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First evidence of viable progeny from three interspecific parents in sturgeon

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Abstract Polyspermy is the most commonly observed cause of embryonic abnormalities in fertilization, often resulting in death. In sterlet (*Acipenser ruthenus*), however, polyspermic embryos have high survival (similar to a control group) and morphological development is similar to monospermic larvae. Ploidy of these individuals is n/2n mosaic (whereas the normal state for *A. ruthenus* is a functional diploid). This study was undertaken to test whether sturgeon eggs can be fertilized by several spermatozoa from different species to produce viable offspring from three interspecific parents: *A. ruthenus* (2n), *A. gueldenstaedtii* (4n), and *A. baerii* (4n). Four trials were performed: (1) and (2) *A. baerii* eggs were fertilized with a mixture of *A. ruthenus* and *A. gueldenstaedtii* sperm; (3) *A. gueldenstaedtii* eggs were fertilized with a mixture of *A. baerii* and *A. ruthenus* sperm; and (4) *A. gueldenstaedtii* eggs were fertilized with a mixture of *A. gueldenstaedtii* and *A. ruthenus* sperm. Fertilized embryos with abnormal cleavage (3, 5, 6, 7, 9, and 10 cells) were collected and kept separately until 14 days post-fertilization. Ploidy level of 25 larvae (hatched from abnormal cleaved embryos) was evaluated by flow cytometry. Forty-four percent of observed hybrids had a mosaic 2n/3n ploidy. Five larva were processed further with microsatellite analysis and demonstrated that three specimens were heterospecific polyspermic larvae, containing alleles from three parents, and two specimens were conspesific polyspermic larvae from two parents. This astonishing phenomenon was emphasized by the fact that it was generated without any significant intervention.

Keywords Sturgeon · Polyspermy · Mosaicism · Hybridization

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Introduction

Sturgeons (Acipenseridae) are an ancient family (Bemis and Grande 1997) with unique biological characteristics that can be challenging for research (Carmona et al. 2009). Sturgeon eggs have multiple micropyles, and the number varies among females of different species and between eggs of individual females (Dettlaff et al. 1993): *Acipenser ruthenus* eggs contain five to 13 micropyles (Zalenskii 1878); up to 52 micropyles in *A. gueldenstaedtii* eggs (Dettlaff and Vassetzky 1991); and *A. baerii* eggs have two to 16 micropyles (Debus et al. 2002).

Ploidy in sturgeon fishes also varies and can be categorized into three classes depending on chromosome number: functional diploids (2n) have ~ 120 chromosomes (*Huso huso*, *A. sturio*, *A. ruthenus*); functional tetraploids (4n) have ~ 240 chromosomes (*A. baerii*,

A. gueldenstaedtii, *A. naccarii*, *A. mikadoi*); and functional hexaploids (6n) have ~360 chromosomes (*A. brevirostrum*) (Ludwig et al. 2001; Fontana and Colombo 1974; Fontana 1994; Kim et al. 2005; Birstein and Vasilev 1987; Vasil'ev 2010). However, sturgeons hybridize more easily than other vertebrates (Birstein et al. 1997) between species with the same and/or different ploidy level (Billard and Lecointre 2001) and demonstrate interspecific and intergeneric hybridization in the wild and under artificial conditions (Havelka et al. 2011).

Multiple micropyles, which are typical for sturgeon eggs, and fertilization with high concentrated sperm suspensions are associated with the occurrence of polyspermy (Dettlaff et al. 1981). It is believed that polyspermy is the most commonly observed reason for embryonic abnormalities in fertilization, often resulting in death (Boveri 1901; Wang et al. 2003). However, recent research by Iegorova et al. (2018) demonstrates that sturgeon polyspermic embryos are unique in their ability to survive and to develop into morphologically normal fry after abnormal cleavage as 3, 5, 6, 7, 9, and 10 blastomeres at the 2- to 4-cell stage. It was described that ploidy level of *A. ruthenus* larvae from abnormally divided embryos (abnormally divided embryos = AD embryos) was n/2n, and *A. gueldenstaedtii* x *A. ruthenus* hybrids had 2n/3n mosaic ploidy. That was the reason of fusion of one sperm pronucleus with the egg pronucleus generating a zygote, and the accessory sperm pronucleus or pronuclei developed separately from the zygote (Iegorova et al. 2018).

Based on these characteristics of sturgeon, we tested the possibility of producing a polyspermic interspecific hybrid by using gametes from three parents.

Broodfish and gamete collection

Two *A. baerii* females, two *A. gueldenstaedtii* females, four *A. ruthenus* males, three *A. gueldenstaedtii* males, and one *A. baerii* male were held in 4 m^3 tanks at 15 °C in the hatchery of the Research Institute of Fish Culture and Hydrobiology in Vodnany, Czech Republic. To induce spermiation, sturgeon males were injected with acetone-dried homogenized carp pituitary extract (CPE) at a dose of 4 mg/kg body weight (BW). Using a catheter, sperm was collected from the urogenital papilla at 42 h post-injection, transferred to a 250 ml cell-culture container, and stored at 4 °C until fertilization. Sperm concentration was estimated using a Burker cell hemocytometer (Meopta, Czech Republic) at 20× magnification on a Nikon ECLIPSE Ci-S phase contrast microscope (Nikon, Japan). Ovulation in sturgeon females was induced with two doses of CPE by intramuscular injection: the first was given 36 h before stripping (0.5 mg/kg BW) and the second 24 h before stripping (4.5 mg/kg BW) (Dettlaff et al. 1981). Ovulated eggs were collected by microsurgical incision of oviducts as described by Podushka (1999).

Fertilization and identification of AD embryos

Four separate fertilizations were completed: (1) Eggs from one *A. baerii* (4n) female were fertilized with a mixture of sperm from one *A. ruthenus* (2n) and one *A. gueldenstaedtii* (4n) male; (2) eggs from one *A. baerii* (4n) female were fertilized with a mixture of sperm from one *A. ruthenus* (2n) and one *A. gueldenstaedtii* (4n) male; (3) eggs from one *A. gueldenstaedtii* (4n) female were fertilized with a mixture of sperm from one *A. baerii* (4n) and one *A. ruthenus* (2n) male; and (4) eggs from one *A. gueldenstaedtii* (4n) female were fertilized with a mixture of sperm from one *A. gueldenstaedtii* (4n) and one *A. ruthenus* (2n) male. Approximately 450 eggs (10 g) were fertilized for each group, using 2.3312 10^9 spz × 1 ml. To remove the stickiness of the outer layer, fertilized eggs were treated with 0.1% tannic acid solution (three times in 10 min). Embryos with 3, 5, 6, 7, 9, and 10 blastomeres at the 2- to 4-cell stage were considered as AD embryos. AD embryos and normally dividing embryos were separated and counted.

Survival and ploidy analysis

Embryos were incubated in dechlorinated water at 15 ± 1 °C. After 14 days, survival was noted and ploidy level of 25 larvae, developed from ADs, were randomly chosen from all four groups in total and evaluated by flow cytometry (Paa Partec CCA I; Partec GmbH, Münster, Germany) using 4′, 6-diamidino-2-phenylindole (CyStain DNA 2step kit; Partec GmbH) according to manufacturer's instructions. Normally developed hybrids were used as controls, and their expected ploidy was 3n and 4n.

Microsatellite genotyping

To prove an existence of several interspecific spermatozoa in the egg, five randomly chosen AD larvae were processed for microsatellite genotyping: two larvae from *A. baerii* x *A. ruthenus* x *A. gueldenstaedtii*: AD1 and AD2; two larvae from *A. gueldenstaedtii* x *A. baerii* x *A. ruthenus* fertilizations: AD3 and AD4; and one larva from *A. gueldenstaedtii* x *A. gueldenstaedtii* x *A. ruthenus* group: AD5. Genomic DNA was extracted using a DNA extraction kit (GenElute Mammalian Genomic DNA Miniprep Kit; Sigma-Aldrich®) according to manufacturer's instructions. Presence of multiple spermatozoa in AD larvae was estimated by parentage-like assignment using seven informative microsatellite markers, i.e., *AfuG_135* (Welsh et al. 2003), *Aox_27*, *Aox_45* (King et al. 2001), *Spl_101*, *Spl_107*, *Spl_163*, *and Spl_173* (McQuown et al. 2000). Amplification was carried out according to the protocol described by Havelka et al. (2013). Microsatellite fragment analysis was performed on an Applied Biosystems SeqStudio Genetic Analyzer using a GeneScan LIZ 600 size standard (Applied Biosystems), and genotypes were scored in GENEIOUS 8.1.9, using a Microsatellite Plugin 1.4.4. The complexity of the duplicated sturgeon genome and the nature of current microsatellite genotyping make it impossible to reliably determine allele dosage behind a specific peak. Hence, peak patterns were treated as dominant data and interpreted as "allele phenotypes" (Rodzen et al. 2004). In several cases, we were able to estimate a real genotype at a fully heterozygote loci. Alleles that were not mutually shared by males, and by males and female were identified private alleles and tracked in allele phenotypes of AD larvae.

Results

Survival rate of three species hybrids

AD embryos were detected in all groups. The percentage of abnormally divided embryos ranged from 5 to 10% in each group. Hatching was observed at 10 dpf (days post-fertilization). Survival of abnormally cleaved embryos was up to 49% at 4 dpf; 15–42% at 7 dpf; and 15–27% of AD survived to 14 dpf. Survival of normally cleaved embryos was 77–90% at 4 dpf; 76–88% at 7 dpf; and 76–81% by 14 dpf (Table 1, Fig. 1).

Ploidy level of obtained AD embryos

From 25 analyzed larvae, 88% of AD embryos were mosaic: 44% had 2n/3n ploidy (three-species progenies) (Fig. 2a), 40% had 2n/4n ploidy (polyspermic *A. baerii* x *A. gueldenstaedtii* larvae), and 4% had 2n/5n ploidy (unexpected ploidy). The remaining 12% of AD embryos consisted of 4% of diploids, 4% of triploids, and 4% of tetraploids (Table 1). Controls contained triploids and tetraploids, as it was expected (Fig. 2b).

Microsatellite genotyping

Fertilization *A. baerii x A. ruthenus x A. gueldenstaedtii*. Allele phenotypes of sample AD1 showed the presence of private alleles of both *A. gueldenstaedtii* male and

Table 1 Survival rate of the AD embryos and ploidy level of 25 analyzed larvae that developed from AD embryos

Trial	Status of embryo	Total number of embryos	4 dpf	7 dpf	14 dpf	Ploidy level of AD larvae					
						2n	3n	4n	2n/3n	2n/4n	2n/5n
1	AD*	45	22 (49%)	19 (42%)	10 (22%)	1	1	1	0	3	0
	Normal	110	85 (77%)	84 (76%)	84 (76%)						
2	AD	156	34 (22%)	33 (21%)	33 (21%)	0	0	0	9	5	1
	Normal	410	330 (80%)	328 (80%)	323 (79%)						
3	AD	22	7 (32%)	6 (27%)	6 (27%)	0	0	0	1	0	0
	Normal	412	364 (88%)	364 (88%)	333 (81%)						
4	AD	20	9 (45%)	3 (15%)	3 (15%)	0	0	0	1	2	0
	Normal	424	380 (90%)	350 (82%)	332 (78%)						

AD means "atypically divided" at the 2- to 4-cell stage

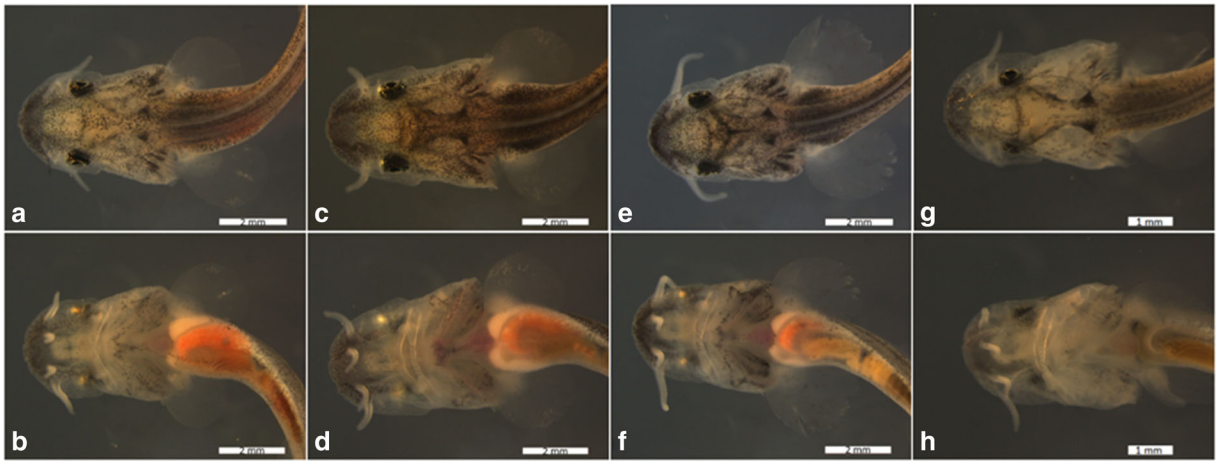

Fig. 1 Demonstration of 22-day post-fertilization larvae, (dorsal and ventral view) from the combination: *A. baerii* eggs x mix of sperm from *A. ruthenus* and *A. gueldenstaedtii*. **a**, **b** Produced three species larvae. Scale bar, 2 mm. **c**, **d** *A. gueldenstaedtii* larva. Scale bar, 2 mm. **e**, **f** *A. baerii* larva. Scale bar, 2 mm. **g**, **h** *A. ruthenus* larva. Scale bar, 1 mm

A. ruthenus male at five informative loci *AfuG_135*, *Aox_27*, *Aox_45*, *Spl_101*, and *Spl_107*. At the most informative locus *Aox_45*, the specimen had five alleles clearly showing that the specimen originated by five genomes, two from diploid oocyte (with genotype 164/167) of tetraploid *A. baerii* female, two from diploid spermatozoon (with genotype 134/158) of tetraploid *A. gueldenstaedtii* male, and one from haploid spermatozoon (with genotype 155) of diploid *A. ruthenus* male. It clearly shows that specimen AD1 originated from heterospecific polyspermy (Table 2).

Allele phenotypes of the second specimen in this group (AD2) had only private alleles transmitted from *A. gueldenstaedtii* male with no private alleles from *A. ruthenus* male. At two informative loci *AfuG_135* and *Aox_27*, the specimen presented three phenotypic

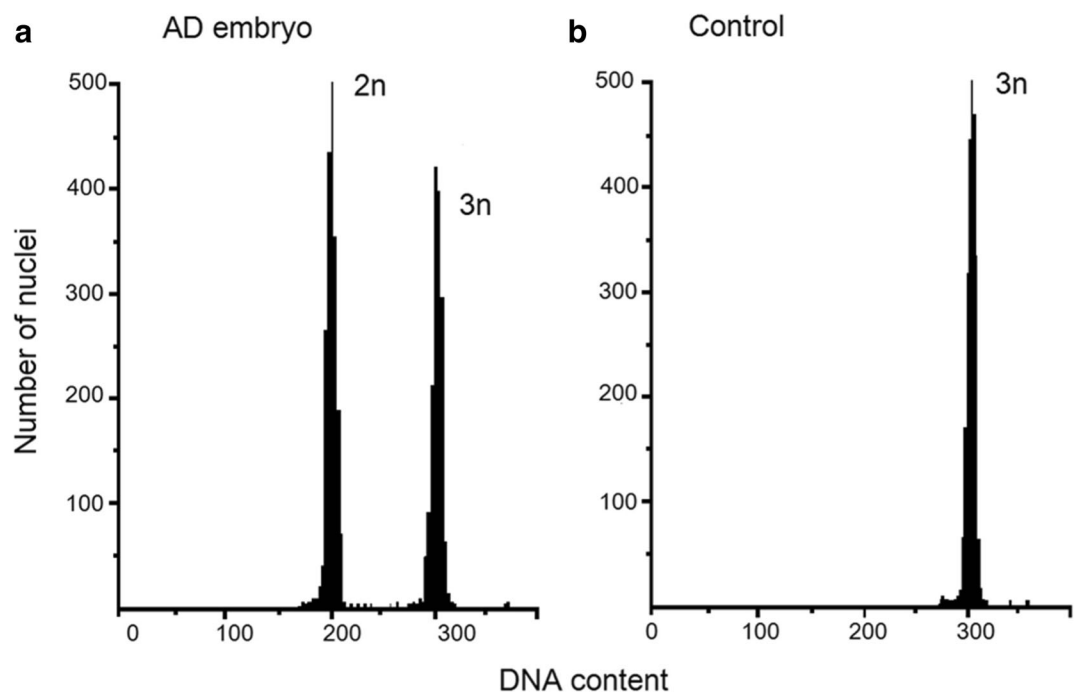

Fig. 2 Flow cytometry analysis showed three-parent AD embryos to be diploid/triploid mosaics. **a** The AD embryo had a diploid (2n) and a triploid (3n) peak. **b** A normally divided embryo at the hatching stage had a single triploid (3n) peak (*A. baerii* x *A. ruthenus*)

alleles transmitted from *A. gueldenstaedtii* male (Table 2). It shows that the specimen originated from conspecific polyspermy.

Fertilization *A. gueldenstaedtii x A. baerii x A. ruthenus*. Allele phenotypes of specimen AD3 showed the presence of private alleles of both *A. baerii* male and *A. ruthenus* male at four informative loci, i.e., *Aox_45*, *Spl_101*, *Spl_163*, and *Spl_173*. On contrary, allele phenotypes of the second specimen in this group had only private alleles derived from *A. ruthenus* male with no private alleles from *A. baerii* male. At the most informative locus *Spl_173*, the specimen AD3 inherited phenotypic allele 254 from diploid oocyte (genotype 254/254) of tetraploid *A. gueldenstaedtii* female, alleles 266 and 270 from diploid spermatozoon of tetraploid *A. baerii* male, and one allele 236 from haploid

Table 2 Fertilization *A. baerii* ♀ x *A. ruthenus* ♂ x *A. gueldenstaedtii* ♂

Sample	Marker	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8
A. baerii	AfuG135	200				216			
A. ruthenus	AfuG135			208	212				
A. gueldenstaedtii	AfuG135		204		212		232		
AD1	AfuG135	200	204		212	216	232		
AD2	AfuG135		204		212	216	232		
A. baerii	Aox27			150		158	164	167	
A. ruthenus	Aox27		134						
A. gueldenstaedtii	Aox27	130		150	154		162		
AD1	Aox27	130	134	150			162		
AD2	Aox27	130		150	154		162		
A. baerii	Aox45		146				164	164	
A. ruthenus	Aox45		146		155				
A. gueldenstaedtii	Aox45	134		152		158			
AD1	Aox45	134			155	158	164	167	
AD2	Aox45	134				158	164		
A. baerii	Spl101			316	336	340			358
A. ruthenus	Spl101		300					352	
A. gueldenstaedtii	Spl101	296					350		
AD1	Spl101		300		336	340	350		
AD2	Spl101	296			336	340			
A. baerii	Spl107				308				
A. ruthenus	Spl107	296	300						
A. gueldenstaedtii	Spl107		300	304	308	312			
AD1	Spl107	296			308	312			
AD2	Spl107		300	304	308	312			
A. baerii	Spl163				224	264			
A. ruthenus	Spl163		212	220					
A. gueldenstaedtii	Spl163	200	212	220					
AD1	Spl163		212	220	224	264			
AD2	Spl163	200	212	220	224	264			
A. baerii	Spl173	236		254		262			
A. ruthenus	Spl173	236					266		
A. gueldenstaedtii	Spl173	236	246	254	260				
AD1	Spl173	236	246	254		262			
AD2	Spl173	236	246		260				

spermatozoon of diploid *A. ruthenus* male (Table 3). Specimen AD4 inherited two alleles 254 and 258 from diploid oocyte of tetraploid *A. gueldenstaedtii* female and two alleles 236 and 266 from two haploid spermatozoa of diploid *A. ruthenus* male (Table 3). It shows that the specimen AD3 originated from heterospecific polyspermy while the specimen AD4 from conspecific polyspermy.

Fertilization *A. gueldenstaedtii x A. gueldenstaedtii x A. ruthenus*. Allele phenotypes of the specimen AD5 showed the presence of private alleles of both *A. gueldenstaedtii* male and *A. ruthenus* male at three informative loci, i.e., *AfuG_135*, *Aox_45*, and *Spl 163*. The most informative was locus *Aox_45*. At this locus, the specimen AD5 had five alleles clearly showing that the specimen originated from five genomes, i.e., two

Table 3 Fertilization *A. gueldenstaedtii* ♀ x *A. baerii* ♂ x *A. ruthenus* ♂

Sample	Marker	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8
A. gueldenstaedtii	AfuG135			216	224	228			
A. baerii	AfuG135	208		216					
A. ruthenus	AfuG135	208	212						
AD3	AfuG135	208		216	224				
AD4	AfuG135	208	212		224				
A. gueldenstaedtii	Aox27	130	134	162					
A. baerii	Aox27	130		162					
A. ruthenus	Aox27		134						
AD3	Aox27	130	134						
AD4	Aox27	130	134						
A. gueldenstaedtii	Aox45	134	140				158		173
A. baerii	Aox45				149	155		164	
A. ruthenus	Aox45			146		155			
AD3	Aox45				149	155	158		173
AD4	Aox45		140	146		155			173
A. gueldenstaedtii	Spl101			320	324	328			
A. baerii	Spl101	316					332		360
A. ruthenus	Spl101		300					352	
AD3	Spl101	316		320				352	360
AD4	Spl101			320	324			352	
A. gueldenstaedtii	Spl107			296	300	304	320		
A. baerii	Spl107	264	272		300				
A. ruthenus	Spl107			296	300				
AD3	Spl107	264	272	296	300		320		
AD4	Spl107			296	300	304			
A. gueldenstaedtii	Spl163	204			224	228	236		
A. baerii	Spl163					228		264	
A. ruthenus	Spl163		212	220					
AD3	Spl163			220	224	228	236	254	
AD4	Spl163	204	212				236		
A. gueldenstaedtii	Spl173			254	258	264			
A. baerii	Spl173		250				262	266	270
A. ruthenus	Spl173	236						266	
AD3	Spl173	236		254				266	270
AD4	Spl173	236		254	258			266	

from diploid oocyte (with genotype 140/158) of tetraploid *A. gueldenstaedtii* female, two from diploid spermatozoon (with genotype 134/176) of tetraploid *A. gueldenstaedtii* male, and one from haploid spermatozoon (with genotype 146) of diploid *A. ruthenus* male (Table 4). It clearly showed that specimen AD5 originated from heterospecific polyspermy. Other loci were not fully informative for the estimation of polyspermy; however, no locus was contradictory to the conclusion.

Discussion

This study is a first report about three interspecific parent fertilization in the animal kingdom. Here, we demonstrate sturgeon fertilization characteristics, great plasticity for hybridization between species and survival.

Our previous research described physiological polyspermy in sturgeons. Cytologically analyzed *A. ruthenus* and *A. baerii* embryos demonstrated high number of spermatozoa in the cytoplasm right after fertilization and that number of supernumerary spermatozoa is significantly decreasing before first cleavage. At the same time, sometimes, additional spermatozoa were participating in the development in a way "karyogamy with additional plasmogamy." A sperm nucleus or nuclei destined to form an additional blastomere began developing independently and probably 1 cycle later than a zygote (Iegorova et al. 2018). Our present work

Table 4 Fertilization *A. gueldenstaedtii* ♀ x *A. gueldenstaedtii* ♂ x *A. ruthenus* ♂

Sample	Marker	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8
A. gueldenstaedtii	AfuG135			216	224	228			
A. gueldenstaedtii	AfuG135				224		240		
A. ruthenus	AfuG135	208	212						
AD5	AfuG135	208			224	228	240		
A. gueldenstaedtii	Aox27	130	134	162					
A. gueldenstaedtii	Aox27	130							
A. ruthenus	Aox27		134						
AD5	Aox27	130	134						
A. gueldenstaedtii	Aox45	134	140			158		173	
A. gueldenstaedtii	Aox45	134				158	170		176
A. ruthenus	Aox45			146	155				
AD5	Aox45	134	140	146		158			176
A. gueldenstaedtii	Spl101		320	324	328				
A. gueldenstaedtii	Spl101		320		328	332			
A. ruthenus	Spl101	300					352		
AD5	Spl101		320	324	328		352		
A. gueldenstaedtii	Spl107	296	300	304	320				
A. gueldenstaedtii	Spl107		300	304		368			
A. ruthenus	Spl107	296	300						
AD5	Spl107	296	300	304		368			
A. gueldenstaedtii	Spl163		204			224	228	236	
A. gueldenstaedtii	Spl163	188				224	228	236	
A. ruthenus	Spl163			212	220				
AD5	Spl163	188	204		220			236	
A. gueldenstaedtii	Spl173		254	258	264				
A. gueldenstaedtii	Spl173		254	258					
A. ruthenus	Spl173	236				266			
AD5	Spl173		254	258	264	266			

demonstrates that no significant intervention was needed to generate interspecific three-parent hybrids, where two species gave rise to a hybrid zygote and the third species contributed supplementary sperm-derived cells.

In Fig. 3, we list all possible variations that could be obtained using gametes from three species (as an example fertilization of A. baerii (4n) eggs with a mixture of sperm from A. ruthenus (2n) and A. gueldenstaedtii (4n) as an example). Monospermic fertilization of *A. baerii* egg by a single *A. ruthenus* spermatozoon would produce a normally divided triploid embryo. If *A. baerii* egg was fertilized by a single *A. gueldenstaedtii* spermatozoa, the embryo should be a normally divided tetraploid. Due to multiple micropyles, *A. baerii* egg could be fertilized by several spermatozoa. Polyspermic fertilization of *A. baerii* egg with several *A. ruthenus* spermatozoa should result in abnormally divided mosaics with ploidy level 1n/3n. Polyspermic fertilization of *A. baerii* egg by several *A. gueldenstaedtii* spermatozoa would produce an abnormally divided embryo with a mosaic ploidy of 2n/4n. Using gametes from three parents, and fertilizing *A. baerii* egg with *A. gueldenstaedtii* spermatozoa and *A. ruthenus* spermatozoa, would produce either 2n/3n or 1n/4n mosaic hybrid. The 2n/3n mosaics would be created if the *A. ruthenus* sperm pronucleus fused with *A. baerii* egg pronucleus (3n) and the accessory *A. gueldenstaedtii* sperm pronucleus developed singly as 2n. However, 1n/4n mosaics would appear if *A. gueldenstaedtii* sperm pronucleus fused with *A. baerii* egg pronucleus (4n) and the accessory *A. ruthenus* sperm pronucleus developed singly as 1n.

In our study, 88% of AD embryos displayed mosaic ploidy: 44% of polyspermic embryos from three species: *A. baerii* x *A. gueldenstaedtii* x *A. ruthenus*, 40% of polyspermic embryos from two species: *A. baerii* x *A. gueldenstaedtii*, and 4% of 2n/5n embryos, which could be an unexplained fusion of pronuclei. Triploids and tetraploids could be obtained due to asymmetrical division of blastomeres, as described by Dettlaff et al. (1993), which we could consider as AD embryos. Their ploidy suggests that they were monospermic progeny from two species. Besides mosaics, triploid and tetraploid was detected one diploid fish. Probably it was occurred due to androgenotes: one decondensed sperm nuclei from *A. baerii* or *A. gueldenstaedtii* became a male pronuclei and continued development without fusion with female pronucleus and produced diploid larva. Successful amplification of DNA fragments from three sturgeon species proved the heterospecific polyspermy: three larvae contained alleles from three parents.

Survival of AD three parents' hybrid embryos

During the last few decades, it was believed to be fatal if more than one spermatozoa fused with the oocyte: polyspermic embryos develop abnormally and perish before hatching or can develop into abnormal larvae that subsequently die. However, Iegorova et al. (2018) showed that polyspermic embryos had almost the same

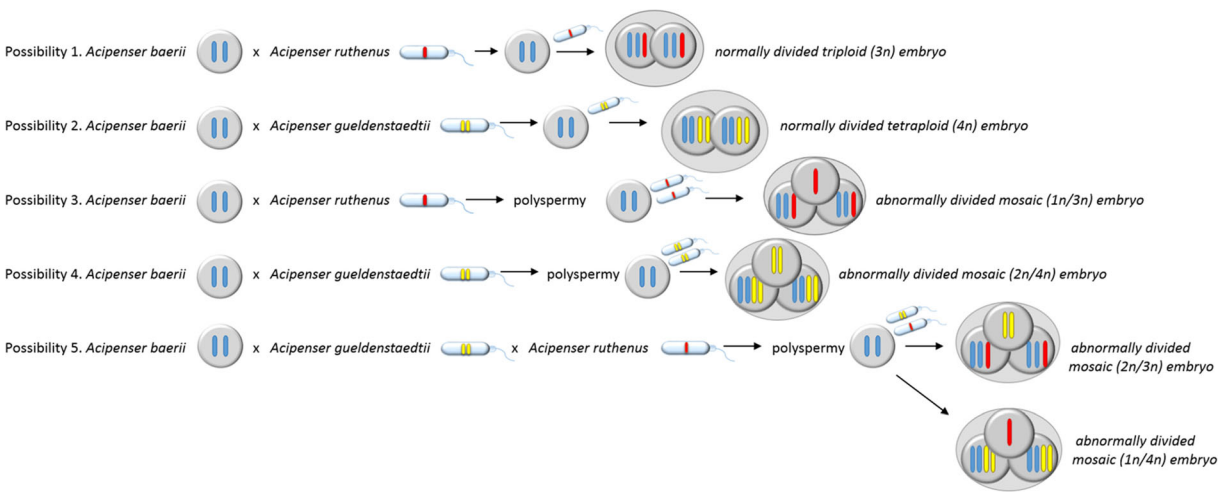

Fig. 3 A schematic illustration of all possible variations during fertilization of gametes from three parents using an example of *A. baerii* insemination by *A. gueldenstaedtii* and *A. ruthenus* spermatozoa

survival rate as controls. Our present work demonstrates that three-parent hybrids still have high survival, although the survival rate was lower than the control (27 vs 81% at 14 dpf), which indicated that probably some combinations of interspecific pronuclei can be lethal. Interestingly, we did not find any 1n/4n mosaics (*A. baerii* x *A. gueldenstaedti* hybrid with *A. ruthenus* accessory sperm pronuclei) in our trials, which could represent a lethal combination.

Do AD embryos produce clonal gametes?

Morishima et al. (2004) have shown a diploid/triploid loach (*Misgurnus anguillicaudatus*) males produce clonal unreduced diploid spermatozoa. When normal diploid female was crossed with mosaic diploid/triploid male, only triploid progeny appeared and exhibited microsatellite genotypes with two alleles identical to the clonal genotype and one allele derived from female. When UV-irradiated eggs were fertilized by diploid spermatozoa from the mosaic male, they gave rise to the occurrence of androgenetic diploids and they exhibited microsatellite genotypes and DNA fingerprints, absolutely identical to those of the natural clones. These results of Morishima et al. (2004) clearly concluded that the diploid-triploid mosaic male generate clonal diploid spermatozoa, with genetically identical genotypes.

In our previous research (Iegorova et al. 2018), we produced mosaic 1n/2n sterlet (*A. ruthenus*). We found that haploid cells were distributed among all germ layer derivatives, including gonads. Hypothetically, additional blastomeres produced by supernumerary spermatozoa exhibit the paternal genome exclusively. Thus, if these haploid-derived germ cells produce gametes, the gametes must carry only the paternal genome, and all haploid gametes will be clonal. If AD fish can produce clonal gametes, induction of multiple-sperm mosaicism might be a useful tool for production of isogenic strains in sturgeons in a short time.

Conclusion

Our findings indicated that sturgeons present a developmental pattern unique in the animal kingdom. This study clearly refutes statements about fatality of organisms if more than one spermatozoa fertilizes the oocyte, especially when gametes belong to interspecific animals. Here, we demonstrate a great plasticity in sturgeon hybridization, and easy ploidy manipulations, which could be an important strategy in aquaculture for mass production of clonal gametes.

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Compliance with ethical standards All experimental procedures were performed in accordance with national and institutional guidelines on animal experimentation and care and were approved by the Animal Research Committee of the Faculty of Fisheries and Protection of Waters in Vodnany, Czech Republic.

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