Theor Appl Genet (1994) 89:193–197
© Springer-Verlag 1994

N. B. Cherfas · Y. Peretz · N. Ben-Dom
B. Gomelsky · G. Hulata

Induced diploid gynogenesis and polyploidy in the ornamental (koi) carp, *Cyprinus carpio* L.

4. Comparative study on the effects of high- and low-temperature shocks

Received: 12 July 1993 / Accepted: 25 November 1993

Abstract The effects of high- and low-temperature shock treatments, applied at different phases of the 2nd meiotic division within the limits of 0.05–0.60 τ_0 (τ_0 = relative unit of embryological age) in order to induce gynogenesis in the common carp, were studied. A remarkable difference in the effect of two temperature treatments applied at the same biological age after insemination (expressed in τ_0) was revealed. The curves of embryo survival and diploid gynogenetic larva output showed a bimodal response in cold-shocked gynogenetic progenies, with the highest level of diploid larva output at the periods 0.05–0.10 τ_0 and 0.30–0.40 τ_0 (after insemination), separated by a period of high sensitivity to cold shock (0.15–0.25 τ_0). In contrast to this, the curves of embryo survival and diploid gynogenetic larva output showed a single, narrow, peak corresponding to 0.15–0.25 τ_0 in heat-shocked gynogenetic progenies. The results obtained are in general accord with those of previous experiments on induced gynogenesis and triploidy in common carp, in which either cold- or heat-shock was used.

Key words Common carp · Chromosome-set manipulations · Heat shock · Cold shock · Induced gynogenesis

Introduction

Temperature shocks are common agents used to induce suppression of cell division for chromosome-set manipulations in fish (e.g., Thorgaard 1983). Two types of temperature treatment, i.e., long cold shock and short heat shock, are used for suppression of the 2nd meiotic division in eggs, in order to induce meiotic gynogenetic or triploid fish progenies. In spite of a large number of experiments on this subject, there is limited information concerning the comparative effect of these two types of temperature-shock treatment on embryo development. The first indications of differential high- and low-temperature-shock effects were obtained in experiments on induced gynogenesis in the loach *Misgurnus fossilis* L. (Romashov and Belyaeva 1965). Further data were obtained for other fish, including the tilapia species *Oreochromis aureus* and *O. niloticus* (Don and Avtalion 1988; Hussain et al. 1991). Results of experiments on induced gynogenesis and triploidy in the common carp have also indicated some differences in the effects of high- and low-temperature shocks on embryo development, mainly concerning the optimum shock timing for interference with the 2nd meiotic division (Nagy et al. 1978; Hollebecq et al. 1986; Taniguchi et al. 1986; Linhart et al. 1987, 1991; Komen et al. 1988; Gomelsky et al. 1989; Cherfas et al. 1990, 1994). No direct comparison of the effects of high- and low-temperature shocks in the common carp has, however, yet been carried out. The present investigation was, therefore, aimed at obtaining accurate data on the comparative effects of differential temperature shocks applied to common carp eggs at the 2nd meiotic division, with a view to the optimization of chromosome-set manipulation techniques and an improvement in our understanding of the nature of temperature influences on cellular processes.

Communicated by G. Wenzel

N. B. Cherfas (✉) · Y. Peretz · N. Ben-Dom · B. Gomelsky · G. Hulata
Agricultural Research Organization, Fish and Aquaculture Research Station, Dor, Mobile Post Hof Hacarmel 30820, Israel

Materials and methods

The experiments were conducted at the fish and Aquaculture Research Station, Dor, during 1991–1992. The experimental procedures were identical to those described by Cherfas et al. (1993, 1994), with the specific required modifications. Eggs, fertilized with irradiated sperm, were separately exposed to either a high- or low-temperature shock, or left unshocked as a control. Temperature shocks were initiated within the period of the 2nd meiotic division, the timing of shock initiation being expressed in τ_0 (Detlaff and Detlaff 1961; Cherfas et al. 1993, 1994). In the present experiments, temperature shocks were initiated (at 0.5 τ_0 intervals) from 0.05 to 0.40 τ_0 after insemination, in exp. 20/91 and form 0.05 to 0.60 τ_0, in exp. 1/92 and 2/92. The exact

temperatures, as well as the durations and timings of shocks used in each experiment, are presented in Table 1. After completion of temperature shock, the eggs were returned to incubation at an ambient water temperature of 20–23 °C. Eggs (150–450) were shocked and incubated, attached to Petri dishes placed on trays, within a circulated water system.

Active surviving larvae of normal appearance were assumed to be gynogenetic diploids. The results were expressed as per-cent survival prior to and 2–3 days following hatching.

Results

The rates of fertilization and embryo survival prior to hatching in control batches varied in the ranges of 81–96% and 72–90%, respectively, indicating that eggs used were of good quality (Table 1). A majority of the embryos died at or around hatch, presumably due to their haploid condition. A few diploid larvae were found in all control groups, the rate of spontaneous diploidization varying between 0.5–0.9% of the number of eggs inseminated. All descendants (both in control and shocked progenies) had the recessive (transparent) body color, confirming their gynogenetic origin and demonstrating the efficacy of the sperm-irradiation procedures.

The pattern of embryo survival following the exposure of eggs to temperature-shock treatment varied markedly between the two shock types (Table 1, Fig. 1). Cold-shock treatments were characterized by two per-

Table 1 Results of the experiments with temperature shocks applied at the 2nd meiotic division: embryo survival and diploid larvae production in gynogenetic progenies

Shock initiation τ_0	heat shock		2n-larvae		Cold shock		2n-larvae	
	No. of eggs	Survival prior to hatching %[a]	No.	%[a]	No. of eggs	Survival prior to hatching %[a]	No.	%[a]
Experiment 20/91	(39.4 °C, 2.5 min)				(1.4–2.6 °C, 45 min)			
0.05	217	68	14	6.5	167	74	22	13.1
0.10	313	62	12	3.8	295	28	8	2.7
0.15	242	73	22	9.0	317	20	6	1.9
0.20	260	81	23	8.8	364	26	11	3.0
0.25	280	76	46	16.4	291	5	0	0.0
0.30	347	63	27	7.2	291	39	5	1.7
0.35	291	53	12	4.1	271	56	30	11.1
0.40	306	44	3	0.9	277	45	19	6.9
C[b]	325	90	1	0.3				
Experiment 1/92	(39.5 °C, 1.8 min)				(3.6–3.8 °C, 30 min)			
0.05	236	38	9	3.8	272	49	6	2.2
0.10	287	39	18	6.3	407	53	2	0.4
0.15	206	81	55	26.7	398	19	2	0.7
0.20	300	77	101	33.7	463	12	1	0.2
0.25	260	61	35	13.5	339	3	0	0.0
0.30	285	49	9	3.2	371	27	15	4.0
0.35	272	42	2	0.7	269	77	9	3.3
0.40	293	23	2	0.7	312	73	1	0.3
0.45	280	22	0	0.0	446	83	0	0.0
0.50	291	26	1	0.3	246	72	0	0.0
0.55	267	37	0	0.0	288	68	3	1.0
0.60	353	33	0	0.0	189	53	0	0.0
C[b]	369	72	2	0.5				
Experiment 2/92	(39.8 °C, 2.0 min)				(3.2–3.5 °C, 30 min)			
0.05	257	11	0	0.0	316	63	14	4.4
0.10	205	20	1	0.5	358	28	1	0.3
0.15	171	16	4	2.3	247	2	2	0.8
0.20	231	25	14	6.0	254	2	0	0.0
0.25	283	11	3	1.1	268	17	5	1.9
0.30	197	14	0	0.0	236	52	24	10.2
0.35	237	4	0	0.0	207	49	5	2.4
0.40	213	2	0	0.0	256	52	5	1.9
0.45	229	3	0	0.0	273	53	1	0.4
0.50	225	6	0	0.0	304	66	2	0.7
0.55	219	5	0	0.0	308	62	0	0.0
0.60	229	7	0	0.0	190	47	1	0.5
C[b]	276	84	2	0.7				

[a] From the total number of inseminated eggs

[b] No-shock gynogenetic control

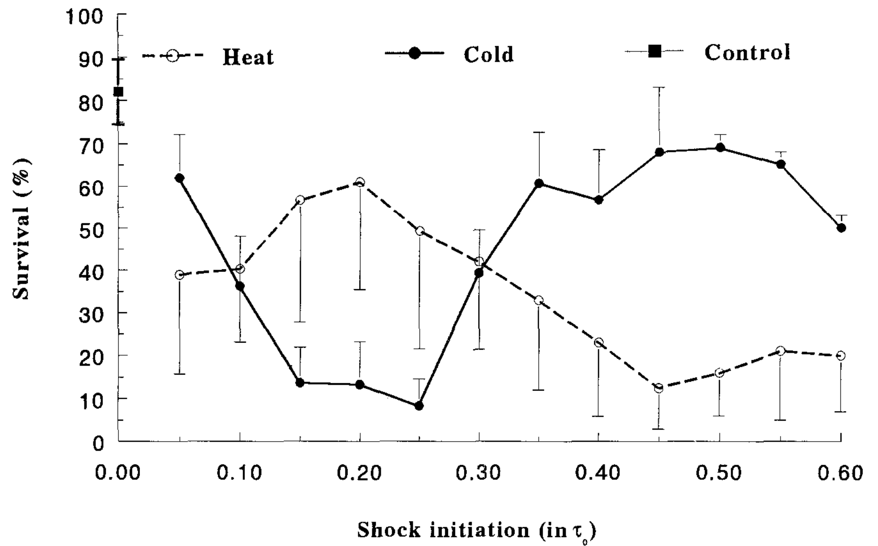

Fig. 1 Survival (% from number of inseminated eggs) prior to hatching in heat- and cold-shock-treated gynogenetic progenies (means of three experiments ± SEM)

iods of high embryo survival (corresponding to 0.05–0.10 τ_0 and 0.30–0.55 τ_0). Embryo survival was similar within these two periods, but 15–30% lower than in control batches. A drastic decrease in embryo survival was registered in the period 0.10–0.30 τ_0 after insemination, indicating extremely high sensitivity to low temperature during this period.

Embryo survival following heat-shock treatments was distinctively different from that seen in the, simultaneously applied, cold-shock treatments (Table 1, Fig. 1). A single peak within 0.15–0.25 τ_0 after insemination was seen, in which survival was close to that of the control groups in experiments 20/91 and 1/92. In experiment 2/92 embryo survival was very low, apparently due to a too-high shock temperature.

The rate of production of 2n-gynogenetic larvae following cold-shock treatments also showed a bimodal response (Table 1, Figs. 2, 3), the optimum timing for cold-shock initiation corresponding to either 0.05 τ_0 or 0.30–0.40 τ_0. Gynogenetic diploid larvae were infrequently recorded when cold shocks were initiated at other timings, even when high embryonic survival had been observed (later than 0.40 τ_0). The yield of diploid larvae was similar in these two effective periods, being 6.6% (at 0.05 τ_0) and 5.3–5.6% (at 0.30–0.35 τ_0) when calculated from the total number of inseminated eggs (Fig. 2), and 9.8% and 12.9–9.7% of the number of live embryos prior to hatching (Fig. 3).

Although heat shocks were more variable in their effect (Table 1), a single peak was observed in the production curves of diploid gynogenetic larvae following their application (Figs. 2, 3). The highest numbers of diploid larvae were obtained when heat shocks were initiated at 0.15–0.25 τ_0. The optimum timing corresponded to 0.20 τ_0, when the mean frequency of diploid larvae was 16.2% of the total number of inseminated eggs (Fig. 2), or 26.3% when calculated from the number of live embryos prior to hatching (Fig. 3).

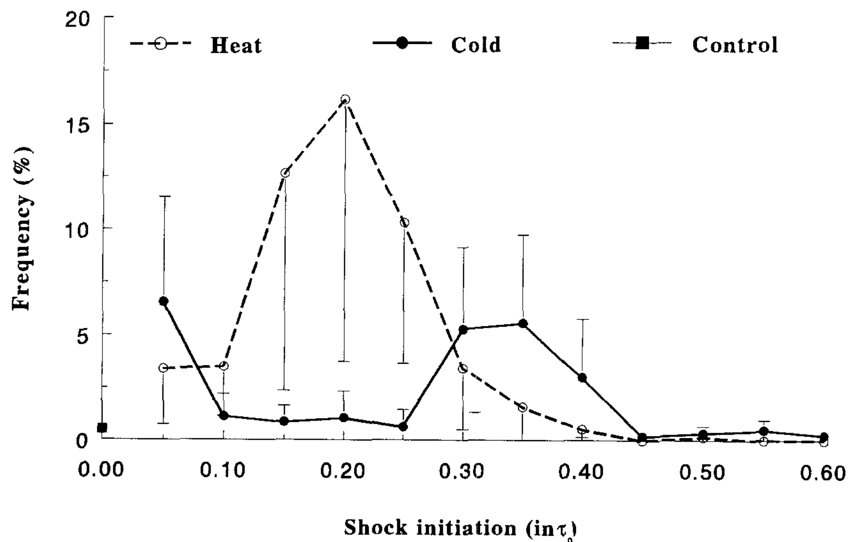

Fig. 2 Production of diploid larvae (% from number of inseminated eggs) in heat- and cold-shock-treated gynogenetic progenies (means of three experiments ± SEM)

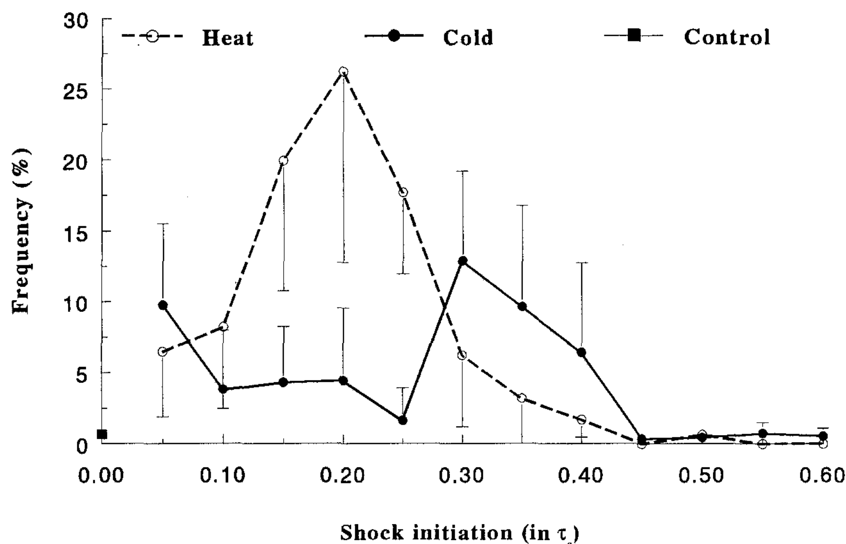

Fig. 3 Production of diploid larvae (% from number of live embryos prior to hatching) in heat- and cold-shock-treated gynogenetic progenies (means of three experiments ± SEM)

Heat shock was more effective than cold shock (according to the output of diploid gynogenetic larvae) in exp. 20/91 and 1/92, while no difference was found between cold- and heat-shock series in exp. 2/92. The mean production of diploid larvae at the effective periods was higher in heat-shock than in cold-shock treatments (Figs. 2, 3), although the differences were not significant [$t_{[df=4]} = 1.3-1.4$].

Discussion

The results of the present experiments revealed differences between the effects of cold- and heat-shocks on both embryo survival and chromosome-set diploidization, with a peak in the cold-shock curve when a sharp drop occurred in the heat-shock curve, and vice versa.

The features of the response of diploid larva production to cold- and heat-shock treatments are independent of embryo survival, since the pattern does not change when it is calculated relative either to the number of inseminated eggs or to that of live embryos prior to hatching (Figs. 2, 3). Based on the data of Saat (1991) the optimum timing for cold shock corresponds to early anaphase-II (the first peak) and telophase-II (the second peak), while optimum timing for heat shock corresponds to middle-late anaphase-II. The period from telophase-II to the separation of the 2nd polar body ($0.30\,\tau_0$–$0.48\,\tau_0$ after insemination) is ineffective for both type of temperature-shock treatment. These results are in agreement with previous published data. Bimodal response to cold shock of embryo survival and the production of diploid gynogenetic (or triploid) larvae was described by Nagy et al. (1978), Taniguchi et al. (1986) and Komen et al. (1988). According to our calculations, the optimal timing for cold shocks observed in these investigations corresponded to $0.08\,\tau_0$ and 0.34–$0.42\,\tau_0$ (Taniguchi et al. 1986), to $0.05\,\tau_0$ and $0.39\,\tau_0$ (Komen et al. 1988) and to $0.17\,\tau_0$ and $0.51\,\tau_0$ (Nagy et al. 1978). The results of the first two studies coincide with those of the present investigation. Similar data were also obtained by Cherfas et al. (1990) when shock application was examined shortly after insemination. The highest number of diploid gynogenetic larvae was obtained in that study when the shock was initiated within 0.03–$0.07\,\tau_0$ after insemination, while a sharp decrease in embryo survival and diploid gynogenetic larval production was observed when cold shock was applied later. The best results reported from other cold-shock experiments on induced gynogenesis and triploidy in the common carp (Gervai et al. 1980; Ueno 1984; Linhart et al. 1987, 1991) suggested shock initiation within a wide range of 0.06–$0.27\,\tau_0$ after insemination, but a true optimum timing could not be calculated because cold shocks were initiated at wide time intervals.

In contrast, high-temperature 2nd meiotic interference treatments are characterized by having a single optimum (Hollebecq et al. 1986; Gomelsky et al. 1989; Sumantadinata et al. 1990; Cherfas et al. 1994) occurring within 0.10–$0.25\,\tau_0$ after insemination (Cherfas et al. 1994 and this study).

In experiments on induced gynogenesis in the loach (Romashov and Belyaeva 1965) the curves for the production of diploid larvae also had a different optimal timing for cold- and heat-shock initiation. A pattern similar to that of the common carp was observed, and the effective interval after insemination corresponded approximately to the single heat-shock and the second cold-shock peaks of the present study. In experiments on induced triploidy in tilapia (Don and Avtalion 1988) cold shock was effective within a long period after insemination, whereas only a narrow range therein was also effective for heat shock. Some indications of different shock timing for low and high temperatures (similar to those reported for the loach) were obtained in a further investigation on tilapia (Hussain et al. 1991). It may be safe to conclude, therefore, that the optimal timings for the application of cold- and heat-shocks in order to suppress the 2nd meiotic division are different.

Acknowledgments This research was supported by the Center for Absorption in Science, Minstry of Absorption, State of Israel, and by Grant No IS-1612-89R from BARD, the United States-Israel Binational Agricultural Research and Development Fund. We thank an anonymous reviewer for comments that helped us improve the presentation of the results.

References

Cherfas NB, Kozinsky O, Rothbard S, Hulata G (1990) Induced diploid gynogenesis and triploidy in ornamental (koi) carp *Cyprinus carpio* L. 1. Experiments on the timing of temperature shock. Isr J Aquacult – Bamidgeh 42:3–9

Cherfas NB, Hulata G, Kozinsky O (1993) Induced diploid gynogenesis and polyploidy in ornamental (koi) carp, *Cyprinus carpio* L. 2. Timing of heat shock during the first cleavage. Aquaculture 111:281–290

Cherfas NB, Peretz Y, Ben-Dom N, Gomelsky B, Hulata G (1994) Induced diploid gynogenesis and polyploidy in ornamental (koi) common carp, *Cyprinus carpio* L. 3. Optimization of heat shock timing during the 2nd meiotic division and the 1st cleavage. Theor Appl Genet

Dettlaff TA, Dettlaff AA (1961) On relative dimensionless characteristics of the development duration in embryology. Arch Biol 72:1–16

Don J, Avtalion RR (1988) Comparative study on the induction of triploidy in tilapias, using cold- and heat-shock techniques. J Fish Biol 32:665–672

Gervai J, Peter S, Nagy A, Horvath L, Csanyi V (1980) Induced triploidy in carp, *Cyprinus carpio*. J Fish Biol 17:667–671

Gomelsky BI, Rekoubratsky AV, Emelyanova OV, Pankratyeva EV, Lekontzeva TI (1989) Obtaining the carp diploid gynogenetic offspring by heat shock on developed eggs. (in Russian) Voprosy Ichtiology 28:168–178

Hollebecq MG, Chourrout D, Wohlfarth G, Billard R (1986) Diploid gynogenesis induced by heat shock after activation with UV-irradiated sperm in common carp. Aquaculture 54:69–76

Hussain MG, Chatterji A, McAndrew BJ, Johnstone R (1991) Triploidy induction in Nile tilapia, *Oreochromis niloticus* L., using pressure, heat-and cold-shocks. Theor Appl Genet 81: 6–12

Komen J, Duynhouwer J, Richter CJJ, Huisman EA (1988) Gynogenesis in common carp (*Cyprinus carpio* L.). I. Effect of genetic manipulation of sexual products and incubation conditions of eggs. Aquaculture 69:227–239

Linhart O, Slechtova V, Kvasnicka P, Rab P, Kouril J, Hamackova J (1987) Rates of recombination in LDH B_1 and MDH loci phenotypes after "pb" and "m" gynogenesis in carp, *Cyprinus carpio* L. In: Tiews K (ed) Selection, hybridization, and genetic engineering in aquaculture. Heeneman Verlagsgesellschaft, Berlin, pp 335–345

Linhart O, Flajshans M, Kvasnicka P (1991) Induced triploidy in the common carp (*Cyprinus carpio* L.): a comparison of two methods. Aquat Living Resour 4:139–145

Nagy A, Rajki K, Horvath L, Csanyi V (1978). Investigations on carp, *Cyprinus carpio* L., gynogenesis. J Fish Biol 13:215–224

Romashov DD, Belyaeva VN (1965) The increase in the yield of diploid gynogenetic larvae in loach (*Misgurnus fossilis* L.) achieved by temperature shocks. (in Russian with English summary) Bull Mosc O-va isp Prir Biol Part 60:47–59

Saat T (1991) The time-course of fertilization of fishes and amphibians (in Russian with English summary). Ontogenez 22:63–70

Sumantadinata K, Taniguchi N, Sugama K (1990) The necessary conditions and the use of ultraviolet-irradiated sperm from different species to induce gynogenesis of Indonesian common carp. In: Hirano R, Hanyu I (eds) Proc 2nd Asian Fisheries Forum. Asian Fish Society, Manila, pp 539–542

Taniguchi N, Kijima A, Tamura T, Takegami K, Yamasaki I (1986) Color, growth and maturation in ploidy-manipulated fancy carp. Aquaculture 57:321–328

Thorgaard GH (1983) Chromosome-set manipulation and sex control in fish. In: Hoar WS, Randall DJ, Donaldson EM (eds) Fish physiology IXB. Academic Press, New York, pp 405–434

Ueno K (1984) Induction of triploid carp and their haematological characteristics. Jpn J Genet 59:585–591