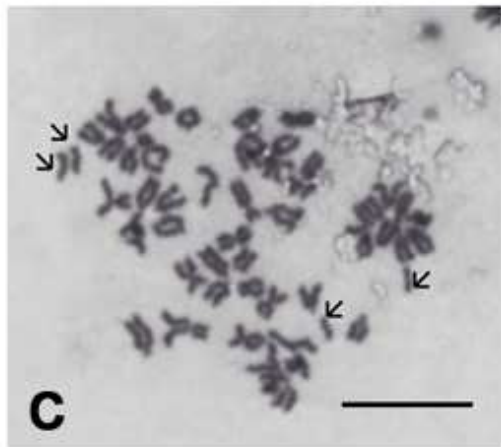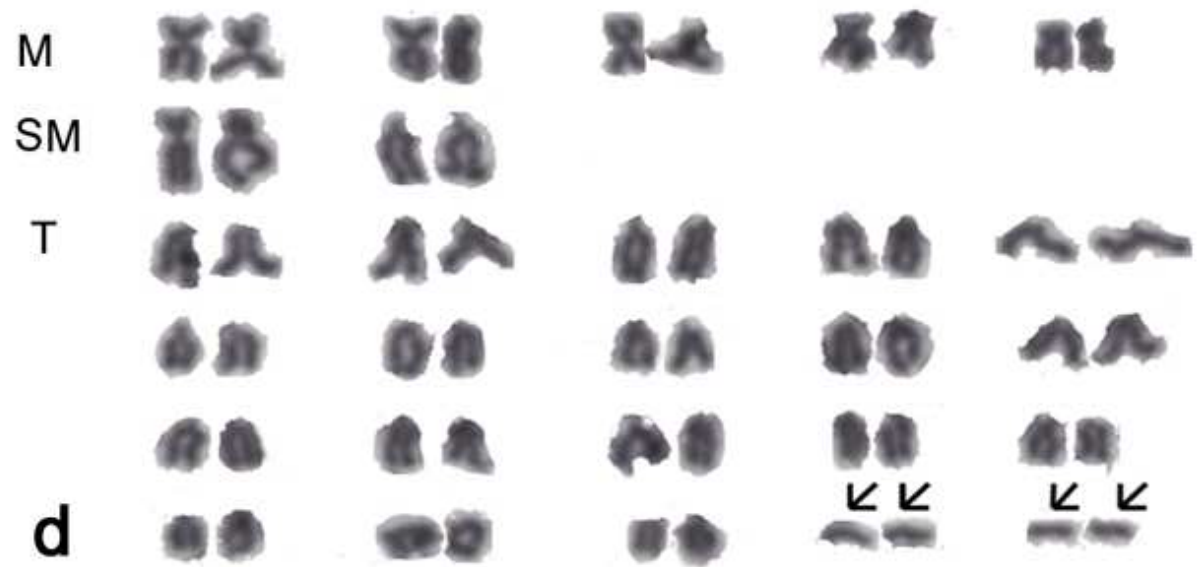| Peak	Mean	Area%	CV%
3'	109.75	87.35	2.51

M
SM
T

a
b

M
SM
T

c
d

Table 1. Developmental capacity and ploidy status in progenies derived from sperm of normal 2N, neo-4N and hyper-4N males.

Putative progeny	Cross		Fertility (%)	Hatch (%)	Normal (%)	Sample no.	Ploidy range		
	Female*	Male					1n	2n	3n
Control-2n	2N	2N	98.4	90.6	97.5	10	0	10	0
3n	2N	neo-4N	94.3	92.2	94.5	10	0	0	10
3n	2N	hyper-4N-#1	90.6	85.0	84.3	10	0	0	10
3n	2N	hyper-4N-#2	95.8	86.5	92.4	10	0	0	10
3n	2N	hyper-4N-#3	95.5	90.3	92.8	10	0	0	10
Andro-1n	(2N)**	2N	90.3	28.3	0	10	10	0	0
Andro-2n	(2N)**	neo-4N	95.1	35.6	90.4	10	0	10	0
Andro-2n	(2N)**	hyper-4N-#1	90.7	30.4	0	10	0	10	0
Andro-2n	(2N)**	hyper-4N-#2	80.2	25.2	0	10	0	10	0
Andro-2n	(2N)**	hyper-4N-#3	90.5	34.7	0	10	0	10	0

* Intact or UV-inactivated eggs were obtained from the same diploid female.

** UV-irradiated

Table 2. Chromosome numbers in progenies derived from control cross (2N×2N) and androgenetes derived from sperm of neo-4N and hyper-4N males

| Cross | | | Chromosome number | | | | | | | | | | | |
Female	Male	n	45	46	47	48	49	50	51	52	53	54	55	56
2N	2N	30	1	1	2	4	22							
(2N)*	neo-4N	41		1	1	1	8	27	2	1				
(2N)*	hyper-4N-#1	25							1	1	7	15	1	
(2N)*	hyper-4N-#2	9								1	4	4		
(2N)*	hyper-4N-#3	34								1	9	21	2	1

* UV-irradiated