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The genetic control of sexual maturation in the teleost, *Xiphophorus maculatus* (Poeciliidae) ; a review

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Summary. A sex-linked gene which controls the age at which the gonadotropic zone of the pituitary gland develops and becomes physiologically active has been identified in the platyfish. The alleles for early (P^e) and late (P^l) differentiation are linked to pigment genes that serve as genetic markers. The average age of sexual maturation was 12.5 weeks (range, 10-15) for $P^e\ P^e$ males, 20 weeks (range, 16-29) for $P^e\ P^l$ males and 26.5 weeks (range, 18-40) for $P^l\ P^l$ males. $P^e\ P^e$ females matured between 10 to 14 weeks of age and $P^e\ P^l$ females between 16 to 20 weeks. No overlap in maturation time is found when fish of different genotypes are raised under identical conditions. The presence or absence of the gonadotropic zone is well correlated with the differentiation of the gonad. However, even in the absence of a gonadotropic zone oocytes may proceed up to the yolk droplet stage and testis will form spermatogonia. In males, androgen from the developing testis, in turn, controls the metamorphosis of the anal fin into a gonopodium. The growth rate of immature males and females is the same, however it declines sharply in males (but not females) at the time of sexual maturity. Thus, early-maturing males are significantly smaller than late-maturing ones. Female genotypes do not exhibit size differences and eventually become larger than all males.

This polymorphism effecting gonadotrop differentiation is a natural component of wild populations and of laboratory stocks derived from them.

Intraspecific variation in a variety of endocrine parameters including those dealing with the hypothalamo-hypophysial-gonadal (HPG) axis are known to be under genetic control. Most of our information comes from studies of laboratory animals and genetically controlled aberrant endocrinological conditions in man. Many of the differences between laboratory stocks are merely of a quantitative nature and thus make the understanding of the basic mechanisms involved difficult. Recently, we have discovered in the platyfish, *X. maculatus*, a gene that controls an all-or-nothing response of the pituitary-gonadal axis.

A sex-linked gene, *P*, affects the onset of sexual maturity in *X. maculatus* by controlling directly or indirectly the age or size at which the gonadotrops differentiate and become physiologically active. Five *P* alleles have been identified in natural populations and laboratory stocks (Kallman and Borkoski, 1978). The various *P* factors are closely linked to a number of color genes that serve as genetic markers. The same *P* allele may be associated with a variety of color genes. The existence of platyfish with

« early » and « late » genotypes that differ only by the sex chromosomes they carry but are genetically identical in all other respects (platyfish have 23 pairs of autosomes), has provided a model system to study how the genotype controls the development of the pituitary-gonadal axis, how the pituitary gland regulates gonadal structure and function, and how age and size separately affect gonad maturation.

Originally, it was found that in the Belize stock males homozygous for a certain pigment gene, red iris (*Ir*), matured at 13 weeks and at a size of 24 mm, and males homozygous for a second pigment gene, red body (*Br*) matured at 26 weeks and at 31 mm (table 1). Heterozygous males were intermediate for both traits (Kallman and Schreibman, 1973 ; Schreibman and Kallman, 1977 ; Kallman, Schreibman and Borkoski, 1973). The *P* allele linked to *Ir* was subsequently described as P^3 and the one linked to *Br* as P^4. Males and females homozygous for P^1 mature at approximately 8 weeks and at 21 mm whereas 50 p. 100 of the females homozygous for P^5 still had undeveloped ovaries at 60 weeks, although they exceeded 40 mm in length (Kallman and Borkoski, 1978).

TABLE 1

Age of sexual maturity and adult size of 3 genotypes of male platyfish, Xiphophorus maculatus *(Belize stock)*

n	Maturity weeks s. e.	Size mm s. e.	n	Maturity weeks s. e.	Size mm s. e.
	Ir P^3 *Ir* P^3 males *			*Ir* P^3 *Br* P^4 males *	
11	13.3 ± 0.57	24.4 ± 0.83	24	18.4 ± 0.74	27.9 ± 0.67
17	12.6 ± 0.33	23.3 ± 0.23	19	19.7 ± 0.52	27.7 ± 0.43
	Ir P^3 *Br* P^4 males **			*Br* P^4 *Br* P^4 males **	
40	20.2 ± 0.53	27.0 ± 0.42	46	26.5 ± 0.60	31.3 ± 1.43

* From W - + Y-*Ir* female × Y-*Ir* Y-*Br* male (Kallman and Schreibman, 1973).
** From W - + Y-*Br* female × Y-*Ir* Y-*Br* male (Schreibman and Kallman, 1977).

Neonatal platyfish have pituitary glands that, with the exception of the gonadotrops, contain all the cell types present in mature fish (Schreibman, 1964). In immature animals, regardless of age, the gonadotropic zone is represented by only a few chromophobes in the peripheral caudal pars distalis. The activity of the *P* gene is correlated with the age and size at which the chromophobes proliferate and differentiate to form a well-developed zone of active gonadotrops. Depending upon genotype this event may occur as early as 5 weeks or not until the fish are well past one year of age. Ultimately the region of gonadotrops in early and late developers are indistinguishable. Whether this gene operates directly on the pituitary gland, the hypothalamus or some other level of the endocrine or sensory systems is still unknown and under investigation. We are especially interested in the events that occur in the hypothalamus concomitantly with the maturation of the hypophysis.

The development of the gonadotropic zone precedes, and is essential for, complete gonadal maturation. In the absence of a functional zone oocytes develop through the oil

droplet stage and are surrounded by active follicle cells and a prominent zona pellucida. Yolk deposition, however, does not occur. Spermatogenesis proceeds up to the spermatocyte stage and efferent ducts display little activity.

The anal fin of male platyfish undergoes a complex transformation into an intromittent organ. Each of the 6 clearly-defined successive stages of this process are dependent upon increasing levels of androgens. Thus the stage of development of the anal fin serves as an indicator of sexual maturation and of sex steroid levels. It permits us to postulate the course of steroidogenesis as indicated in figure 1. All males, but not females, enter into stage one at 5 weeks of age. The transition of the unmodified anal fin into stage 1 is not contingent upon the presence of a Y chromosome, since the same transformation occurs in males with the exceptional genotypes, XX (Kallman, 1968). All males remain in this stage until the pituitary-gonadal axis becomes active which, depending upon genotype, may occur as early as 5 weeks in P^1 P^1 males or as late as 25 weeks in P^2 P^5 males. The rate of anal fin metamorphosis (stage 2-stage 6) is directly related to the age at onset of maturity ranging from 3.2 weeks for P^1 P^1 to 7 weeks for P^2 P^5 males (Kallman and Borkoski, 1978).

Androgens are also responsible for retarding the rate of growth of poeciliid fish (Pickford and Atz, 1957). Immature males and females grow at the same rate, however this rate declines sharply in males (but not females) at the time of sexual maturity. This leads to significant differences in the final adult size that are a permanent phenotypic expression of the *P* locus and of activity in the HPG axis.

Figure 1 illustrates our findings that several processes associated with sexual maturation are retarded in the later-developing genotypes. Gonopodial differentiation, a sensitive indicator of androgen (Turner, 1942) and indirectly of gonadotropin production, takes almost twice as long to go from stage 2 to stage 6 when early and late genotypes are compared (Schreibman and Kallman, 1977). Similarly, histological observations indicate that the development of the gonadotropic zone and of the gonads proceeds more slowly in the late genotypes. Ultimately all fish, regardless of their genetic makeup, have histologically indistinguishable pituitaries and gonads.

The relationship between age and size in determining the onset of sexual maturity was studied in three genotypes of females. P^1 P^1 (early) females matured at 21 mm regardless of age. Some P^5 P^5 (late) females initiated maturity after week 34 regardless of size, but even at 60 weeks, half of them were still immature although they exceeded 40 mm in length. An inverse relationship between age and size existed for P^1 P^5 females. They had ripe ovaries as early as 11 weeks provided they had attained a size of 30 mm, but they became mature as small as 23 mm at 25 weeks of age. Keeping age and size constant, females homozygous for P^5 always had a smaller number of eggs than females of the other two genotypes. Up to a size of 30 mm, P^1 P^1 females had significantly more ova than P^1 P^5 females. This suggests that fecundity and the gonadosomatic index are genotype specific (Kallman and Borkoski, 1978).

Our analysis of the HPG axis raises a number of obvious questions that go beyond the study of the platyfish. Investigation of these problems may provide some insight into general endocrinological mechanisms and phenomena.

1. What triggers the *P* gene ? How is it related to size and age ? How do internal and external stimuli affect activity of the various *P* alleles ?

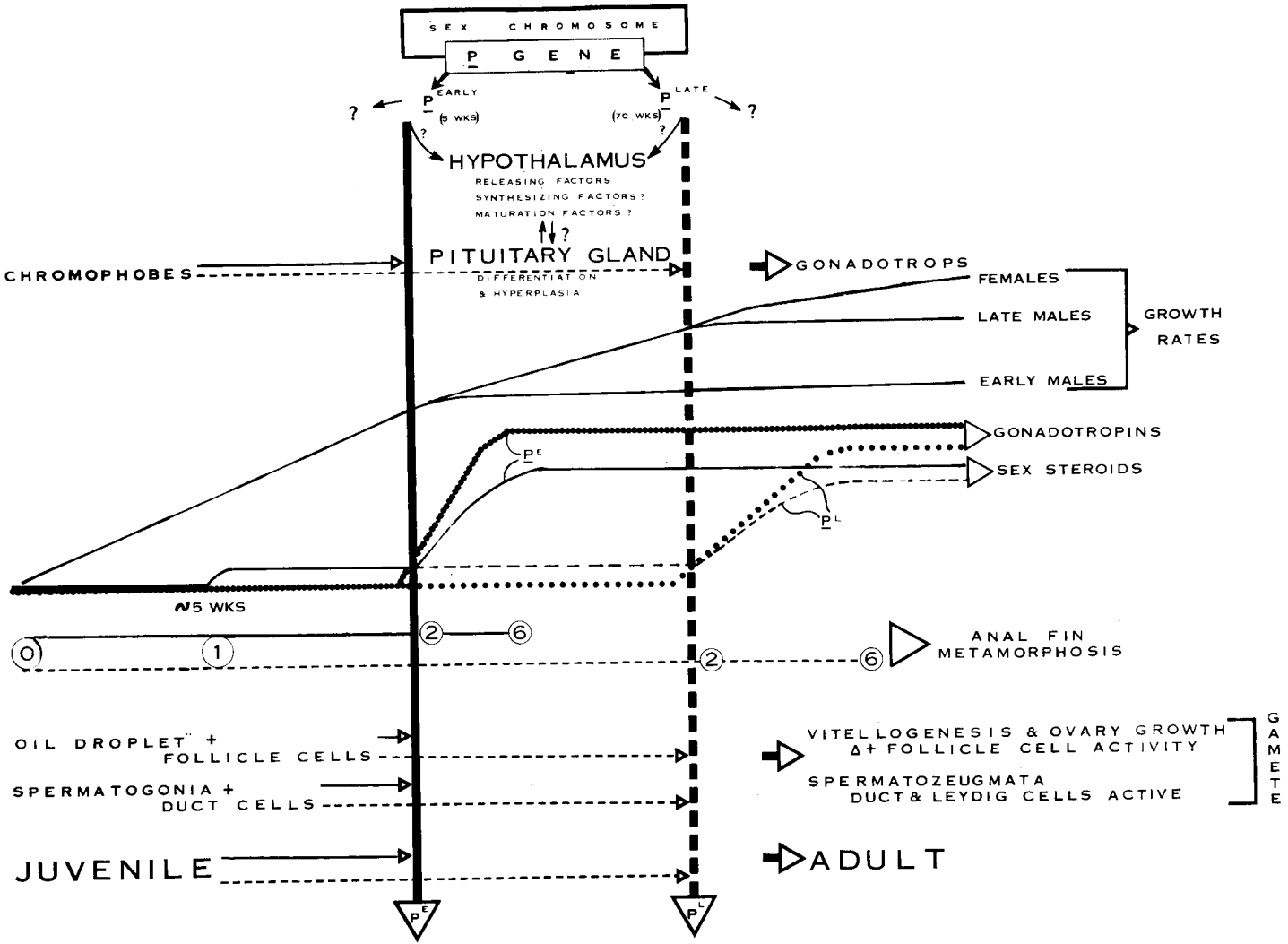
SEX CHROMOSOME
P GENE
P EARLY
(5 WKS)
P LATE
(70 WKS)
?
HYPOTHALAMUS
RELEASING FACTORS
SYNTHESIZING FACTORS ?
MATURATION FACTORS ?
PITUITARY GLAND
DIFFERENTIATION
& HYPERPLASIA
CHROMOPHOBES
GONADOTROPS
FEMALES
LATE MALES
EARLY MALES
GROWTH RATES
GONADOTROPINS
SEX STEROIDS
PE
PL
~5 WKS
0
1
2
6
ANAL FIN METAMORPHOSIS
OIL DROPLET + FOLLICLE CELLS
SPERMATOGONIA + DUCT CELLS
JUVENILE
VITELLOGENESIS & OVARY GROWTH
Δ+ FOLLICLE CELL ACTIVITY
SPERMATOZEUGMATA
DUCT & LEYDIG CELLS ACTIVE
GAMETE
ADULT

FIG. 1.

2. What is the site and mechanism of action of the *P* gene ? It is important to know if the gene turns on a single event which then serves to trigger successive processes in a « chain-reaction » type of phenomenon or if it affects several organs or processes simultaneously. Does it dictate when other genes become activated ?

3. What is the role of the hypothalamus in determining the onset of sexual maturation ? Although it is clear that the hypothalamus produces releasing and inhibiting factors, its role in regulating the rate of synthesis of pituitary hormones is somewhat less lucid (Vale *et al.*, 1977). Does the hypothalamus develop at approximately the same time as the pituitary or does it contain releasing factors long before the gonadotrops differentiate. Perhaps a hypophysiotropic substance is produced that *induces* maturation of the gonadotropic zone (a gonadotrop-maturing factor ?). This problem may be analyzed by transplanting pituitary glands and hypothalami from genetically early maturing fish to genetically late fish and vice versa. The availability of isogenic stocks (Kallman, 1975) makes this feasible.

4. What is the mechanism by which the size of the gonadotropic zone increases ? Recent examination of a homozygous late-maturing (P^5) female suggests that proliferation of the chromophobes occurs prior to their activation. This requires additional study.

5. The structural changes that take place in the anal fin at approximately five weeks of age in all males signals the beginning of gonopodial stage one. Since this occurs in the absence of a gonadotropic zone it suggests that low levels of androgens may already be present months before the gonadotrops develop. Since we could not demonstrate active Leydig cells with our methods the question arises as to the origin of the steroids. It also suggests that androgens do not initiate the maturation of the HPG axis.

6. What is the explanation for the protracted development of various segments of the HPG axis and of the maturation process in general ? Is it simply a case of older tissues having lost some of their responsiveness (loss of hormone receptors ?) or do the various *P* genes produce their effects at different rates ?

This polymorphism which affects maturation of the HPG axis is a natural component of wild populations and laboratory stocks derived from them. This system is developing as an important model for investigating the genetic control of endocrine gland structure and function and for the study of basic endocrine mechanisms.

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Résumé. Un gène lié au sexe qui contrôle l'âge auquel la zone gonadotrope de l'hypophyse se développe et devient physiologiquement active a été identifié chez le Xiphophore. Les allèles pour la différenciation précoce (P^e) et tardive (P^l) sont liés à un gène de pigmentation qui sert de marqueur génétique. L'âge moyen à la maturation sexuelle est 12,5 semaines (extrêmes 10-15) pour les mâles $P^e\,P^e$, 20 semaines (16-29) pour les mâles $P^e\,P^l$ et 26,5 semaines (18-40) pour les mâles $P^l\,P^l$. Les femelles $P^e\,P^e$ maturent entre 10 et 14 semaines d'âge et celles $P^e\,P^l$ entre 16 et 20 semaines. Aucun chevauchement dans l'époque de maturation n'a été trouvé lorsque les poissons des différents génotypes ont été

élevés dans des conditions identiques. La présence ou l'absence de zone gonadotrope est bien corrélée avec la différenciation des gonades. Cependant même en l'absence de zone gonadotrope, les ovocytes peuvent se développer jusqu'au stade de globule lipidique et les testicules jusqu'au stade spermatogonie. Chez les mâles, les androgènes du testicule en développement à leur tour contrôlent la métamorphose de la nageoire anale en gonopode. Le taux de croissance des mâles et femelles immatures est le même, mais diminue fortement chez les mâles (mais pas chez les femelles), lors de la maturité sexuelle. Donc les mâles précoces sont significativement plus petits que ceux à maturité tardive. Le génotype femelle ne montre pas de différence de taille et finalement devient plus gros que tous les mâles.

Ce polymorphisme affectant la différenciation gonadotrope est un composant naturel des populations sauvages et des populations de laboratoire qui en dérivent.

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