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# 紫外线诱导栉孔扇贝雄核发育的研究

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**摘要**:研究了利用紫外线诱导栉孔扇贝雄核发育的条件。表明紫外线(254 nm)对卵子染色质失活是有效的。在强度为 2.8mW·cm$^{-2}$·s$^{-1}$的紫外线下照射 20 s 的卵子与正常精子混合后能保持一定的受精率(60.2 %),且 D 形幼虫发生率为 0。染色体检查结果显示此时单倍体率最高(49.2 %)。说明在强度为 2.8mW·cm$^{-2}$·s$^{-1}$的紫外线下照射 20 s 是获得雄核发育单倍体的适宜条件。研究发现受精率和 D 形幼虫发生率随照射时间的增加而下降,遗传失活的卵子与正常精子受精后其胚胎发育至 D 形幼虫前期停止。实验中各处理组均出现非整倍体。出现的原因可能由于紫外线照射剂量对卵子染色体遗传失活的作用程度不同以及 DNA 的光修复。

**关键词**:栉孔扇贝;紫外线照射;雄核发育;遗传失活
**中图分类号**:S917 **文献标识码**:A

# Induction of androgenesis in *Chlamys farreri* by ultraviolet irradiation

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**Abstract**: Androgenesis is defined as all-paternal inheritance. Viable androgenetic diploids can be generated by inhibiting the first cleavage to double paternally derived chromosomes, after fertilization of genetically inactivated eggs with normal sperm. It is a technique that could facilitate the production of completely homozygous isogenic lines, examine sex determination, make genetic analysis, and protect endangered species. Gamma and X-rays inactivation are the usual methods used to inactivate egg nuclei. However, since special facilities are required to manage radioactivity safety, these methods are not practical for routine induction of androgenesis. In many fish species, efficient procedures of genetically inactivating eggs and restoring diploidy have been studied and achievements of successful diploid androgenesis were reported. In contrast, in mollusks, there were only a few reports of cytobiological studies on one species of spontaneous androgenetic *Corbicula leana*, artificial induction of androgenesis has rarely been studied. *Chlamys farreri* is a main cultured mollusk species in China, as well as an important object of study in mollusk breeding science. In this study, optimum conditions of ultraviolet (UV) ray irradiation for genetic inactivation of eggs were examined to develop simple and safe techniques to induce haploid androgenesis in *C. farreri*. Mature cultured *C. farreri* were collected locally in late March and early May from the coast of Weihai, Shandong. Eggs and sperm were obtained by artificially inducing spawning with the

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stimulations of dryness and raising water temperature from 16 to 20 ℃. Discharged eggs were collected by suction and rinsed in filtered seawater several times. Suspensions of sperm and egg were prepared at concentrations of 1.0 $\times 10^6$ sperm per mL and 1.0 $\times 10^4$ egg per mL by dilution with filtered seawater. Egg suspension (4 mL) was spread on a plastic Petri dish and treated with the UV rays (254 nm) at intensity of 2.8 mW·cm$^{-2}$·s$^{-1}$ for 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, or 70 s. On the completion of irradiation, egg suspension was mixed with normal sperm (0.5 mL) and transferred to a baker for culture at temperature of 19 - 20 ℃. During the culture, the fertilization and development rates of each group were recorded and samples of the larvae (trochophores) were collected to determine the ploidy of embryos. The result showed that the ultraviolet ray was effective for inactivating egg chromosomes. The fertilization rate was relatively high (60.2 %) and the developmental rate became zero when eggs were irradiated for 20 s at UV intensity of 2.8 mW·cm$^{-2}$·s$^{-1}$ and mixed with normal sperm. The results of chromosome observation showed that under this condition the rate of haploid was the highest (49.2 %). This result indicated that irradiation for 20 s at UV intensity of 2.8 mW·cm$^{-2}$·s$^{-1}$ was the optimum dose to achieve haploid androgenesis in this kind of scallop. The fertilization rate decreased with increasing irradiation time. This is probably because with the increasing of irradiation dose, the degree of damage of some important factors that control the fertilization potency of eggs and the development of embryos increased. It was also observed that the development of D-shaped larvae decreased with increasing irradiation time, and the development of the genetically inactivated eggs fertilized with the normal sperm terminated before reaching the D-shaped stage. Aneuploids were found in this study. Because ultraviolet irradiation is known to cause pyrimidine dimerization in the DNA helix, which prevents the replication of genome, the occurrence of these aneuploids is probably attributed to different degrees of maternal chromosomal inactivation by UV irradiation and/or DNA repair.

**Key words**: *Chlamys farreri*; ultraviolet irradiation; androgenesis; genetic inactivation

雄核发育是指卵子只依靠雄核进行发育的特殊的有性生殖方式。人工雄核发育的诱导,是利用γ射线、X射线、紫外线和化学诱变剂使卵子遗传失活,而后通过抑制第一次卵裂使单倍体胚胎的染色体加倍发育成雄核二倍体个体[1]。由于雄核发育后代的遗传物质完全来自父本,加倍后各基因位点均处于纯合状态,因而可以用于快速建立纯系[2-5]。对于一些濒危动物,通过将冷藏精子与遗传灭活的亲缘关系较近的卵子"受精",可以使这一物种得到恢复[6-9]。因此,雄核发育技术可以成为物种保护的重要手段之一。此外,雄核发育还可以用于判别性别决定机制以及进行遗传分析[3,4]。

在许多鱼类中,已有学者成功地诱导了雄核发育单倍体[10-13],并获得一定比率的雄核发育二倍体。Parsons 和 Thorgaard[14], Scheerer 等[15]利用$^{60}$Co-γ射线灭活卵子遗传物质与静水压抑制第一次卵裂的方法获得了虹鳟雄核发育单倍体及纯合二倍体个体。Bongers 等使用紫外线照射与热休克结合的方法成功地诱导出鲤(*Cyprinus carpio*)雄核发育二倍体[16]。在泥鳅(*Misgurmus anguillicaudatus*)[17-19]、大鳞副泥鳅(*Paramisgurnus dabryanus*)[20]、马苏大麻哈鱼(*Oncorhynchus masou*)[3,21]、溪红点鲑(*Salvelinus fontinalis*)[22]和鲫(*Carassius auratus*)[10]等种类中也有类似的研究报道。然而在贝类中,目前只有关于一种生活在河口区的天然雄核发育的蚬类(*Corbicula leana*)的细胞生物学研究方面的报道[23-25],人工诱导贝类雄核发育的研究在国内外还未见报道。

栉孔扇贝(*Chlamys farreri*)是我国贝类养殖的主要种类,也是目前贝类育种研究的重点对象之一。本文首次研究了紫外线照射栉孔扇贝卵子使其遗传失活,成功地诱导出雄核发育单倍体。

## 1 材料和方法

### 1.1 精卵的采集

成熟的栉孔扇贝(壳高 6.70 ±0.37 cm;壳长 6.20 ±0.30 cm)取自山东威海北海养殖海区。采用阴干升温的促排方法获得精卵。精卵分别用海水稀释至浓度为 1.0 $\times 10^6$ mL$^{-1}$ 和 1.0 $\times 10^4$

$mL^{-1}$。用四级砂滤海水,培养温度为 19～20 ℃。

### 1.2　紫外线处理卵子和受精

卵悬液(4 mL)置于直径 9.0 cm 的塑料培养皿中,轻微振荡使卵均匀分散于培养皿底部。将培养皿置于 15 W 紫外杀菌灯下 15 cm 处,用紫外线强度测定仪测得此条件下紫外线强度为 2.8 $mW \cdot cm^{-2} \cdot s^{-1}$。卵子在紫外线下分别处理 0、5、10、15、20、25、30、35、40、45、50、60 和 70 s。照射结束后,每个培养皿中加入 0.5 mL 精悬液,充分混合后转移至烧杯培养。

受精率和幼虫成活率分别在受精后 4 h 和 35 h 通过计算分裂卵数占总处理卵数的百分比以及 D 形幼虫数占受精卵数的百分比获得。实验重复 3 次。

### 1.3　倍性检查

胚胎的倍性通过检查受精后 24h 担轮幼虫的染色体标本而获得。收集每组的担轮幼虫样品,浓缩后加入含 0.1 %秋水仙素的海水中处理 2h,然后加入 0.075 $mol \cdot L^{-1}$ KCl 溶液低渗 30min,去掉低渗液,用 Carnoy 氏液(甲醇∶冰醋酸 = 3∶1)固定,反复固定 3 次。滴片前去掉固定液,加入 50 %冰醋酸,用吸管轻轻吹打解离成单细胞。样品滴到已加热的载玻片上,空气干燥后经磷酸缓冲液(pH 为 6.8)稀释的 10 %Giemsa 染色。观察、计数分散较好的中期分裂相以获得染色体数。

## 2　结果

### 2.1　照射剂量对受精率和幼虫成活率的影响

卵子被照射 15 s 时受精率还没有受到显著影响,但照射时间超过 20s 后受精率随照射时间的增加急剧下降,照射 70 s 时,受精率变为 0。随着照射时间的增加,D 形幼虫发生率明显降低,照射 20 s 时,其值变为 0(图 1)。

### 2.2　染色体观察结果

栉孔扇贝幼虫细胞有丝分裂中期分裂相如图 2 所示。

图 3 表示第一次实验中 0 (对照组)、5、10、15、20、25、30、35、40、45 和 50 s 各处理组幼虫细胞的染色体数目的频率分布。对照组即栉孔扇贝二倍体的染色体数目为 38(图 2-1)。在 5s 和 10s 照射组中,N = 38 的幼虫仍占一定比率,染色体数目介于单倍体和二倍体之间的非整倍体细胞大量出现(图 2-2)

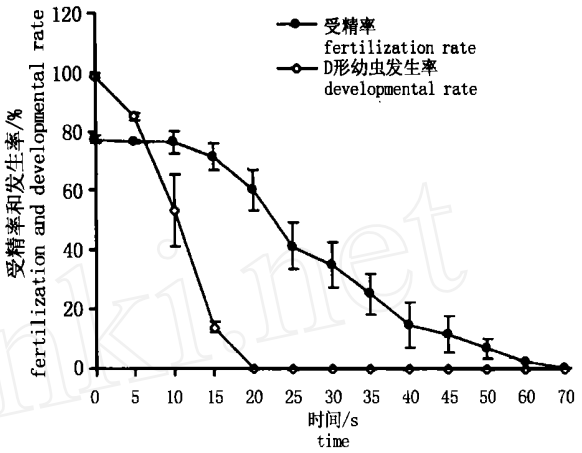


图 1　栉孔扇贝受精率和 D 形幼虫发生率与紫外线照射时间之间的关系

Fig. 1　Relationships between ultraviolet irradiation duration and the rates of fertilization and development of D-larvae in *Chlamys farreri*

在 15 s 照射组中,染色体数目为 19 的单倍体细胞比率明显上升(图 2-3),此时仍有一部分细胞的染色体为二倍体的 38 条。20、25 和 30s 照射组中,大部分细胞的染色体数目为单倍体的 19,而且没有二倍体细胞。35、40、45 和 50s 照射组中,N = 19 已不具有代表性,染色体数目比较分散。

第 2、3 次实验结果与第 1 次实验基本相同。

## 3　讨论

研究发现利用紫外线(波长 254 nm)使栉孔扇贝卵子 DNA 遗传失活从而诱导雄核发育是有效的。根据染色体数目分析结果,可以看出强度为 2.8 $mW \cdot cm^{-2} \cdot s^{-1}$的紫外线照射 20～30s 均能成功地诱导出栉孔扇贝雄核发育单倍体,但卵子的照射时间超过 20s 后受精率就会明显下降。

由于有效地诱导雄核发育既需要卵子遗传物质失活、又需要保证受精率较高,因此强度为 2.8 $mW \cdot cm^{-2} \cdot s^{-1}$的紫外线照射 20s 可以认为是使栉孔扇贝卵子遗传失活的适宜剂量。贝类中还没有关于人工诱导雄核发育的相关报道,但在已报道的人工诱导鱼类雄核发育的研究中,不同紫外线照射强度与照射时间的适宜组合均能有效地诱导雄核发育。有关泥鳅的研究表明,强度为 1.25$mW \cdot cm^{-2} \cdot s^{-1}$的紫外线照射 15～120s 或强度 0.7 $mW \cdot cm^{-2} \cdot s^{-1}$的紫外线照射 60～300s 均能有效地诱

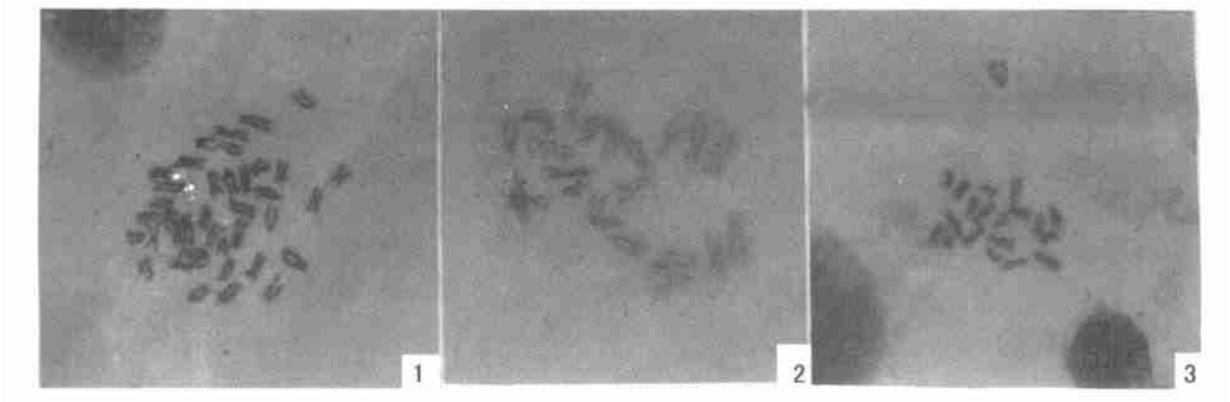


**图 2 栉孔扇贝幼虫细胞有丝分裂中期分裂相**

Fig. 2 Mitotic metaphase plates from the larval cells of *Chlamys farreri*

1. 对照组中的二倍体细胞,染色体数目为 38, ×330;2. 15 s 照射组中的非整倍体细胞,染色体数目为 21, ×330;
3. 20 s 照射组中的单倍体细胞,染色体数目为 19, ×330

1. A diploid cell with 38 chromosomes from the control group, ×330;
2. An aneuploid cell showing 21 chromosomes from the 15s irradiation group, ×330;
3. A haploid cell with 19 chromosomes from the 20 s irradiation group, ×330

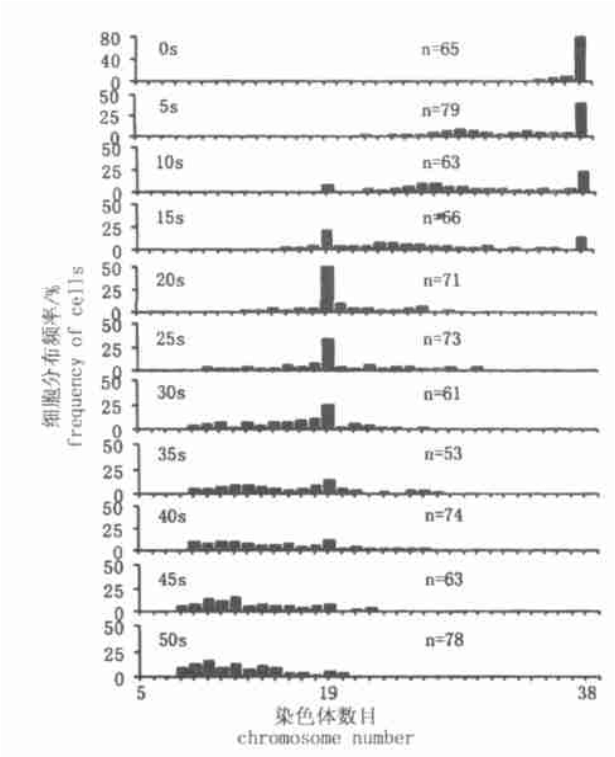


**图 3 实验 1 中不同紫外线照射时间幼虫细胞染色体数目分布频率(*n* 代表观察的细胞总数)**

Fig. 3 Frequency distribution of chromosome number in larval cells by various durations of ultraviolet irradiation in experiment 1 (*n* means total number of cells observed)

导该品种的雄核发育[12]。对鲤的研究指出,强度为 1.1 mW·cm$^{-2}$·s$^{-1}$的紫外线照射 135～270s 能够获得雄核发育单倍体后代[16]。尽管紫外线照射剂量因种类不同而不同,并取决于卵悬液的密度、体积,但在这次实验中,经紫外线照射而使栉孔扇贝卵子遗传失活这一处理的重复性很强。

与人工诱导鱼类雄核发育的研究结果相比较,可以发现以栉孔扇贝为研究对象,利用紫外线作为失活卵子 DNA 的手段,后者所需紫外线照射剂量要比前者低。在紫外线照射装置方面,鱼类多采用双向照射,而栉孔扇贝则只需单向照射。这可能是因为鱼类的卵子相对较大,而紫外线属于非电离辐射,穿透能力弱,因此失活鱼类卵子遗传物质时所需剂量大。贝类卵子相对鱼类卵子要小得多,因而较低剂量的紫外线就能够使卵细胞 DNA 失活,有效地诱导雄核发育。

本次实验的各照射组中均出现了非整倍体。Arai 等关于紫外线照射诱导泥鳅雄核发育的研究中也有关于非整倍体的报道[12]。紫外线照射能够使卵子遗传失活的原理在于它能够使 DNA 氢键断裂,同一链上相邻的或相对应的两条链上的胸腺嘧啶之间形成胸腺嘧啶二聚体,从而使双螺旋的两链间的键减弱,使 DNA 结构局部变形,从而严重影响 DNA 的正常复制和转录[1]。因此紫

外线照射剂量决定了卵子染色体失活的程度。经不同剂量紫外线照射的卵子在胚胎发育过程中母本染色体的排除可能存在以下两种情况:第一,母本染色体完全失活,不与父本染色体融合,不参与有丝分裂过程中的核分裂并在胚胎发育过程中被逐渐排除。这种情况下,卵子只依靠精子 DNA 发育,胚胎的染色体数目为 19,是雄核发育单倍体。第二,母本染色体部分失活,被紫外线照射损伤的母本染色体可能与父本染色体粘连而参与有丝分裂,并在以后的胚胎发育过程中依各染色体着丝点的损伤程度而逐渐被排除[26]。此外,由紫外线照射引起的 DNA 损伤可以在特定的酶作用下被光修复[27],这也可能导致母本染色体不同程度地参与核分裂,从而产生非整倍体。实验中 40～50 s 照射组的细胞中还出现了较多 6～9 条这种小数目的染色体。这可能是由于雄核发育卵子发生了异常的核分裂或在染色体制片过程中因人为因素造成部分染色体丢失而引起的。

研究发现,随着照射剂量的增加受精率逐渐下降,这与 Arai 等关于紫外照射诱导泥鳅雄核发育的研究结果相似[12]。说明随着照射剂量的增加,卵细胞质中一些在受精、胚胎发育过程中起重要作用的因子受到破坏的程度可能增大,从而减弱了卵子的受精能力,造成受精率逐渐下降这一现象。

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