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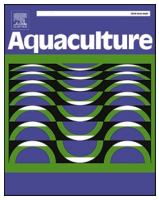

Androgenesis or polyploidization? The ploidy induction trend of different meiotic cold shock conditions in red crucian carp (2n, ♀) × tetraploid carp (4n, ♂)

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ABSTRACT

The application of meiotic cold shock treatment (MCST) to inhibit meiosis II of fertilized egg can simultaneously induce androgenesis and polyploidization. The reason is that, after the failure of meiosis II, the second polar body and the female nucleus form a meiotic complex, which may be retained in the fertilized egg to form a polyploid, or extruded out the fertilized egg to form an androgenetic individual. However, androgenesis or polyploidization? The induced trends for different MCST conditions are not yet known. The crossing between red crucian carp (2n, ♀) × tetraploid carp (4n, ♂) is a suitable object to study this problem, because both the androgenetic diploids and the hybrid tetraploids induced by MCST can develop normally. In this study, three gradient variables were set, namely post-fertilization time (0, 5, 10 min, the time before MCST after fertilization), shock temperature (0, 4, 6, 8 °C) and shock time (20, 40, 60 min). We found that the shorter the post-fertilization time, the lower the shock temperature and the longer the shock time, the greater the likelihood of meiosis failure, leading to the production of diploids or tetraploids. When the post-fertilization time is 5 min, the shock temperature was 4 °C and the shock time was 60 min, 100 % diploids can be detected in surviving individuals. Importantly, we found that the longer the post-fertilization time, the higher the shock temperature and the longer the shock time, the higher the proportion of diploids in these individuals with meiosis failure. Conversely, the higher the proportion of tetraploids. We speculate that under MSCT, the outward progression of meiotic complex is not completely stop, and the higher the temperature, the faster it progresses. Therefore, we think that prolonged post-fertilization time, increased shock temperature, and prolonged shock time can all increase the outward distance of meiotic complex before the end of treatment. The more the outward distance, the easier it is for the meiotic complex to be extruded from the fertilized egg (inducing androgenesis). Conversely, the less the outward distance, the easier it is for the meiotic complex to remain in the fertilized egg (inducing polyploidization). This study may provide new insights into androgenesis, polyploidy and gynogenesis breeding in fish.

1. Introduction

The application of meiotic cold shock treatment (MCST) to inhibit the extrusion of the second polar body (PB2) in the fertilized eggs is commonly employed in fish triploid breeding (Komen and Thorgaard, 2007; Piferrer et al., 2009; Xu et al., 2015). However, in numerous studies of the creation of artificial triploid fish through MCST in diploid fish, it has been commonly observed that abnormal embryos with external characteristics resembling those of haploid individuals often appear shortly after fertilization. The presence of haploids in these aberrant embryos was subsequently confirmed through cytogenetic method in stickleback (Swarup, 1956, 1959), salmonids (Ueda et al., 1988), loach (Suzuki et al., 1985), yellowtail tetra (Adamov et al., 2017) and so on. Subsequent studies confirmed that these haploids were androgenetic haploids by interspecific hybridization or crossing with male individuals exhibiting recessive mutant phenotypes, such as

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yellowing or albinism mutations, whose homozygous formation results in the loss of melanin during embryonic development (Kaspar et al., 2022; Morishima et al., 2011; Ueda and Aoki, 1995). Through histological section on fertilized eggs, it was discovered that MCST can not only lead to the retention of PB2 and female nucleus (FN) together to form polyploids, but also result in the extrusion of PB2 and FN together to form androgenetic individuals (Hou et al., 2015; Morishima et al., 2011; Zhou et al., 2018; Zhou et al., 2019). MCST induced polyploidy and androgenesis often occur together (Gu et al., 2024; Morishima et al., 2011; Zhou et al., 2018). However, the occurrence of androgenesis is seldom observed when subjected to meiotic heat shock and pressure shock. Pressure shock probably involve an effect of pressure acting on the oolemma, literally resisting the extrusion of the PB2 (Piferrer et al., 2009). Compared to cold shock, the microtubules implicated in chromosome migration exhibit greater sensitivity to heat shock (Dustin, 1984), which potentially disrupting the migration of FN and PB2, leaving them only to remain in the fertilized egg.

The conditions for MCST generally include three variables: post-fertilization time (the time before MCST after fertilization), shock temperature and shock time. In triploid breeding, the post-fertilization time is generally 2–7 min in temperate or warmwater fish, and 15–20 min in coldwater fish. Shock temperature is generally around 0–4 °C. Shock time is generally around 2–20 min in temperate or warmwater fish, 35 min-3 h coldwater fish (Piferrer et al., 2009). In hybrid grouper, the post-fertilization time with the highest triploids rate was 6 min (range: 4–8 min), and the triploid rate tends to increase with prolonged shock time (range: 10–20 min). However, the triploid rate was minimally affected by the shock temperature (range: 1–9 °C) (Zhang et al., 2023). In bluefin tuna, the induction of triploid can only be achieved with a post-fertilization time of 5 min (5, 10, 15, 20 min), and the triploid rate tends to increase with decreasing shock temperature (range: 3–7 °C) and prolonged shock time (range: 5–15 °C) (Hayashida et al., 2021). In red tilapia, the optimal triploid rate can be obtained by 9 °C treatment (range: 6–15 °C), and the triploid rate tends to increase with prolonged shock time (range: 15–35 °C) (Pradeep et al., 2014). The above examples demonstrate that the post-fertilization time, the shock temperature and the shock time all have an impact on the final triploid rate. It is worth noting that the above treatment may also induce a large number of lethal androgenetic haploids, whose deaths may be confused with physical damage caused by MCST. Therefore, reducing the occurrence of androgenesis is one of the important factors to increase triploid yield. Similarly, the above phenomenon may also be present in artificial gynogenesis of fish by MCST chromosome doubling.

Some studies have shown that following MCST, mitotic heat shock treatment can be used to double the paternal genome and obtain androgenetic double haploids (Hou et al., 2014, 2015; Kaspar et al., 2022). About 10 % induction rate of androgenetic double haploids in loach (*Misgurnus anguillicaudatus*) can be achieved by applying a heat shock treatment, initiated 35 min after the completion of MCST, at 42 ± 0.5 °C for 2 min (Hou et al., 2014). However, only 1.10 % induction rate in zebrafish (*Danio rerio*) by heat shock treatment, initiated 13 min after MCST, at 41.4 ± 0.5 °C for 2 min; less than 1 % induction rate in common carp (*Cyprinus carpio*) by heat shock treatment, initiated 40 min after MCST, at 40 °C for 2 min (Kaspar et al., 2022). The activity of androgenetic double haploids is extremely low, which may be due to physical or genetic damage from mitotic shock (Piferrer et al., 2009; Zhang and Onozato, 2004), high homozygosity (Komen and Thorgaard, 2007), and so on. Fortunately, tetraploids to provide diploid sperm may be a great way to address the low activity of androgenetic diploids, as it avoids all the above problems from doubling paternal genome (Gu et al., 2024; Thorgaard et al., 1990; Zhou et al., 2018).

In previous study, we obtained fertile tetraploid carp (4n = 200) through distant hybridization (Wang et al., 2019). The tetraploid carp possess two sets of nuclear genomes from crucian carp (*Carassius auratus*, 2n = 100) and two sets of nuclear genomes from common carp (2n = 100) (Gu et al., 2024). In our recent study, applying MCST after hybridization between red crucian carp (♀) and tetraploid carp (♂) can induce diploids (failed meiosis, androgenetic diploids induced by the simultaneous extrusion of PB2 and FN), triploids (normal meiosis, hybrid triploids) and tetraploids (failed meiosis, hybrid tetraploids induced by the simultaneous retention of PB2 and FN) (Gu et al., 2024). However, only one MCST condition (post-fertilization time: 2 min; shock temperature: 4–6 °C; shock time: 30 min) was used in this study (Gu et al., 2024). What are the preferred MCST conditions for inducing tetraploids (polyploidization) or diploids (androgenesis)? This aspect remains unclear (Fig. 1a).

After MCST, diploid parents may produce haploids, diploids, and triploids by self-crossing (the both parents come from the same species), but haploids are lethal and only diploids and triploids exist in surviving offspring. After MCST, tetraploid carp may produce diploids, tetraploids and hexaploids by self-crossing, but these hexaploids may die due to excessive ploidy, and only diploids and tetraploids exist in surviving offspring (Gu et al., 2024). Secondly, the egg quality of tetraploid carp is poor, and the yield of normal larva is very low by self-crossing (Gu et al., 2024). Therefore, diploid fish or tetraploid carp self-cross is not a good research object for this study. However, the hybridization between red crucian carp (♀) and tetraploid carp (♂) is an excellent combination for studying the above problems, as all offspring of diploid, triploid, and tetraploid can develop normally (Gu et al., 2024). In this study, we mainly tested the ploidy of offspring under different MCST conditions and found an induced trend between androgenesis and polyploidization. This study will provide a meaningful reference for triploid, gynogenesis, and androgenesis breeding in fish.

2. Method

2.1. Ethics statement

The research was granted ethical approval by the ethics committee at the Institute of Experimental Animals, located in Hunan Province, China. Prior to sample preparation, the fish utilized in the experiment were subjected to anesthesia using MS-222 (100 mg/L, 3-Aminobenzoic acid ethyl ester methanesulfonate).

2.2. Experimental design

Three key variables in this study were studied: post-fertilization time, shock temperature, and shock time. This study used dry fertilization, and the moment when the sperm and eggs were mixed and activated by adding fresh water was defined as the moment of fertilization. Based on previous experience and preliminary experimental results, we conducted a univariate gradient experiment centered around 5–4-40 (the numbers represent the post-fertilization time (min), the shock temperature (°C), and the shock time (min) respectively in order). This study set up a total of eight experimental groups, namely 5–4-40, 0–4-40, 10–4-40, 5–0-40, 5–6-40, 5–8-40, 5–4-20, 5–4-60, and one control group without treatment. The post-fertilization time of 0 min refers to the start of MCST within 10 s after fertilization. The above nine sets of treatments were completed in the one fertilization event (including three pairs of parents). The experiment was independently repeated three times (Fig. 1b).

2.3. Fish production and cold shock treatment

Red crucian carp and tetraploid carp were obtained from the State Key Laboratory of Developmental Biology of Freshwater Fish. In breeding season, the sexually mature female red crucian carp and male tetraploid carp were injected with suitable HCG (human chorionic gonadotropin, NSHF, China), LRH-A2 (luteinizing hormone-releasing hormone A2, NSHF, China) and domperidone (NSHF, China). When the water temperature is between 20 and 24 °C, red crucian carp can be extruded into mature eggs after about 6–12 h. After the eggs (collected

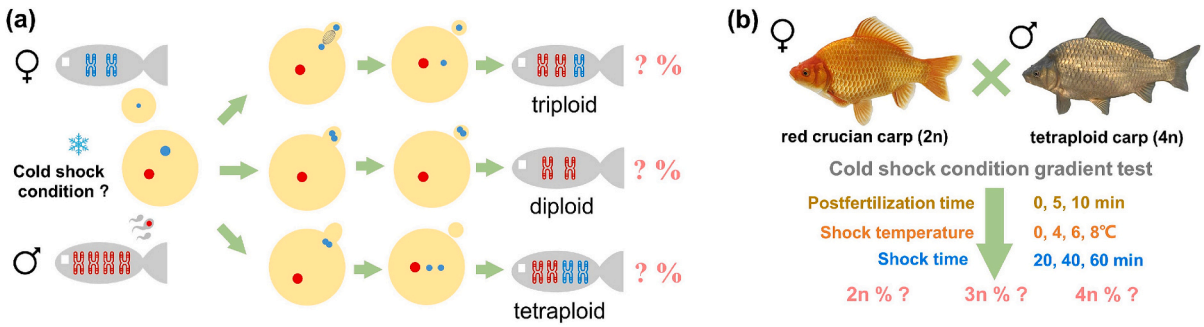

Fig. 1. The scientific question and technical roadmap of this study. (a) The scientific question of this study. What are the preferred MCST conditions for inducing tetraploids (polyploidization) or diploids (androgenesis)? The red circle represents the male nucleus; The big blue circle represents secondary female nucleus; The small blue circle represents female nucleus or second polar body. Different chromosomal colors are used only to distinguish between maternal and paternal lines. (b) The technical roadmap of this study. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

form three red crucian carp) were mixed with the semen (collected form three tetraploid carp), the eggs were evenly distributed among nine round petri dishes (diameter 18 cm, each was covered with about 1000 to 2000 sticky eggs) containing room-temperature fresh water (22 ± 1 °C) to activate fertilization. Then, each petri dish was treated according to the experimental design. The shock temperature was controlled by water bath. In the treatment of 4–8 °C, the water temperature was controlled by controlling the proportion of ice and room-temperature water. In the treatment of 0 °C, the water bath tank was placed in the −20 °C freezer, when the surface began to freeze, the water temperature was maintained at about 0 °C. After MCST, the eggs were transferred to room-temperature to hatch. These experiments were repeated three times in one week. The fertilization rate (FR), hatching rate (HR), malformation rate (MR) and non-malformed yield rate (NMYR) of each group were calculated by the following formula:

$$FR = number\ of\ fertilized\ eggs / number\ of\ eggs \times 100\%$$

$$HR = number\ of\ hatched\ larvaes / number\ of\ fertilized\ eggs \times 100\%$$

$$MR = number\ of\ malformed\ larvaes / number\ of\ hatched\ larvaes \times 100\%$$

$$NMYR = FR \times HR \times (100\% - MR)$$

Eggs that can develop to the late stage of the gastrula (about 24 h, at this point, the unfertilized egg deteriorates and appears white, which can be distinguished from the fertilized egg by the naked eye) are defined as fertilized eggs. Due to the small number of eggs used in the experiment, we did not adopt sampling statistics, but instead counted all eggs and larvaes in each culture dish.

2.4. Ploidy level determination

All groups of 2-week-old larvaes were sampled for DNA content determination.

Each larva was gently anesthetized using MS-222 and positioned at the center of a 200-mesh square nylon mesh (40 mm × 40 mm). Subsequently, the nylon mesh was folded diagonally, and a small quantity of ACD (anticoagulant citrate dextrose) solution was applied to moisten the body. Using tweezers, the larvae were then ground and rinsed with ACD solution to obtain a single cell suspension (0.4 mL), which was collected in a 1.5 mL EP tube. Following this, DAPI solution (Biotium, USA) measuring 0.4 mL was introduced into the EP tube and allowed to stain in darkness for a duration of 20 min. Finally, employing a flow cytometer (Cell Counter Analyser, Partec, Germany), we measured the DNA contents of all samples. The ploidy level can be determined by assessing its fluorescence level (FL), wherein diploid specimens exhibit an FL approximately around 100, triploid ones around 150, and tetraploid ones around 200; these values correspond consistently with their respective chromosome numbers.

2.5. Statistical analysis

Data are presented as the mean ± standard deviation of the mean (SDM). Significant differences among the different groups were determined using one-way analysis of variance (ANOVA), followed by Duncan's comparison test ($P < 0\ 0.05$). Pearson's correlation was used to analyze the relationship among three type gradient indicators and other data; $0.0 < |r| \leq 0.25$ indicated no correlation, $0.25 < |r| \leq 0.5$ indicated a weak correlation, $0.5 < |r| \leq 0.75$ indicated a moderate correlation, and $0.75 < |r| \leq 1.0$ indicated a strong correlation (Bae et al., 2006). Statistical analyses were performed using IBM SPSS Statistics (version 21.0, Armonk, USA).

3. Result

3.1. Effect of cold shock condition on larva yield

When the shock temperature was 4 °C and the shock time was 40 min, with the increase of post-fertilization time (0 → 10 min), the value of FR ($r = 0.90$, $p = 0.001$), HR ($r = 0.29$, $p = 0.448$), NMYR ($r = 0.82$, $p = 0.007$) showed an increasing trend, and MR ($r = -0.50$, $p = 0.169$) showed a decreasing trend (Table 1 and Fig. 2a). It can be seen that shortening post-fertilization time mainly affects fertilization rate and ultimately leads to a decrease in normal larva yield.

When the post-fertilization time was 5 min and the shock time was 40 min, with the increase of shock temperature (0 → 8 °C), the value of FR ($r = 0.72$, $p = 0.008$), HR ($r = 0.29$, $p = 0.368$), NMYR ($r = 0.60$, $p = 0.038$) showed an increasing trend, and MR ($r = -0.59$, $p = 0.044$) showed a decreasing trend (Table 1 and Fig. 2c). It can be seen that decreased shock temperature mainly affects fertilization rate and malformation rate, and ultimately leads to a decrease in normal larva yield.

When the post-fertilization time was 5 min and the shock temperature was 4 °C, with the increase of shock time (20 → 60 min), the value of FR ($r = -0.98$, $p < 0.001$), HR ($r = -0.88$, $p = 0.002$), NMYR ($r = -0.89$, $p = 0.001$) showed a decreasing trend, and MR ($r = 0.30$, $p = 0.427$) showed an increasing trend (Table 1 and Fig. 2e). It can be seen that increased shock time mainly affects fertilization rate and hatching rate, and ultimately leads to a decrease in normal larva yield.

3.2. Effect of post-fertilization time on ploidy level

Triploids were derived from normal meiosis, diploids and tetraploids were derived from failed meiosis (androgenesis and polyploidization, respectively). 100 % triploids were detected in the control group (Table 1). When the shock temperature was 4 °C and the shock time was 40 min, with the increase of post-fertilization time (0 → 10 min), the

Table 1
Comparison results of three univariate gradient experiments.

Items	FR (%)	HR (%)	MR (%)	NMYR (%)	3n/N (%)	2n/(2n + 4n) (%)	N
0–4-40	20 ± 6^{a}	51 ± 22^{ab}	35 ± 17^{b}	7 ± 3^{a}	16 ± 3^{a}	34 ± 3^{a}	45
5–4-40	44 ± 4^{b}	39 ± 7^{a}	39 ± 19^{b}	11 ± 5^{a}	5 ± 5^{a}	79 ± 5^{b}	50
10–4-40	52 ± 8^{c}	62 ± 11^{ab}	16 ± 2^{ab}	28 ± 9^{b}	69 ± 11^{b}	83 ± 17^{b}	54
C	67 ± 3^{d}	73 ± 15^{b}	7 ± 1^{a}	46 ± 8^{c}	100	/	59
5–**0**-40	49 ± 8^{a}	49 ± 24^{a}	30 ± 2^{bc}	18 ± 12^{ab}	0 ± 0^{a}	32 ± 11^{a}	49
5–**4**-40	44 ± 4^{a}	39 ± 7^{a}	39 ± 19^{c}	11 ± 5^{a}	5 ± 5^{a}	79 ± 5^{b}	50
5–**6**-40	63 ± 6^{b}	73 ± 13^{a}	21 ± 4^{ab}	36 ± 1b^{c}	24 ± 7^{b}	80 ± 5^{b}	59
5–**8**-40	74 ± 7^{b}	57 ± 24^{a}	6 ± 4^{a}	40 ± 16^{c}	94 ± 6^{c}	100 ± 0^{c}	52
C	67 ± 3^{b}	73 ± 15^{a}	7 ± 1^{a}	46 ± 8^{c}	100	/	59
5–4-**20**	59 ± 3^{c}	62 ± 9^{b}	23 ± 10^{ab}	28 ± 5^{b}	69 ± 5^{b}	41 ± 8^{a}	56
5–4-**40**	44 ± 4^{b}	39 ± 7^{a}	39 ± 19^{b}	11 ± 5^{a}	5 ± 5^{a}	79 ± 5^{b}	50
5–4-**60**	28 ± 4^{a}	28 ± 8^{a}	33 ± 11^{b}	6 ± 3^{a}	0 ± 0^{a}	100 ± 0^{c}	32
C	67 ± 3^{d}	73 ± 15^{b}	7 ± 1^{a}	46 ± 8^{c}	100	/	59
5–4-60*	8 ± 4	21 ± 13	28 ± 15	1 ± 1	0 ± 0	100 ± 0	13
C*	74 ± 4	60 ± 16	8 ± 5	42 ± 15	100	/	57

FR: fertilization rate; HR: hatching rate; MR: malformation rate; NMYR: non-malformed yield rate; N: the total number of individuals tested for ploidy; 2n, 3n, 4n indicate the detected diploid, triploid and tetraploid, respectively. Different letter superscripts (a, b) indicate significant differences between groups ($P < 0.05$), based on Duncan's test. * indicates repeated experiments.

triploid ratio ($r = 0.76$, $p = 0.017$) showed an increasing trend (Table 1, Figs. 2b and 3a). The triploid ratio of 0–4-40 (16 ± 3 %) and 5–4-40 (5 ± 5 %) was significantly lower than that of 10–4-40 (69 ± 11 %) ($p < 0.05$) (Table 1). It can be seen that meiosis II may have been mostly completed about 10 min after fertilization, resulting in a significant increase in triploid proportion than 0 and 5 min. This result is consistent with other triploid breeding studies in diploid fish, the best post-fertilization time for triploid induction is generally less than 10 min (Hayashida et al., 2021; Meng et al., 2023; Pradeep et al., 2014; Vasconcelos et al., 2022). However, the shortened post-fertilization time may also lead to a decrease in normal larva yield.

When the shock temperature was 4 °C and the shock time was 40 min, with the increase of post-fertilization time (0 → 10 min), the value of 2n/(2n + 4n) ($r = 0.85$, $p = 0.004$) showed an increasing trend (Table 1, Figs. 2b and 3a). This result indicates that after meiosis failure, the likelihood of the meiotic complex being extruded increases with the prolongation of post-fertilization time.

3.3. Effect of shock temperature on ploidy level

When the post-fertilization time was 5 min and the shock time was 40 min, with the increase of shock temperature (0 → 8 °C), the ratio of triploid ($p = 0.81$, $r = 0.001$) showed an increasing trend (Table 1, Figs. 2d and 3a). These results indicate that the lower the shock temperature, the better the inhibition of meiosis II, which is consistent with other research results (Hayashida et al., 2021; Hussain et al., 1991; Piferrer et al., 2000; Vasconcelos et al., 2022). However, the reduced shock temperature may also lead to a decrease in normal larva yield.

With the increase of shock temperature (0 → 8 °C), the value of 2n/(2n + 4n) ($p = 0.94$, $r < 0.001$) also showed an increasing trend (Table 1, Figs. 2d and 3a). This result indicates that after meiosis failure, the likelihood of the meiotic complex being extruded increases with the increase of shock temperature.

3.4. Effect of shock time on ploidy level

When the post-fertilization time was 5 min and the shock temperature was 4 °C, with the increase of shock time (20 → 60 min), the ratio of triploid ($r = -0.89$, $p = 0.001$) showed an decreasing trend (Table 1, Figs. 2f and 3a). These results indicate that the longer the shock time, the better the inhibition of meiosis, which is consistent with other research results (Hayashida et al., 2021; Pradeep et al., 2014; Vasconcelos et al., 2022). However, the prolonged shock time may also lead to a decrease in normal larva yield.

With the increase of shock time (20 → 60 min), the value of 2n/(2n + 4n) ($r = 0.97$, $p < 0.001$) also showed an increasing trend (Table 1, Figs. 2f and 3a). This result indicates that after meiosis failure, the likelihood of the meiotic complex being extruded increases with the increase of shock time.

4. Discussion

Once the mature egg is ovulated and activated, the meiosis II starts quickly in the edge of egg membrane. As meiosis transitions from mid to late stages, chromosomes continue to be pulled towards the two poles, and the outer chromosomes protruding from cell membrane surface, ultimately forming a PB2 (Dekens et al., 2003; Hu, 2016; Wang, 2011; Wolenski and Hart, 1987). This process of the extrusion of PB2 is usually completed within 10 min in eurythermal fish, which is why the maximum post-fertilization time was set at 10 min in this study. After entering the egg, sperm gradually transform into a male nucleus, and the centrosome it carries gradually emits starlight, and the zygote is formed with the FN about 30 min later in eurythermal fish (Hu, 2016; Wang, 2011). MCST can inhibit meiosis II, resulting in the formation of a meiotic complex (including the PB2, FN and disabled spindle body), which may be extruded from the fertilized egg to induce androgenesis, or it may be retained in the fertilized egg to induce polyploidy (Gu et al., 2024; Morishima et al., 2011; Zhou et al., 2018).

Our results showed that the less the post-fertilization time, the lower the shock temperature and the longer the shock time, the greater the damage to the fertilized eggs in MCST, and the higher the death and malformation rates of the embryos. In addition, the greater the damage caused by MCST to the fertilized eggs, the better the effect of inhibiting the meiosis II, resulting in an increase in the proportion of diploid and tetraploid offspring (Fig. 3b). Conversely, the less the damage caused by MCST to the fertilized eggs, the weaker the effect of inhibiting the meiosis II, resulting in an increase in the proportion of triploid offspring (Fig. 3b).

Our results showed that the ratio of diploid increases with an increase in post-fertilization time in individuals experiencing meiosis failure. As meiosis II progresses, the polar side gradually protrudes outward (Hu, 2016; Wang, 2011). Therefore, as the post-fertilization time increases, the meiotic complex is further outward after meiosis failure. We speculate that the outward distance of the meiotic complex is the key to inducing androgenesis or polyploidization. The more outward the meiotic complex is, the more likely it is to be extruded (inducing androgenesis), while the more inward the meiotic complex is, the more likely it is to be retained (inducing polyploidization). The ratio of diploid increases with an increase in shock temperature in individuals experiencing meiosis failure. This result indicates that the outward progression of meiotic complex is not completely stop during MCST and can still proceed slowly, and the higher the temperature, the faster it progresses. Therefore, we speculate that the distance of the meiotic complex is further outward with increasing shock temperature after meiosis failure, adding the likelihood of being extruded (inducing androgenesis). The ratio of diploid increases with an increase in shock time in individuals

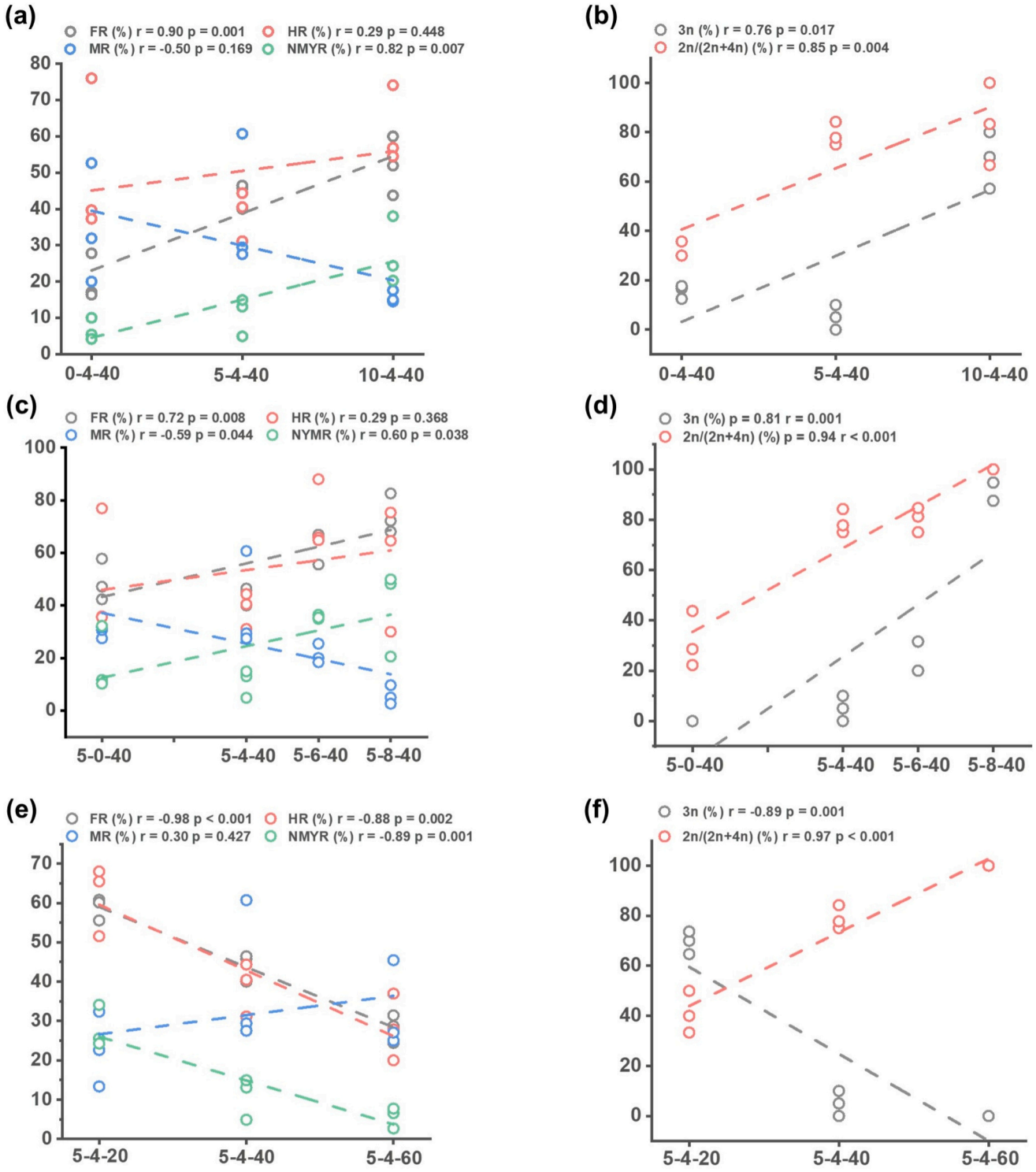

Fig. 2. The trend relationship between different meiosis cold shock conditions and each indicator. (a-b) The trend relationship between postfertilization time and each indicator. (c-d) The trend relationship between shock temperature and each indicator. (e-f) The trend relationship between shock time and each indicator. FR: the fertilization rate; HR: the hatching rate; MR: the malformation rate; NMYR: the non-malformed yield rate; 2n: diploid; 3n: triploid; 4n: tetraploid.

experiencing meiosis failure. Since the outward progression of meiotic complex is not completely stop, we speculate that the outward distance of the meiotic complex is further outward with increasing shock time, adding the likelihood of being extruded (inducing androgenesis).

In summary, with the increase of post-fertilization time, shock temperature and shock time, the meiotic complex may further protrude after meiotic failure, thereby increasing the possibility of meiotic complex extruded from the fertilized egg and more likely leading to androgenesis. We suggest that the outward distance of the meiotic complex is a key factor in inducing androgenesis or polyploidization after meiosis failure. Here we propose a “total distance” hypothesis to explain the above phenomenon. We compare the outward extending distance of the meiotic complex during the untreated time after fertilization to the “starting distance”, which relates to the post-fertilization time. The longer the post-fertilization time, the farther the “starting distance”. We compare the outward extending distance of the meiotic complex during

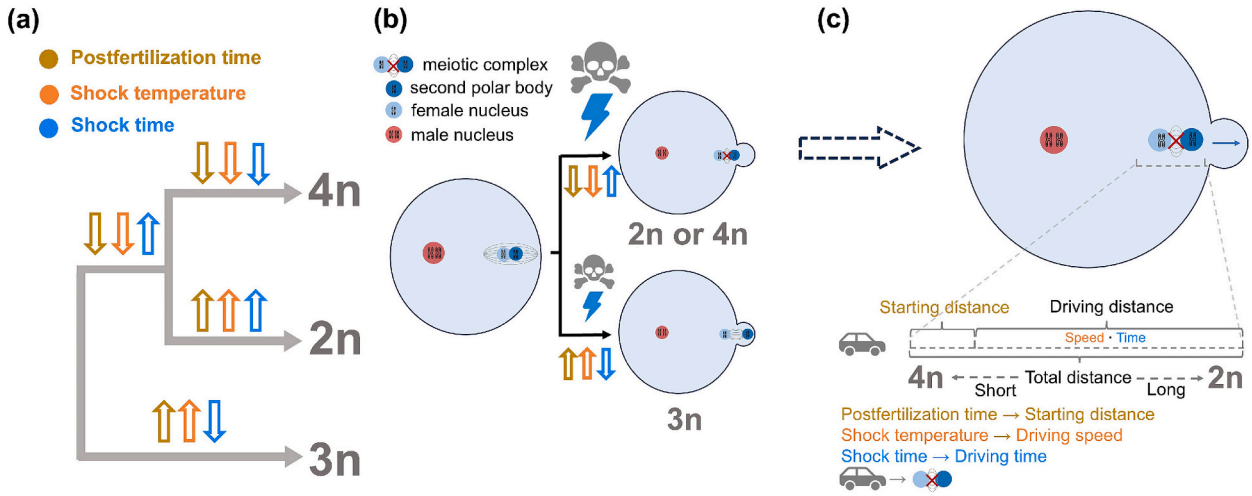

Fig. 3. Summary and hypotheses of this study. (a) Summary of ploidy inducing trends in three variables. The color of variables corresponds to arrows of the same color; the up arrow indicating a positive correlation and the down arrow indicating a negative correlation. (b) A model explains the inducing trends between 3n and 2n (or 4n). The blue lightning represents shock stress, and its size represents strength. The skull represents the proportion of dead and deformed individuals, and its size indicates the proportion. (c) A "total distance" hypothesis explains the inducing trends between 2n and 4n. Postfertilization time is compared to "Starting distance"; shock temperature is compared to "Driving speed"; shock time is compared to "Driving time". "Driving distance" is positively related to "Driving speed" and "Driving time". All three variables are positively correlated with "total distance". (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

MCST to the "driving distance", which relates to the shock temperature and shock time. We compare the shock temperature to the "driving speed", and the higher the shock temperature, the faster the "driving speed". We compare the shock time to the "driving time", and the longer the shock time, the longer the "driving time". In summary, the "total distance" is positively related to "starting distance", "driving speed" and "driving time". This hypothesis suggests that after meiosis failure, the shorter "total distance", the easier it is for the meiotic complex to remain in the fertilized egg, and the proportion of tetraploids increases. The longer the "total distance", the easier it is for the meiotic complex to be extruded from the fertilized egg, and the proportion of androgenetic diploid increases (Fig. 3c).

This study may provide valuable references into androgenesis, triploid and gynogenesis breeding in fish, because these techniques will use MCST. For androgenesis, we found MSCT can induce a large number of androgenetic individuals, and the condition of 5–4-60 that the androgenetic individuals can be 100 % detected in surviving individuals, which may save a lot of screening time and cost. However, the death rate of embryos under the condition is extremely high. Moreover, most aquaculture fish are diploids, and after MSCT induces androgenetic haploids, it is necessary to double genome to obtain male androgenetic double haploids, but they are difficult to develop into adults, which is an important factor limiting the application of androgenesis (Piferrer et al., 2009; Komen and Thorgaard, 2007). In gynogenesis and triploid breeding through MCST doubling genomes, the post-fertilization time, shock time and shock temperature can be appropriately reduced to decrease the occurrence of androgenetic haploids. However, reducing post-fertilization time and shock temperature can also lead to a decrease in normal larva yield, so appropriately reducing shock time may be a better option. Due to the negative correlation between normal larva yield and meiosis inhibition rate, shock conditions need to be balanced according to different experimental purposes. For example, if only a small number of doubled individuals or androgenetic individuals need to be obtained, stricter cold shock conditions can be set to increase meiotic inhibition rate, thereby reducing the workload of subsequent screening. Instead, the cold shock conditions should be appropriately relaxed if the yield of target fish is to be considered.

5. Conclusion

In this study, we used the crossing between red crucian carp (2n, ♀) × tetraploid carp (4n, ♂) as the research object, and different MCST conditions were set to explore the ploidy induction trend. We found that the shorter the post-fertilization time, the lower the shock temperature and the longer the shock time, the greater the likelihood of meiosis failure, leading to the occurrence of androgenesis and polyploidy. Further, we found that the longer the post-fertilization time, the higher the shock temperature and the longer the shock time, the higher the proportion of androgenetic individuals in these individuals with meiosis failure. We propose a "total distance" hypothesis to explain these results.

CRediT authorship contribution statement

Haoran Gu: Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Yalan Zhang:** Investigation. **Yuxiang Zhang:** Investigation. **Xin Wang:** Investigation. **Qilong Liu:** Investigation. **Rurong Zhao:** Resources. **Conghui Yang:** Writing – review & editing. **Qizhi Liu:** Resources, Investigation. **Kaikun Luo:** Resources. **Shi Wang:** Writing – review & editing, Resources, Funding acquisition. **Shaojun Liu:** Writing – review & editing, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

Data will be made available on request.

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