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COURTSHIP BEHAVIOR IN THE MALE GUPPY (*Poecilia reticulata*): A GENETIC ANALYSIS

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ABSTRACT: Courtship behavior in the male guppy (*Poecilia reticulata*) was analyzed employing 13 behavioral sequences measured in standardized situations. The stability of these measurements allowed a systematic study of individual differences and of their origins. Factorial analyses lead to two distinguishable main categories of behaviors: one was related to gonopodial swinging; the other to sigmoïd display. Variables used to describe gonopodial swinging have only environmental correlates. The additive genetic component was null when estimated by two independent genetic analyses and this result was confirmed by a nonresponse to directional selection. On the contrary, most of the variables used to describe sigmoïd display exhibit significant additive genetic components and this was confirmed by the incidence of males exhibiting vs. not exhibiting this behavior in a replicated directional selection. The adaptive significance of the variables is discussed in the light of genetic and observed correlations between the phenotypes.

RÉSUMÉ: Le comportement de cour du male guppy a été analysé à partir de 13 séquences comportementales relevées dans une situation standardisée. La stabilité des mesures autorise une étude systhématique des différences individuelles et de leurs origines. Des analyses factorielles conduisent à dégager deux catégories de comportements: l'une est relative aux mouvements du gonopode, l'autre au développement de sigmoides. 1) Les variables décrivant les mouvements du gonopode semblent n'être affectées que par les facteurs d'environnement. Deux analyses indépendantes, conduisant à l'estimation des composantes génétiques additives, fournit des valeurs nulles. Ce résultat est confirmé par l'absence de réponse à la sélection pour la variable nombre de mouvements du gonopode, au cours d'une expérience de sélection directionnelle. 2) Au contraire, la plupart des variables mesurant les caractéristiques des sigmoides sont affectées par une part de variance génétique additive, significativement differente de zéro. Conformément à l'hypothèse attendue dans le cas ou la variance génétique additive est significative, le trait *présence* opposé à *absence* de sigmoide durant le test, répond à la selection. La valeur adaptive de ces traits est discutée à la lumière des corrélations phénotypiques et génotypiques.

Twenty years ago, Barlow (1981) lamented the lack of knowledge concerning the genetic correlates of "modal action patterns" that he defined

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as "a subset of patterned motor outputs" or the "chunks of behavior animals perform that have a statistically recognizable pattern such as displays, specialized feeding behavior and some actions involved in bodily care." Since then, the situation has hardly changed, if we except species such as *Drosophila* (Sokolowski, 1992; Ringo, Dowse, & Barton, 1986; Ringo, Dowse, & Lagasse, 1987; Ricker & Hirsch, 1988). However, species such as fishes in which the modal action patterns are particularly well documented, provide an ethological framework for the study of individual differences and their genetic or environmental correlates. Data are generally limited to crosses between hybridizing species: territoriality in *Macropodus opercularis* and *M. opercularis concolor* (Vadasz, Kiss, & Csanyi, 1978), social dominance in *Salvelinus namaycush* and *S. fontinalis* (Ferguson & Noakes, 1982), and courtship behavior between *Xiphophorus helleri* and *X. maculatus* (Clark, Aronson, & Gordon, 1948, 1954) or between *Lepomis gibbosus* and *L. macrochirus* (Ballantyne & Colgan, 1978). Indeed interspecific hybrids provide an analytical perspective otherwise unavailable and, moreover, are informative about phylogenetic relationships (Hirsch & McGuire, 1982). These crosses, thus, provide useful information that is complementary to intraspecies genetic analysis, particularly with long term selection that enlightens the evolutionary factors.

There have been very few attempts at intraspecies behavior-genetic analysis in fishes, in spite of several elegant studies: locomotor behavior (Gerlai, Crusio, & Csanyi, 1990), social behavior (Francis, 1984) in *Macropodus opercularis,* aggression in *Gasterosteus aculeatus* (Bakker, 1985, 1986), and social composition or interaction between males in *Poecilia reticulata* (Farr, 1983). The small fish called the fanciers' Guppy, or *Lebistes reticulatus* (now *Poecilia reticulata*) is a good model for genetic analysis. It has a long history both in genetics and behavior studies. A Y-linked inheritance has been demonstrated for a color marker (Schmidt, 1920). Other Y, X and autosomal genes correlated with colored and morphological variants were discovered by Winge (1923), Winge and Didlesen (1947) and the list has been lengthened since these pioneering works (see Yamamoto, 1975 for a review and Phang, Ng, & Fernando, 1989 for complex inheritance of color patterns). On the other hand, the behavior of *P. reticulata* has been extensively described by ethologists, particularly for courtship behavior in males (Haskins & Haskins, 1950; Baerends, Brouwer, & Waterbolk, 1955). Liley (1966) described for *P. reticulata* several courtship displays that are not present in the sympatric species *P. vivipara, P. parae* and *P. picta.*

The purpose of the present paper is to analyze the structure of individual differences in the male courtship behavior of *P. reticulata* and their genetic correlates: genetic components of the variation and response to selection.

EXPERIMENT 1: INDIVIDUAL DIFFERENCES IN MALE COURTSHIP BEHAVIOR

The previously published ethologic descriptions provide many variables describing specific courtship behavior in *P. reticulata* males. The prerequisite of the genetic analysis is the detection of the variables that are susceptible to disclose stable differences between males in a standardized test.

Animals. The males came from a heterogeneous population developed for the experiments presented here. Eight populations were at its origin: seven stocks having been selected for independent history were provided by fanciers and one population was directly imported from Guyana. The eight populations were crossed two by two, giving four F_1s; their crosses gave two F_2s; these were used to produce an F_3 heterogeneous population that included 422 males used in the different experiments.

Breeding Conditions. As a general rule, the females were isolated from males in an individual tank after fecundation and removed as soon as parturition was observed. Each tank (39 × 21 × 19 cm) was individually supplied with filters under sand, aeration and plants: *Elodea canadensis* and *Lemna polyrhisa. Fontinalis antipyretica* was added when necessary to provide a protective device for the fry against female cannibalism. Pregnant females were fed with living Artemia, experimental subjects with standardized commercial dry food and fry with hard-boiled egg yolk. Temperature was kept at 24.9 ± .9°C, pH 7.5 to 7.8, photoperiod 11/13 hours with light on at 7 pm.

Behavioral Observations. These were performed during the light period with 180 ± 7 day old males maintained by separated pairs of brothers from the age of 30 days until the test (except in Experiment II) to avoid possible effects due to isolation. A glass tank having three compartments was used for the observations. The two lateral compartments, each divided into four opaque boxes, were assigned to males selected for the observations. The central part (40 × 30 × 18 cm) was used for behavioral testing. Each of the eight boxes was connected to the central part by holes (30 mm in diameter) located 40 mm under the surface of the water, and closed by a trap. The physical conditions were identical to the rearing tanks except that there were neither plants nor food.

The female was placed in the central part. Only females 4–29 days after parturition were chosen since the physiological state of the female has been shown to affect courtship frequency of the male in this species (Liley, 1966). One hour later the entrance gate of the first hole was gently lifted so that the male could enter. The behavioral recordings lasted 20 min starting when one of the behavioral sequences described below appeared.

Prior to the beginning of courtship, the male follows the swimming female positioned 2 to 3 cm behind the female. This first variable labelled "time spent following the female" is abbreviated: I. *Time following.* The male then assumes a position in front of the female before beginning the display, and then makes a more or less complete circle around the female. The "number of circles around the female" is abbreviated: II. *Nb. circles.* Another pattern of behavior has been described (Baerends et al., 1955) for this stage of courtship. The general axis of the male's body is directed toward the body of the female. The male is watching the female, the dorsal and caudal fins being folded and slight quiverings of the male's body may be observed (labelled "number of watching positions": III. *Watching*).

Two other behavioral sequences are of the greatest importance for the present paper: gonopodial swinging and sigmoïd display, interrupted by orientation patterns.

Gonopodial swinging forward is performed independently of copulation or copulation attempts. Generally the movement is performed when the male is motionless, and the snout directed toward the snout of the female. The movement was either performed alone by the male: (IV. *Nb. simple gonopodial swingings*) or associated with incurvation of the body and with spreading the fins: V. *Nb. complex gonopodial swingings.* The reciprocal of the first gonopodial swinging latency, either simple or complex (VI. *1/first gonopodial latency*) and a composite score summing variables IV. and V. (XIII. *Nb. gonopodial swingings*) will be used in further steps of genetic analysis.

The sigmoïd display was first described by Clark, Aronson & Gordon (1948, 1954): the body is arched, both tail and head away from the female. This posture is held for 5 to 50 sec in some individuals. The body, the pectoral, and caudal fins quiver rapidly. Generally, the male remains in the same place but may on occasion move backward and forward. During the sigmoïds, the fins may be more or less spread. The frontal sigmoïd begins when the male is in front of the female, the male body perpendicular to the axis of the female's body. The lateral sigmoïd starts when the male is parallel to the female, the head being in the same or in the opposite direction. These two behaviors were pooled (VII. *Nb. sigmoïds*) since there are many intermediate patterns and a lateral sigmoïd may become frontal and vice-versa during the display. The total durations of these sigmoïds during the observation (VIII. *Duration sigmoïds*) and the reciprocal of the latency of the first sigmoïd, either lateral or frontal (IX. *1/latency first sigmoïd*) were noted. Furthermore, we calculated the average duration of all the sigmoïds, either lateral or frontal (X. *Average duration sigmoïds*) except the weak. The variable XI. *Nb. weak sigmoïds* refers to sigmoïds with a low intensity form, the body being in "an arched and not very pronounced sigmoïd shape" (Liley, 1966). Thrusting, copulation and copulation attempts are characterized by gonopodial contact or attempted contact (XII. *Nb. copulations*). Whether insemination is accomplished or not has not been considered and consequently the post-

TABLE 1
Reliability of Variables Used to Measure Courtship Behavior

	Variables	*Split half*	*Test retest*
I	Time following	.17	.16
II	Nb. circles	.01	.05
III	Watching	.59	.45
IV	Nb. simple gonopodial swingings	.62	.63
V	Nb. complex gonopodial swingings	.91	.77
VI	1/first gonopodial latency	ND	.89
VII	Nb. sigmoïds	.84	.97
VIII	Duration sigmoïds	.67	.78
IX	1/latency first sigmoïd	ND	.85
X	Average duration sigmoïds	.77	.81
XI	Nb. weak sigmoïds	.62	.71
XII	Nb. copulations	ND	.63
XIII	Nb. gonopodial swingings (IV + V)	.86	.93

ND: not done.

ejaculation jerk (Clark, Aronson & Gordon, 1948, 1954) has not been taken into account here.

Statistics. Split half (between the two parts of the first test, except for latencies), and test/retest (25 ± 5 days later) were computed to test for the reliability of the measurements. Fifty males were tested twice. A factor analysis (principal components method) was performed on their scores at the first test after exclusion of the least reliable variables. In all cases scale transformations were carried out to ensure normality of the distribution scores to allow the use of product moment correlations and R^2 as diagonal values. In order to confirm the factorial structure of the variables selected for the genetic analysis, a new sample of 115 males was observed. The following variables were included in the factor analysis: the composite variable XIII. *Nb. gonopodial swingings* (sum of variables IV. and V.), VI. (*1/first gonopodial latency* either simple or complex gonopodial swinging), VII. (*Nb. sigmoïds*), VIII. (*Duration sigmoïds*), and IX. (*1/latency first sigmoïd*), X. *Average duration of sigmoïds* (excluding the weak sigmoïds).

Results and Discussion. Most of the reliability coefficients exhibit values that are high enough to ensure behavioral or genetic analysis (Table 1).

The first factor analysis (Table 2) performed with all the variables (excluding variables I and II, having insufficient reliability) led to the extraction of two factors. After rotations (maximization of loadings as

TABLE 2
Loadings in the First Factor Analysis of Variables Used to Measure Courtship Behavior

	Variables	F_1*	F_2	F_1'	F_2'	*Communalities*
III	Watching	.42	−.06	.37	−.20	.18
IV	Nb. simple gonopodial swingings	.64	.62	.84	.29	.79
V	Nb. complex gonopodial swingings	.58	.55	.73	.33	.64
VI	1/first gonopodial latency	.19	.32	.30	.22	.14
VII	Nb. sigmoïds	.72	−.33	.54	−.59	.63
VIII	Duration sigmoïds	.88	−.25	.70	−.60	.85
IX	1/latency first sigmoïd	.56	−.34	.38	−.54	.44
XI	Nb. weak sigmoïds	.70	−.22	.73	.40	.27
XII	Nb. copulations	.13	.50	.33	.40	.27

*F_1 F_2 before and F_1' F_2' after rotations.

criteria) the first factor can be interpreted as a general factor taking 38% of the variance into account, with all the loadings being higher than .30. The second factor (18% of the variance) was defined by the opposition between variables describing sigmoïd display on the one hand (VII. *Nb. sigmoïds,* VIII. *Duration sigmoïds*) and gonopodial swinging on the other (IV. *Nb. simple gonopodial swingings,* V. *Nb. complex gonopodial swingings,* VI. *1/first gonopodial latency*), and XII. *Nb. copulations.* In the second analysis, the first factor (49% of the variance) has only positive loadings, whereas the second (33%) only presents loadings higher than .30 for gonopodial swinging measurements (Table 3).

Some differences appear in the percentages of variance explained by

TABLE 3
Loadings in the Second Factor Analysis of Measures of Courtship Behavior (Rotated Factors)

	Variables	F'_1	F'_2
XIII	Nb. gonopodial swingings	.54	.39
VI	1/first gonopodial latency	.27	.44
VII	Nb. sigmoïds	.82	−.20
VIII	Duration sigmoïds	.91	−.08
IX	1/latency first sigmoïd	.69	−.14

each of the factors in the two analyses. This was expected due to differences in the selected variables. However, the two factorial structures can be interpreted in the same way: first, they show a common part of variance for the variables used to describe the behavioral sequences observed during courtship and, moreover, suggest a distinction between variables measuring gonopodial movements on the one hand and sigmoïd display on the other. The variables used for the second analysis, covering these two categories of behaviors, were hence considered for environmental and genetic analyses.

EXPERIMENT II: EARLY ENVIRONMENTAL VARIATION AND ADULT COURTSHIP BEHAVIOR

Do environmental variations such as the size of the brood and the density of population during the early postnatal period affect courtship in adult males?

Environmental Modifications. Males were taken from the heterogeneous stock and two rearing conditions were considered: first the size of the brood: 30 males from the small (less than 20 offspring) and 30 from large broods (greater than 20 offspring) were compared; second the length of exposition to high population density: two groups of 15 subjects each were constituted by taking individuals from pairs separated from the brood at 25 days or another separated at 45 days of age. The six behavioral variables defined at the end of the first experiment were measured in the previously defined conditions and compared by a t test.

Results and Discussion. The size of the brood only affects VII. *Nb. sigmoïds*: Males from small broods had a higher performance than those from large broods (20.26 ± 1.97 vs. 17.03 ± 2.13, $p < .05$). As concerns age at separation from the brood, subjects separated earlier showed significantly more courtship behavior than the others: 25.94 ± 3.20 vs. 12.11 ± 4.34 for VII. *Nb. sigmoïds* ($p < .01$), 199.31 ± 7.56 vs. 80 ± 4.23 for XIII. *Nb. gonopodial swingings* ($p < .01$). The other variables present tendencies in the same direction without reaching the $p < .05$ significance level.

EXPERIMENT III: GENETIC COMPONENTS OF THE VARIATION

The genetic method was imposed by the features of the species: no inbred strains (in the narrow sense) at the beginning of the experiment and difficulty in performing individual diallic crosses since the females keep spermatozoa alive for several months.

Genetic Design. Two genetic designs were performed to estimate the main components of variation: an offspring-one parent (O-O.P) and a half-sib (H-S) design. These two methods can provide an accurate estimation of the additive component (Jinks & Broadhurst, 1965; Dawson, 1965; Kearsey, 1965; Mather & Jinks, 1971), that could be of particular interest in the present study since some of them will be used to predict the success of directional selections. Moreover, full-sib correlations were calculated.

For the O-O.P design, 34 males were mated with 34 virgin females; for each variable, the male value and the averaged score of its male progeny were analyzed according to the methods described by Falconer (1960, 1963). For the H-S design, 118 males were individually mated each with 3 virgin females. In the two experiments every subject was taken from the heterogeneous population: males came from different families and were also mated with non-sister females. The males came from pairs separated from broods including between 15 and 25 individuals at 30 ± 3 days.

The scores of the offspring in the H-S design were analyzed according to Falconer (1960, 1963) and Mather and Jinks (1971) with an ANOVA method for nested designs with unequal size (Snedecor & Cochran, 1967) because not all the females were fecundated and the numbers of males differ across their progenies. The genetic correlations among the 6 previously described behaviors were calculated by the cross-covariance method (Falconer, 1960). Appropriate transformations were made to fulfil the technical requirements of ANOVA for individual scores ($\log_{10}(C + x)$), C being different for each variable.

Test for Independence of Genotypic and Environmental Effects. The different models assume that the interaction between the genotype of the population and environment to which they are exposed in this population has no effect upon the observed variance. When a population is in Hardy-Weinberg equilibrium, the gene frequencies can be assumed to be identical from one generation to the next, particularly in the father and son generation of our populations. Since small variations could occur in the environment from one generation to the other, changes in observed variance in the father and son generations could indicate an interaction effect between genotype and environment. Moreover, when similar genotypes or identical randomly selected pools of genes are borne by individuals reared under different experimental environments (as in Experiment II) the unequality of variance for the same variable can provide information concerning the genotype and environment interaction effect.

Behavioral Measures. The rearing conditions and behavioral tests were identical to those described in the first experiment except that the duration of observation for each subject was 15 min. The two variables dealing with swinging (IV and XI) and the four variables dealing with sigmoïd display (VII, VIII, IX and X) were measured as defined in Experiment I.

Results and Discussion. Interaction between genotype and environ-

TABLE 4
Comparison of Variances in the Populations of Male Fathers and Male Offspring

	Variables	*Father* $N = 18$	*Offspring* $N = 118$	F
XIII	Nb. gonopodial swingings 10 $g_{10(7+x)}$*	.8816	.8840	1.083 NS
VI	1/first gonopodial latency 10 $g_{10(9+x)}$	.2991	.3220	1.076 NS
VII	Nb. sigmoïds 10 $g_{10(8+x)}$	.0692	.0443	1.562 NS
VIII	Duration sigmoïds 10 $g_{10(9+x)}$	.2200	.1992	1.104 NS
IX	1/latency first sigmoïd 10 $g_{10(24+x)}$	.2956	.3619	1.224 NS
X	Average duration sigmoïds 10 $g_{10(10+x)}$	.0720	.0560	1.285 NS

*Transformation.

ment was first tested on the 6 variables. Transformed scores were employed to compare variances: 1) between two successive generations fathers vs. sons; 2) between groups from large vs. small brood and 3) between groups of individuals separated from the brood at 25 vs. 45 days of age. We failed to show an interaction effect between the genotypes and the categories of the environment that we had defined, since none of the F values reaches a $p < .10$ level. The estimation of the genetic parameters for the different variables was thus performed (Table 4) admitting that this interaction was negligible but the likelihood of this assumption must be discussed in the light of the other experiments.

The variable XIII. *Nb. gonopodial swingings,* will be considered first (Table 5). The VA/VP ratio is either null (H-S) or nonsignificant (O-OP). Using the first method, the component of the variation attributed to the fathers was negative (−.0003) and thus VA was considered to be zero. This lack of additive variance can be explained by several technical factors: 1) the true variance for number of gonopodial swingings is too small; 2) the inadequacy of the model; 3) the computational method for the components of variation. The first explanation cannot be retained because the reliability of the variables is high (Table 1). The second explanation is not pertinent because it has been demonstrated that the interaction G × E is not significant. In addition, the sources of variation are independent, the females being randomly affected by males and every male offspring in the litter being observed. The third explanation would

TABLE 5
VA/VP Ratios and Standard Deviations for 6 Variables in Male Courtship Behaviors Estimated by Regression of Offspring on Father Value $b_{(O\text{-}OP)}$ and Intraclass Correlation of Half Sibs $t_{(H\text{-}S)}$.

	Variables	*VA/VP* $b_{(O\text{-}OP)}$ ± *SE**	*VA/VP* $t_{(H\text{-}S)}$ ± *SE**
XIII	Nb. gonopodial swingings	.18 ± .78	0
VI	1/first gonopodial latency	0	0
VII	Nb. sigmoïds	.38 ± .15	.40 ± .09
VIII	Duration of sigmoïds	.32 ± .27	.29 ± .15
IX	1/latency first sigmoïd	.56 ± .11	.51 ± .14
X	Average duration sigmoïds	.39 ± .09	.68 ± .23

*Computed according to Falconer's method (1963).

suggest that the zero value of the component attributable to males may be the consequence of the computation of causal components by successive subtractions. In fact, the high standard deviation (.0078 ± .003) of the component attributable to the females may have led to a possible overestimation of this component and consequently to an underestimation of the VA. However, the nonsignificant value of VA/VP in the other design where this subtractive computational method is not used, strongly suggests that the number of gonopodial swingings does not have additive genetic correlates in this population. The fact that the variable VI. *1/first gonopodial latency* has a null additive component in the two designs suggests that the conclusion for variable XIII is also true for variable VI.

The two variables VII. *Nb. sigmoïds* and VIII. *Duration sigmoïds* have similar genetic architecture. The component attributed to the females was negative and thus considered to be zero, whereas the VA/VP ratios were in complete agreement in the two designs. For X. *Average duration sigmoïds,* the component attributable to the female differs significantly from zero in the half-sib design. The higher VA/VP ratio in the H-S than in the O-OP design is compatible with an epistatic effect due to linked segregating units, since, as a general rule, the ascendent/descendent covariance is not affected by epistasis when the segregating units are linked, whereas it is affected when computed from full-sib or half-sib designs. The results for X. *Average duration sigmoïds* are compatible with a model including IX. *1/latency first sigmoïd*: the VA/VP ratios do not differ from the O-OP and H-S designs. Moreover, another estimation of the ratio from the O-OP design (Kempthorne & Tandon, 1953) provides a similar value (.506 ± .117). As the component attributable to the females is different from zero, it is possible to consider VA from a full-sib design. In this case, VA/VP = .572 ± .137, differs from zero and is similar to the other coefficients. This suggests that in this population and for IX. *1/latency first sigmoïd* the deviation due to dominance and the common environmental variation that often inflate VA in full-sib designs are null.

The genetic correlations between these variables are reported in Table 6. As a general feature, the agreement is good between the values estimated from the two designs. The variables describing gonopodial swinging (VI and XIII) are not correlated, in spite of their high reliability and good commonalities (see Tables 1 and 2). The correlation between X. *Average duration sigmoïds* and VII. *Nb. sigmoïds* must not be overemphasized because they are not independent by construction. On the contrary, the common genetic correlates of VIII. *Duration sigmoïds* and VII. *Nb. sigmoïds* should be stressed. The correlations with IX. *1/latency first sigmoïd* are either null or weak when compared to their standard deviation. The components of variation and consequently VA/VP and genetic correlation coefficients were estimated here from relatively small samples. An accurate estimation of the coefficient values would need larger samples, but with small populations, indeed, we have demonstrated that the coefficients differ significantly from zero. Moreover, the highly similar estimates obtained from the two independent designs with different populations lend additional weight to the results.

EXPERIMENT IV: DIRECTIONAL SELECTION

When conclusions from biometrical genetics are taken into account, a response to selection is expected for the variables describing sigmoïd display and not for variables related to gonopodial swinging.

Material and Methods. The subjects were derived from the heterogeneous population. Over the generations of selection, 565 male guppies served as subjects. The conditions for breeding and observation of behaviors were identical to those presented in the first and third experiments.

Genetic Design. Two behaviors were measured during the test (15 min) and considered for selection: *Nb. gonopodial swingings* as described above and a new variable for sigmoïd display: *Incidence of sigmoïd display* (presence vs. absence). Individual selections were independently performed in duplicate for the two variables, leading to eight selected lines:

1) males exhibiting sigmoïd display at least once and labelled S+ and S+′;
2) males never exhibiting the display: S− and S−′;
3) males performing the highest number of gonopodial swingings: G+ and G+′;

TABLE 6

Sexual Behavior in Male Guppy: Observed Correlations in Parent plus Offspring Males (Under the Diagonal); Genetic Correlations and Standard Errors (Above the Diagonal), First Line from Half-sib Design; Second Line from one Parent-offspring Design

	Variables	*XIII*	*VI*	*VII*	*VIII*	*IX*	*X*
XIII	Nb. gonopodial swingings		0 0	0 .09 ± .13	0 .01	0 0	0 .01
VI	1/first gonopodial latency	.40		0 0	0 0	0 0	0 0
VII	Nb. sigmoïds	.27	−.02		.71 ± .12 .86 ± .11	.88 ± .04 .73 ± .10	−.11 ± .17 −.01 ± .20
VIII	Duration sigmoïds	.40	.13	.73		.31 ± .27 .13 ± .31	0 0
IX	1/latency first sigmoïd	.29	−.04	.16	.64		.11 ± .21 .02 ± .15
X	Average duration sigmoïds	.13	−.07	.57	.41	.18	

4) males performing the lowest number of gonopodial swingings: G− and G−′.

An unselected line bred from the basic population was used as a reference for each of the 8 other lines: UNS.

Twenty percent of the males (either high or low scores) of the observed population were included in each line in each generation. One half was used as progenitors and the sisters of the other half were used for mating. In each line and each generation the numbers of males observed were high enough to yield at least ten male progenitors. The selection was performed over four generations in every line, and was relaxed at generation 3. The continuous variable *N. gonopodial swingings* was analyzed according to an ANOVA procedure. For the dichotomized variable *Incidence of sigmoïd display,* the frequencies were compared with a log linear model (Sokal & Rohlf, 1981) and computed with the SAS CATMOD procedure (SAS Institute Inc., 1987); all the main effects and interactions were included in the model.

Results and Discussion

N. gonopodial swingings was considered first. The replicated lines G+ and G+′ on the one hand and G− and G−′ on the other hand do not differ ($F < 1$ in each comparison). There is no response to selection either for G+ (G+ and G+′ being pooled or analyzed separately) or for the G− (the replicated lines being either pooled or independently analyzed) since the generation effect is always comprised between $p < .20$ and $p < .40$. In any case, the selected lines differ from the UNS line (Figure 1).

Incidence of sigmoïd display (Figure 2) was then analyzed. Neither S+ and S+′ nor the S− and S−′ lines differ among each other ($\chi^2_{(1)} = .31$ and 2.14, respectively), so that the replicated lines were pooled. The S+ and S− generally differ ($\chi^2_{(1)} = 74.49$) and the difference is increased with the number of generations ($\chi^2_{(3)} = 13.84$ for the interaction between lines and generation). The difference reaches the $p < .05$ level as soon as generation 1 and remains significant for generations 2 and 4. The relaxation of the selection at the 3rd generation does not modify the difference between the lines. The upper line S+ differs from the UNS line ($\chi^2_{(1)} = 74.49$) but the difference is acquired at the first generation and remains constant at the 2nd and 3rd generations. The lower line also differs from the UNS line ($\chi^2_{(1)} = 24.23$) but the $p < .05$ significance level is only reached at the second generation. The reduction of the incidence of sigmoïd display across the generations reaches the $p < .003$ value ($\chi^2_{(3)} = 12.64$). Generally, there is no asymmetry between the S+ and S− lines when each of them is compared to the UNS. The *Incidence of sigmoïd display* is no more than a dichotomization of *Nb. sigmoïds* and the observed response to selection for this variable is in agreement

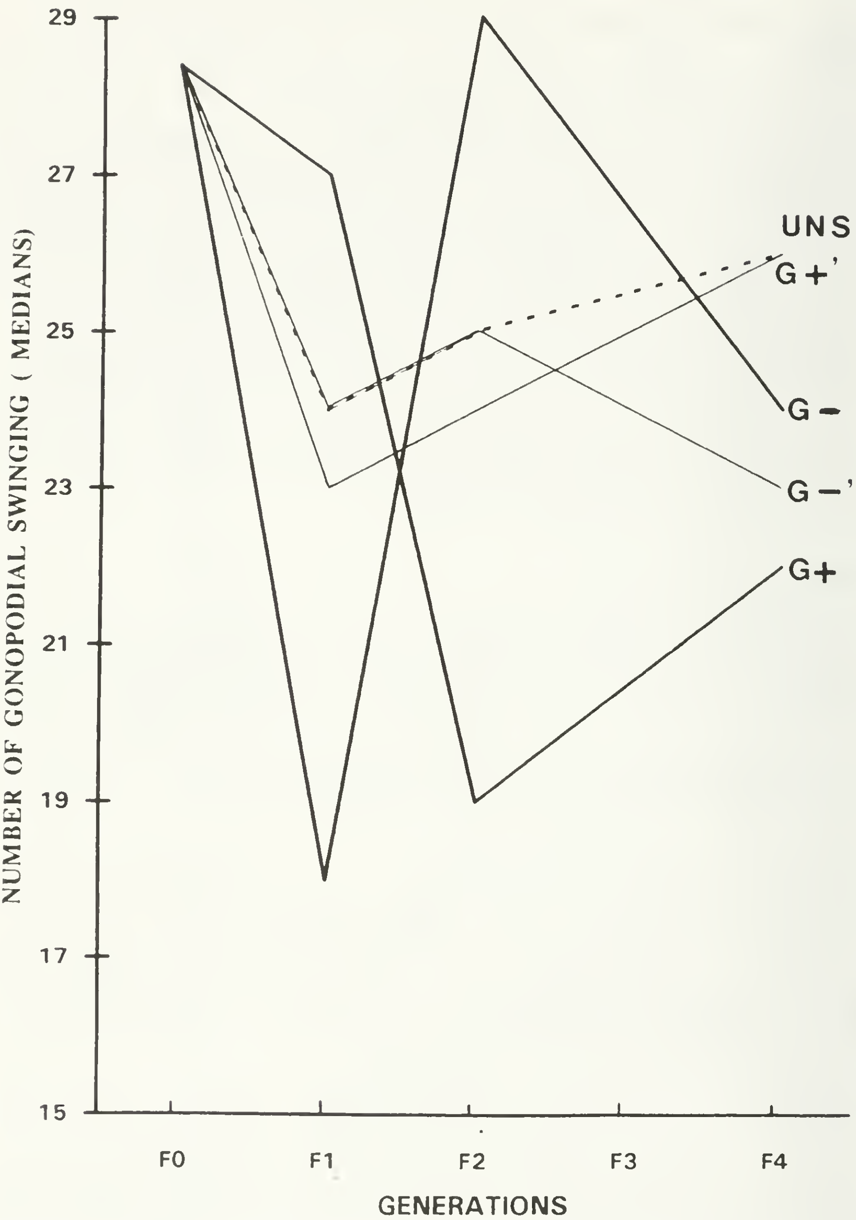

FIGURE 1. Response to selection for the number of gonopodial swingings (selection relaxed at generation 3); see the text for the abbreviations.

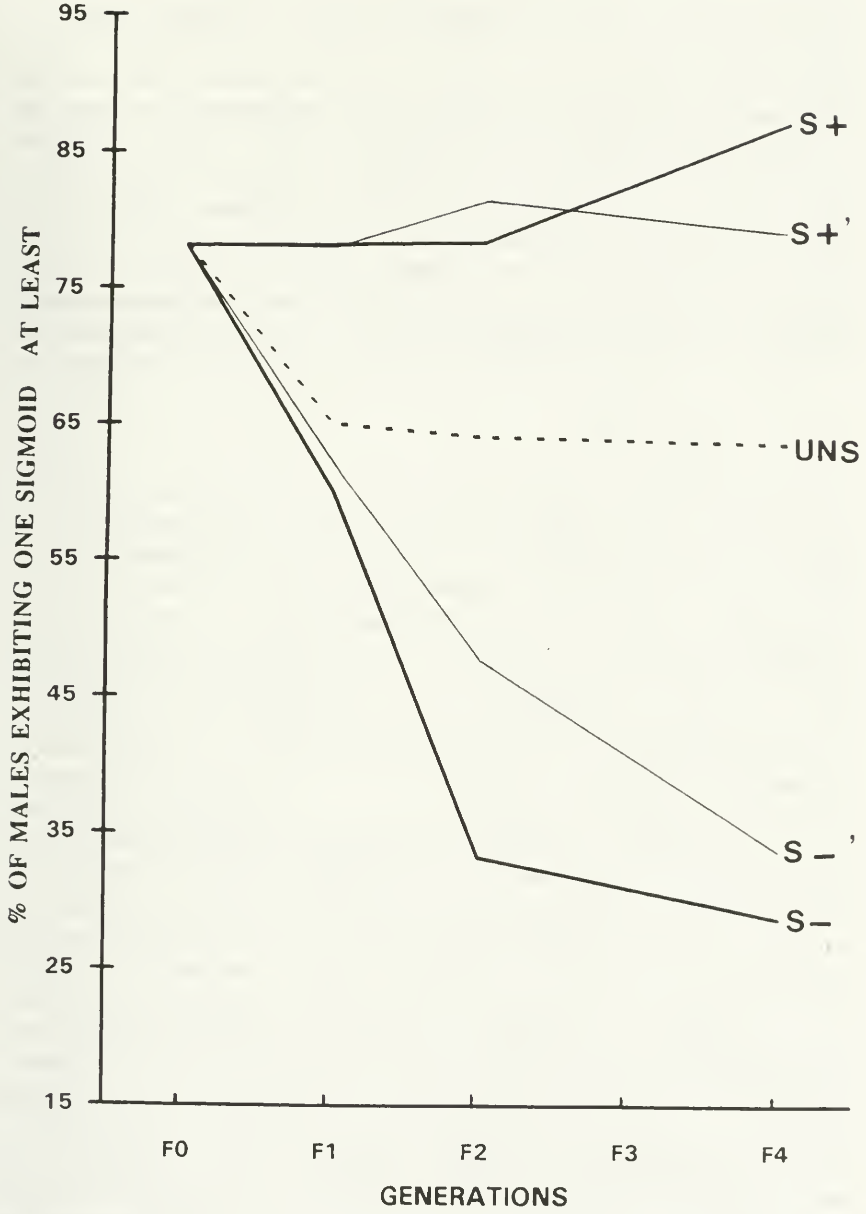

FIGURE 2. Response to selection for the presence vs. absence of sigmoïd display (selection relaxed at generation 3); see the text for the abbreviations.

with the significant VA/VP values obtained from the quantitative genetic analyses.

The correlated responses to selection were examined for the variables considered in Experiment III. There is obviously no correlated response for the *Nb. gonopodial swingings*. The S+ and S− lines were then considered. In the S+ line *Average duration sigmoïds* is higher (F = 5.83) and *1/latency first sigmoïd* shorter (F = 7.85) than in the S−. The correlated responses to selection reveal a structure of the observed sexual behavior in the male guppy that is compatible with the conclusion drawn from the quantitative genetic analysis. The measures of the gonopodial swingings and sigmoïd display appear to be genetically independent whereas in this last group the variables have common additive correlates.

GENERAL DISCUSSION

Individual differences were observed for variables measured during a standardized test for sexual behavior in male *Poecilia reticulata*. The factorial structure of individual differences is stable. A general factor appears and the second factor allows two categories of behavior to be distinguished. The first deals with gonopodial swinging and attempt to copulate, the second with characteristics of the species-specific display called "sigmoïd." The quantitative genetic analysis (with both male offspring-father and half-sibs designs) leads to an additive estimate for the variables employed to measure gonopodial swinging which is not significantly different from zero. On the contrary, most of the variables related to sigmoïd display have additive components that differ significantly from zero in the two designs. The conclusions from the directional selection experiment are those expected from the estimated values of the quantitative genetic analysis. We were not able to obtain a response for the number of gonopodial swingings whereas selected responses in duplicate were effective both for absence and presence of sigmoïd display (presence vs. absence). This agreement confirms the fitness of the biometrical models we have employed and the fact that the data were compatible with the requirements for these models. The test for an interaction between genotype and environment raises particular problems with these models and this species. The nonsignificant value found by the test we had employed for this source of variation is supported by both the agreement of the quantitative genetic estimates with the results of the selection and by the similar range of variation within each line for each generation.

The lack of additive variance for the variables used to measure the characteristics of gonopodial swinging and our failure to obtain a response to any selection does not exclude the contribution of other sources of genetic variation that are undetectable with the available methods. The absence of detectable additive genetic correlates for the measures of gonopodial swingings could be interpreted according to two hypotheses. First, it would be the consequence of the natural selection pressure for the trait, having an obvious functional usefulness for the survival of the species. It could be considered as being fixed, the allelic forms correlated with the unadaptive low number of gonopodial swingings having been eliminated. Second, the variation observed for the variables VI and XIII could only have environmental correlates. We have no evidence favoring the first hypothesis deduced from the synthetic evolution dogma. On the other hand, the high reliability, found for these variables, confirms that their observed variations are not random, but correspond to stable individual characteristics. The values reached by these coefficients and the high loadings in the factorial analysis indicate the large margin of their true variations. Moreover, we have demonstrated the susceptibility of variable XIII. *Nb. gonopodial swingings* to the effects of early environment but other sources of early environmental modifications could also be efficient as suggested by Laming and Loughin (1987).

The most surprising conclusion deals with the measures of sigmoïd display, a species-specific characteristic of this behavior (that is not found in other *Poecilidae* (Liley, 1966). We could thus expect a null genetic additive variation as a consequence of a selection against the genotypes correlated with low frequencies of the display. This is not the case here, as confirmed both by quantitative genetic analysis and selection. Unfortunately, the biological material and subsequently the biometrical models available for their analysis did not provide the means to test for other genetic components. The symmetry in the S+ and S− lines does not lead to the suggestion of a dominance effect. Moreover, a directional dominance hypothesis with the effect of recessive alleles associated with the reduction of sigmoïd display can be eliminated; it would not fit with the absence of inbreeding depression that we had found for frequency of sigmoïd behavior (Roubertoux, unpublished). But the hypothesis of directional gene frequencies cannot be excluded since this only has effects on long term selection and would not be detectable at the fourth generation. The significant additive genetic source of variation, the only source proven at present in this research, could be interpreted in two ways. First, it could be an indicator of a weak adaptive value of this species-specific trait, the sigmoïd display, thus leading to cautious interpretations of behavior-genetic analysis in evolutionary perspectives. Second, the result of a possible stabilizing selection could be invoked to explain the genetic additive variability underlying sigmoïd behavior.

The differences between the conclusions from an analysis of the observed and genetic correlations is not surprising since the first are the results of genetic and environmental covariations and of their interactions (Hirsch, 1967, who points out the misinterpretations of correlational conclusions). The observed correlations between gonopodial swinging and sigmoïd measurements are not due to genetic factors but to environmental ones. The sexual behavior pattern appears to be composed of sequences, some of them being heritable, but the coordination of these sequences has environmental correlates. This fact prevents any simple link between additive genetic variance and evolution as far as species-specific behaviors are considered.

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