热休克法诱导日本沼虾四倍体的初步研究

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摘 要 以热休克抑制受精卵第一次卵裂，进行日本沼虾四倍体诱导实验。热休克温度在38、39、40和41℃，于产卵210～230分钟后，处理1～2分钟均可获得四倍体胚胎，40℃处理1.5分钟，四倍体胚胎诱导率达36.8%，38和39℃处理2分钟所获得的嵌合体胚胎比率大于四倍胚胎比率，而热休克温度40℃时，四倍体率显著高于嵌合体比率。41℃处理时由于有丝分裂异常导致胚胎死亡率高，更适宜的处理时间有待进一步研究，以便提高四倍体育成率。

关键词 日本沼虾，四倍体，热休克

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评《中国淡水鱼类种质资源和保护》

我国是生物多样性十分丰富的国家。种类众多的动、植物，幅员广阔和多变的地理条件形成了多种多样的生态系统。保护生物多样性，就是保护人类自己。保护好鱼类资源，就是保护了人类生存所必需的水产动物蛋白质生产源泉。

我国是淡水渔业大国，淡水养殖产量居世界首位。丰富的淡水生物多样性是我国淡水养殖发展的物质基础。由于生产的迅速发展，向自然界索取生物资源的强度越来越大，已开始威胁到他们的持续利用。上海水产大学李思发教授编著的《中国淡水鱼类种质资源和保护》一书，从遗传多样性及其永续利用角度，我国淡水鱼类资源的种质资源及其保护问题进行了阐述，是我国在这一领域的首部书籍。全书分五章，着重介绍了我国淡水鱼类种质资源遗传差异、保护和利用的途径与措施，种质鉴定和评介方法。书中有7幅彩照，书后附有140篇国内外参考文献。可供水产、生物、环保等方面的科技人员、行政管理人士阅读参考。

《中国淡水鱼类种质资源和保护》一书，由中国农业出版社1996年出版。全书186页，定价21.20元。

（卢 怡）

A PRELIMINARY STUDY ON INDUCTION OF TETRAPLOIDY IN THE FRESHWATER PRAWN *MACROBRACHIUM NIPPONENSE* BY HEAT SHOCK

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ABSTRACT Heat shocks were used in this experiment for inducing tetraploids through inhibition of the first cleavage division in the freshwater prawn *Macrobrachium nipponense*. Tetraploidy prawn embryos had been produced by treating eggs at either 38、39、40 or 41 ℃ for 1 ~ 2 minute duration from 210 ~ 230 minutes shortly after spawning. At 40 ℃, shock lasting 1.5 minutes yield 36.8% tetraploidy embryos. The percentage of mosaics were significantly higher than that of tetraploids at shock of either 39 ℃ for 2 minutes. With shock 41 ℃, the mortality of embryos was high due to abonormal karyokinesis at mitosis. Optional treatment time is needed for further investigating in order to yield high percentages of tetraploids.

KEYWORDS *Macrobrachium nipponense*, Tetraploidy embryos, Heat shock

1 INTRODUCTION

Chromosomal engineering was suggested as a genetic method of improving aquatic cultured species. Polyploidy was as useful in aquaculture industry as in plant breeding. Triploids were reported to display a faster growth rate and a larger body size due to their sterility [Cassani et al. 1984, Purdom 1972], but it was difficult for them to be applied in large-scale commercial production because of methodological problems. Tetraploids, which were expected to be fertility, would produce sterile triploids offspring by crossing with normal diploids, so the benefits of triploids would be made more practical [Bidwell et al. 1985]. To date, a variety of tetraploid fishes had been successfully induced [Bidwell 1985, Hong 1990, Refstie 1981, Thorgaard et al. 1981, Valenti 1975]. However, in decapod, the only artificial tetraploidy shrimp were those reported by Xiang et al. [1992] in *Penaeus chinensis*. In the case of freshwater prawn *Macrobrachium nipponense*, Qiu et al. [1994] had previously studied the chromosome and karyotypes. In this paper, we present the results of experiment on the induction of tetraploidy in this species.

2 MATERIALS AND METHODS

2.1 MATERIALS

Sexually mature prawn used in this experiment were purchased from local markets in April

1995—06—23 Received

1993for our laboratory experiments. They were kept in well aerated water at 25 ℃ and were fed daily with earthworms. When females finished copulating, the gravid females with spermatophores were isolated in different aquariums and induced to spawn by raising the water temperature to 28 ℃. The females would begin to spawn within 4 ~ 18 hour.

2.2 HEAT SHOCK TREATMENT

2.2.1 DETERMINATION OF INITIAL TIME OF HEAT TREATMENT

The most important parameter to produce tetraploids was the initial heat shock time for suppression of the first cleavage division. The optimum treatment time was determined by the following experiments in which the events of early development were investigated. Fertilized eggs were collected in 120 ~ 250 minutes immediately after ovulation, fixed in Bouin' s fixing solution, then dehydrated in a graded series of ethanol and embedded in paraffin. Continuous sections were stained with Delafield' s hematoxylin and eosin, and examined under an Olympus microscope. The first mitotic karyokinesis of the fertilized eggs was often observed in 210 ~ 240 minutes soon after spawning at 28 ℃.

2.2.2 HEAT SHOCKS

The fertilized eggs which attached to the pleopods of the maternal were subjected to heat shock at either 38、39、40 or 41 ℃ for 1, 1.5 or 2 minute duration from 220 ~ 230 minutes post spawning. Following the treatment, the maternal with treated eggs were separately put back to aquarium with temperature of 25 ℃ for hatch. If eggs came off the pleopods or maternal died in the treatments, the eggs were transferred into a 1 000 mL glass beaker for incubation. The dead eggs were removed at regular intervals. The controls received no treatment of heat shock.

2.2.3 CHROMOSOME EXAMINATION

Chromosome preparations were made at blastula stage and gastrula stage. Aliquots embryos from experimental and control groups were placed in a 0.05% colchicine solution for 3 or 4 hours, hypotonized in 0.1 M KCl solution for 30 minutes, and then fixed in two changes of fresh Carnoy' s fixative (3 ∶1 methanol to acetic acid) at 4 ℃ for 1 hour or longer. Fixed embryos were placed on a clear slide, macerated with one or two drops of 60% glacial acetic acid for duration of 1 ~ 3 minutes, and minced gently with fine forceps. The preparations were kept at room temperature for air dried, then stained in 10% Giemsa solution (made in pH 7.2 phosphate buffer) for 30 minutes. Chromosomes were counted to determine ploidy level.

3 RESULTS

3.1 CHROMOSOME EXAMINATION

Tetraploid embryos were successfully induced by blocking the first cleavage division with heat shock in this experiment. The chromosome numbers of tetraploid embryos in tested groups were about 208 (Plate-2), while in the control embryos only diploids were found with 104 chromosomes (Plate-1). The chromosomal behavior was almost not the same in a tetraploidy cell at mitosis. There were about 104 rod-shaped chromosomes and 104 spot-shaped chromosomes present simultaneously in a tetraploidy cell (Plate-3). Abnormal karyokinesis occurred when the shock temperature raised to 41 ℃. A large number of uncountable chromosomes was observed in

chromosome preparations (Plate-4, Table 1).

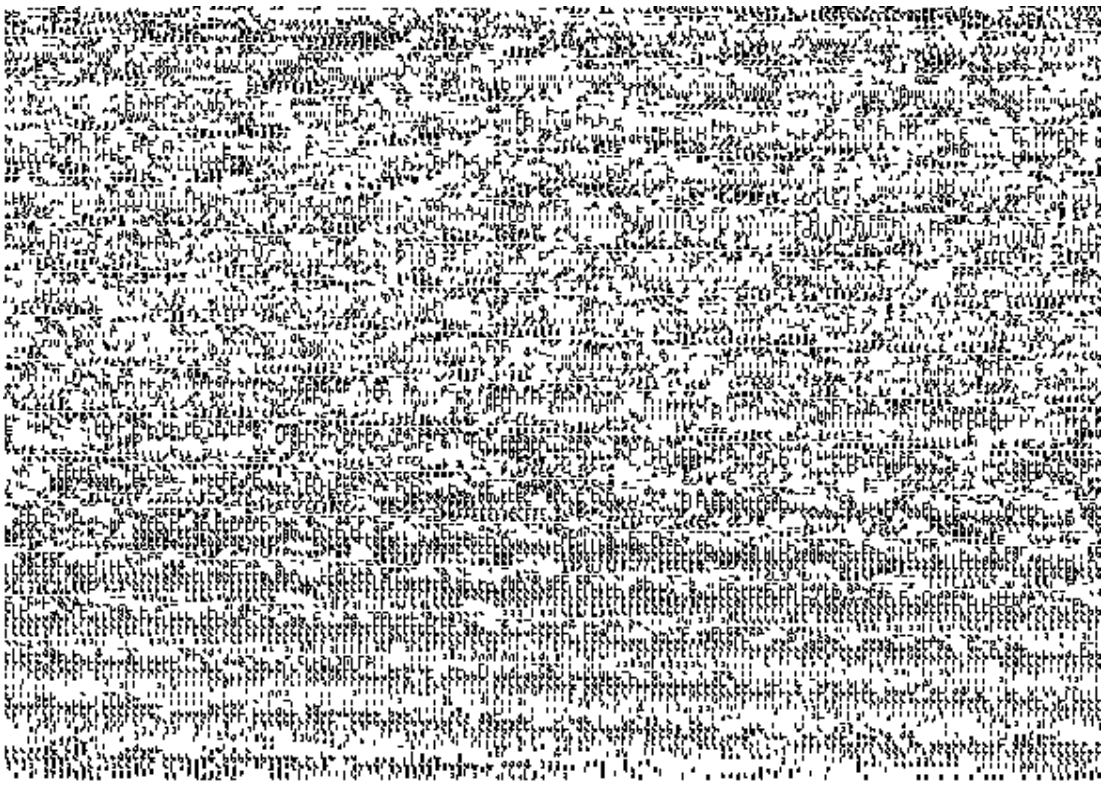

Plate　图版

1. Metaphase from diploid embryos of *M. nipponense* with about 104 chromosomes (2n = 104); 2. Metaphase from tetraploid embryos induced by heat shock in *M. nipponense* (4n=208); 3. Two types of chromosomes in shape present simultaneously in a tetraploidy cell; 4. A large number of chromosomes occurred when shock temperature increased to 41 ℃

3.2 INDUCING RATE OF TETRAPLOIDY

The frequency of tetraploid embryos induced by heat shock are given in Table 1. Tetraploids were detected in all experimental groups. The temperature shock of 40 ℃ for duration of 1.5 mimutes resulted in the highest percentage of tetraploid embryos (36.8%). There were only a little variation of the percentage of tetraploid when the duration time of treatments increased from 1.5 to 2 minutes in Group 3.

When the temperature of shock rose from 40 ℃ to 41 ℃, the embryos would display a high rate of mortality if the length of shock time was longer than 1 minute. Mosaics were also produced in test groups. The frequency of mosaics was significantly greater than that of tetraploids both in Group 1 shocks for 2 minutes and in Group 2 shocks for 1 minute (Table 1).

Neither mosaics nor tetraploids were observed in control groups.

Table 1 Frequency of tetraploid embryos induced by heat shock in *Macrobrachium nipponense*

Group	Treatment		Cell Counts	Ploidy(%)		
	Temp.(℃)	Dura.(min)		2N	4N	Mosaic
1	38	1	30	100	0	0
		2	42	81	7.1	11.9
2	39	1	11	73.7	8.1	18.2
3		1	43	79.1	20.9	0
	40	1.5	19	57.9	36.8	5.3
		2	28	69.2	30.8	0
4	41	1	6	83.3	16.7	0
		2	*			
Control			35	100	0	0

* chromosome number was uncountable

4 DISCUSSION

Polyploidy had been induced in a wide variety of fish and bivalve molluscs [Lincolin et al. 1974, Stanley et al. 1981, Swarup 1956]. In decapod, similar research work made a slow progress because of technological difficulties [Qiu 1996]. Until recently, only two cultured species polyploidy had been produced [Dai et al. 1993, Lu et al. 1993, Xiang et al. 1992]. In this study, we had successfully induced tetraploidy embryos of another commercially important species, *M. nipponense*, using heat shock treatment.

The freshwater prawn *M. nipponense*, like most of decapod crustaceas, the females incubated their embryos on pleopods on the abdomen until hatching. If the fertilized eggs did not attach themselves to pleopodal setae, they would deteriorate soon. In order to overcome this difficulty in manipulation of chromosome, we developed a method in this study as below: In the treatments, only the abdomen of the maternals with eggs were immersed in water at different temperature. After heat shocked, the maternals incubated their eggs on pleopods as before. The results indicated that the technique employed in this experiment was feasible for the freshwater prawn in chromosome manipulation. Afterwards, Chen et al. (1997) applied this technique to induce successfully triploidy and tetraploidy embryos in Chinese mitten-handed crab *Eriocheir sinensis* (private communication). The long interval between fertilization and the first mitotic karyokinesis of eggs might make it more convenient to induce tetraploidy in freshwater prawn and crab than in fish and molluscs.

The first cleavage division of fertilized eggs in *M. nipponense* was confined to karyokinesis, so heat shock blocking the first cleavage division essentially prevented karyokinesis. In some chromosome preparations, two types of chromosomes in shape were detected in a tetraploidy cell at mitotic activity (Plate-3). With shock 41 ℃ for longer time than 1 minute, the eggs were damaged and displayed high mortality. Abonormal karyokinesis occurred in some tetraploidy cells (Plate-4). The same result was those reported by Dai et al. [1993] in the induction of triploid in shrimp *Penaeus orientalis*. The different behavior and abonormal divide of chromosomes might result in high mortality of embryos. In addition, heat shock treatment induced tetraploidy was also

(1) Chen L Q, et al. Studies on the polyploid induction in the Chinese mitten-handed crab, *Eriocheir sinensis*. I. induction of triploidy and tetraploidy embryos in *Eriocheir sinensis* by cytochalasin B.

accompanied by mosaic in the present experiment as in earlier work[Bidwell et al. 1985, Xiang et al. 1992] .The percentage of mosaics were significantly higher than that of tetraploids with shock of either 38℃ for 2 minutes or 39℃ for 1 minute, but significantly lower percentage or no mosacis were found with shock of 40℃ for 1~2 minutes (Table 1).

The cause for the low induction rate of tetraploidy was that the development of fertilized eggs was not synchronic. Some of the eggs would have finished the first mitotic karyokinesis before the heat treatment was applied. It is necessary to establish the technique of artificial fertilization *in vitro* to solve this problem. Another reason for the low induction rate was that the duration time of treatment was too short (1~2 minutes). Further experiment will be needed to be carried out for determination of optional time of the treatment.

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REFERENCES

Dai J X, et al. 1993. Studies on the triploid induction in *Penaeus orientalis* : I. Temperature shocks. Herditas (Beijing). 15(5): 15~18.

Bidwell C A, et al. 1985. Polyploid induced by heat shock in channel catfish. Aquaculture. 51: 25~32.

Cassani J R, et al. 1984. Morphological comparison of diploid and triploid hybrid grass carp, *Ctenopharngodon idella* × *Hypopthalmichthys nobillis*. J Fish Biol, 24: 133~338.

Hong Y H. 1990. Tetraploidy induction by heat shock in bighead carp, *Aristichthys nobilis*. Acta Zool Sinica, 36(1): 70~75.

Lincolin R F, et al. 1974. Attempted triploid in Atlantic salmon (*Salmo salar*) using cold shocks. Aquaculture, 4: 287~297.

Lu R H, et al. 1993. An experiment on induction of triploidy in *Eriocheir japonicus hepuensis* Dai by heat treatment. Chin J Oceanol Limnol, 11(1): 21~24.

Purdom C E. 1972. Induced polyploidy in plaice (*Pleuronectes platessa*) and its hybrid with the flounder (*Platichythys flesus*). Heredity, 29: 11~24.

Qiu G F. 1996. The status and problems of studies on the polyploidy breeding in shrimps and crabs (Decapoda). J East China Normal University (Nature science special issue of Zoology), 105~110.

Qiu G F, et al. 1994. ? Chromosomal and karyological studies on the freshwater prawn *Macrobrachium nipponense*. Ocean et Limono Sinica, 25(5): 493~498.

Refstie T, et al. 1977. Induction of polyploidy in salmonids by cytochalasin B. Aquaculture, 10: 65~74.

Stanley J G, et al. 1981. Polyploidy induced in the ? American oyster, *Crassostrea virginica*, with cytochalasin B. Aquaculture, 23: 1~10.

Swarup H. 1956. Production of heteroploidy in the three-spined stickleback *Gasterosteus aculeatus* (L). Nature, 178: 1124~1125.

Thorgaard G A, et al. 1981. Polyploidy induced by heat shock in rainbow trout. Trans Amer Fish Soc, **110**: 546~550.

Valenti R F. 1975. Induced polyploidy in *Tilapia awrea* Steindachner by means of temperature shock treatment. J Fish Biol, 7: 519~528.

Xiang J H, et al. 1992. Induction of the tetraploids of the Chinese shrimps, *Penaeus chinensis*. Chin Marine Science, 4: 55~61.