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NILE TILAPIA GAMETE MANAGEMENT FOR CHROMOSOME MANIPULATION

Eighth Work Plan, Reproduction Control Research 1B (RCR1B)
Progress Report

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ABSTRACT

Artificial propagation is an important component of chromosome manipulation. Spawning of Nile tilapia (*Oreochromis niloticus*) was manipulated by photoperiod and temperature control and through hormonal intervention. Four males and ten females produced 86 natural spawns, 41 of which developed to hatching. Artificial propagation resulted in 87 pairings, which were successfully used in 23 tau estimates and 11 UV experimental trials. A tau-curve was developed over temperatures ranging from 20.6 to 28.7°C with mitotic intervals from 73.8 to 30.1 min, respectively. A UV dose of 300 to 500 J m^{-2} appears to be sufficient to inactivate the DNA of Nile tilapia eggs. Genetic color markers were identified by progeny testing.

INTRODUCTION

Artificial propagation of fishes is a requisite for chromosome manipulation. Control of spawning can be by habitat manipulation or through direct hormonal intervention (Shelton, 1989). A suitable temperature and controlled photoperiod will maintain reproductive activity in tilapias throughout the year, but hormone induction of spawning can improve predictability of the collection of freshly released gametes. Chromosome manipulation includes ploidy alteration or euploidy induction with a single parent genomic contribution. Retention of the first polar body permits induction of gynogenotes (meiogynotes) or triploid progeny while interference with first karyokinesis can result in gynogenotes (mitogynotes) or tetraploids. Diploidization of the paternal genome, or androgenesis, also is accomplished by interference with first mitosis. In the case of androgenesis and gynogenesis, either the female or male DNA, respectively, must be neutralized before the egg is activated, while in the case of ploidy manipulation both parental genomes are left intact.

Chromosome manipulation involves two basic treatments after obtaining fresh gametes (Thorgaard and Allen, 1986). For androgenesis, the female genome is inactivated by ultraviolet (UV) irradiation. Treated eggs are activated by normal spermatozoa, then diploidized by shocking to interrupt the first mitotic karyokinesis. In order to prevent chromosome separation, the shock is timed to coincide with metaphase and must be sufficiently severe to disrupt microtubule and spindle fiber formation. Selection of the type of shock (thermal—cold or hot—or pressure) depends on effectiveness and ease of application. Pressure shock is more complicated than thermal treatment. Thus, shock intensity, duration, and time of application must be optimally combined into a protocol for maximum yield of progeny. Further, because the rate of development is inversely temperature dependent, either the preshock incubation temperature must be standardized or the shock time must be calibrated to the temperature effect. Absolute shock time (minutes post-activation) can be transformed with reference to an index of development rate or mitotic interval, tau (τ_0), also in minutes (Dettlaff and Dettlaff, 1961). Shock time (τ_s) can be related to tau (τ_s/τ_0) to report shock protocol in a dimensionless index which is temperature-compensated (Dettlaff, 1986). Finally, evaluation of chromosome manipulation is verified by the use of a genetic marker that carries a phenotypic expression. A visual genetic marker greatly facilitates chromosome manipulation studies. Nile tilapia (*Oreochromis niloticus*) possess two color mutations, the blond and red phenotypes (McAndrew et al., 1988). One method for verification of chromosome manipulation of the Nile tilapia is to use normal-color females and blond-mutant males. This homozygous recessive trait, blond phenotype, would usually be appropriate; however, the Ivory Coast stock of Nile tilapia to be used for this study (at the University of Oklahoma) has had some introgression of the blond gene and must be progeny-tested before use. Alternatively, the red phenotype, which is a dominant gene, can be used as an egg source with normal-color males as sperm donors.

This report describes the following:

1) Efforts to improve the efficiency of collecting freshly ovulated eggs from Nile tilapia;
2) The development of a tau curve over the spawning temperature range;
3) Preliminary data on UV treatment of eggs for induction of androgenesis; and
4) Progeny tests to identify genetic markers in the broodstock of Nile tilapia kept at the University of Oklahoma.

METHODS AND MATERIALS

Spawning

The stock of Nile tilapia kept at the University of Oklahoma originated in Ivory Coast; however, it has been hybridized to an unknown level with the Egyptian Lake Manzala stock. The Egyptian strain carries the blond and red genes (McAndrew et al., 1988). Spawning of Nile tilapia was maintained throughout the year by photoperiod management. Four to six females and one male were stocked in each of four large aquaria (550 l).

Water was aerated and was circulated at a rate of one turnover per day. Temperature was cycled; it was maintained at 26 ± 2°C for approximately one week, then elevated to 29 ± 2°C for one week. The light cycle was started by adjusting a timer to turn on the overhead lighting at 0100 h; the natural sunset regulated the end of the photoperiod, which totaled 20L:4D. Tilapia spawn eight to ten hours after the beginning of the light cycle (Myers and Hershberger, 1991). Ovulation and spawning readiness can be judged by courtship behavior, coloration, and papilla erection (Rothbard and Pruginin, 1975; Rothbard, 1979). Preliminary trials of hormone-induced ovulation were based on information from Gissis et al. (1988) who used a commercial gonadotropin-releasing hormone analogue (GnRHa) product (Dagin), which includes a dopamine antagonist. The injection level was 10 mg kg^{-1} and was compared with a luteinizing hormone-releasing hormone analogue (LHRHa) (without a dopamine antagonist) at the same dose and human chorionic gonadotropin (HCG) at 3500 IU kg^{-1}. Females were stripped after initiation of spawning or as judged from the courtship behavior characteristics described above.

Fertilization

Eggs were collected in a clean container, milt was expressed over the eggs, and water was added directly to initiate activation. Fertilized eggs were placed in incubators within 2 to 3 min at controlled temperature ± 0.2°C. Incubation temperature was regulated closely to document the developmental rate. Eggs were subsequently incubated in downflowing units (1-l capacity) with sufficient flow to gently tumble the developing embryos.

Tau Estimates

Developmental rate is defined as the duration of one mitotic cycle during early synchronous cleavage (Dettlaff and Dettlaff, 1961). The mean interval between the initiation of the first and third mitoses in 5 to 10% of the eggs was recorded at temperatures within the usual developmental range (20 to 30°C). Twenty to thirty eggs were examined under magnification at 5-min intervals. Tau curves have been used to facilitate chromosome manipulation studies in various fishes (Shelton and Rothbard, 1993; Shelton et al., 1997) and are a primary criterion in timing the shock for Nile tilapia.

UV treatment

Freshly ovulated eggs were stripped from females and allocated into six subsamples for differential UV exposures. Two to four hundred eggs were placed in each of six 10-cm petri dishes with enough water to provide slight buoyancy. Five dishes were placed in a UV crosslinker (FisherBiotech, FB-UVXL-1000) and exposed to 100 to 500 J m^{-2}. One subsample was not exposed to UV. The UV-treated eggs were then activated with freshly collected spermatozoa as described above, and after 30 min they were transferred to individual flow-through incubators. Development was monitored through hatching and swim-up stages.

Progeny Testing

Progeny from pair-spawned, individually tagged broodstock were hatched in the system described above. Swim-up fry were nursed in individual small-mesh hapas until pigment patterns were developed. Parental genotype for color mutations was inferred from ratios of progeny color pattern.

RESULTS AND DISCUSSION

Photoperiod manipulation shifted spawning activity to midday. Females ovulated/spawned between 8.7 and 14.5 h (mean = 11.4 h, n = 33) after the light-on cycle. There was no apparent correlation between the latent period and water temperature ranging from 23 to 28°C. Spawning was less frequent when temperatures were maintained below 25°C, but appeared more frequent and synchronized under the alternating high end of the temperature cycling.

During the 1997-98 period, eggs were collected from 86 natural pair spawnings, and an additional 87 pairings were made through stripping and artificial fertilization. Fertilization and hatching rate were variable for natural spawning and artificial fertilization; zero hatch data are not included for natural spawning or artificial fertilization. From 41 natural spawnings an average of 44% hatched compared to 18% hatch from 11 groups of eggs that were artificially fertilized; 16% of the larvae from natural spawning survived to swim-up, while only 9% of the larvae from artificial spawning survived to swim-up. Initiation of intraovarian egg resorption is common among tilapias and although it does not prevent ovulation of the deteriorated eggs, fertilization and hatching are poor (Peters, 1983). Some of the problem may also be in the type of incubation and the developmental stage of the egg when incubation outside the buccal cavity began. Hatch rate in down-flow incubators is better than in upwelling ones (Rana, 1986), but neither is as efficient as the natural system. Eggs removed from the buccal cavity early in development generally have lower survival than eggs left in the buccal cavity until nearer to hatching. Eggs fertilized in vitro must be incubated in artificial systems, and thus lower survival is expected.

Ovulation based on the light cycle has been a reasonable means of anticipating stripping time. Hormonal induction of ovulation would seem to be a logical extension, but cichlids have responded poorly to gonadotropic therapy (Rana, 1988), with the exception of the data from Gissis et al. (1988). In preliminary studies, seven tests with Dagin had a mixed response. In the first trial with six females, five ovulated; however, each subsequent trial was less successful. In two trials with Dagin, LHRHa, and HCG, only HCG increased ovulation rate over the control group. While controlled ovulation for the Nile tilapia is not too encouraging, the physiological characteristics of tilapia gametes provide advantages for chromosome manipulation. In contrast to most fishes, tilapia eggs are fertile for 3 to 6 h post-immersion in water (Myers et al., 1995) and sperm remain motile in water for several hours (Yeheskel and Avtalion, 1986). Even though the fertility of gametes should not deteriorate during UV treatment in comparison with other fishes, the relatively low fertilization and hatch rate for untreated eggs may neutralize these attributes.

Estimates of tau were made for 23 trials with temperatures ranging from 20.6 to 28.7°C (Figure 1). The calculated tau curve is described by the following equation:

$$\tau_0 = 10^{5.4167}\, C^{-2.7009} \quad (r^2 = 0.90)$$

where

C = temperature in Centigrade.

The tau-temperature relationship was inverse and ranged from 73.8 to 30.1 min in the observed temperature range. Time to

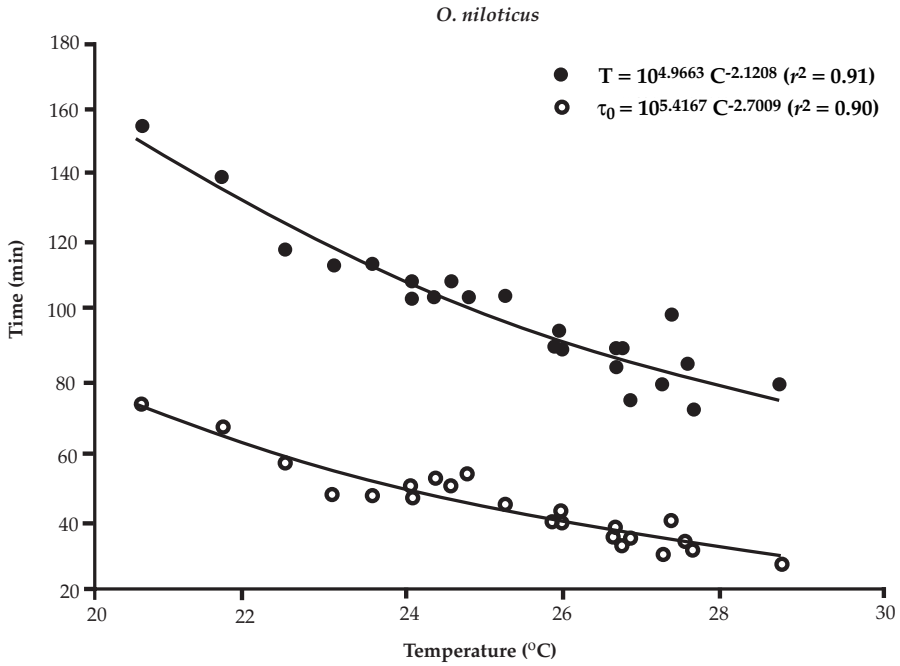

Figure 1. Mitotic interval (τ_0) and time to first mitosis (T) for *Oreochromis niloticus*.

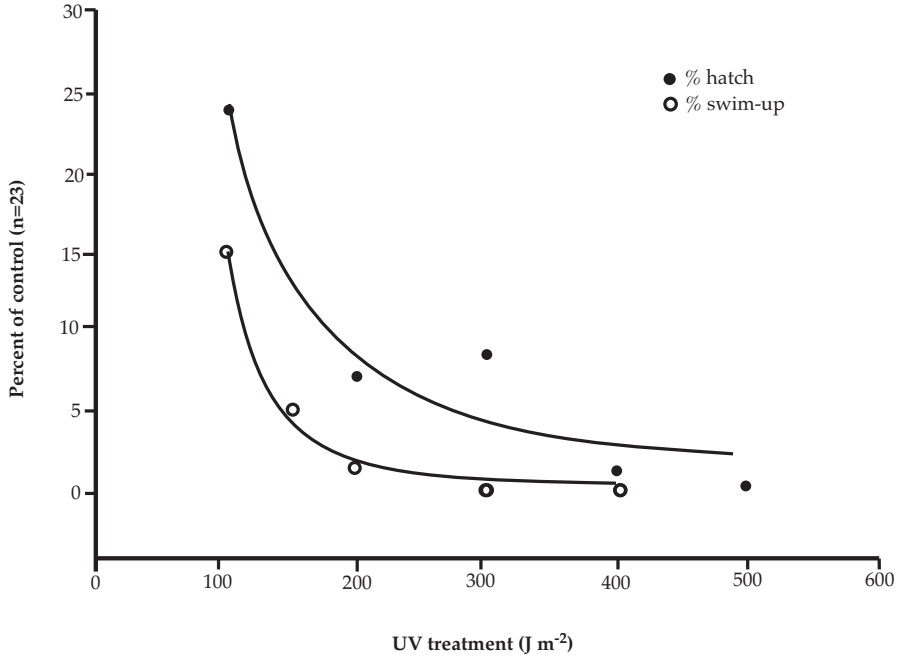

Figure 2. Effect of ultraviolet (UV) dose on hatch and swim-up rate for *Oreochromis niloticus*.

first mitosis (T) is also included because the relative time to first metaphase will be important in late shock protocol. The empirical relationship is characterized by the curve:

$$T = 10^{4.9663}\ C^{-2.1208}\ (r^2 = 0.91)$$

These working curves can be used to standardize shock treatments.

The UV treatment of eggs was tested in 23 trials, but useable data were obtained from only 11 tests. Hatch rate was generally reduced to zero at a dose of 400 to 500 J $m^{-2,}$ but survival to swim-up was zero at 300 to 400 J m^{-2} (Figure 2). Note the apparent rise in percent hatch between 200 and 400 J m^{-2} , which might be evidence of a Hertwig effect (Chourrout and Itskovich, 1983). Again, relatively low levels of fertility (16% hatch and 8% survival to swim-up in the controls) complicate confidence in the

relationship. These data are in general agreement with the dosage level of 400 to 600 J m^{-2} reported by Myers et al. (1995) to inactivate the ova genome in Nile tilapia.

Functional genetic color markers were examined for 31 pair spawnings between four males and ten females. Progeny tests indicated that two of the males were not carrying the recessive gene for the blond phenotype and five females were homozygous for normal color. Thus, eggs from these females can be used in androgenic trials with blond males.

Tilapia of the Ghana strain, which do not carry the blond gene, were received during this season and can be used as egg donors in the future. In addition, a founder stock of a strain of Nile tilapia with the red phenotype was obtained from Israel this year. This strain also can be used in the androgenetic studies, but from a different orientation because the red phenotype is dominant (McAndrew et al., 1988).

The most effective type of shock, thermal or pressure, must be addressed. Thermal shocks will be used for practical reasons because effectiveness is as good as or better than pressure shock. Don and Avtalion (1988a) used cold shock for tetraploid induction based on a comparison of hot and cold shocking for triploid induction (Don and Avtalion, 1988b). Heat or pressure shock to induce tetraploidy and mitotic gynogenesis should also provide an alternative for endomitotic protocol (Mair, 1993; Hussain et al., 1993; 1998); however, only one study has developed androgenetic protocol for tilapia (Myers et al., 1995). The timings for shock reported by Myers et al. (1995) at 27 min post-activation (28°C) and by Hussain et al. (1993) at 47 min (28°C) sharply contradict the optimal shock times of 78 min (28°C) and 92 min (26°C) post-activation reported by Shirak (1996) and Don (1989), respectively, even when adjusted for temperature. Therefore, these reported optimal times and shock types will be examined using the tau information acquired in this study.

ANTICIPATED BENEFITS

Development of androgenesis for *O. niloticus* should directly provide an alternate means of producing YY-males for monosexing through breeding.

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