bound radioactive GABA. In the presence of nonradioactive GABA, release is not abolished but is slightly reduced, perhaps because of dilution of internal GABA pools or blockage of its own release. Another possible artifact not commonly considered is that the increase in GABA collected on stimulation might arise from blockage of reuptake processes. To study this possibility Ca-dependent release was measured under conditions that inhibit reuptake. Removal of Na ions from the medium and replacement with choline is known to block GABA uptake (*13*). In sodium-free media, Ca-dependent release was little altered. Thus, artifacts from either surface exchange or reuptake blockage do not account for Ca-dependent release.

In summary, we have described a system that rapidly releases GABA from synaptosomes when stimulated by Ca and that meets a number of criteria of stimulus-secretion coupling processes not shown in previous similar studies (*1, 2*). In addition we have shown that (i) the release phenomenon is specific to synaptosomes and not due to several possible artifacts and (ii) exogeneous GABA accumulated by synaptosomes can be released, which means that synaptosomes in vitro possess the capability to package, store, and release neurotransmitters. In this regard, it is particularly interesting that subcellular fractionation has failed to reveal GABA in synaptic vesicles (*14*), possibly owing to the nonphysiological conditions required for isolation. Our system should be suitable for further detailed analysis of packaging, storage, and release of GABA. The rapidity of the measurements makes the system ideal to study the effects of various agents on release of transmitters independent of many metabolic effects. An individual measurement requires only a few micrograms of tissue, and synaptosomal beds previously used (*1*) which require a few milligrams of tissue are not necessary. The system is sensitive enough to be used with specific brain regions and possibly even biopsy samples. At present a simple mammalian system to study the properties of GABA secretion is not available for analysis, and our system appears unique in its potential for studying the secretion properties of this major mammalian inhibitory transmitter.

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Genetic Control of Gonadotrop Differentiation in the Platyfish, Xiphophorus maculatus (Poeciliidae)

Abstract. *A sex-linked gene controlling the age at which the gonadotrops of the pituitary gland differentiate has been discovered in* Xiphophorus maculatus. *Males homozygous for early differentiation become sexually mature between 10 and 16 weeks; those homozygous for late maturation between 22 and 40 weeks. Heterozygotes are intermediate. Since growth rate decreases when testes mature, the two classes of males differ significantly in adult size.*

A sex-linked gene controlling the age at which the gonadotropic zone of the adenohypophysis differentiates has been identified in the teleost *Xiphophorus maculatus* (Guenther) native to southern Mexico and adjacent parts of Central America. Within the "Belize" stock (*1*) males homozygous for the sex-linked factor *Ir* (red iris) reach sexual maturity several weeks prior to males homozygous for the sex-linked factor *Br* (red body). Males homozygous for *Ir* or heterozygous for *Ir* and *Br* (there is no evidence that the two color genes are alleles) were obtained from matings of the type W Y-*Ir*♀ × Y-*Ir* Y-*Br*♂ (*2*). Homozygous *Br* males and heterozygotes originated from the following cross: W Y-*Br*♀ × Y-*Ir* Y-*Br*♂.

Since environmental factors (*3*) can serve as triggering devices for the onset of sexual maturity and since we were interested in measuring the differences in the age of sexual maturation between the two types of males, all fish of a given brood were raised in the same aquarium. Thus each brood served as both control and experimental group. Only pedigree 2964 (born 1/26/72) was separated into two tanks (a and b) when 1 day old because of the large number of offspring (Tables 1 and 2). Intrabrood comparisons are more meaningful than comparisons between groups. Fish were raised in the laboratory equipped with a skylight under standard conditions (*4, 5*).

The anal fin of immature male platyfish is fan-shaped. Under the influence of androgenic hormone from maturing testes, the anal fin transforms into a rodlike gonopodium. This metamorphosis (*6*) requires several weeks and has been divided into six easily recognizable stages. The structure of the anal fin can be used as a precise index of sexual maturity.

Within the Belize stock, homozygous *Ir* males become sexually mature before their *IrBr* sibs and these in turn mature at a younger age than homozygous *Br* males (Table 1). Within the five broods of pedigrees 2828, 2918, and 3030 the difference between the two classes of males is absolute. In the four groups of pedigree 2964 only one *Br Br* male (2964-11, mature at 18 weeks) differentiated as early as any of its *IrBr* sibs. Overlap between broods and pedigrees is primarily related to the number of fish raised per liter of water.

Since the pituitary gland controls testicular maturation and, indirectly, gonopodial differentiation (*7*), we examined both the hypophyses and the testes of *Ir Ir* and *Ir Br* males (pedigree 2828, born 2/27/71). In addition to eight females this sibship consisted of five *Ir Ir* and ten *Ir Br* males. At 9 weeks of age, the anal fins of the homozygous *Ir* males and of one *Ir Br*

male (2828-32) had begun to differentiate. At this time the Ir and five Ir Br males (but not 2828-32) were killed. The pituitary gland of homozygous *Ir* males possessed a typical (*8*), clearly defined, well-formed zone of gonadotrops containing varying amounts of granules that surrounded a large nucleus and a prominent dark-staining nucleolus. The gonadotropic zone of the *Ir Br* males, however, was either nonexistent or was composed of only a few chromophobes containing small dark-staining nuclei. No conspicuous differences in other regions of the pituitary gland were noted.

The testes of *Ir Br* males were undeveloped with small clusters of spermatogonia embedded in connective tissue and surrounding poorly developed ducts. Their anal fins were unmodified. By contrast the testes of *Ir Ir* males were well developed and filled most of the coelom when viewed in cross section. Their gonopodia were partially differentiated. The remaining four *Ir Br* males were killed at 15 weeks of age after their anal fins had begun to differentiate. At this time their gonadotropic zones and testes were as well developed as those of their *Ir Ir* sibs at 9 weeks.

Male 2828-32 was the only *Ir Br* male that became sexually mature as early (9 weeks) as Ir males without Br. We postulated that this male is a crossover between *Br* and a locus controlling gonadotrop differentiation. We have termed this the "*P*" locus at which two alleles have been identified: P^e, for early differentiation, which is linked to *Ir*, and P^l, for late differentiation, to *Br*. This possibility was tested by mating male 2828-32 (Y-*Ir* Y-*Br*) to a W Y-*Ir* female. Although only two small broods were obtained (pedigree 2992), it is evident that the mechanism that led to early maturation of 2828-32 is heritable since there was no difference in age of maturation between its *Ir Ir* ($N = 4$) and *Ir Br* ($N = 5$) progeny. Both groups reached sexual maturity between 9 and 12 weeks. This cross demonstrates that the age of sexual maturity is not controlled by the pigment genes, but by another sex-linked locus.

The same kind of differences between *Ir* and *Br* males was also observed among the progeny (pedigree 2974) of a Y-*Ir* Y-*Br* Belize male and a X-*Sp* X-*Dr* female of the Jamapa stock inbred since 1939 (*5*). Since X-Y-*Ir* males became sexually mature at an age essentially identical with that of *Ir Ir* males and since X- Y-*Br* males are comparable to *Ir Br* males (Table 1), the factor on the X chromosome of Jamapa controlling age of sexual maturity is probably the same as the one associated with Y-*Ir*.

The *P* locus is probably not a structural gene that specifies the amino acid sequence of gonadotropins. Rather its function may be of a regulatory nature by determining when other genes become activated. Of particular significance is the observation that heterozygotes are truly intermediates for the age at which the gonadotrops differentiate. At 9 to 12 weeks, when Ir males homozygous for P^e attain sexual maturity, Br males, P^e P^l, do not possess a functional gonadotropic zone and synthesize no gonadotropins. Based upon physiological and morphological evidence, males homozygous for *Br* (P^lP^l) show no indication of sexual maturation and presumably lack a differentiated gonadotropic zone at a time the gonadotrops of P^eP^l (*BrIr*) heterozygotes are fully functional. Moreover, there is no evidence that the differentiation of gonadotrops begins at the same age in P^eP^e and P^eP^l fish and then proceeds at different rates. As judged by anal fin metamorphosis, heterozygous males differentiate just as fast as homozygous males once the process begins. Whether this also holds true for homozygous P^lP^l (*BrBr*) males, however, has not yet been established.

These observations cannot be taken as evidence that P^e is inactive in heterozygotes at a time when P^eP^e males mature. P^e may produce a certain substance necessary for gonadotrop differentiation, but it is only after this substance has reached a certain critical concentration that morphological and physiological changes in the gonadotropic zone can be recognized. The system studied here could represent a polymorphism at a promoter site; at P^e, messenger RNA synthesis proceeds faster than at P^l. Alternately, P^e and

Table 1. Age of sexual maturity of male platyfish with different genotypes. No asterisk indicates that fish are P^eP^e; asterisk indicates P^eP^l; double asterisk indicates P^lP^l.

Birth date	Total fish: Y Y / *IrIr*	Y Y / *IrBr**	Y Y / *BrBr***	Number of fish reaching sexual maturity at age (in weeks): 10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32 · · · 36 · · · 40
Pedigree 2828																										
2/27/71	1	4			1			3*			1*															
Pedigree 2918																										
6/25/71	2	6				1				1						5**										
9/30/71	5	2				3			2			1*						1*	1*							
Pedigree 3030																										
2/15/72	1	3						1			3*															
3/17/72	8	4		1			7			3*			1*													
Pedigree 2964																										
9/20/71		3	3								2*			1*			1**			2**						
10/20/71		3	5							1*			2*			2**			1**			1**				1**
1/26/72 a		6	6							4*		1**	2*			2**			1**				2**			
1/26/72 b		6	6							2*					2*			2*	2**		1**			1**	1**	1**
X- Y-*Ir* X- Y-*Br**																										
Pedigree 2974																										
11/1/71	13	15				3			10			5*			5*			4*			1*					

Table 2. Size (standard length, millimeters) of adult male platyfish with different genotypes.

Birth date	P^eP^e			P^eP^l			P^lP^l		
	Y-*Ir* Y-*Ir*			Y-*Ir* Y-*Br*			Y-*Br* Y-*Br*		
	Mean	Range	*N*	Mean	Range	*N*	Mean	Range	*N*
				Pedigree 2828					
2/27/71	28		1	32	30–34	4			
				Pedigree 2918					
6/25/71	24.5	23–26	2	31	28–33.5	6			
9/30/71	22.1	19–25	5	27.3	24.5–30	2			
				Pedigree 3030					
2/15/72	25		1	29.3	28.5–31	3			
3/17/72	22.7	21–23.5	8	27.4	27–29	4			
				Pedigree 2964					
9/20/71				30.5	29.5–31	3	36.6	35–38	3
10/20/71				25.5	22.5–27	3	31.8	28–34.5	5
1/26/72 a				25.8	24–28	6	28.3	24.5–32	6
1/26/72 b				24.1	22–26	6	29	27.5–30.5	6
	X-Y-*Ir*			X-Y-*Br*					
				Pedigree 2974					
11/1/71	24.7	23–26.5	13	29.9	27.5–32	15			

P^l may specify two proteins differing in their efficiency in initiating morphogenic changes. The *P* locus probably exerts its effect directly on the pituitary gland, although the role of the hypothalamus should also be evaluated.

The *P* gene also has an indirect effect on the adult size of male platyfish. Since growth rate decreases with increasing androgen production (*9*), early maturing males are significantly smaller than late maturing ones. Size differences between the two classes of males were absolute within seven broods, whereas in one (9/30/71) the largest *IrIr* male surpassed the smallest *IrBr* male by 0.5 mm (Table 2). The exceptional *BrBr* male (2964-11) that matured at 18 weeks (Table 1), is also the smallest homozygous *Br* male obtained so far. Small and large fish grow at the same rate, but the former stop at an earlier age when they become sexually mature. Since these observations did not differ throughout the year, natural daylight, which was not controlled, cannot account for the differences in growth and age of sexual maturity.

The *P* factors have manifested themselves both in the offspring of intra- and interstrain crosses. This polymorphism is a natural component of wild populations and is apparently widespread. We have found it in platyfish stocks collected from four river systems (*10*). The sex chromosome constitution of the males, XY or YY, has nothing to do with adult size and age of sexual maturation. P^e and P^l can be both X- and Y-linked. An X chromosome with P^l has recently been identified from the Belize population (*10*).

Similar variations in size and age of sexual maturation have been reported for a number of other species of *Xiphophorus* (*11*), but little is known about the genetics of these differences. The polymorphism at this locus may have been important for the evolution of the genus, since various body parts show allometric growth and large males, for example, those of *X. pygmaeus*, not only assume a different habitus, but also develop structures not present in the smaller morphs (*12*). The allele for early gonadotrop differentiation has become fixed in *X. pygmaeus pygmaeus* inhabiting the Rio Axtla, and has led to a uniform population of small males. However, *X. pygmaeus nigrensis* in the Rio Choy is polymorphic at the *P* locus resulting in two kinds of males (*13*). Elmination of P^e from the Rio Choy would leave two populations: *X. pygmaeus pygmaeus* homozygous for P^e (small size) and *X. pygmaeus nigrensis* homozygous for P^l (large size), differing completely in breeding structure and appearance.

The *P* locus of *X. maculatus* is of general significance not only for pituitary gland function in teleosts, but for all vertebrates including man. This system may become an important model for studying the genetic control of endocrine structure and function.

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Perfluorocarbons Having a Short Dwell Time in the Liver

Abstract. *Perfluorinated organic liquids are useful as high capacity oxygen and carbon dioxide solvents. After intravenous infusion most of these perfluorinated emulsions are deposited in the liver and spleen in a matter of days, where they remain for the lifetime of the animal. Hence, while they may be useful as isolated organ perfusion media their value as artificial blood is limited. A family of perfluorocarbons has now been discovered, which, although deposited in the liver after circulation in the blood, leave the liver to be excreted via the lungs and skin in a matter of days without apparent harm to the animal.*

Since 1966, when organic liquid breathing was first reported, there have been over 100 publications concerning the use of inert fluorochemicals (*1*) in physiological research. These include reports of liquid breathing (*2*), of organ perfusion (*3*), of infusion in whole animals (*4*), and as radiographic