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# Courtship and Female Choice Behavior in the Male-Polymorphic High-Backed Pygmy Swordtail, *Xiphophorus multilineatus*

(Poeciliidae) \*)

by

Johannes H. SCHRÖDER & I.S. OFTEN \*\*)

# Balz und Weibchenverhalten beim männchenpolymorphen Hochrückigen Zwergschwertträger, Xiphophorus multilineatus

(Poeciliidae)

Synopsis: Five male size and color morphs exist in *Xiphophorus multilmeatus* nuales: Small blue (SB), small yellow (SY), intermediate 1 (H), intermediate 2 (I2) and large (L) males. These differences are inherited paternally through genetic variation at a Y-linked locus. Despite the fact that all females are genetically identical, because no such variation exists on the X-chromosome, the sisters of these five morphs were designated here according to their brothers as SB, SY, H, I2 and L, respectively. Ten females of each of these five groups had to choose between 2 male morphs presented at the same time. An aquarium was subdivided by panes of glass into three equal compartments. The test female was located in the center. The frequency and duration the female spent at a pane separating her compartment from those of the two competing males was recorded both with no male touching the pane at the same time and doing so. To cancel the effect of passible side-preferences of the female, the position of the two males was switched after 15 min of observation. All 10 possible combinations involving two different male morphs were tested. There was a complex network of non-linear rank order in female preferences. While only L und H females clearly preferred L males over all other four morphs, 12, SB and SY females preferred either H od 12 males over all other types. However, while I2 males were slightly prefered over L males by H females, L males were preferred by the same females over H males (except H) preferred SB over SY males. Taking all significant comparisons together, a rank order of preferences through female choice has been established as follows:  $12 \ge L > 11 > SB = SY$ .

Concomitantly with these female-choise experiments, male courtship and female response behavior was recorded quantitatively in 1 male/1 female matings during 1 h of observation. Neither corralling nor nipping behavior and copulation attemps were observed for L-males. While II- and I2-males exhibited high courtship activity with both forward and backward corralling and low frequencies of nipping. SB- and SY-males behaved as sneakers following and chasing the females with low frequency and duration of corralling but relatively high activity in nipping and thrusting the gonopodium toward the female. Sequencing all behavioral transitions, communication between males and females by exchange of signals was analysed. All niale morphs provoked a distinct female response behavior.

Under competition in large mating group (5 male morphs, 11 virgin females), the L, 11 and 12 males were the mostcourting and aggressive males, while no attacks were delivered by the small SB and SY males.

#### 1. Introduction:

In earlier investigations, several instances of male size polymorphism have been described in poeciliid fishes. In two species of pygmy swordtails, *Xiphophorus nigrensis* and *X. multilineatus*, large and small males ex-

<sup>\*)</sup> Dedicated to Prof. Dr. h.c. Klaus Dieter Kallman, New York, on the occasion of his 70th birthday.

<sup>\*\*)</sup> Author's adresses: Prof. Dr. J.H. Schröder and Mdme, I.S. Otten, Institut für Säugetiergenetik, GSF-Forschungszentrum für Umwelt und Gesundheit, D-85756 Neuherberg bei München, BRD.

hibit different mating stategies (KALLMAN 1984, 1989; RYAN 1988; ZIMMERER & KALLMAN 1988, 1989). Differences in adult male size and age at sexual maturity of X. multilineutus are controlled by genetic variation at a Ylinked locus which determines four genetic size-classes (table 1). Mating behavior of the males of the three largest size-classes consist exclusively of an elaborate courtship display, whereas that of the genetically small males ranges from display to a sneak-chase behavior. Females prefer the display of large males. In mating-competition experiments (two females with one large male and one small male), the large males are dominant and generally deny the small males access to females. From 20 such experiments, 601 large-male and 200 smallmale progeny were obtained, indicating that the switch to sneak-chase behavior by small males is not particularly effective in overcoming the large-male advantage. By using the largest males of the genetically smallest size class and the smallest males of the genetically next-larger size-class, size was kept constant, whereas genotype was varied. When these males were tested in competition with genetically large males, only the males of the genetically smallest size class showed sneak-chase behavior. These observations suggest that the difference in mating behavior is not an indirect developmental effect of size but, rather, is under genetic control (ZIMMERER & KALLMAN 1989). In the present study, we completed the previous studies of ZIMMERER & KALLMAN (1989) and thus provided more detailed information on the sexual behavior patterns of both males and females of X. multilineatus.

Table 1: Average adult size, standard length of the four genotypes of male X. multilineatus (according to ZIMMERER & KALL-MAN 1989). Y-linked color patterns at the yellow (flavus) locus (nomenclature adapted from C.D. ZANDER 1968) are as follows: con = flavus concolor (solid yellow); cm = flavus caudimarginatus (vental and dorsal margins of caudal fin yellow); cp = flavus caudipinna (caudal fin yellow); + = absence of yellow (solid blue). Tow lines each of 12 and L males were maintained with cp and cm patterns, respectively. In the experiments, all L males were cm, and all 12 males were cp.

Phenotypic size-class	Genotype	Color pattern	N	Standard lengt (mm)		
(line)		Color patient		Mean ± SD	Range	
Small (SB + SY)	X - s / Y - s	con, +	302	25.3 ± 1.20	22 - 28	
Intermediate-1 (I1)	X - s / Y - I	cm	135	$27.4 \pm 0.12$	25 - 32	
Intermediate-2 (12)	X - s / Y - II	cp	216	$32.4 \pm 2.06$	29 - 38	
Large (L)	X - s / Y - L	cm	154	$37.5 \pm 2.05$	32 - 42	

# 2. Materials and methods:

## 2.1. Maintenance and origin of the fish:

The high-backed pygmy swordtait, *Xiphophorus multilineatus* from the RÍo Pánuco basin, San Luis Potosi, Mexico, has a long history of systematic revisions (fig. 1). This species possesses five male morphs, designated as small blue (SB, fig. 2), small yellow (SY, fig. 3), intermediate 1 (II, fig. 4), intermediate 2 (I2, fig. 5) and large males (L, fig. 6; KALLMAN 1989). According to genetic experiments (ZIMMERER & KALLMAN 1989), all five male size and color morphs are inherited strictly patroclinally, i.e. via the Y-chromosome (table 1). This male polymorphism constitutes a chromosomal polymorphism of the Y-chromosome.

The fish were kept in aquaria of 25, 50, 100, or 200 liter volume at a temperature varying between 25° - 28° C. They were fed on TetraMin<sup>®</sup>, nauplia of *Artemia salina*, and *Daphnia* spec. Illumination by neon lights was set to a 12-h cycle (7.00 - 19.00 hs), but daylight was not excluded. All tanks were filled with Munich tap water (pH 7.5) and were artifically aerated. Only observation tanks contained gravel on the bottom.

Each morning all basins containing mated pairs or gravid females were checked for newborn fry or dead fish. Because the broods were sometimes born over a period of several hours and because some females might have been cannibalistic, all fry were immediately removed to small glass vessels and later transferred to rearing



Fig. 1: Systematics of Xiphophorus multilineatus.

aquaria. Aquatic plants, *Fontinalis* spec. and *Cryptocoryne* spec., were grown in aquaria in which broods were anticipated to enable newborn fish to hide in the vegetation.

In a specific stage of their ovarian cycle (SICILIANO 1972), virgin females are receptive to male sexual courtship behavior. To obtain virgin females, immature females were separated from their male sibs at the time when the males' anal fin began to transform into a gonopodium, the male copulatory organ. Although no size polymorphism is known for females (fig. 7), they were designated in this study according to the morphs of their brothers as SB, SY, I1, I2 and L.

All fish used in these experiments were obtained from Prof. Dr. Dr. h.c. Klaus D. Kallman, New York Aquarium and American Museum of Natural History, who collected their ancestors in RÍo Coy, RÍo Pánuco basin, Mexico.

#### 2.2. Description of sexual behavior activities:

After recognizing a female, the male approaches (A) and begins to nip (N) ("nipping": SCHLOSSBERG et al. 1949 and CLARCK et al. 1954; "Maultupfen": WICKLER 1957; "biting": BAERENDS et al. 1955) her genital pore by repeatedly touching it with his snout (table 2). The male then tries to "corner" the fleeing female by presenting himself in front of her and/or slightly to her side with all his fins rigidly spread, sigmoid curving of the body and bending his swordlike appendage toward the female. Should the female swim forward and/or try to escape, the male will respond with rapidly alternating backwards and forwards movements. It seems as if the male attempts to restrict the female's forward movement. Following a suggestion of FAVOR (pers. comm.), HEINRICH & SCHRODER (1986) have introduced the term corralling to describe this behavior. Sometimes during corralling the male may switch his position from one side of the female to the other (alternating, Al). Corralling is wide-spread among the species of *Xiphophorus*, but the intensity and velocity of the forward (FC) and backward (BC)

Activity Male morphs							
ACUVI	.ty	$L(n=5)^{-1}i$	$+11 (n = 9)^{(2)}$	$12(n=9)^{31}$	$SB(n = 14)^{(4)}$	$SY(n=4)^{-5}$	
Aď	F	60.28 <sup>a</sup>	37.19	56.71 <sup>b</sup>	49.88	57,49 <sup>a, b</sup>	
	D	70.41	54.12	73.51°	58.02	75.88 <sup>c</sup>	
Nơ	F	0.63 <sup>d</sup>	0.16 <sup>d</sup>	1.05	5.71	2.47	
	D	0.17 <sup>e, f</sup>	0.04 <sup>e</sup>	0.28 <sup>f</sup>	2.88	0.76	
FCơ	F	0.07	6.64	1.92	0.55	0.10	
	D	0.02	9.66	1.78	0.30	0.06	
BCơ	F	0.09	1.51	0.78	0.51	0.10	
	D	0.02	1.03	0.47	0.24	0.02	
Po	F	0.61	10.05	9.19	5.69	2.73	
	D	0.11	2.52	3.24	2.29	0.56	
Alď	F	0.14	0.15	0.02	n.o.	0.03	
	D	0.06	0.03	0.01	n.o.	0.01	
BSLơ	F	n.o.	n.o.	n.o.	0.97	n.o.	
	D	n.o.	n.o.	n.o.	1.44	n.o.	
10,	F	1.44	0.67	1.07	1.96	3.37	
	D	0.21	0.11	0.23	0.48	0.62	
Τơ	F	1.33	0.26	0.44	1.03	2.41	
	D	0.24	0.07	0.11	0.29	0.92	
Gơ	F	0.34	0.15	0.37	0.41	0.45	
	D	0.17	0.04	0.17	0.22	0.15	
Hơ	F	n.ə.	8.32	n.o.	n.o.	<b>n.</b> 0.	
	D	n.o.	3.61	n.o.	n.o.	` n.o.	
1HQ,	F	n.o.	n.o.	3.29	n.o.	n.o.	
	D	n.o.	n.o.	1.04	<b>n.</b> ə.	n.o.	
Zơ	F	n.o.	n.o.	n.o.	0.80	n.o.	
	D	n.o.	n.o.	n.o.	1.08	n.o.	
Аð	F	26.89	27.93	18.04	27.64	26.76	
	D	25.98	26.15	16.75	30.50	20.00	
₽Q	F	6.94	4.01	6.31	3.37	1.51	
	D	1.93	1.27	2.04	1.62	0.26	
JQ	F	0.77	1.71	0.44	0.95	2.25	
	D	0.12	0.51	0.07	0.25	0.44	
0Q	F	0.09	0.76	0.27	0.04	0.03	
	D	0.03	0.30	0.18	0.02	0.03	
Ag♀	F	n.o.	n.o.	<b>n</b> .o.	n.o.	n.v.	
	D	n.o.	n.o.	n.o.	n.o.	n.o.	
Eo'/♀	F	n.o.	n.o.	<b>n.</b> 0.	0.45	n.o.	
	D	n.o.	n.o.	n.o.	0.32	n.o.	
PS♂/♀	F	0.41	0.22	0.07	0.03	0.32	
	D	0.53	0.14	0.10	0.05	0.29	

Table 2: Percent frequency (F) and duration (D) of 20 different behavioral activities spent relative to the total activity of the n couples in 1 male/1 female matings.

n.o. not observed

1) 4441 events lasting 3419.9 sec

- 8913 events lasting 6925.6 sec;
- 3) 16420 events lasting 6103.7 sec;
- 4) [494] events lasting 7562.3 sec;
- 5) 3117 events lasting 2356.6 sec

 a - f: non-significant; all other differences between the 10 possible intermorphic combinations are significant (p < 0.01) as computed by 2x2-contingency table and adjusted by the Bonferroni-Holm procedure (BHP) for simultaneous multiple comparisons.

phases of the behavior differ significantly among species. The earliest references to this behavior can be found in ARNOLD (1909), SCHOLZ (1909) and LANGER (1913). Corralling was previously described in greater details as "Wiegen" (LATTERMANN 1957; FRANCK 1964, 1968): "arcing" (CLARK et al. 1954). "Rückwärtsschwimmen" (WICKLER 1957) and "Forward and Backward Swimming" (HEINRICH & SCHRÖDER 1985).

*Xiphophorus* males exhibit significantly more often gonopodial swinging (G) in the presence of females than in their absence. Therefore, we assume that this behavior expresses sexual irritability of the males rather than presenting a comfort movement (BAERENDS et al. 1955). This behavior consists of a downward and forward rotation of the gonopodiam (ROSEN & GORDON 1953). The term gonopodial swinging was coined by CLARK et al. (1954), but the behavior had already been previously described as "isolated flexion of the gonopodium" (SCHLOSSBERG et al. 1949).

After numerous episodes of approaching, corralling and nipping, a receptive female may approach the male and present herself accompanied by jerking (J) which consists of rapidly performed up and down movements of the anterior part of the body (SCHRODER & HEINRICH 1985). If the malc responds by jerking, both fish may swim parallel (PS) to each other for relatively long distances. Sometimes the female swims forward slowly and abruptly for short distances (offering). The male then attempts to copulate by positioning himself behind the female, swimming forward, stanting his body and pointing his gonopodium forward at an angle in excess of 90° toward the female's genital pore, ultimately to insert the gonopodial tip into it (gonopodial thrusting, T; BAERENDS et al. 1955). Copulation attemps occur more frequently than copulations. Because Xiphophorus females store sperm, and sperm may survive in the folds of the ovarian tissue for many months thus being available for the next set of fertile eggs in the spontaneous ovarian cvcle (SICILIANO 1972), a single insemination may result in many broods. True copulations are recognizable by the postcopulatory jerking movements of the male. Gravid females are not receptive and may attack males who try to copulate with them (FARR 1980b). Accordingly in 1 male/1 female matings aggressive behavior (Ag) is directed only toward males. Females are in the receptive phase of their ovarian cycle only during the first five days after delivering a brood and than accept the copulation attempts of the male (FARR 1976, FARR 1980a). According to SICILIANO (1972), fertile eggs are available in the ovary of Xiphophorus females during eleven days.

Receptive females often "offer" (O) themselves to the male by slowly performed jerky forward swimming for short distances to the male. Offering seems to be a typical female response behavior which looks like a ritualized flight. Sometimes males try to inseminate females withouth their cooperation. Then the fleeing female hides in the vegetation or on the bottom. After discovering her, the male begins to encircle (E) the female as if to bring her to a position where copulation attempts may be successful. As was pointed out by CLARK et al. (1954) and FRANCK (1964, 1968), pecking (P) seems to be a replacement movement but may also play a role in the synchronization between male and female: SCHRÖDER & HEINRICH (1985) observed that increased synchronization of pecking sometimes leads to a completely synchronous duet of pecking in pairs of platyfish. *Xiphophorus maculatus*.

Some other behaviors occur more rarely and seem to be restricted to a particular male morph. Thus, headup and forward swimming (H) was only observed in 11 males, while a combination of jerking and forward swim-



Figs. 2 - 7: Xiphophorus multilineatus. (2) Small blue (SB) male, ca. 1.5 x nat. size. – (3) Small yellow (SY) male with female, ca. 2 x nat. size. – (4) Intermediate 1 (I1) male, nat. size. – (5) Intermediate 2 (I2) male, nat. size. – (6) Large (L) male, nat. size. – (7) Female, ca. 1.5 x nat. size.

ming, correspondingly named jerky head-up and forward swimming. JH, was seen in 12 males. Zigzag swimming (Z) describes a very rapid movement of SB males chasing a female, thereby transversing a zigzag route. Copulation jumps (CJ) which were observed in II males (table 2), occur rarely after copulation or copulation attempts when the male leaves rapidly the female by darting.

#### 2.3. Scoring sexual behavior activities:

All 1 male/1 female experiments were carried our for 1 hour in 37.5-liter tanks with water-moss (*Fontinalis* spec.) at a temperature of  $(25 \pm 1)^\circ$  C by direct observation. Both frequency, duration, and sequences of the 16 behavior activities were scored and analysed with an event recorder (EV. 24) the hard- and software of which was manufactured and equipped by Ingenieurbüro Erbacher, Buchenain nr. Munich, Germany.

#### 2.4. Female choice behavior:

A 37.5-1 aquarium was subdivided by two panes of glass into three equal compartments (fig. 13). Each virgin test female in the center compartment had to choose between one male morph at one side of her compartment and a different male morph on the other. The frequency and duration the female spent at a pane separating her compartment from those of the two competing males was recorded for thirty minutes both with no male touching the pane at the same time and doing so. To avoid the effect of possible side bias by the female, the position of the two males was reversed after the first 15 minutes of observation. The 10 possible male combinations were always tested with females of different origin (SB, SY, 11, 12, and L).

#### 2.5. Competition experiment:

The competition experiment was performed in a large mating group of eleven virgin females and five males belonging to the five morphs. The sizes of the five males of this experiment are given in table 4. All observations were carried out at a mean temperature of 25° C in a 120-L tank equipped with an aeration and filtration system producing an outflow of water at a velocity of 0.11 m/sec. There were, however, also areas of stagnant water. Aquatic plants were either grown in the gravel (*Vallisneria, Spyrogyra*) or floating (*Fontinalis, Myriophyllum*). Illumination by neon lights was set to a 12-h cycle providing about 1.000 Lux at the surface, but daylight was not excluded.

Sexual and agonistic behavior was scored three times for each of the five morphs during a 24-day observation period. The observation time for one session varied between 402 and 508 sec. The total observation time (sec) for each of the morphs reads as follows: 1525.2 (L), 1426.5 (11), 1198.6 (I2), 1469.6 (SB), and 1205.1 (SY). Because each male was observed seperately from the others, the total observation time amounted to 113.4 min. The results of the competition experiment is presented in figs. 15 and 16.

The male sexual and agonistic activities were obtained by observations of each of the five males for 25 min in 5 and 10-min sessions. The sexual activities as given by fig. 15 were calculated as percent of the sum of all sexual plus pecking activities of the respective male morph. Sexual and agonistic behaviors were compared for all possible combinations of the five morphs by the 2 x 2 contigency chi-square table, adjusted for multiple comparisons by the Bonferroni-Holm procedure (HOLM 1979). Accordingly, the symbol ">" means a significant difference (p < 0.05) between two successive (and the following) morphs, while "=" stands for no significant (p > 0.05) difference.

#### 2.6. Statistical testing treatments:

After analysing all experimental data for normal distribution by the Kolmogorov-Smirnov test, parametric and non-parametric methods were used by the aid of STATGRAPHICS and the test procedures as given by



Fig. 8: Percent frequency (F) and duration (D) of "Approaching" (A) spent relative to the total activity of every n pairs.

SIEGEL (1956) and SACHS (1973). Multiple comparisons were adjusted by the Bonferroni-Holm procedure (HOLM 1979).

#### 3. Results:

#### 3.1. Genetic determination of male size:

Like in most poeciliid species, males and females of Xiphophorus cannot be distinguished phenotypically at birth. But between three and five weeks of age the sexes can be told apart by differences in the shape of the anal fin. At sexual maturity, males cease their growth, whereas the genetically uniform females continue to grow. ZIMMERER & KALLMAN (1989) found that differences in age at sexual maturity and adult male size of Xiphophorus multilineatus are controlled by genetic variation at a Y-linked locus. Four genetic size-classes have been identified (table 1). In the present study, we determined the size and some body dimensions of males and females at the time of the courtship tests. The comparison of mean values (t test, p < 0.05) confirmed the size differences between the male morphs as were expected according to the measures by ZIMMERER & KALLMAN 1989 (table 1). However, since females should be genetically uniform, so significant size differences were expected. Contrary to this expectation, size differences were found for the total and standard length between I2 and SB females as well as for the greatest depth of the body between L and I2 females on the one hand and between I2 and SY females on the other. As to the body dimensions of males, the body shape of L and I males was more highbacked than that of small males which therefore appear more slender. The sword lenght of L and I2 males relative to the standard length was found to be larger than that of small SB males which mostly lack a swordlike appendage as is also characteristic for SY males. Otherwise, the length of the gonopodium of SB males relative to their standard length was greater than that of I2 males.



Fig. 9: Percent frequency (F) and duration (D) of "Corralling" behavior (FC = forward corralling, BC = backward corralling) spent relative to the total activity.

## 3.2. Male and female sexual behavior patterns:

The occurence of 17 different behaviors in 1 male/1 female matings of the five morphs is listed as percent of the total activity of the pairs under investigation (table 2). Perhaps because of the use of virgin females, no female attacks were registered. Apart from the behaviors which were seen only in one morph (H, JH, E, and CJ), both phases of corralling (FC and BC) play a major role for 12 males, while thrusting (T) and female offering (O) are most prominent for SY. The histograms of figs. 8 - 11 represent the percent frequency and duration of behaviors spent relative to the total activity of the respective pairs. Fig. 12 gives the ratios of some selected behaviors in order to analyse the communication signals exchanged between males and females. As to approaching (A; fig. 8), all males more frequently followed the females and correspondingly spent more time in approaching than did females. The frequency ratio of approaching males to approaching females (fig. 12) elucidates that this value is near unity only for II pairs. Both forward (FC) and backward corralling (BC) was the most common behavior and took most time in 11, followed by 12, SB, and SY. L males spent least time corralling (fig. 9). Because of this low rate of corralling, the ratio of female offering (O) to both phases of male corralling (FC + BC) was highest for L males followed by SY males (fig. 12). Although these ratios are considerably lower for 11 and 12 males, they perhaps reflect a better communication between males and females. Fig. 10 presents frequency and duration of thrusting (T) behavior which was highest for SY males, followed by L males. Intermediate values were recorded for I2 and SB males, whereas I1 males showed the lowest values. The ratio of T to FC + BC (fig. 12) then reveals the great importance of thrusting for SY, followed by L males. As expected for the sneaking-small SB and SY males, nipping (N) (fig. 11) is much more important than for all other morphs. The ratio of T to N (fig. 12) again demonstrates how meaningful both T and N are in particular for SY males. The ratio of jerking males to jerking females approaches unity only in SB (fig. 12) thus emphasizing the sig-



Fig. 10: Percent frequency (F) and duration (D) of "Thrusting" (= Copulation Attempt) behavior spent relative to the total activity.

nificance of intersex communication for this morph, followed by I2 and SY. It seems of less importance for L and I1.

Table 3 presents the percent behavioral sequences which occurred within intervals of 0.5 sec. Only those activities were considered which amounted to at least 5 % of all 0.5-sec. transitions. The transitions of male approaching follows female approaching and vice versa was found to be most important, in a decreasing rank order, for L, SY, 12, 11, and SB pairs. However, no transition was found for 11 with respect to female approaching follows male approaching follows nale approaching follows nale approaching occured only in SB. Male FC follows BC was only found in 12, and BC follows FC only in 11, thus again emphasizing the importance of corralling behavior for 11 and 12. Both male headup and forward swimming follows female approaching and vice versa occurred only in 11 where it seems to serve the communication between male and female. Summarizing all transitions in which female and male behavior activities follow each other, a decreasing rank order of intersex exchanges would be read as follows: L (76.8 %) > SY (62.2 %) > I2 (57.0 %) > I1 (43.1 %) > SB (37.4 %). Thus, the SB morph which represents a very pronounced type of "sneaker" exhibits remarkably the least cooperation between male and female.

#### 3.3. Competition experiment:

Whereas the above observations were performed in 1 male/1 female matings in which no inter-male competition can occur, in the large mating group of eleven females and five males, each of the five morphs (table 4), the males compete for access to females. Ranking in decreasing order the all-over sexual activity both as frequency and duration, the following sequence was obtained:  $L > 11 \ge 12 = SB = SY$ . However, a significant dif-

		Morph					
Transitions	L))	[]2)	I2 <sup>31</sup>	SB <sup>4</sup> )	SY <sup>5)~</sup>		
A♂ follows A*♀	49.0 a, b, c	30.8 a. d. e	38.7 h.d.f	26.0 c. f. g	41.6 a. g		
Ao' follows No	() h. l	() j. k	()1.m ,	21.8 h. j. l. n	12.5 i. k. m. n		
No <sup>r</sup> follows Ao <sup>r</sup>	0 <sup>0</sup>	0 P	0 d	20.4 o. p. q. r	0 r		
FCO' follows BCO'	0 `	o <sup>t</sup>	5,3 s. t. u. v	() u	0 v		
BCo <sup>*</sup> follows FCo <sup>*</sup>	0 *	6.2 w. x. y. z	0 × .	0 У	0 z		
H♂ follows A*♀	0α	6.6 α, β, τ, δ	0β	0 t	0δ		
Ao' follows A*o'	27.8 + * *	0+****	18.3 <b>V. 4</b> . §	<u>11.</u> 4 ♦. ♦. §. π	20.6 <sup>#. π</sup>		
A♀ follows H*♂	0 µ	5,7 μ, ε. Θ, Φ	0 с	0 0	0Φ		

Table 3: Percent behavioral sequences appearing within intervals of 0.5 sec. Only those behavioral activities were considered which amount to at least 5 % of all 0.5-sec transitions.

\*) communicative interachtions between ♂ and ♀

- 1) 353 0.5-sec transitions
- 2) 679 0.5-sec transitions
- 618 0.5-sec transitions
- 4) 2285 0.5-sec transitions
- 5) 296 0.5-sec transitions

Significant differences as determined by the 2 x 2-contingency table adjusted by the Bonferroni-Holm procedure (BHP) for simultaneous multiple comparisons:

 $p < 1.3 \times 10^{-2}$ a - g  $p < 1.5 \times 10^{-3}$ h - n 0 - 1  $p < 10^{-5}$  $p < 4.6 \times 10^{-4}$ s - v p < 10<sup>-4</sup> w - z  $p < 5 x 10^{-5}$  $\alpha - \delta$ +, ♥, ♦, ♣, ♠, #, §, π  $p < 6.2 \times 10^{-3}$ μ, ε, Θ, Φ  $p < 2.3 \times 10^{-4}$ 

Table 4: Standard length,	sword length,	gonopodial	length and	greatest	depth (m	nm) of the	five male	morphs in	the con	mpeti-
tion experiment.										

Male competitor	Standard length	Sword	gonopodium	greatest depth	
L	37	15	5	12	
12	37	8	5	12	
11	27	0	3	8	
SB	25	0	3	7	
SY	26	0	3	7	

ference between I1 and I2 (" $\geq$ ") was only found, if the Bonferroni-Holm procedure was neglected. Pecking as a measure for the non-sexual activity of males under consideration ranks as follows: I1 = SB > SY = L > I2.

The agonistic behavior of the five competing male genotypes was recorded as attacks (true or feigned bites) delivered and received for each of the males in the same sessions in which sexual activities were scored. Fig. 16 presents the data in percent of the sum of all bites delivered by the competing males which amounts to 154 attacks in 55.3 sec. The agonistic behavior follows a decreasing rank order of L > II > SB = SY. This means,



Fig. 11: Percent frequency (F) and duration (D) of "Nipping" behavior spent relative to the total activity.

under competition, the large L, II, and I2 males were the most courting and aggressive males, while no attacks were delivered by the small SB and SY males.

#### 3.4. Female choice behavior:

There was a complex network of a non-linear rank order in female preference (fig. 14). While only L and II females clearly preferred L-males over all other morphs, I2, SB and SY females preferred either II or I2 males over all other types. However, while I2 males were slightly preferred over L males by II females, L males were preferred by the same females over 11 males which on their part were overridden by I2 males. The lowest preference was exhibited for SY males by all females. Without exception females preferred SB over SY males. Apart from size, there perhaps was a slight preference for males with whom the females were raised together. Taking all significant comparisons together, a rank order of preferences through female choice was established as follows:  $I2 \ge L > II > SB > SY$ .

# 4. Discussion:

The percent analysis of sexual behavior in 1 male/1 female matings (tables 2 - 3, figs. 8 - 12) revealed that in SB and SY males almost no courtship display (FC + BC) occurs. It is replaced by a high rate of thrusting and nipping behavior which only scarcely appeared in 11 which courted at a high rate. The 12 males also courted at a higher rate then small males but at a significantly lower frequency than 11 males. Correspondingly, thrusting and nipping rates of 12 males were higher than those of 11. Unexceptedly, also L males courted at a very low rate similar to that of SY males. Their nipping rate was only slightly enhanced as compared to that of 11 males but was considerably lower than that of 12 and small (SB + SY) males. On the other hand, thrusting rate of L males



Fig. 12: Morph-specific male sexual and female response behavior (ratios of arithmetic means).

was higher than that of 11, 12, and SB males but lower than that of SY males. Accordingly, the most pronounced "sneaking" behavior was observed for small males, followed by L males. Although there are hundreds of fish under natural conditions, the question should be allowed of whether or not the large males' handicap of both



Fig. 13: Design for female choice experiments.

a low rate of courtship and of sneaking behavior perhaps may be compensated by a better communication between the sexes. Indeed, there was a decreasing rank order of transitions of male and female social behavior activities following each other within 0.5 sec (table 3) which runs as follows: L > SY > I2 > I1 > SB. Thus, the exchange of signals between males and females seems to control the sexual behavior in L and SY pairs while it plays only a minor role in SB. However, the social behavior of males competing for the access to females in large matings groups consists almost entirely of the sexual and agonistic behavior of the larger males (L, I) and I2), while small SB and SY males do not contribute to the social behavior of the group (figs. 15 and 16). Like competing guppy males (FARR 1980), also males of *X. multilineatus* seem to maximize their courtship activities only in the presence of competent competitors. Although no prediction on the reproductive success of the five male morphs is possible from the present experiments. ZIMMERER & KALLMAN (1989) found that at least under the condition of a small mating group (one large and one small male), 75 % of all offspring were sired by the large and only 25 % by the small males.

Despite the finding of a complex network of non-linear rank order of female preferences (fig. 14), significant differences suggest an overall rank order of preferences through female choice as follows:  $I2 \ge L > II > SY$ . Accordingly, SY males are least preferred by females while only a borderline difference was observed between L and I2 males. However, regarding the preference behavior of females originating from broods with various male morphs, always a slight preference for the "own" morph, i.e. the male morph belonging to the brood with which the females were raised, could be registered (fig. 14). Since all females used in the experiments were separated from their brothers only at the onset of sexual maturation, the females may have been conditioned to the respective male morphs. Preferential (or assortative) mating systems in teleosts might also have



Fig. 14: Network of female preferences with respect to five male morphs. ">" indicates significance at the 5 % level, ">>" at the 1 % level, and " $\geq$ " stands for borderline significance ( $p \ge 5$  %).

been established through imprinting effects (p.e. MCKAYE & BARLOW 1976). However, it is rather unlikely that preferential mating may play any role in natural habitats of X. multilineatus.

The results of the competition experiment agree with those of the female-choice behavior. The large L, II, and I2 males were the most courting and aggressive males, while no attacks were delivered by the small SB and SY males. However, small males may also exhibit the entire repertory of the social behaviors in 1 male/1 female matings (figs.  $8 \cdot 12$ ), whereas in mating groups with large males present the social activity of small males was repressed. Under these conditions small SB and SY males exhibited neither corralling behavior nor delivered attacks (figs. 15 - 16).

As in *Poecilia peruguiae* (ERBELDING-DENK et al. 1994; SCHARTL et al. 1993), the sneaking-chase behavior of small males is also correlated with different body shape. The different size-morphs of *X. multilineatus* associated with different body proportions may perhaps result from allometric growth (ROSEN 1960). Because large and intermediate males of *X. multilineatus* are relatively more deep bodied than small males, large males appear relatively broader and shorter (figs. 2 - 6). The sword length of L and I2 males relative to the standard length was found to be larger than that of small males which mostly lack a swordlike appendage. Besides this, the length of the gonopodium of SB males relative to their standard length was longer than that of I2 males.

It cannot be decided without knowledge of the social behavior of this species in its natural environment whether or not differences between the environment in natural habitats and the artificial conditions in the laboratory tanks account for some findings of the present study. Thus, the question remains still open as to the biological meaning of five male morphs in a poeciliid species. As we know from other *Xiphophorus* species such as *X. gordoni*, one male morph is sufficient for the survival of the species. Perhaps the split into different size and/or color morphs is the first indication of the onset of further speciation. The previous history of the systematics of the pygmy swordtail (fig. 1) favors this view.



Fig. 15: The sexual activities of competing male morphs were calculated in percent of the sum of all sexual plus pecking activities of the respective male morph.

#### 5. Zusammenfassung:

Bei Xiphophorus multilineatus gibt es fünf Männchenmorphen, die sich in Größe und Färbung unterscheiden: Kleine blaue (SB), kleine gelbe (SY), intermediäre 1 (I1), intermediäre 2 (I2) und große (L) Männchen. Diese Unterschiede werden paternal durch genetische Veränderung an einem Y-gebundenen Locus vererbt. Da eine solche Variabilität am X-Chromosom nicht existiert, sind alle Weibchen genetisch identisch. Trotzdem wurden hier auch die Weibchen gemäß der Morphe ihrer Brüder, mit denen sie in einem Wurf aufgewachsen waren, mit SB, SY, II, I2 und L gekennzeichnet. Jeweils 10 Weibchen dieser fünf Gruppen hatten zwischen zwei Männchenmorphen zu wählen, die zur selben Zeit dargeboten wurden. Dazu wurde ein Aquarium durch Glasscheiben in drei gleiche Abteile unterteilt. Das zu untersuchende Weibchen wurde in das mittlere Abteil gesetzt. Die Häufigkeit und Dauer der Zeit, die das Weibchen an den Scheiben verbrachte, die ihr Abteil von denen der beiden konkurrierenden Männchen trennte, wurde registriert, und zwar sowohl die Zeit, in der neben dem Weibchen sich auch das betreffende Männchen an der Scheibe aufhielt, als auch die Zeit, in der sich das Weibchen allein an der Scheibe befand. Um die mögliche Bevorzugung einer Seite durch das Weibchen auszuschließen, wurde die Position der beiden Männchen nach 15 Minuten Beobachtungsdauer ausgetauscht. Alle 10 möglichen Kombinationen mit jeweils zwei unterschiedlichen Männchenmorphen wurden getestet. Es ergab sich ein komplexes Beziehungsgefüge einer nicht-linearen Rangordnung der weiblichen Präferenzen. Im Vergleich bevorzugten nur die L- und II-Weibchen eindeutig L-Männchen gegenüber den anderen vier Männ-

# **XIPHOPHORUS MULTILINEATUS**

Agonistic behavior of male morphs under competition



Fig. 16: The agonistic behavior of competing male morphs was determined as attacks (true or feigned bites) delivered and received for each of the five males in the same sessions in which sexual activities were scored. The data are presented in percent of the sum of all bites delivered by the five competing males which amounts to 154 attacks in 55.3 sec.

chenmorphen, wohingegen von den 12-, SB- und SY-Weibchen entweder 11- oder 12-Männchen bevorzugt wur den. Während jedoch die 11-Weibchen 12-Männchen im Vergleich zu L-Männchen geringfügig bevorzugten, gab es eine Präferenz derselben Weibchen für L-Männchen im Vergleich zu I1-Männchen, die ihrerseits im Vergleich zu 12-Männchen benachteiligt waren. Alle Weibchentypen zeigten die geringste Präferenz für SY-Männchen. Mit Ausnahme der 11-Weibchen bevorzugten alle anderen Weibchen SB- gegenüber SY-Männchen. Wenn man alle signifikanten Vergleiche zusammenfasst, ergibt sich die folgende Rangordnung für die Präferenzen der Weibchen bei der Wahl der fünf Männchen:  $I2 \ge L > I1 > SB = SY$ .

Gleichlaufend mit den Weibchenwahlversuchen wurde die Balz der Männchen und das Antwortverhalten der Weibchen in 1 Männchen/1 Weibchen-Paarungen für die Dauer von 1 Stunde quantitativ registriert. Weder Wiegeverhalten ("Corralling") noch Nippen und Kopulationsversuche konnten bei L-Männchen beobachtet werden. Während 11- und 12-Männchen eine hohe Balzaktivität mit vollständigem Wiegeverhalten (Vorwärts- und Rückwärtsphase) bei geringer Häufigkeit von Nippen zeigten, verhielten sich die SB- und SY-Männchen als Anschleicher ("sneakers"), welche den Weibchen folgten oder sie jagten bei einer gleichzeitig geringen Häufigkeit und Dauer von Wiegeverhalten, das durch eine relativ hohe Aktivität von Nippen und Gonopodialstoßen am Weibchen kompensiert wurde. Die Sequenzanalyse aller Verhaltensübergänge erlaubte eine Untersuchung der Kommunikation zwischen Männchen und Weibchen durch Austausch von Signalen. Alle Männchenmorphen konnten bestimmte Antwortverhalten der Weibchen auslösen.

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Request for reprints: Prof. Dr. J.H. Schröder Mariastein 8 A-6322 Kirchbichl

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