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Effects of gene dosage and hormones on the expression of Dr in the platyfish, *Xiphophorus maculatus* (Poeciliidae)*

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SUMMARY

Platyfish, *Xiphophorus maculatus*, are polymorphic for the patterns red-dorsal (Dr) and spotted-dorsal (Sd) fins, both controlled by closely linked loci on the *X* chromosome of Jamapa strain, Jp 163A. The intensity of red pigment looks the same in males and females, but spectrophotometric analysis of dorsal fin extracts showed that heterozygous intact males have significantly more red pigment (drosopterin) than homozygous or heterozygous females or castrated males. The mechanism of Dr expression in Jamapa is, thus, similar to the one present in the Belize stock, where a sex difference is readily apparent that is known to be under androgenic control. The Sd phenotype is identical in both sexes. *Sd* and *Dr* are not restricted to the *X* chromosome, and no evidence for gene dosage compensation has been obtained. Within the Jamapa stock the expression of Sd and Dr are best described in terms of dominance and recessiveness. Dr is strongly augmented by a testicular hormone. *Dr* and *Sd* have been separated by crossing-over. In natural populations both genes can occur by themselves, linked to each other or to other pigment genes. The development of the Sd macromelanophores is not contingent upon the presence of pterinophores (Dr) in the dorsal fin or elsewhere in the body.

1. INTRODUCTION

Poeciliid fishes provide two outstanding examples of pigmentary polymorphism. Natural populations of the guppy, *Poecilia reticulata*, and the southern platyfish, *Xiphophorus maculatus*, exhibit more pigment patterns or combinations of patterns than any other vertebrate known. The polymorphism of the guppy is mainly expressed in males while females are largely cryptic (Haskins *et al.* 1961; Winge, 1922, 1927). Restriction of the patterns to males is achieved by hormonal (androgenic) control. Recently, Haskins *et al.* (1970) found that several 'absolutely' *Y*-linked pigment genes were closely linked to a recessive lethal factor, thus assuring male heterozygosity.

The pigmentary polymorphism of the platyfish, which is controlled by incompletely sex-linked genes, is different in one important respect. Females are almost as variable as males. There are two types of pattern: those that are composed of

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macromelanophores and others that range from yellow to red and are caused by pterin and carotenoid pigments, often within the same cell (Öktay, 1964). The black patterns look basically identical in both sexes except in the case of Sp^8 (Kallman, 1970*a*), but the expression of the red pigment genes may range from zero expression in females to a condition in which there is no visible difference between males and females. The two extremes, however, are quite rare and in most cases females are of a lesser hue than males. It is interesting to note that although the intensive study of the two species began at approximately the same time (guppy since 1920; platyfish since 1922), the first report about sex-limited inheritance in *X. maculatus* was not published until recently (Kallman, 1970*a*). Wherever the differences in the expression of the red patterns between males and females (mostly Belize River stock) have been analysed, they were found to be due to androgenic control (R. J. Valenti, unpublished).

The sex-linked red-dorsal pattern (Dr) of *Xiphophorus maculatus* has been analysed in some detail. The Dr pattern of the Belize River population is quite variable in females, but always of lesser intensity than in males. Several cases of non-penetrance of *Dr* in females of this stock are known. The expression of Dr in males (homozygous or heterozygous) is always strong (Kallman, 1970*b*). However, the Dr pattern of the Rio Jamapa (Jp) population as present in stocks Jp 163A and Jp 30 is visibly identical in both sexes; females are homozygous, males heterozygous. The factors responsible for this pattern in the Jamapa and Belize populations are not identical (Kallman, 1970*b*). Of particular interest is that in F_1 hybrids between *X. maculatus* (Jamapa) × *X. couchianus* a sex difference in the expression of *Dr* of Jp has been observed that could be under androgenic control. The red coloration is intense in the male hybrids but subdued in females (Kallman & Schreibman, 1971).

Anders & Klinke (1967) and Anders (1967*a*, *b*) discussed the lack of sex difference in the expression of *Dr* (and of *Sd*, spotted-dorsal, a macromelanophore gene closely linked to it) in the Jamapa strain in terms of gene dosage (*X-DrSd X-DrSd* ♀♀; *X-DrSd Y-* ♂♂) and special genes for gene dosage compensation. The same authors also state that *Dr* and *Sd* form a functional unit with the same mechanism for gene dosage compensation. Since in the Belize stock Dr females are heterozygous and have less pigment than males homozygous or heterozygous for *Dr* (*W* − + *Y-Dr* ♀♀; *Y-Dr Y-* or *Y-Dr Y-Dr* ♂♂) (Kallman, 1970*b*) Anders' hypothesis for gene dosage compensation in *X. maculatus* may not be directly applicable to stocks other than Jamapa.

Because of the obvious differences in the expression of the Dr phenotype in the two populations, we have studied the effects of gene dosage and hormones on this pattern in the Belize and Jamapa stocks. Of particular interest with respect to the evolution of the patterns and of how differences between populations can arise, is the question whether the apparent differences in hormonal control are a property of the various pigment factors themselves (*Dr* of Jamapa and *Dr* of Belize) or are due to genetic background. A number of observations are also described that demonstrate that *Sd* and *Dr* can be separated through crossing-over.

2. MATERIALS AND METHODS

The Jamapa fish available for this investigation (Table 1) belong to one inbred strain, Jp 163A, and to pedigree 2299, which was derived from crossing Jp 163A with a second Jamapa stock, Jp 163B (*X-Sp X-Sp*♀♀, *X-Sp Y-Ar Sr* ♂♂). The detailed ancestry of the Jamapa stocks maintained by brother-to-sister matings since 1939 and of ped. 2299 has been presented elsewhere (Kallman, 1970*b*). The *Y-Sp* chromosome of ped. 2299 can be traced to a crossover in a male of Jp 163B (Kallman, 1970*b*). Females of Jp 163A are homozygous for the *X*-linked factors *Dr* and *Sd* while males and the fish of ped. 2299 are heterozygous:

Jp 163A ♀♀ *X-Dr Sd X-Dr Sd*
♂♂ *X-Dr Sd Y-Ar Sr*
ped. 2299 ♀♀ *X-Dr Sd X-Sp*
♂♂ *X-Dr Sd Y-Sp*

Some fish of ped. 2299 were also homozygous for *Sp*, but these were not used in this experiment, because they did not carry any red colour gene. Males of Jp 163A which are heterozygous for *Dr* and *Ar* were excluded from the experiments as a precautionary measure to preclude the possibility that Ar has a slight effect on dorsal fin pigmentation. The fish of the Belize stock (Table 2) with *Dr* belong to pedigree 2237 and arose from a mating of the type $W-+$ *Y-IyAy* ♀× *Y-Dr* $Y-+$ ♂. The detailed ancestry of ped. 2237 has been presented elsewhere (Kallman, 1970*b*). Pedigrees 1921 and 1970 (Table 3) represent F_1 hybrids between strain Jp 163A and the Belize stock. The reasons for these crosses and all other pertinent information can be found in Kallman (1970*a*). *Sp* and *Sr* are different spotting factors which, like *Sd*, belong to the complex macromelanophore locus. *Ar* (anal red) may be allelic to *Dr*. Mr (lower jaw and jugular region red), Ay (yellow spot above anal fin) and Iy (yellow iris) are patterns controlled by sex-linked genes, neither allelic to each other nor to *Dr*.

Since water conditions, light intensity, sex, age and social interactions could affect the intensity of the red pigmentation, Jamapa fish homozygous (the third brood of the 39th generation of Jp 163A, 24 fry, born 12/2/68) and heterozygous (the third brood of ped. 2299, 34 fry, born 11/29/68) for *Dr* were raised under identical conditions.

Following established laboratory procedure, fish are born to pair matings in 16 l aquaria. To avoid overcrowding the fish were placed at the age of 1 week in two 36 l tanks (A and B), each tank receiving one half of each brood (A: 12 Jp 163A, 19 ped. 2299; B: 12 Jp 163A, 18 ped. 2299). At 7 weeks the fish were scored for sex and patterns; the Jp 163A males and those fish of ped. 2299 that were homozygous for *Sp* and lacked *Dr* were removed. This left in tank A 5 Jp 163A ♀♀ and 6 ♀♀ and 4 ♂♂ of ped. 2299, and in tank B 6 Jp 163A ♀♀ and 6 ♀♀ and 4 ♂♂ of ped. 2299. The developing gonopodia of the males in both tanks were amputated to prevent fertilization of the females. It was felt that pregnancy could influence the intensity of the pattern.

The first two broods of ped. 2299, consisting of 42 fry born 9/30/68 and 39 fry born 10/30/68 were used for castration experiments. The fish of each brood were evenly distributed among two 3 l aquaria and raised under standard laboratory conditions (Gordon, 1950; Kallman, 1965) until sex and patterns could be differentiated. All females and homozygous *Sp* males were then removed and the heterozygous *Dr* males, 11 from the 9/30/68 brood and 10 from the 10/30/68 brood, were then placed in two 16 l aquaria according to birth date. These males were castrated at a stage when the anal fin began to elongate, approximately at two months of age. Seven fish died within a week after the operation, six eventually regenerated a testis (sham-operated), leaving eight castrated males.

Belize fish (ped. 2237; Kallman, 1970*b*) were treated similarly, but since in this stock differences in Dr expression between males and females are always apparent to the unaided eye, only heterozygous fish were examined. The Dr fish of the third brood of ped. 2237 were set aside to determine the amount of pigment in males ($N = 8$) and females ($N = 7$), the sexes being raised in separate aquaria. Dr males of the second and fourth brood were used as sham-operated ($N = 8$) and castrated ($N = 5$) animals. The two broods were kept in separate aquaria, but sham and castrated fish of the same age were maintained together.

Immature males were castrated according to the method of Grobstein (1947). After the operation the fish are placed in conditioned aquarium water to which commercial sea salts have been added to make a 0·9% salt solution. Wound healing occurs within 2–3 weeks, at which time the fish are returned to freshwater aquaria. Castrated males retain an undifferentiated anal fin for life while sham-operated males (controls) – those which regenerate a testis from a small fragment left behind – develop a normal gonopodium within 2–4 months of the operation.

At 1 year of age the dorsal fins of *Dr* fish were carefully removed with a scalpel and placed in individual beakers containing 4 ml of a 1% ammonium hydroxide solution to extract the red pigment (Goodrich, Hill & Arrick, 1941). Fins were so treated for 3 hrs in darkness to keep photo-oxidation to a minimum.

Previous investigation of the red patterns of *X. maculatus* had identified the red pigment as a drosopterin compound (Valenti, unpublished). Using a non-fluorescent cellulose base, thin-layer chromatography paper and a solvent system of *n*-propanol–1% ammonium hydroxide (2:1, v/v), the red pigment, which appeared as a visible orange spot and fluoresced orange at the base of the chromatogram, was found to be similar to drosopterin from the eyes of *Drosophila melanogaster* (Matsumoto, 1965). A quantitative method was developed to yield a comparative measure of the red pigment, using visible spectrophotometry. On a visible absorption spectrum drosopterin was found to peak at 500 nm. Absorbance readings were taken for each 4 ml of extract using a Beckman DU spectrophotometer. Serial dilutions of two extracts showed absorbance readings and quantity of red pigment to be in a linear relationship. It was not possible to calibrate the readings with a known quantity of drosopterin due to its unavailability. The figures listed in Table 1 represent the average of three absorbance readings taken in succession.

3. RESULTS

Jamapa stock: At 1 year of age the red pigment in the dorsal fin of all homozygous and heterozygous females and heterozygous males appeared to be of the same intensity to the unaided eye. Castrated males had visibly less pigment than sham-operated males or intact males and females. Results of spectrophotometric absorbance readings of the red pigment extracts (Table 1) agreed with overall visual observations of the extracts just prior to obtaining absorbance values. The results obtained from tanks A and B were essentially the same (Table 1) and therefore the data have been combined for statistical analysis (Table 2).

Table 1. *Mean absorbance values of red pigment extracted from the dorsal fin of* Xiphophorus maculatus

	Tank A			Tank B		
	N	Mean	S.E.	*N*	Mean	S.E.
♀♀ Jp 163A *Dr Dr*	5	0·061	0·001	6	0·063	0·008
♀♀ ped. 2299 *Dr* +	6	0·051	0·003	6	0·067	0·007
♂♂ ped. 2299 *Dr* +	4	0·092	0·008	4	0·130	0·002

Table 2. *Mean absorbance values* (M) *and statistical analysis* (*analysis of variance and Duncan's multiple range test**) *of red pigment extracted from the dorsal fin of* Xiphophorus maculatus, *homozygous or heterozygous for* Dr (*Dorsal-red*)

A. Jamapa stock. Analysis of variance†

Source	D.F.	*MS*	*F*
Treatment	4	0·0082	16
Error	40	0·0005	

♀♀		♂♂ (heterozygous)		
Heterozygous	Homozygous	Castrated	Intact	Sham
Pedigree 2299 *X-DrSd X-Sp*	Strain Jp 163A *X-DrSd X-DrSd*	Pedigree 2299 *X-DrSd Y-Sp*	Pedigree 2299 *X-DrSd Y-Sp*	Pedigree 2299 *X-DrSd Y-Sp*
M ... 0·059	0·062	0·044	0·111	0·116

B. Belize stock (pedigree 2237). Analysis of variance†

Source	D.F.	*MS*	*F*
Treatment	3	0·004069	482
Error	24	0·000008	

	♂♂		
♀♀	Castrated	Intact	Sham
W-+ Y-Dr	*Y-IyAy Y-Dr*	*Y-IyAy Y-Dr*	*Y-IyAy Y-Dr*
M ... 0·0364	0·0244	0·0730	0·0728

* Steel & Torrie (1960).

† Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

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No statistically significant difference was found between homozygous and heterozygous females. Both kinds of female had significantly less pigment than heterozygous males. Females had also slightly more pigment than castrated males, but this difference was significant only in the case of the heterozygous females. Sham-operated and intact males had more than twice as much pigment as castrates.

Table 3. *Documentation of a crossover between* Dr (*dorsal-red*) *and* Sd (*spotted-dorsal*) *in the Platyfish,* Xiphophorus maculatus

Pedigree and genotypes of parents			
	♀♀		♂♂
1921-1*	*X-AySr X-DrSd*	1970-12*	*X-DrSd Y-Mr*
Jp 163B	*X-Sp X-Sp*	2010-11*	*X-AySr Y-Sd*
		2120-16*	*X-Sp Y-Sd*
		2120-13*	*X-Sp Y-Sd*
		2120-12*	*X-Sp Y-Sd*
		2120-11*	*X-Sp Y-Sd*
		2120-32*	*X-Sp Y-Sd*
		2120-33*	*X-Sp Y-Sd*
		2120-31*	*X-Sp Y-Sd*
		2308-11*	*X-Sp Y-Sd*

Pedigree and phenotypes of offspring			
Ped.	♀♀		♂♂
2010	12 Dr, 15 DrAySr,	1 + †	14 DrMr, 10 AySrMr 1 *AySr*
2120	14 SpAySr		12 Sp, 4 SpSd
2306	9 Sp		8 Sp, 1 SpSd
2307	8 Sp		12 Sp, 2 SpSd
2308	14 Sp		6 Sp, 2 SpSd
2310	14 Sp		7 Sp, 3 SpSd
2311	11 Sp		6 Sp, 3 SpSd
2313	8 Sp		8 Sp, 2 SpSd
2328	9 Sp		2 Sp, 5 SpSd
2776	2 Sp		—, 6 SpSd

* Sd not expressed in these fish, scored at 1 year of age.
† Presumably non-expression of *Dr* which was often weak in the other females.

A series of crosses are described in Table 3 that show clearly that *Dr* and *Sd* can be separated through crossing-over. Both progenitors of ped. 2010 were F_1 hybrids between Belize and Jamapa, the *X-DrSd* chromosome of each parent having been derived from Jp 163A (for details see Kallman, 1970*a*). The exceptional Ay Sr male of ped. 2010 that lacked Mr (italicized in Table 3), resulted from a crossover between *Dr* and *Mr* (Fig. 1). As subsequent breeding tests showed (ped. 2120–2776, Table 3), this male (2010-11) had the genotype *X-AySr Y-Sd*. However, *Sd* in this male as in all Dr fish of ped. 2010 was not expressed. Non-penetrance of *Sd* of Jp is the rule in such hybrids, as discussed in detail by Kallman (1970*a*, *b*). A penetrance

of 25 % for *Sd* of Jp in ped. 2120 is in good agreement with an earlier rough estimate of 28 % for the first backcross generation to Jamapa (Kallman, 1970*b*). It must be noted, however, that pedigree 2010 represents an F_2 generation, and therefore it is not known whether ped. 2120 corresponds more to a F_1 or backcross generation. Because of the critical importance of establishing that *Sd* is *Y*-linked and not associated with any red colour factor, the *Y-Sd* chromosome was carried through two more backcrosses to Jamapa. The penetrance of *Sd* of Jp has returned to 100 % in ped. 2776, and the expression of Sd on a clear, non-red background is just like that in strain Jp 163A.

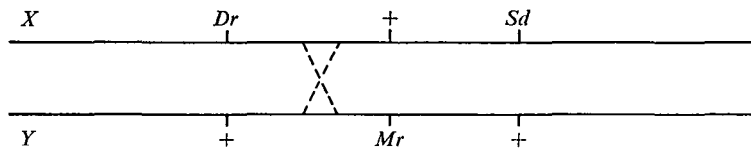

Fig. 1. Sex chromosomes of *X. maculatus* with arrangement of some of the pigment loci. The sex locus or sex differential segment is assumed to be at extreme left. Crossover occurred between *Dr* and *Mr*.

Belize stock: Results of spectrophotometric absorbance readings and visual observations with the unaided eye are in good agreement. Statistically significant differences exist between sham-operated and intact males on one hand and females and castrated males on the other (Table 2). Intact heterozygous males have about twice as much pigment in the dorsal fin as heterozygous females. The differences between castrated males and females is significant, but may result from the small sample size for castrated males.

4. DISCUSSION

No fundamental difference exists in the hormonal control of the red dorsal pattern of *X. maculatus* of the Belize and Jamapa populations. The amount of pigment is higher in males than in females and experiments with castrated fish suggest that a testicular hormone augments strongly the expression of *Dr* in both stocks. The essential difference between the Jamapa and Belize populations is that in the former the amount of dorsal fin pigment in females is sufficiently high so that no difference between the sexes or individuals can be detected by the unaided eye. The hormonal control of Dr is apparently an intrinsic property of the pigment genes rather than attributable to genetic background, for when the *Dr* factors are introduced into foreign genotypes, hormonal control is maintained. *Dr* males have visibly more pigment than females in the F_1 offspring of Jamapa × Belize, in which the expression of *Dr* of Jp becomes reduced (Kallman, 1970*b*). A striking sex difference involving *Dr* of Jp is also observed in the F_1 hybrids of *X. maculatus* (Jamapa) × *X. c. couchianus*, where the pattern extends over much of the body in both sexes but is of a much brighter coloration in males (Kallman & Schreibman, 1971). Similar observations have been made on the Dr and Ar patterns of Bp after introduction into a Jamapa gene pool (Kallman, 1970*b*).

Thus no separate evolution towards genetic systems for androgenic control of the Dr patterns need be postulated for the different populations. The amount of pigment produced is a function of the primary pigment genes and the population specific modifier systems. It must be recalled here that the identical Dr pattern of the Jamapa and Belize populations of *X. maculatus* have a different genetic basis (Kallman, 1970*b*). They are caused by different *Dr* factors, presumably alleles, interacting with population-specific modifier systems. The phenotypes produced by the two *Dr* factors are not the same in the F_1 of Jamapa × Belize and also differ from those of either parental stock. *Dr* of Jamapa after introgression into Belize becomes suppressed, while the expression of *Dr* of Bp in Jamapa is greatly increased and the pigmentation extends over much of the body (Kallman, 1970*b*).

The testicular hormone affects the Dr pattern primarily by influencing the amount of pigment within the pterinophores rather than by controlling their numbers and distribution. This can be best demonstrated in Jamapa × Belize hybrids with *Dr* of Bp, where Dr extends over much of the body in both sexes, but the cells are only faintly pigmented in females.

The sex difference in the intensity of the pigment patterns of *X. maculatus* and *P. reticulata* is not unique for poeciliid fish. On the contrary, it is a general phenomenon that has received scant attention. In the majority of poeciliids (sex-linked pigmentary polymorphisms are known from less than ten species) males have a brighter background coloration than females. Presumably selection is for cryptic coloration in females and – under certain conditions – for bright coloration in males, but direct evidence is lacking. Baird (1968) reported varying degrees of coloration in males of *P. latipinna* and thought this likely to be important for the position in the hierarchy. Earlier, McAllister (1958) found that in *Gambusia hurtadoi* the dominant males exhibited the brightest colours. In none of these cases is anything known about possible hormonal or nervous control of the increased pigmentation. Haskins *et al.* (1961) suggested that selection in the guppy may be for those colour patterns that develop early in male ontogeny, and which might be those that attain a high intensity of colour later on. *X. maculatus* may just be beginning to evolve pigment patterns that are under androgenic control. Another mechanism, the *W–X–Y* sex-chromosome system, exists in certain platyfish populations that ensures the presence of a larger number of pigment genes in males (Kallman, 1970*a*, and unpublished).

Anders (1967*a*, *b*) and Anders & Klinke (1967) discussed the expressivity of *Dr* and *Sd* in the Jamapa stock of *X. maculatus* in terms of gene dosage and gene-dosage compensation. Repressor genes which decrease the expressivity of pigment factors in the homozygous and heterozygous conditions to the same level are made responsible for the lack of difference between the sexes.

Anders & Klinke based their theory upon the well-known fact (Anders, Anders & Klinke, 1963; Atz, 1962; Gordon, 1948; Kosswig, 1929; Zander, 1969) that in hybrids between *X. maculatus* and *X. helleri* the manifestation of *Dr* and *Sd* is greatly increased and the pigmentation extends over much of the body (loss of

repressor genes). They reported that hybrids homozygous for *Dr* and *Sd* were more heavily pigmented and developed tumours sooner than heterozygous ones.

The idea of gene-dosage compensation in the pure species resulting in a normal pattern, and the absence of dosage compensation in species hybrids resulting in atypical pigment cell growth and melanomas, is a very important one. But we find for example, that, contrary to Anders, heterozygous fish (males) of the Jamapa stock have not the same amount of red pigment but significantly more than homozygous and heterozygous ones (females). This difference is not observed by mere visual observations; it can only be detected by the extraction and absorption techniques. The quantity of pigment in the Jamapa stock is unrelated to gene dosage or gene-dosage compensation, but instead is a function of gonadal control. Heterozygous males that were castrated have significantly less pigment than intact heterozygous males and slightly lesser amounts than homozygous and heterozygous females. These observations suggest that a testicular hormone, presumably an androgen, strongly augments the expression of the red-dorsal (pterin) pigmentation.

It seems to us that the term 'gene-dosage compensation' should not be used in this context; this term has been applied to those cases where a gene is present on the *X* chromosome but where no equivalent locus is to be found on the *Y*. This is clearly not the situation with *Sd* and *Dr* of platyfish, which can be found on both *X* and *Y* chromosomes (Gordon, 1947; Kallman, 1965, 1970*a*, *b*; Kallman & Schreibman, 1971; Öktay, 1964). As already pointed out by Cock (1964), no need exists for dosage compensation for partially sex-linked genes in fishes (and other organisms) when an allele is present on the *Y* chromosome. Within the Jamapa population the expression of *Dr* and *Sd* is best described in terms of dominance and recessiveness. To be sure, there are modifier genes that have a powerful effect on the final patterns, since in a foreign genotype the expression of *Dr* and *Sd* varies greatly. Some of these modifiers may be comparable to the suppressor genes of Anders.

The combination of *Dr* and *Sd* on the *X* chromosome of *X. maculatus* (Jamapa stock) is regarded as a functional unit by Anders (1967*a*, *b*) and Anders & Klinke (1967). In support of their view they cite among other evidence the apparent absence of crossing-over between the two genes and the fact that macromelanophores always differentiate in areas of the dorsal fin occupied by erythrophores controlled by *Dr*. Anders' observations, however, are based upon a single closed laboratory stock of *X. maculatus*. The lack of published information concerning the frequency and occurrence of pterinophore genes in natural populations of *X. maculatus* and their linkage relationships with macromelanophore factors makes it difficult to determine whether Anders' theory is of general validity. Recently Kallman (1970*a*) has provided part of this missing information for the Belize population, where *Sd* and *Dr* may occur by themselves or linked to other pterinophore or macromelanophore genes. Although the particular combination *DrSd* was not present in the 1966 sample, it was subsequently found in a collection taken in 1969 (Kallman, unpublished). Similarly, unpublished information for the Jamapa popu-

lation (the records of the 1939 collection and a recent one from 1971) indicates that the situation in the Rio Jamapa is not much different from Belize. Macromelanophore spotting controlled by *Sd* can develop in the absence of pterinophores in the dorsal fin or elsewhere on the body and there is no evidence that the phenotypic expression of *Sd* is contingent upon the presence of a closely linked pterinophore gene. Crossing-over between the pterinophore and macromelanophore loci as well as between these and the sex-differential segment have been reported by Fraser & Gordon (1929), Gordon (1937), Kallman (1970*a*) and Kallman & Schreibman (1971). Most pertinent is the crossover between *Dr* and *Sd* on the *X* chromosome of Jamapa as a result of which *Sd* became *Y*-linked and not associated with any red factor (Table 3). The expression of *Sd* did not change. A similar crossover was reported by Öktay (1964), but no details were provided. The position of *Sd* is also not immediately adjacent to *Dr*; at least one gene, *Mr*, is located in between (Fig. 1).

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