CHAPTER 2

Literature reviews

2.1 Sex determination of Nile tilapia

The gonads of fish are vary labile with respect to sex determination. Sex determination (SD) system is a biological system that determines the development of sexual characteristics in an organism and is controlled by the actions of a variety of biochemical pathways such as transcription factors, steroidogenic enzymes and second messenger systems involving several proteins. The interacting biochemical process of sex determination can lead to sex cell determination and differentiation. The genetic sex determination mechanisms have two sex systems with male heterogamety (XY) and female homogamety (XX) in which males produce X and Y sperm, females produce X ovary only and either XX or XY zygotes are fertilizations yield. XX zygotes become female and XY zygotes become male are the maintenance of this system show in **Figure 2.1.** Female heterogemety, indicated as ZW female and WW male, differ from male heterogemety only in a reversal of the heterogametic sex. (Bull, 1983).

Figure 2.1 The sex genetic system of sex determination.

In Nile tilapia *(Oreochromis niloticus)*, the sex chromosome system, similar to mammals, the classical karyotype analysis did not show any dimorphic differences between males and females (Majumdar and McAndrew, 1986; Mair *et al.*, 1991; Bezault *et al.*, 2001). The application of gynogenesis and progeny testing following sex inversion by hormone administration indicated that Nile tilapia has a predominantly

monofactorial genotypic system, but the cytogenetic between X and Y is not difference (Müller-Belecke and Hörstgen-Schwark, 1995; Mair *et al*., 1997). However, it is possible to be male or female heterogametic sex determination system in this fish spicies, female in *O. hornorum, O. aureus, O. karongae, and Tilapia mariae* is heterogametic with WZ/WW system whereas Male in *Oreochromis. niloticus* and *T. zillii* is heterogametic with XY/XX system (Mair *et al*., 1991; Desprez *et al.*, 2003; Cnaani *et al*., 2008). In Nile tilapia, Cnaani *et al.* (2008) demonstrated that the sex chromosome in fish species appears at an early stage of differentiation but sex determination mechanism is not yet fully understood. The complex sex determination mechanism in Nile tilapia is controlled by the interaction of a complex genetic sex determination systems (GSD) with a major determinant locus and some minor factors as autosomal or environmental sex determination (ESD) such as hormone, pH or temperature (temperature-dependent sex determination; TSD) (Shirak *et al.*, 2006; Lee and Kocher, 2007; Wessels and Hörstgen-Schwark, 2007;2011; Cnaani *et al*., 2008; Baroiller *et al.*, 2009a;b; Eshel *et al*., 2010). Thus, Nile tilapia is might be the one of documented model fish species to exhibit a complicated sex determination system combining both genetic (sex chromosome and multi-locus autosomal triggers) and environmental (temperature) factors as showed in **Figure 2.2** (Baroiller and D'Cotta, 2001;Devlin and Nakahama, 2002; Bezult *et al*., 2007; Baroiller and D'Cotta, 2001).

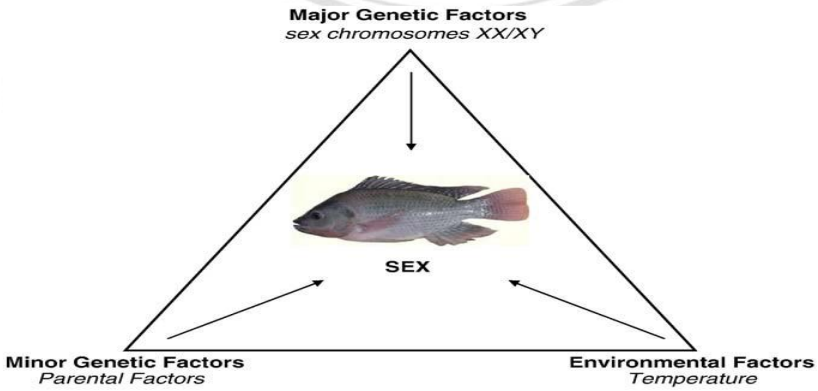

Figure 2.2 The three factors influencing sex: the genetic sex determination carried by the XX/XY sex chromosomes, the minor genetic factors which are parental, and temperature, the environmental factor

Source: Baroiller *et al*., 2009

2.1.1 Genetic sex determination system (GSD)

The two types of fish sexuality as hermaphroditism and gonochrolism. Hermaphroditism in a population produce both sperm and eggs The major genetic factor in Nile tilapia is male heterogamety (XX/XY), similar to mammals, even though Nile tilapia cannot found the karyotypic of sex chromosome and no different dimorphic between males and females (Majumdar and McAndrew,1986; Mair *et al.*, 1991; Bezault *et al.*, 2001). In **Figure 2.3A**, the current genome release to the actual number of 22 chromosomes and size of chromosome found that each chromosome had a different size (Sofy *et al*., 2008). The terminal region of the largest chromosome, which is chromosome 1, was identified as the candidate sex-determination region and confirmed as the sex chromosome (XX/XY) in Nile tilapia by analysis of meiotic chromosomes (Foresti *et al.*, 1993; Carrasco *et al*., 1999; Harvey *et al*., 2002; Cnaani *et al*.,2008, Lühmann *et al*., 2012). However, the inhibition of pairing and synapsis was showed only in the XY genotype, not shows in XX or YY genotypes (Harvey *et al*., 2002). The genetic mapping of Nile tilapia with 545 microsatellite markers was present on 24 linkage group (LG) (Lee *et al*., 2005). The sex-linked markers on LG1 in XX/XY system (**Figure 2.4B**), LG 3 in ZZ/ZW system (**Figure 2.4C**) and LG 23 (**Figure 2.4D**) were identified as the important sex-determiner (Lee *et al*.,2003; 2005; Shirak *et al.*, 2006; Cnaaci *et al*., 2008; Palaiokostas *et al*., 2013; 2015). The major sex-determining region has been previously located on LG 1, fine mapped in a region of approximately 1.2 Mb (Lee *et al*., 2005; Palaiokostas *et al*., 2013), that were linked with the phenotypic sex and indicated XX/XY male heterogamety as Nile tilapia (*Oreochromis niloticus*) and redbelly tilapia (*Tilapia zilli*). The markers that link to the phenotypic sex in ZZ/ZW female heterogamety, as *Oreochromis karonkae* (*Cichlidae*) and spotted tilapia (*Tilapia mariae*) were located on LG 3. There are also described the complex pattern in blue tilapia (*Oreochromis aureus*), had ZZ/ZW female heterogamety associated on LG 3 (Lee *et al*., 2003; Palaiokostas *et al*., 2013). Angienda *et al*. (2010) described that there are significant differences in genotypic frequency between male and female Nile tilapia for markers on LG1 and the major sex determiner has been mapped on LG1 between microsatellites UNH 104, UNH 995 and GM 201 in **Figure 2.4B** (Lee *et al*., 2003; Cnaani *et al.*, 2008; Lühmann *et al*., 2012; Palaiokostas *et al.*, 2013). Moreover,

previous study found that the transcripts on LG1 are enriched for 16 pathways, among which were two pathways with a possible role in sex determination (Böhne *et al.*, 2014). Several studies of sex determination of Nile tilapia found that the major genetic sex determination factor cannot explain all of the variation of sex ratios (Mair *et al.*, 1991). Either the experiments using mating between YY-males and XX-females or mating between pseudomales (XX) and normal females (XX) did not exist 100% males or 100% females as the expected sex ratio (Calhoun and Shelton, 1983; Mair *et al.*, 1991; Müller-Belecke and Hörstgen-Schwark, 1995; Mair *et al.*, 1997). Therefore, the studies on the genetic of sex determination system in Nile tilapia have shown that there might be an interaction between major genetic factor and two or more minor genetic factors (autosomal). In **Figure 2.4D**, LG 23 has also been found to harbor QTL for temperature-dependent sex determination. A microsatellite UNH 898 has been mapped near *amh* on LG 23, which shows a strongly expression at 14 days post fertilization (dpf) before initial stage of gonadal differentiation in Nile tilapia (Poonlaphdecha *et al*., 2010; 2014; Lühmann *et al*., 2012, Palaiokostas *et al*., 2015). UNH 898 also has a strong association with the mixed sex phenotypic (XX/XY) Nile tilapia populations (Eshel *et al.*, 2010, 2012). In the present study of 2015, Palaiokostas *et al*. showed the first evidence for a sex-determinant region on LG 20 which possible to involve in sex reversal for male proportion in Nile tilapia. Various candidate genes such as *amh, dmrta2, sox14* on LG 23 (Shirak *et al*., 2006; Eshel *et al*., 2010) and *cyp1a, dm0, wt1b* on LG 1 and 3, respectively (Lee and Kocher, 2007) were mapped to LG as putative master key regulators of sex determination of Nile tilapia.In the GSD of Nile tilapia for sex ratio, minor genetic factors can lead to bias the sex ratio and the sex determination mechanism can determine that is controlled by the interaction of many factors as the polyfactorial system. The polyfactorial system of sex determination can be assumed to define of three criteria of sex ratio such as a large sex ratio variance, parental and maternal effective and a response to selection for sex ratio (Bull, 1983; Wohlfarth and Wedekind, 1991; Tuan, 1999). The heritability (h^2) of sex ratio in Nile tilapia was estimated of 0.26 (Lester *et al.*, 1989). In additional, the sex ratios have a strong genetic background with low influence of family variation and parental factors (Lozano *et al*., 2011).

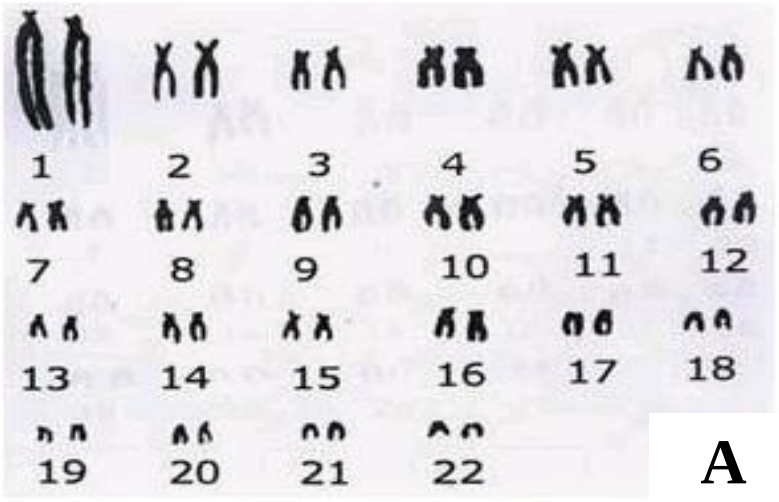

Figure 2.3 The chromosome of Nile tilapia (*Oreochromis niloticus*)

Source: www.researchgate.net

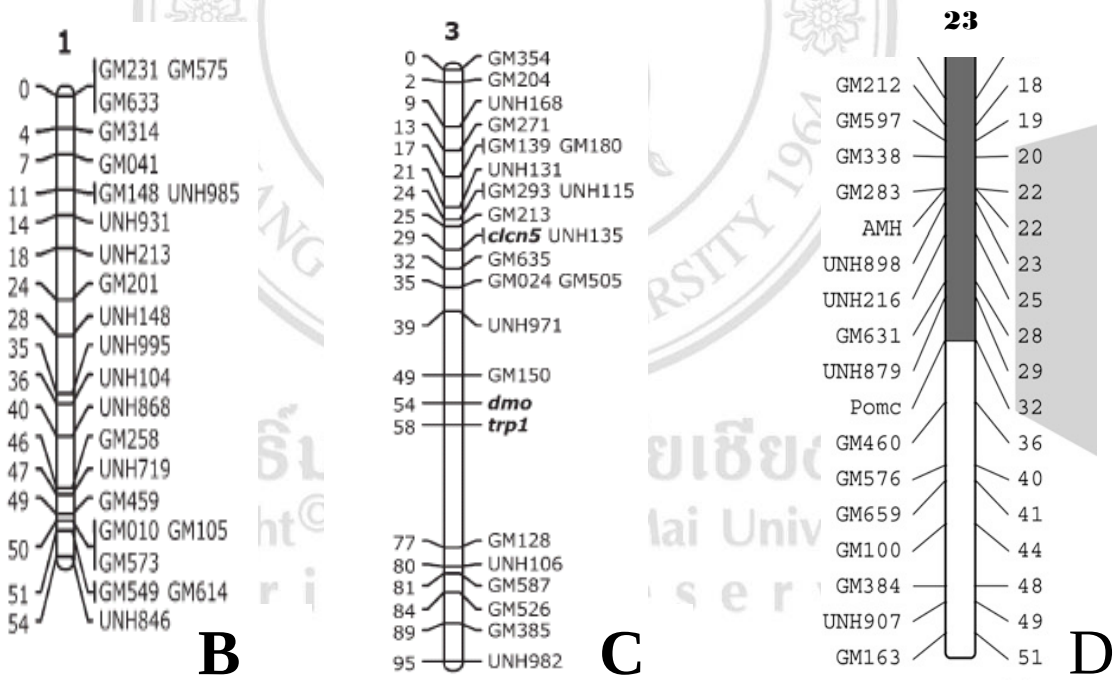

Figure 2.4 The kaoryotype of Nile tilapia (*Oreochromis niloticus*). (B), (C) and (D). The Linkage group 1 (LG1), Linkage group 3 (LG3) and LG 23 (LG23) of Nile tilapia genome, respectively.

Source: adapted from Lee *et al*., 2005 and Sofy *et al*., 2008

2.1.2 Environmental sex determination system (ESD)

In fish species, the environment factors such as density, pH, high water temperature, can influence the phenotypic sex.

1) Density

Many studies investigated the environment effect by density in eels. The eel densities were affected skewed sex ratios. Most eels strain reproduce in marine water and migrate to freshwater in larvae before back to the sea in adult eels. Degani and Kushnirow (1992) showed that the male proportion of elvers (*A. Anguilla*) rearing communally was higher than rearing isolatation with a significantly and Roncarati *et al*. (1997) also described that the density-dependent may be affect the eel (*A. Anguilla*) proportion of males. Tesch (2003) demonstrated that the low density of eel in the river tending to develop high female proportion, whereas the high density favoring male proportion. In addition, the warm temperature was determined in eel sex determination and found that is the first sex determiner factor to induce testis differentiation, but they show the opposite result in long period (Holmgren and Mosegaard, 1996). Thus, density condition could be the major of environment factor that involved the sex determination and sex differentiation in eel (Degani and Kushnirow, 1992; Roncarati *et al*., 1997; Tesch, 2003; Davey and Jellyman, 2005)

2) pH

In some fish species, pH influences the skewed sex ratio. In *Apistogramma,* indicated that the acidic water (pH <6) effect the male differentiation and female in neutral water (pH 7.0). Similar to the 50-80 broods fish of *Pelvicachromis*, showed yield in acidic (pH 4-5) with 90% of males and 90% female in neutral water (pH 7.0) and the similarly result was also found in some fish species as *Xiphophorus helleri*, *Poeciliidae* and *Apistogramma caetei* (Heiligenberg, 1965; Rubin, 1985; Oldfield, 2005).

3) Exogenous steroids

The manipulation of sex differentiation in fish can be controlled with exogenous steroids (Al-ablani and Phelps, 2002; Devlin and Nagahama, 2002; Kalil *et al*., 2011). Some studies explored the mechanism of hormone administration action to produce monosex fish population, but the mechanisms of hormone applications action during sexual ability are still unclear. The androgenic were used for masculinization of genotypic females and estrogenic steroids were used for feminization of genotypic males. The skewed sex ratio can assume that depend on the androgen and estrogen ratio and the feedback of hormone applications can be occurred on brain to gonad differentiation (Bogart, 1987; Kah *et al*., 1993). Using hormonal application can be obtained the maximal percentage of male or female population ($\geq$ 90%). In rainbow trout (*Oncorhynchus mykiss*), female grow faster and larger than male and the maturation occurred later than males. This characteristic also seen in other species of this family such as coho samon and Chinook salmon (Devlin and Nakahama, 2002; Piferrer, 2001; Razmi, 2010). The ethynylestradiol-17α (EE_2) was used as the hormone application to produce female rainbow trout populations. Razmi *et al.* (2010) described that use of EE_2 to fry rain bow trout in a 30 day dietary treatment can increase the proportion of female at 94% in dietary treatment of 20 mg/kg feed comparing with control at 38%. In Nile tilapia, the hormone treatmentusing 17α-methytestosterone (17α-MT) can be affected the sex ratio by increase the proportion of male. Many studies have used different does of 17α-MT as dietary treatment to determine the sex reversal of Nile tilapia. Ferdous and Ali (2011) demonstrated that using the dose of 60 mg MT/kg feed of 17α-MT to tilapia fry result the highest of male sex (94.28%). The similar results were showed in several studies as show in **Table 2.1**. In addition, the hormonal application is also used to produce monosex progenies in the YY or ZZ broodstocks (Cnaani and Levavi-Sivan, 2009). However, the hormone administration in Nile tilapia should begin simultaneously period, critical sensitive period for sex determination, and

should be continued at least 21 days with dosage of 40-60 mg of kg feed to gain the high percentage of male.

Table 2.1 Effect of 17α-methytestosterone**,** with different dose**,** using as dietary feed to Nile tilapia (fry) on sex ratio

Species	Treatment	% of male	References
O. Niloticus	Control	57.1	Celik et al. (2011)
	20 mg/kg feed	69.8	
	30 mg/kg feed	69.4	
	40 mg/kg feed	70.9	
	50 mg/kg feed	86.1	
	60 mg/kg feed	93.7	
Species	Treatment	% of male	References
O. Niloticus	Control	48.57	Ferdous and Ali (2011)
	40 mg/kg feed	88.57	
	50 mg/kg feed	91.43	
	60 mg/ kg feed	94.28	
	70 mg/ kg feed	91.43	
O. Niloticus	Control	30	EI-Greisy and EI-Gamal (2012)
	40 mg/kg feed	85	
	60 mg/kg feed	97	
	80 mg/kg feed	93	

Source: adapted from Celik *et al*. (2011); Ferdous and Ali (2011); EI-Greisy and EI-Gamal (2012)

4) Temperature-dependent sex determination (TSD)

In gonochoristic vertebrates, there are no consistent differences between male and female sex. The temperature-dependent sex determination (TSD) is a system in which the temperature determines the sexes of the organisms that hatch. It is classified under the reptile class, but is also used among some birds, such as the Australian Brush-turkey. TSD also happens in some fishes such as salmon, gold fish, tilapia and so on. However, the basis of TSD is still unknown but it may have an effect on the hormone acting during sex differentiation. Ospina-Alvarez and Piferrer (2008) studied in many 59 fish species belong to 13 families of many types of fishes showed that the sex ratio of fish with TSD have been grouped as three pattern of sex ratio response to temperature-dependent 1) more males at high temperature 2) more females at high temperature and 3) more males at extreme high and low temperature (**Figure 2.5**). Many studies can proved that the elevated temperature can be induced in both of ferminizing and masculinzing effect, depend on each sex phenotype (Baroiller and D'cotta, 2001; Kwon *et al*., 2002; Ospina-Alvarez and Piferrer, 2008). The results also showed that higher temperatures invariably result in highly male skewed sex ratios even small changes of just 1-2°C can significantly alter the sex ratio from 1:1 (males:females) up to 3:1 in both freshwater and marine species. The incubation of Atlantic silverside (*M. menedia*) larvae showed the higher male sex than female (Conover and Kynard, 1981) at increasing temperature. Conversely, the increasing temperature produced the high female sex ratio in catfish (*Ictalucus punctatus*) and the sockeye salmon (*Oncorhynchus nerka*) (Patino *et al*., 1996 and Craig *et al*., 1996). Pavlidis *et al*. (2000) described the low temperature (13-15 ^{o}C) treatment applied to European sea bass could increase the high proportion of female (70-73%) at the early stage of sex differentiation.

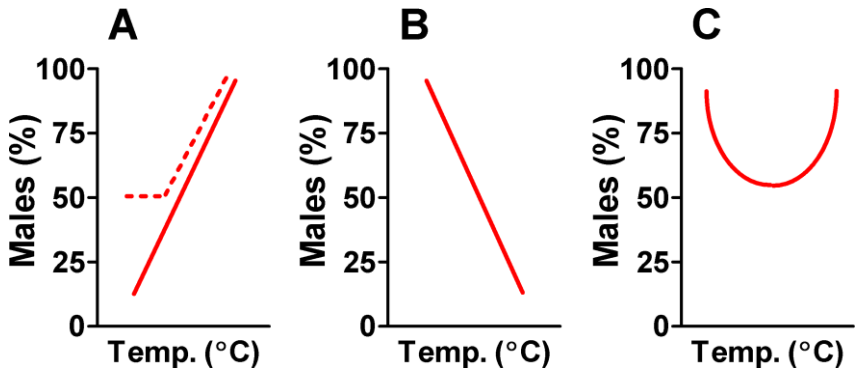

Figure 2.5 The temperature-dependent sex determination (TSD) in fish A) more male at high temperature B) More female at high temperature and C) More male at high and low temperature

Source: Ospina-Alvarez and Piferrer, 2008

In Nile tilapia, despite the basis of major genetic for sex determination that other factors as environment factors especially the temperature which affects on sex differentiation in many species tilapia species. The high water temperature during the juvenile stage of Nile tilapia can override the genetic sex and induces the sex ratio in favor of males. The application of high temperature, more than 34^{o}C, can induce the higher proportion of males during the larval stage in all-female progenies (XX-female). Abucay *et al.* (1999) according to the high temperature treatment (36.54 ± 0.39^{o}C) produced the significantly higher percentage of males in all-female progenies (XX), but lower male percentages of all-male (XY) and YY-male progenies than control treatment (27.87 ± 1.40^{o}C) with significantly ($P<0.05$). The temperature can affect only the XX individuals to produce all male phenotype but not in XY individuals and YY-male (Baroiller *et al.*, 1995a, b; Abucay *et al.*, 1999; Luckenbach *et al.*, 2009). The temperature treatment has applied to the critical sensitive period while the gonad still undifferentiated characteristic. The use of female monosex population (100% XX-female) as the progeny testing in high temperature treatment, above 32^{o}C to skewed male sex ratio corresponding to sex-inversion of the genetic female (XX) to a homozygous functional of thermo-neomales (Δ♂XX), these phenotypic depend on the breeder (Baroiller *et al.*, 1995a; b). Tessema *et al.*, 2006 demonstrated that to applied the temperature

treatment at 36ºC for 10 days treatment duration, start on 10 dpf , in the two strain of Nile tilapia increased the male proportion of 79% in Lake Manzala and 61% males in Lake Rudolph with significantly (P<0.001). However, the most period that most effective to high temperature treatment start from 10th dpf until 20th dpf and continued 10 days treatment duration at 36ºC (Baroiller *et al*., 1995a; b**;** Tessema *et al*., 2006; Wessels and Hörstgen-Schwark, 2007, Lühmann *et al*., 2012). At 36ºC, the percentage of males was higher without survival rate effective. Moreover, the temperature treatment which starts at 7-13th dpf was not successful in case (Baroiller *et al*., 1995a; b**;** Tessema *et al*., 2006). However, the proportions of male also depend on breeding. Wessels and Hörstgen-Schwark (2007) according to the heritability estimation of temperature sensitivity in Nile tilapia (Lake Manzala strain, Egypt) were 0.69 in *high* line and 0.86 in *low* line. In sensitive *high* line showed a male percentage of 90% and 54% of male in sensitivity *low* line (**Table 2.2**). The critical sensitive period for environmental sex determination of Nile tilapia lasts from the 10th day until 19th day post fertilization (dpf) that the gonad still undifferentiated (Baroiller *et al*.,1995a; b; D' cotta *et al*., 2001). The first sign of sex differentiation was observed with the histological data occur approximately 26th until 30th dpf (Nakamura and Nakahama., 1985). The indicator of sex differentiation is *cyp19a* gene (aromatase gene) expression, which codes the *cytochrom p450 aromatase* from 18th until 26th dpf. Aromatase enzyme activity in fry brain showed the sexual dimorphism with higher activity in females but low in males (D' cotta *et al*., 2001; Kwon *et al*., 2001).

2.2 Sex differentiation in Nile tilapia

The first establishment of gender can be triggered by the action of the major sex determining factor, several sex-associated loci and an environment factors as temperature-dependent sex determining when the gonads are undifferentiated or even before they are formed at the pre-gonadal stage. The differences of the proliferative rate of germ cells which can be one of the first effects of sex determining factor. The high rate of germ cell proliferation can produce females and low rate proliferation produce

males, whether genetic or environmental. During this early period, the factors as high temperature can change the sex differentiation, usually from female to male. At the beginning of gonad differentiation, the undifferentiated gonad can transform to testis or ovary. The incorporation of sex steroids, androgens or estrogens can result in female into male or male into female sex reversal in that genotype of male or female develop into male phenotypic female and males, respectively. (**Figure 2.6**)

Table 2.2 Male proportion of temperature treated at 36°C from two strain of Nile tilapia

Strain/Population	Line*	36 °C Males (%)	References
Lake Manzala, Egypt	-	79	Tessema et al. (2006)
Lake Rudolph, Kenya	-	61	
Lake Manzala, Egypt	*high*	90	Wessels and Hörstgen-Schwark (2007)
	low	54	

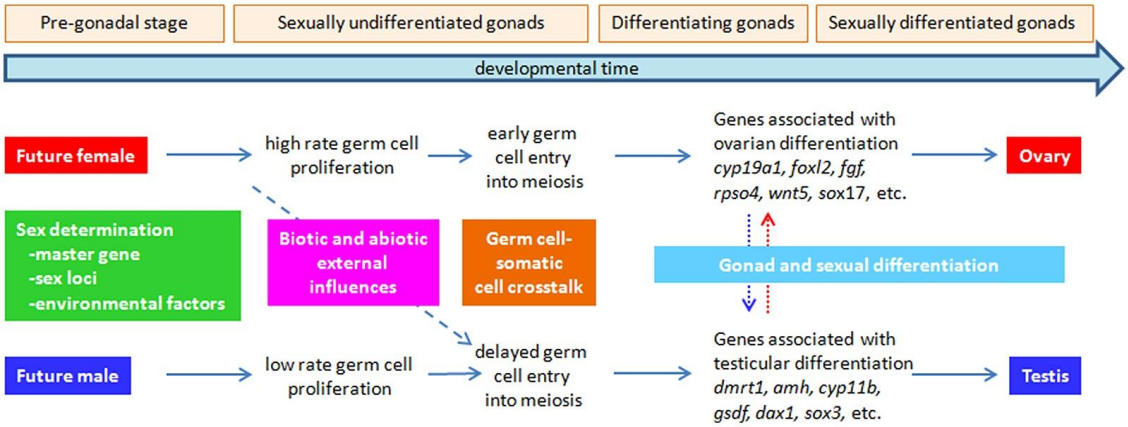

Figure2.6 The main pathway leading to ovarian (female) and testicular (male differentiation in fish, respectively

Source: Martinez *et al*., 2014

2.3 Candidate genes for sex determination

In mammals, SRY (sex determining region of Y) can be seen as the only testis determining factor (Sinclair *et al*., 1990). In fish and other lower vertebrates, no such gene for the initial sex determination process is certainly known to be conserved (Devlin *et al*., 1991; Devlin and Nagahama, 2002). The specification of the phenotypic sex is underlying a much more labile system, which can be influenced by environmental factors, such as temperature. Endogenous steroids influencing the sex determination pathway can be excluded because steroid-producing cells occur just after the differentiation of the gonads (Nakamura *et al*., 1998). Therefore, it can be suggested, that the expression of interacting genes, which are involved in the sex determination and differentiation processes is influenced by the water temperature. Therefore, it can be suggested that temperature driven phenotypic sex has a strong genetic background.Putative master key regulators and candidate genes for sex determination in fish are sex determining genes of mammals. Some homologues genes were found in different lower vertebrates and for some of them, sex dimorphic expression patterns have been observed in tilapia (Guiguen *et al*., 1999; Kwon *et al*., 2001, Ijiri *et al*., 2008; Poonlaphdecha *et al*., 2011). Partially, they have been mapped to the putative sex

chromosomes of *Oreochromis spp*. (Lee *et al*., 2005; Shirak *et al*., 2006; Lee and Kocher, 2007; Cnaani *et al*., 2008).

2.3.1 *Cyp 19a*

Cyp19a (cytochrome P450, family 19, subfamily A) is involved in the onset of female sex differentiation of Nile tilapia by catalyzing the conversion of androgens to 17β-estradiol. Females showed a higher expression of cyp19a just after the stage of sexual lability (Guiguen *et al*., 1999; D'Cotta *et al*., 2001; Kwon *et al*., 2001; Ijiri *et al*., 2008). Bogart (1987) hypothesized the suppression of aromatase by a Y-chromosomal gene for species with a male heterogametic system. However a decrease in aromatase expression has also been found in hormonally (Bhandari *et al*., 2006) and thermally (D'Cotta *et al*., 2001) sex reversed XX-males before the onset of ovarian differentiation. Moreover, the application of aromatase inhibitors leads to an increase of males as well (Kwon *et al*., 2000; Afonso *et al*., 2001). It has been assumed that environmental effects act on the cyp19a expression either directly or by a genetic cascade (Bhandari *et al*., 2006; D'Cotta *et al*., 2001). The expression of *cyp19*a strongly depends on the expres-sion of the genes *amh* (Pieau, 1996) and *foxl2* (Wang *et al*., 2007). This correlation supports the hypothesis of a genetic cascade that involves *cyp19a*. Shirak *et al*. (2006) and Lee and Kocher (2007) mapped *cyp19a* to LG 1 between the microsatellites GM633 and UNH985.

2.3.2 *Foxl2*

The forkhead transcription factor gene foxl2 (forkhead box L2) is involved in the early ovarian development and differentiation and is highly conserved in different species (Cocquet *et al*., 2003; Loffler *et al*., 2003; Baron *et al*., 2004). In Nile tilapia, foxl2 showed higher expression levels in gonads of XX animals from the 5th day after hatching (dah), increasing until the 35th dah. Expression levels of foxl2 in XY gonads remained consistently low (Ijiri *et al*., 2008). FoxL2 has been mapped to 17 cM on LG 14 (Shirak *et al*., 2006). Concerning tilapia, there is no definite proof, that the expression of fox l2 is temperature-dependent. However, Shoemaker *et al*. (2007) provided evidence that foxl2 is involved in the sex

determination of the red eared slider turtle (Trachemys scripta), a species with TSD. Foxl2 is involved in the regulation of *cyp19a* expression as well and temperature-related expression of foxl2 could also be the result of a negative feedback effect of *cyp19a* expression (Pieau, 1996).

2.3.3 *Wt1*

The wilm's tumor gene (WT1) plays a critical role in mammalian gonadal development (Hossain and Saunders, 2001). Therefore, it is a putative key regulator for sex determination in other vertebrates. In Nile tilapia, the *wt1b* gene has been mapped to LG 1 (32.4 cM), flanked by the markers GM201 and UNH995 (Lee and Kocher, 2007). This is the chromosomal region, where the major sex determiner for Nile tilapia has been mapped to (Lee *et al.*, 2003; Lee and Kocher, 2007), but it has been excluded as the major sex determining gene (Lee and Kocher, 2007).

2.3.4 *Sox-gene family*

The SOX (SRY-related HMG box) gene family consists of 20 different genes in mammals and plays an essential role in sex determination. Many orthologues sox genes have been found in different fish species and some of them in *Oreochromis spp*. Sox2 has been mapped to LG 17 close to the microsatellite UNH991 and *sox14* has been mapped to LG 23 between the markers UNH898 and UNH216, just 1 cM away from amh (Cnaani *et al*., 2007). However, only for *sox9*, sexual dimorphic expressions with higher expressions in XY-gonads of *O. niloticus* have been detected until now (Ijiri *et al*., 2008; D'Cotta *et al*., 2007). *Sox9* expression is temperature-dependent in different TSD species (Western *et al*., 1999; Moreno-Mendoza *et al*., 2001; Shoemaker *et al*., 2007). D'Cotta *et al*. (2007) provided a first evidence for temperature-dependent expression of *sox9* in Nile tilapia with an earlier increase in XX gonads compared to XY gonads.

2.3.5 *Dmrt1, dmrta2* and *dmo*

Dmrt1 (doublesex and mab-3-related transcription factor 1) and *dmrta2* (doublesex- and mab-3-related transcription factor A2) belong to the family of doublesex/mab-3 domain genes. Ijiri *et al*. (2008) found an early male-specific expression of *dmrt1* in gonads of *O. niloticus*, six days after hatching, increasing until day 10 after hatching. Also for medaka (*Oryzias latipes*), the closely related gene *dmy* is known to be male specific (Nanda *et al*., 2002). Additionally, dmrt1 expression is up-regulated by male-producing temperatures in different TSD-species (Kettlewell et al., 2000; Torres Maldonado et al., 2002; Hattori et al., 2007) but no respective information is available for tilapia. In *Oreochromis spp*., *dmrt1* has been mapped to 44 cM on LG 12 (Lee *et al*., 2005). Shirak *et al.* (2006) proposed *dmrta2* to be a master key regulator for sex determination in Nile tilapia. They mapped *dmrta* 2 to 5 cM. on LG 23. Additionally, the gene *dmo* is involved in the gonadal development of the ovaries (Guan *et al.*, 2000) and has been mapped to the region between GM150 and UNH106 on LG 3 (Lee *et al.*, 2005). *Dmo* in Nile tilapia represented a novel gene, whose expression was limited to the ovaries (Guan *et al*., 2000).

2.3.6 Anti-Müllerian (*Amh*) gene

The *amh* gene (anti-müllerian hormone gene) is male- specific hormone represses the müllerian ducts during sex differentiation in mammals. *Amh* inhibits the development of the müllerian ducts into ovaries. In fish, *amh* is conserved in fish even fit lacks of müllerian ducts. In Nile tilapia, *amh* is known to be expressed earlier in the gonads of XY males than of XX females but the mechanism of *amh* is still unclear (D'Cotta *et al*., 2007; Ijiri *et al*., 2008, Poonlaphdecha *et al*.,2011). *Amh* is located on LG23 close to microsattelite marker UNH898 and GM283 where a QTL for autosomal SD and close to UNH 898 and GM047 for temperature-dependent sex reversal as showed in **Figure 2.7** (Shirak et al. 2006, Lühmann *et al*.,2012, Eshel *et. al*., 2012). Poonlaphdecha et al. (2011) described that *amh* gene contains 7 exons that were divided by 6 introns but no splice form (**Figure 2.8**). Moreover, the strong sex dimorphic

expression of *amh* in Nile tilapia brain before gonad differentiation was described in Poonlaphdecha *et al*. (2011; 2013). For a temperature-dependent expression of *amh* in sex determination also found in LG23 similar to autosomal sex determination (Shirak *et.al.*, 2007, D'Cotta *et al*., 2007, Eshel et. al.,2012). Poonlaphdecha *et al*., 2013 described that the effect of elevate temperature (~34 ^{o}C) from 10 dpf induce the rapid expression of *amh* but down- regulation of *Foxl2* and *Cyp 19a1a*. The result can suggest that amh might be interact to *Foxl2* and *Cyp 19a1a* expression.

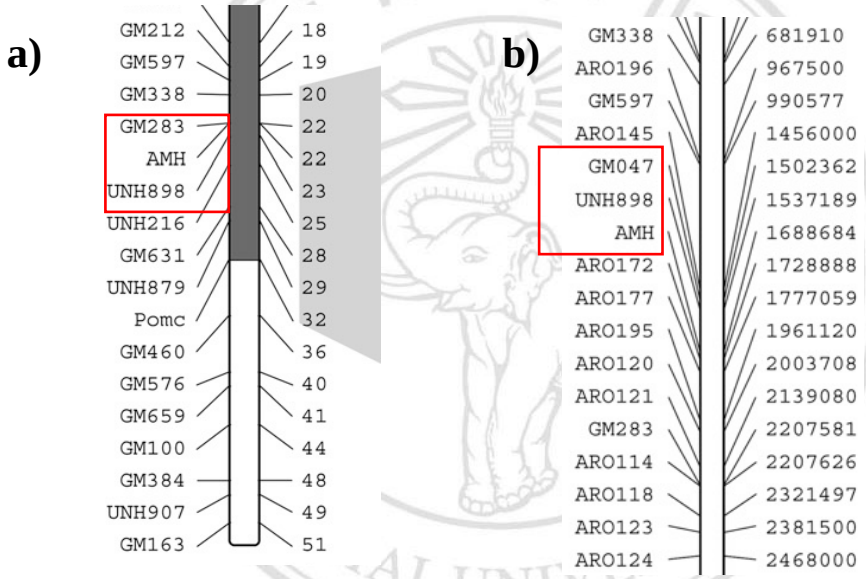

Figure 2.7 QTL mapping on LG 23 a) QTL mapping of autosomal SD on LG 23 b) QTL mapping of sex reversal on LG 23 in Nile tilapia

Source: Shirak *et al*, 2006, Eshel *et al*., 2012

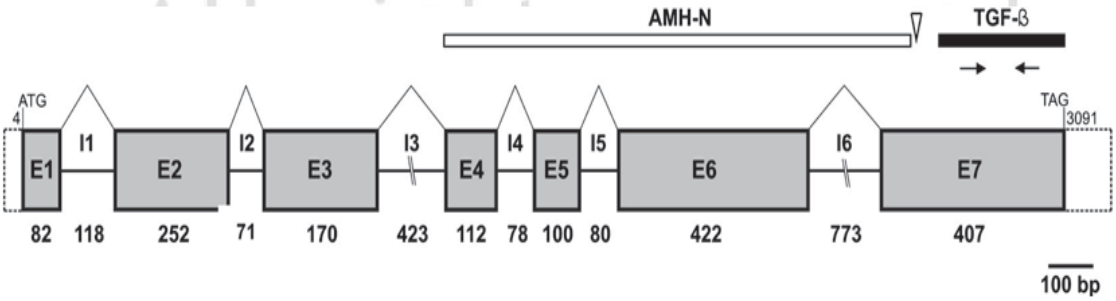

Figure 2.8 Nile tilapia *amh* gene structure with the 7 exons in grey rectangles (E) and introns (I) (Source: adapted from Poonlaphdecha *et al*., 2011)