BACK

EXPRESSION OF THE Y-LINKED COURTSHIP BEHAVIOR GENES LACKS IN XY MALE TO FEMALE SEX REVERSED GUPPIES (*Poecilia reticulata* PETERS, 1859)

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Our research had the main porpose producing of XY females and treir identification without progeny testing. Behavior in the resulted XY females was analysed by direct observation for identification of the male specific Y-linked courtship sequences. The XY females were artificially induced by treatment of both gravid females and newly born fry with estradiol valerate. We concluded the followings: functional XY male to female sex reversed individuals can be induced by treatment with estradiol valerate, XY male to female sex reversed individuals can be identified without progeny testing using as marker the X-linked Nigrocaudatus gene, XY male to female sex reversed individuals have a normal female specific behaviour with no exception, XY male to female sex reversed individuals lacks the Y-linked male courtship behaviour.

COURTSHIP BEHAVIOR IN GUPPY

The guppyfish (*Poecilia reticulata* Peters, 1859) is a species of livebearers with an internal fertilization of the eggs. In the case of this species, matings are not associated with ov iposition sites and they can occur at any place and any time.

A guppy male can inseminate a female with her cooperation, in a „true" copulation which is preceded by courtship, or without courtship or her cooperation using a gonopodial thrust to achieve a sneak copulation (CLARK & ARONSON 1951, BAERENDS ET AL. 1955, LILEY 1966). The courtship display of males is termed the „sigmoid" display, named after the S-shape of the male's body as the display is performed (BAERENDS ET AL. 1955, LILEY 1966, HOUDE 1997).

Males spend much of their time waiting in front or slightly to one side of the female, seemingly for an opportune moment to display. They generally display only when the female has stopped moving or has slowed sufficiently for him to move in front of her. A displaying male bents his body laterally into a sigmoid posture and quivers stiffly. Sometimes the quivering is brief and subtle. When the display continues for a longer time, the male may begin to pulse his body more slowly, moving several millimeters up and down in the vertical axis. The caudal and dorsal fins often remain closed during short displays, but may be spread in larger displays. Displays with open fins appear to be of higher intensity than displays with closed fins (BAERENDS ET AL. 1955, LILEY 166, FARR 1980, HOUDE 1997).

Courtship is ussualy accompanied by changes in the male's color pattern. BAERENDS ET AL. 1955 describe changes in color patterns in response to changes in the motivational state of the male and size or receptivity of the female. In collections of wild guppies, almost every color pattern is different and undergoes slightly different changes during courtship (HOUDE 1997). The black spots of the male color pattern, generally grow larger during courtship, and horizontal black stripes appear on the body along the contours of the colored spots, but the colored spots themselves do not change during courtship. Colors other than black show little if any change during courtship (HOUDE 1997). The black lines seem to accentuate the spots, especially those with orange-red carotenoid colors. Some guppies observed of HOUDE (1997) have the ability to enlarge only a subset of potential black areas one time, so that, for example, an anterior spot may dominate the color pattern a tone time while at another a posterior spot may be the eye-catching feature of the color pattern. These males seem to be able literally to change their spots and dramatically alter their appearance to females (HOUDE 1997).

This initial phase of the male's display may proceed to copulation, or the male may suddenly jump several centimeters away from the female. The function of this jump appears to be to try to led the female away from her current position (BAERENDS ET AL. 1955) or to test her responsiveness. Display sequences are often interrupted by other males, so it is to a male's advantage to lead a female away from a crowded area (HOUDE 1997). Males also tend to avoid displaying when other fish are nearby. Nevertheless, copulations can actually be „stolen" if an intruding male can move in at the crucial moment of the courtship sequence and thrust at the female (HOUDE 1997).

The courtship sequence does not proceed beyond the male's initial displays if the female is unresponsive. Unresponsive females appear simply to ignore the male's diplays and continue feeding or searching for food. A responsive female orients toward the male so that she appears to be looking at him, and then glides smoothly toward him as he displays (HOUDE 1997).

Even when the female responds to the male, the sequence of behavior does not necessarily proceed to copulation. The female may swim away from the males at any point, and the male may terminate the display himself or stop courting the female entirely. Most initially responsive females eventually lose interest in a given male before courtship advances to the point of copulation. When the female stops responding to a male's displays, the male may attempt a sneak copulation, then eventually gives up and moves on to another female (HOUDE 1997).

If the courtship sequence does continue beyond the female's initial response, the male begins to move, still displaying, in a circle around the female. The female turns as the male displays around her. The male then swings his gonopodium forward and attempts to insert it from below and behind the female (HOUDE 1997).

SEX-LINKAGE IN THE GUPPY

Guppy males exhibit many various elaborate secondary sexual characters; several have been shown to be attractive to females: conspicuous coloration, especially bright orange and black spots, large caudal fins, large body size, and high courtship display rate (FARR 1980, BISCHOFF ET AL. 1985, REYNOLDS & GROSS 1992, NICOLETTO 1993, ENDLER & HOUDE 1995, BROOKS & ENDLER

2001, LINDHOLM & BREDEN 2002). Color patterns, caudal fin size and shape, courtship rates, and a composite measure of attractiveness are primarily sex-linked in guppy. Both quantitative genetic and pedigree analyses indicate that most of the attractive male traits are not exclusively Y-linked (WINGE 1927, KIRPICHNIKOV 1981, LINDHOLM & BREDEN 2002). Many of these traits recombine between X and Y chromosomes, denoting a partial homology between the two guppy sex chromosomes.

In 2001, TRAUT & WINKING found some cytological and molecular differentiation between the X and Y chromosomes in *Poecilia reticulata.* Only a half of the Y chromosome pairs with homologous regions of the X in synaptonemal complexes. Furthermore, the orientation of the chromosomes allowed for recombination in only 2 of 49 synaptonemal complexes observed. This fact indicates the fact that recombination is also greatly reduced in the pairing, homologous region. Comparative genomic hybridization reveals a large part of the nonpairing region of the Y chromosome that comprises male specific repetitive DNA. There is structural variation among Y chromosomes in this region (TRAUT & WINKING 2001, LINDHOLM & BREDEN 2002). This fact agrees with results from an in situ hybridization study showing that Y chromosomes, but not X chromosomes, of some domesticated guppies carry large numbers of simple repetitive sequences (NANDA ET AL. 1990, LINDHOLM & BREDEN 2002).

In general, male X-linked traits exclusively express in males, but a few of them can exppress in both male and female organism. Of course, their expresion is weak in females and their intensity cannot be compared with the fully expression of these traits in guppy males. This is the situation of some phenotypic sexually selected traits as: *Nigrocaudatus* I (NYBELIN 1947) , *Nigrocaudatus* II (DZWILLO 1959, NAYUDU 1979), *Flavus* (WINGE & DITLEVSEN 1947, NAYUDU 1979), *Pigmentiert caudalis* (DZWILLO 1959, SCHRÖDER 1969, NAYUDU 1979), red tail (FERNANDO & PHANG 1990, KHOO ET AL. 1999), blue tail (FERNANDO & PHANG 1990, PHANG & FERNANDO 1991), green tail (PHANG ET AL. 1989, PHANG & FERNANDO 1991), variegated tail (KHOO ET AL. 1999), black caudal peduncle (KHOO ET AL 1999) and black tail. The autosomes, have many fewer genes for pigmentation and fin morphology. These autosomal genes which express, as well, in female are: blond (GOODRICH ET AL. 1944), golden (GOODRICH ET AL. 1944), blue (DZWILLO 1959), albino (HASKINS & HASKINS 1948), kalymma (SCHRÖDER 1969), supressor (SCHRÖDER 1969) and elongated (HORN 1972). The Y-linked traits express in males only (as long as female has no Y chromosome). The Y-linked traits are: *Maculatus*-red (SCHMIDT 1920, WINGE 1922, 1927, 1934, WINGE & DITLEVSEN 1938, 1947, HASKINS & HASKINS 1951, HASKINS ET AL. 1970), *Oculatus* (SCHMIDT 1920, WINGE 1927), *Armatus* (BLACHER 1927, 1928, WINGE 1927, HASKINS ET AL. 1970), *Pauper* (WINGE 1927, 1934, WINGE & DITLEVSEN 1938, 1947, HASKINS ET AL 1970), *Sanguineus* (WINGE 1927), *Iridescens* (WINGE 1922, BLACHER 1928, WINGE & DITLEVSEN 1947, DZWILLO 1959), *Aureus* (WINGE 1927), *Variabilis* (WINGE 1927), *Ferrugineus* (WINGE 1927), *Bimaculatus* (BLACHER 1927, 1928), *Reticulatus*

(NATALI & NATALI 1931, apud LINDHOLM & BREDEN 2002), *Trimaculatus* (NATALI & NATALI 1931, apud LINDHOLM & BREDEN 2002), *Viridis* (NATALI & NATALI 1931, apud LINDHOLM & BREDEN 2002), *Bipunctatus* (NATALI & NATALI 1931, apud LINDHOLM & BREDEN 2002, KIRPICHNIKOV 1935), *Doppelschwert* (DZWILLO 1959), *Filigran* (DZWILLO 1959). Beside these, there are a few quantitative traits which are encoded by poligenes. These Y-linked quantitative traits are: black area (BROOKS & ENDLER 2001), fuzzy black area (BROOKS & ENDLER 2001), iridescent area (BROOKS & ENDLER 2001), mean brightness (BROOKS & ENDLER 2001) brightness contrast (BROOKS & ENDLER 2001), mean chroma (BROOKS & ENDLER 2001), attractiveness (BROOKS 2000), tail area (BROOKS & ENDLER 2001), courtship (FARR 1983).

HALF-BLACK RED GUPPY

The Half-Black Red variety is characterized by black color of the second body half, sometimes ¾ of the body present hipermelanism and expression of any other color pattern is physiological delayed in that region. Dorsal and caudal fin are red-brown, often spotted with black or brown. The gene responsible of half-black trait - *Nigrocaudatus* II - is found in our case in X-linked form. Strains which possess that gene in Y-linked form can be easily identified because of their high mortality and low growth rate. There is, also, in the guppy a less frequent variant of *Nigrocaudatus* gene which is not known in Y-linked form - *Nigrocaudatus* I. *Nigrocaudatus* genes are dominant after many authors, or not-complete dominant after other opinions. Which one is the truth, we do not surely know, but sure is the fact that both heterozygote females, with $X_{Ni}X_{ni}$ genotypes, and homozygote ones, with $X_{Ni}X_{Ni}$ genotypes, express the character in a lower dose than the male which is hemizygote ($X_{Ni}Y$).

PURPOSE OF THE RESEARCH

Our research had the main porpose producing of XY females and treir identification without progeny testing. These females are important in YY males' (supermales) technology.

The XY females were produced by treatment of both gravid females and newly born fry with estradiol valerate. Behavior in the resulted XY females was analysed by direct observation for identification of the male specific Y-linked courtship sequences.

MATERIAL AND METHOD

For obtaining of the XY females, three heterozigote $X_{Ni}X_{ni}$ females (*Ni* = abreviation of dominant *Nigrocaudatus* gene, *ni* = represent the wild recesive gene located at the same locus) were used and crossed with a Half-Black Red $X_{Ni}Y$ male (figure 1). Both the hemizygote male and heterozygote females presented the *Nigrocaudatus* character due to its dominance, more intensely in the case of male and weakly in the case of females. The virgin females, and the male repectively, were bred in USAMV biobasis. From these crossing 107 viable fry resulted, which were treated with 400 mg estradiol valerate/kg food, as their mothers. Estradiol valerate (Cyclo-Proginova®, used for the first time of SABADOŞ, BUD AND MAG 2005, unpublished data) was given to gravid guppy females (20 days prior to parturition) with oral administration, and the treatment continued one month after parturition on newly born fry. At three months age, the individuals were sexed.

According to Mendelian Laws, the theoretical chance of occurring of $X_{ni}Y$ (wild type) individuals is 25% (see figure 1). These $X_{ni}Y$ individuals should be wild type males, but the long term treatment with estrogens led to permanent feminization.

RESULTS

Two males and 105 females resulted after sexing. One male presented the half-black trait and one male presented the wild type (gray) body tone. From the total number of females, 21 (20%) were wild type and 84 (80%) were half-black type. We considered the 21 females as $X_{ni}Y$ sex reversed individuals. These male to female sex reversed individuals were observed at the sexual maturity.

The male Y-linked courtship sequences lacked at all in wild type females. Their behavior was a female specific one, with no exception.

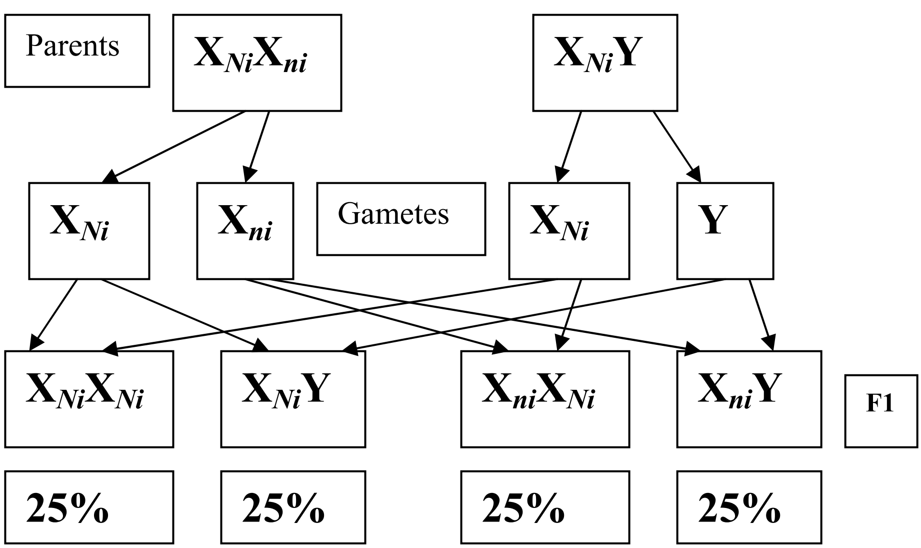

Figure 1. Theoretical chance of occurring of $X_{ni}Y$ individuals

CONCLUSIONS

- Functional XY male to female sex reversed individuals can be induced by treatment with estradiol valerate;
- XY male to female sex reversed individuals can be identified without progeny testing using as marker the X-linked *Nigrocaudatus* gene;
- XY male to female sex reversed individuals have a normal female specific behaviour with no exception;
- XY male to female sex reversed individuals lacks the Y-linked male courtship behaviour.

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