

## PSEUDOMALE BEHAVIOUR AND SPONTANEOUS MASCULINIZATION IN THE ALL-FEMALE TELEOST POECILIA FORMOSA (TELEOSTEI: POECILIIDAE)

by

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(With 5 Figures)  
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### Abstract

Pseudosexual behaviour is a rare phenomenon associated with unisexuality in vertebrates. In the gynogenetic, all-female teleost *Poecilia formosa*, rare individuals occur that resemble males of closely related gonochoristic species both in behaviour and external morphology. These masculinized gynogens and normal gynogens are members of the same clone, as demonstrated by DNA-fingerprinting. The behaviour of these masculinized gynogens is described and compared to the behaviour of the gonochoristic species *Poecilia mexicana*, *P. latipinna* and their hybrid as well as androgen-treated individuals of *P. formosa*. No statistically significant differences were found between masculinized gynogens and hormone-treated individuals nor between the gonochoristic *P. mexicana* and *P. latipinna* males. Differences exist between gonochoristic and unisexual species. Possible causes and effects of masculinized gynogens are discussed.

### Introduction

The evolution and maintenance of sexual reproduction is one of the most challenging questions in modern biology. Unisexual organisms can serve

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here as uniquely suited models (CREWS, 1989). The gynogenetic all-female poeciliid fish *Poecilia formosa* (GIRARD, 1859) is one of the few vertebrates (VRIJENHOEK *et al.*, 1989) with a reproductive mode different from the usual gonochoristic pattern (HUBBS & HUBBS, 1932). Females produce diploid eggs. Embryogenesis is triggered by sperm of males of the closely related gonochoristic species *P. mexicana* STEINDACHNER, 1863 or *P. latipinna* (LE SUEUR, 1821). Normally, males contribute nothing to the genome of the offspring: inheritance is strictly maternal. *P. latipinna* and *P. mexicana* are not only the host species in this mating-system (SCHLUPP *et al.*, 1991) but also most probably the parental species of this natural hybrid species (HUBBS & HUBBS, 1932; TURNER, 1982; AVISE *et al.*, 1991).

Exceptional individuals of *P. formosa* which resembled the phenotype of males of closely related gonochoristic species were first mentioned in a paper by HUBBS & HUBBS (1932), in which they described *P. formosa* as a gynogenetic species. Later HUBBS *et al.* (1959) first described the morphology of a 'phenotypic male' of *P. formosa*. Males of the live-bearing genus *Poecilia* are recognized by their copulatory organ, the gonopodium, a modified anal fin. All *P. formosa* reported as 'males' had a gonopodium and some of them were reported to show aspects of male-typical behaviour (Table 1). Using androgen treatments it has already been possible to induce artificial masculinization in all-female *P. formosa* (SCHARTL *et al.*, 1991; TURNER & STEEVES, 1989).

In one of our laboratory stocks of gynogenetic *P. formosa*, occasionally individuals occur spontaneously with not only the general appearance, but also with the male-typical behaviour of the genus.

In the present paper we compare spontaneously masculinized gynogens to androgen treated individuals and to gonochoristic males of *P. latipinna*, *P. mexicana* and their interspecific hybrid.

We used several techniques to address the following questions: are masculinized gynogens and normal gynogens members of the same clone? Is the spontaneous masculinization induced by free androgens in the aquarium water? Is the behaviour of the masculinized gynogens comparable to the behaviour of hormone-induced 'males' of *P. formosa*? Do masculinized gynogens show behaviour patterns of the presumed parental species, especially the elaborate male courtship display of *P. latipinna*? Do males of the laboratory produced hybrid of *P. mexicana* and *P. latipinna* behave more like masculinized gynogens or their gonochoristic parents?

In preliminary experiments we tried to evaluate effects of masculinized gynogens and factors responsible for the observed masculinization.

TABLE 1. Reports of masculinized gynogens of *Poecilia formosa*

Authors	Year	N	Gonad	Behaviour	Offspring	Origin	Remarks
HUBBS & HUBBS	1932	—	—	—	—	Rio Papaloapan, Mexico	suspected hybrid
HUBBS <i>et al.</i>	1959	1	male	—	—	Brownsville, Texas	collected dead
HASKINS <i>et al.</i>	1960	1	—	—	yes +	Brownsville, Texas	probably a <i>P. sphenops</i> male
KALLMAN	1962	2	—	sexual	no	San Marcos River, Texas	introduced population originating from Brownsville
HUBBS	1964	31*	male/ female	some: sexual	no	Brownsville and San Marcos River, Texas	
DARNELL & ABRAMOFF	1968	2	male or —	—	1 yes + 1 no	Rio Tamesi and Rio Guayalejo, Mexico	born in the laboratory
RASCH <i>et al.</i>	1970	2	not definitive	courting	no	Olimito, Texas	suspected hybrid

\* including HUBBS *et al.*, HASKINS *et al.*, KALLMAN.+ with *P. formosa* females.

— not reported.

## Material and methods

### General housing conditions.

The fish were kept in large tanks at a constant temperature of 25-27°C in a 12:12 light/dark cycle with a salinity of approx. 5‰. *P. formosa* were bred with ornamental black molly males in order to detect any transmission of male genes by black spotting of the offspring (HASKINS *et al.*, 1960; SCHULTZ & KALLMAN, 1968; BALSANO *et al.*, 1989; RASCH & BALSANO, 1989). Fish were fed with commercially available dry food twice a day. *Tubifex* and Cladocerans were added depending on the season. Young fish were reared with brine shrimp nauplia. The origin of the stocks was described by SCHLUPP *et al.* (1991).

### Test animals.

Five types of males were examined (Table 2). To distinguish them we use the following terms: masculinized gynogens are spontaneously occurring *P. formosa* females with male-like behaviour and habitus. Hormone-males are *P. formosa* gynogens treated with testosterone. F<sub>1</sub>-males originate from acrossing experiment with *P. latipinna* and *P. mexicana*. *P. latipinna* and *P. mexicana* males originate from gonochoristic species. While we distinguished 5 types of males, the females tested with masculinized gynogens and hormone-males were of the same type (*P. formosa*), so that only 4 groups go into statistical analysis.

TABLE 2. Combinations used in the behavioural tests

male	female	N
Masculinized gynogen ( <i>P. formosa</i> )	gynogen ( <i>P. formosa</i> )	27
Hormone-male ( <i>P. formosa</i> )	gynogen ( <i>P. formosa</i> )	9
<i>P. mexicana</i>	<i>P. mexicana</i>	11
<i>P. latipinna</i>	<i>P. latipinna</i>	8
F <sub>1</sub> -hybrid	F <sub>1</sub> -hybrid	6

N = number of fish used.

### Behavioural tests.

For the behavioural tests one male and one female fish were chosen randomly from a stock tank and placed into the two equal sized compartments of a 25 l aquarium. The compartments were separated by opaque partitions. After acclimatisation for 24 h the opaque partition was removed and the interactions of the two fish were recorded using an event-recorder for 15 minutes. All pairs were tested twice in an experiment.

Three types of behaviour were recorded for all types of males. 'Following' was defined as every approach and staying in close contact with a female, mostly approaching the female from behind and often followed by 'Nipping', defined as contact of the males mouth region with the females genital region. 'Copulation attempts' or copulatory movements are all male attempts to thrust its gonopodium into the female genital opening. A related element of sexual behaviour is 'Gonopodial thrusting', a copulation like movement of the gonopodium, which was not recorded because it was too infrequent. An elaborate 'Courtship display', performed in front of the female, is only known from *P. latipinna* (PARZEFALL, 1969; see Fig. 1 in SCHLUPP *et al.*, 1991).

All types of females can respond to every male behaviour by accepting the male and 'Remaining motionless' or reject the male aggressively ('Rejection') or flee from the male

('Flight'). The first type of behaviour indicates sexual cooperation, while the other two types are common in situations in which the female is not willing to copulate. Males show all kinds of sexual behaviour towards females all through the female sexual cycle (PARZEFALL, 1973). The activity level, however, is much higher, if female are in attractive status. Therefore, females were in non-attractive status (PARZEFALL, 1973) so that scores were not influenced by female receptivity.

#### *Hormonal masculinization.*

To obtain experimentally induced phenotypic males, juvenile *P. formosa* of a truly all-female clone, in which we never observed masculinized gynogens, were exposed to 1 mg methyltestosterone per liter aquarium water for 24 h once a week (DZWILLO, 1962). After 4-6 treatments the fish had developed a gonopodium and clearly changed into 'phenotypic males'. To distinguish the spontaneously occurring masculinized gynogens, we call them hormone-males.

#### *DNA-fingerprinting.*

For DNA-fingerprint analysis DNA was extracted from pooled organs of single adult *P. formosa* gynogens as described (SCHARTL, 1988). For analysis of DNA fingerprints, in gel hybridization of restriction enzyme (Hae III and Hinf I) digested genomic DNA to radioactively labelled oligonucleotide probes was performed as described (NANDA *et al.*, 1990). The following probes were used under stringent hybridisation and washing conditions: (GGAT)<sub>4</sub>, (GACA)<sub>4</sub>, (GATA)<sub>4</sub>, (GAA)<sub>6</sub> and (CA)<sub>8</sub>.

#### *Research of inducing factors.*

Since the masculinizing effect of testosterone is known (see above), we wanted to find out whether free testosterone was present in the stock tanks in which the spontaneous masculinization occurred. To monitor presence of androgenic steroids females of *Girardinus metallicus*, homozygous for the Vn-gene (Ventral nigra), were added to the stock tanks. This was done twice. If androgenic steroids above a concentration level of 10<sup>-9</sup>M are present in the water, these females develop the male-specific Vn-macromelanophore pattern as early as 5-7 days after introduction (SCHARTL *et al.*, 1982).

#### *Research of possible effects.*

To test whether masculinized gynogens were able to induce embryogenesis in normal gynogens, one masculinized gynogen was kept in a 25 l aquarium together with three normal gynogens (N=4). In a second experiment the masculinized gynogen was replaced by a non-functional black molly male, the gonopodium of which had been removed. This made sperm transfer impossible, but did not hinder other sexual behaviour (N=2). As a control we used one black molly male together with three normal gynogens (N=2).

#### *Statistical analysis.*

For statistical analysis we used the non-parametric Kruskal-Wallis one-way ANOVA (H-test) to state whether the observed variance between all groups could be attributed to one statistical population. If H<sub>0</sub> was rejected we used k-sample pairwise tests (after CONNOVER in BORTZ *et al.*, 1990) to compare differences between groups. Female reactivity was expressed as percent of total activity of males for each type of reaction, because absolute numbers depend on the number of actions directed at the female.

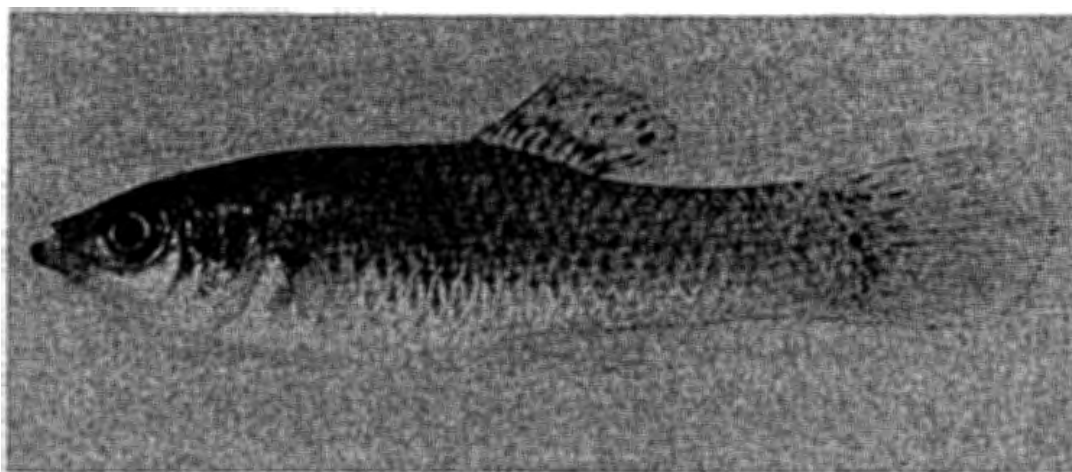


Fig. 1. Spontaneously masculinized gynogen of *Poecilia formosa*.

## Results

### External morphology of masculinized gynogens.

The external morphology of masculinized gynogens resembled the typical appearance of males of the closely related species of shortfin mollies of the subgenus *Mollienesia* (*sensu* MILLER, 1983) (Fig. 1) as well as hormonally induced males of *P. formosa* (SCHARTL *et al.*, 1991). The slender body shape and more intense colouration is also typical for males of *Mollienesia*. A gonopodium was always present, but was sometimes incompletely developed (Fig. 2a, b). Those gonopodia resembled the transforming anal fin of immature males of *Mollienesia*. In the most completely developed gonopodia, only the extent of the gonopodial palp on ray 3 differed from that of *P. mexicana*, *P. latipinna* and F<sub>1</sub>-males (Fig. 2c). For comparison Fig. 2d shows a typical female anal fin.

### Behaviour of masculinized gynogens and males.

Masculinized gynogens of *P. formosa* displayed several behaviour patterns that are otherwise known only from males. In an all-female form this finding is surprising, because the genes coding for male traits are obviously dispensable. 'Following' and 'Nipping' were present in a way typical for both *P. mexicana* and *P. latipinna* males. 'Gonopodial thrusting' was absent and 'Copulation attempts' were rare and incomplete. Apparently the males were not able to move their gonopodium into a forward position. This might be due to lack of the specific muscles.

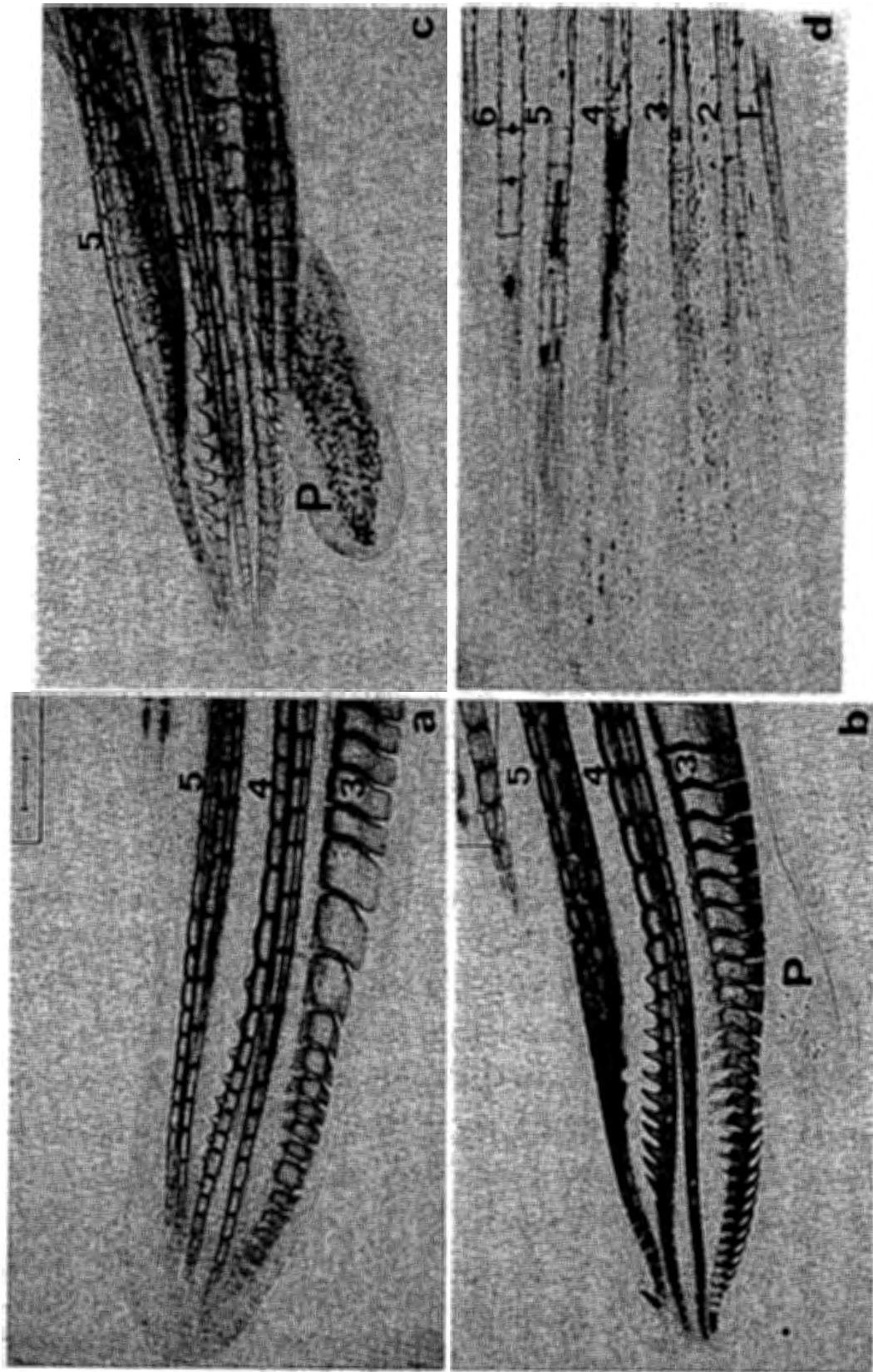


Fig. 2. Gonopodial structures of masculinized gynogens, normal females and males, (a) shows an incompletely, (b) a completely transformed anal fin of a masculinized gynogen, (c) a typical gonopodium of a *P. mexicana* male, and (d) a typical female anal fin of *P. mexicana*. p, gonopodial palp; bar represents 200  $\mu$ m. The numbers refer to the numbers of fin rays.

These types of behaviour are never shown by gonochoristic females and normal gynogens. We classify this as pseudosexual behaviour, *i.e.* male-like behaviour, shown by individuals that are otherwise female (CREWS, 1988). This definition does not include heterotypical behaviour, *i.e.* behaviour usually displayed by the opposite sex, like mounting of female guinea pigs (Goy & Roy, 1991). Heterotypical behaviour is confined to gonochoristic species.

Courtship display as known from *P. latipinna* was absent in masculinized gynogens. This was also true for F<sub>1</sub>-males, although their father was a *P. latipinna* male. Occasionally occurring postures in front of the female shown by masculinized gynogens that resembled an incomplete courtship display could not be distinguished from aggressive display.

The only novel behaviour we observed in masculinized gynogens was a slow crossing underneath the female with fin contact. We never observed this in *P. mexicana* or *P. latipinna*. The function of this remains unknown.

#### Statistical analysis.

Mean and median values for all types of male behaviour were always higher in *P. mexicana* and *P. latipinna*, than in masculinized gynogens or hormone-males (Fig. 3a-c). Masculinized gynogens and hormone-males appeared to be more or less similar. F<sub>1</sub> males have the activity level of gonochoristic males. They were not intermediate in behaviour (Fig. 3a-c).

Using the Kruskal-Wallis H-test we tested for general differences between groups. All results were significant, indicating that the groups were not samples of the same statistical population (Table 3). After this we used k-sample pairwise comparisons to test which groups were different from each other (Fig. 3a-c). Most important is that masculinized gynogens and hormone-males never show statistically significant differences. The same is true for *P. latipinna* and *P. mexicana*. These gonochoristic species are more similar to each other than to masculinized gynogens or hormone-males. No statistically significant differences could be found between F<sub>1</sub>-males and *P. latipinna* and *P. mexicana* males.

Significant differences in 'Following' are only found between F<sub>1</sub>-males and masculinized gynogens and F<sub>1</sub>-males and hormone-males (Fig. 3a). In 'Nipping' and 'Copulation attempts' all pairwise tests including either masculinized gynogens or hormone-males yielded statistically significant results (Fig. 3b, c). This clearly divides the inspected types of males into two groups: masculinized gynogens and hormone-males form one group and the gonochoristic *P. mexicana*, *P. latipinna* and F<sub>1</sub>-males form the other one.



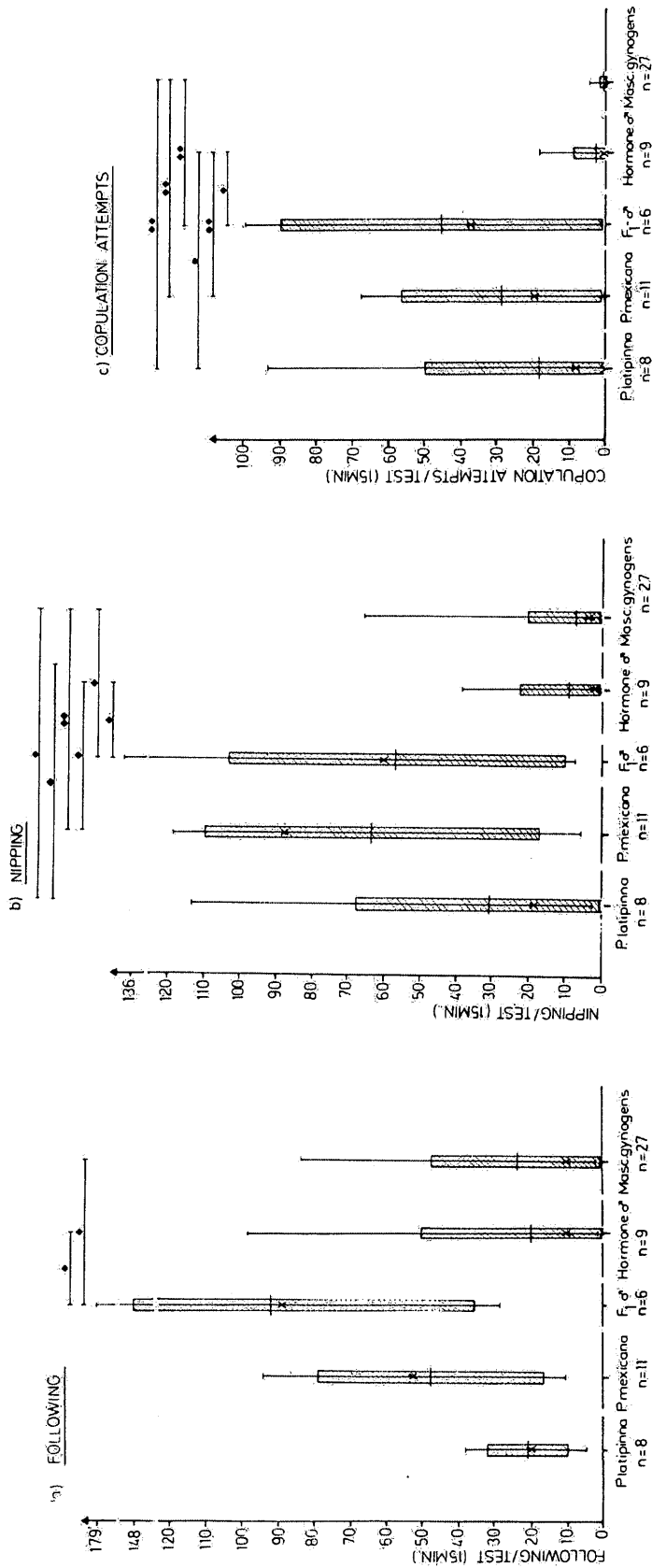


Fig. 3. a: Descriptive statistics for 'Following', showing mean (-), median (x), range (|-|) and SD (hatched). Given is the number of actions per test. b: Descriptive statistics for 'Nipping', showing mean (-), median (x), range (|-|) and SD (hatched). Given is the number of actions per test. c: Descriptive statistics for 'Copulation attempts', showing mean (-), median (x), range (|-|) and SD (hatched). Given is the number of actions per test. One diamond refers to  $p < 0.05$ ; two diamonds are not significant.

TABLE 3. Kruskal-Wallis H - Tests

sex	Behaviour	H-value	df	p-Value	N
male	Following	17.35	4	0.0017	61
male	Nipping	26.12	4	0.0001	61
male	Cop. attempts	36.95	4	0.0001	61
female	Flight	12.38	3	0.0062	59
female	Rejection	10.51	3	0.015	58
female	Remaining	10.36	3	0.016	59

For definitions of behaviour see text. df = degrees of freedom. N = number of fish tested.

### Female behaviour.

Normal gynogens respond to male-like behaviour of masculinized gynogens in a way typical for females of the genus. Because of their dependence on male sperm, gynogens have to be able to recognize males. Gynogens even use an active mating tactic and try to intercept matings of *P. mexicana* (SCHLUPP *et al.*, 1991). Although H-tests indicated significant differences between the groups (Table 3) a pairwise comparison revealed a significant difference only between F<sub>1</sub>-females and *P. mexicana* females in 'Flight' (Fig. 4) and values close to significance in a few more cases. All other comparisons showed no significant differences. Apparently, the H-test provides a less conservative statistical measure than the pairwise comparisons (BORTZ *et al.*, 1990). We used Mann-Whitney U-tests to seek for differences between the female reactions to masculinized gynogens and hormone-males. No significant differences were found ( $z = -1.65, 0.36, 0$  for 'Flight', 'Rejection' and 'Remaining' resp. all  $p > 0.05$ ). All this indicates that normal gynogens recognize masculinized gynogens as 'males'.

### DNA-fingerprinting.

DNA-fingerprinting analysis was performed to investigate whether masculinized gynogens constitute a specific clone different from the phenotypically normal gynogens within our fish population or whether the masculinized gynogens might be aneuploid hybrids with the black molly males and not possessing or not expressing the pigmentation genes. Using two different restriction enzymes for DNA digestion and several different oligonucleotide probes, identical fingerprints were obtained for masculinized gynogens and normal gynogens (Fig. 5).

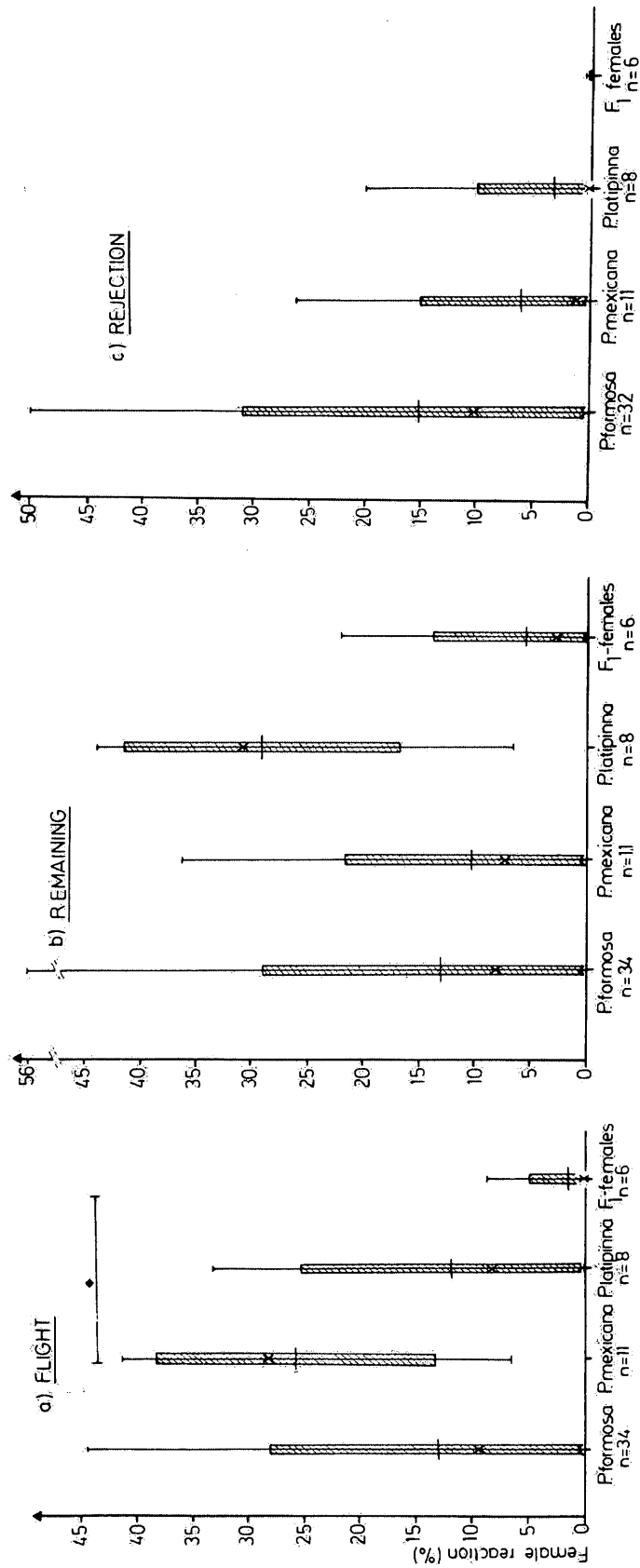


Fig. 4. a: Descriptive statistics for 'Flight', showing mean (-), median (x), range (|-|) and SD (hatched). Given is the percentage of reactions per test. b: Descriptive statistics for 'Remaining', showing mean (-), median (x), range (|-|) and SD (hatched). Given is the percentage of reactions per test. c: Descriptive statistics for 'Rejection', showing mean (-), median (x), range (|-|) and SD (hatched). Given is the percentage of reactions per test. One diamond refers to  $p < 0.05$ . All other differences are not significant.

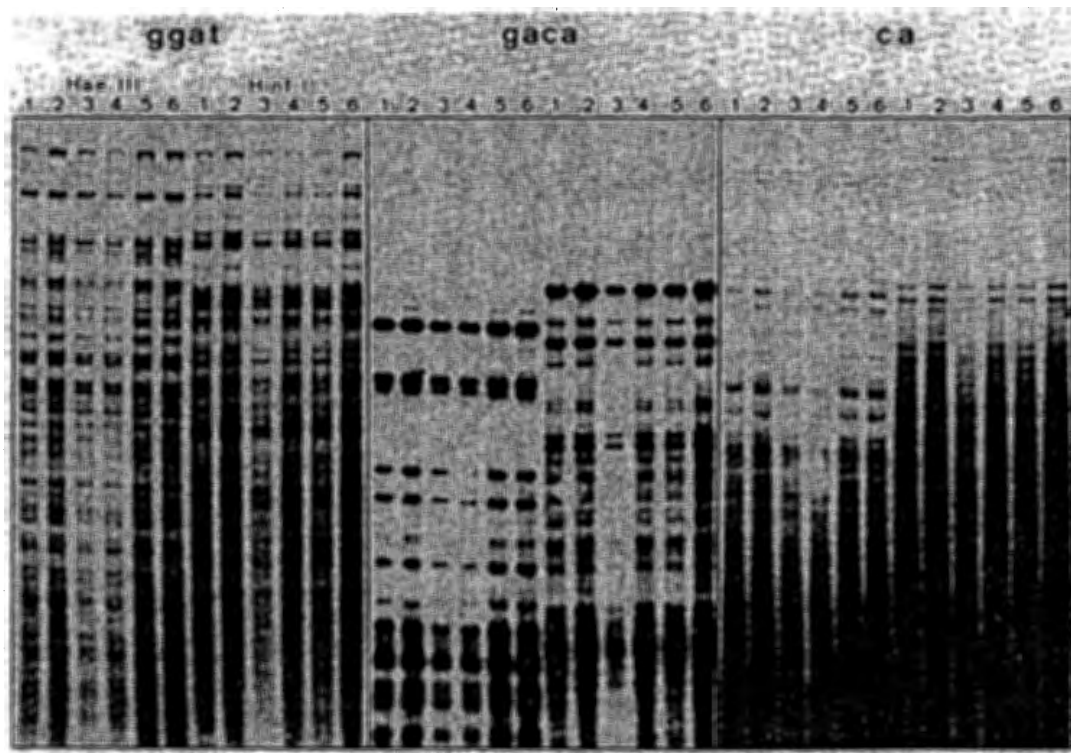


Fig. 5. DNA-fingerprints of masculinized gynogens (1-3) and normal gynogens (4-6) of *Poecilia formosa* obtained with Hae III and Hinf I digested DNA hybridized to the (GGAT)<sub>4</sub>, (GACA)<sub>4</sub> and (CA)<sub>8</sub> probes. The overall identity of the patterns demonstrates the clonal origin and genetic identity of both phenotypic classes. Differences in band intensity are due to loading differences in the DNA amount.

#### Research of inducing factors.

The fact that masculinized gynogens and typical gynogens are members of the same clone indicates that factors as yet unknown are responsible for masculinization. A temperature effect alone, as suggested by HUBBS (1964) is unlikely, because in a second clone of *P. formosa* masculinized gynogens never occurred, although housing conditions were identical. Temperature may, however, interact with other factors. Free testosterone above a concentration of  $10^{-9}$ M in the aquarium water can be excluded. Females of *Girardinus metallicus*, which respond extraordinary sensitively to exogenous androgens, retained the female phenotype when kept in stock tanks with *P. formosa*.

#### Research of possible effects.

In preliminary experiments we tried to evaluate the effects of masculinized gynogens. *P. formosa* gynogens that were kept for six months with

masculinized gynogens did not produce offspring (N=4 replicates). *P. formosa* kept with a male black molly which gonopodium had been clipped did not have offspring either (N=2 replicates), although sexual behaviour was present. Untreated controls *i.e.* black molly males with gonopodium (N=2 replicates) had offspring. This suggests that copulation and sperm is actually needed: the presence of a courting male alone is not sufficient a trigger embryogenesis.

Masculinized gynogens never had offspring during the past two years, although kept in stock tanks with black molly males.

### Discussion

Masculinized gynogens and hormone-males are remarkably similar in behaviour. Clonal identity of masculinized gynogens and normal gynogens, as demonstrated by DNA-fingerprinting, shows that the genes coding for male traits in this all-female fish are remarkably stable and remained functionally intact, as also demonstrated by hormonally induced males (see SCHARTL *et al.*, 1991). Assuming a single hybridisation event that led to *P. formosa* (SCHARTL *et al.*, 1991), the probable age of *P. formosa* is approx. 10000 years (DARNELL & ABRAMOFF, 1968; SCHARTL *et al.*, 1991). This period is by far shorter than the 47 million years calculated by OHNO (1985) as the half time for functionality of dispensable alleles. Both the results on masculinized gynogens and those on testosterone treated individuals are in agreement with OHNO's (1985) findings. Both masculinized gynogens and normal gynogens are members of the same clone, indicating that masculinized gynogens arise due to an as yet unknown stimulus. Free testosterone and temperature alone are unlikely to cause the masculinization. Masculinized gynogens furthermore demonstrate that several male traits can be expressed in an all-female species without experimental manipulation.

The sexual behaviour of masculinized gynogens comprises the typical elements of male behaviour of the parental species. Copulatory behaviour is modified, apparently because of the inability to move the gonopodium. This may be due to the lack of a muscle that moves the gonopodium sideways.

Using mtDNA analysis AVISE *et al.* (1991) demonstrated that *P. mexicana* is likely to be the female and *P. latipinna* to be the male ancestor of *P. formosa*. Courtship display as known from *P. latipinna* is missing in masculinized gynogens, hormone-males and F<sub>1</sub>-males. This does not necessarily mean that these males are unable to show this behaviour. It is

possible that larger males would court. In *P. latipinna* it depends on a male's relative size within a population whether a male courts or not, and only the largest do so (PARZEFALL, 1969; FARR *et al.*, 1986; TRAVIS & WOODWARD, 1989). On the other hand, it is possible that the genetic information for courtship display is lacking. In hybrids of *P. mexicana* and *Poecilia velifera*, a species closely related to *P. latipinna*, courtship was present in nearly all F<sub>1</sub>-males (PARZEFALL, 1989).

The F<sub>1</sub>-hybrids we used were not intermediate in the examined behaviour patterns. Meristic characteristics, however, are intermediate (SCHARTL *et al.*, 1991). They were clearly gonochoristic and statistically not different from the parental species. All reported attempts to produce a gynogenetic strain by crossing *P. latipinna* and *P. mexicana* (rev. TURNER, 1982) resulted in such gonochoristic hybrids.

Individuals of *P. formosa* that appeared to be males have occasionally been reported previously (Table 1). Only two such individuals proved to be able to trigger embryogenesis in *P. formosa* females and 'fathered' some offspring. One of these males was probably a male of the gonochoristic species *Poecilia sphenops* (see HASKINS *et al.*, 1960; HUBBS, 1964), so that only one case of an apparently functional male (DARNELL & ABRAMOFF, 1968) appears to be reliable. This indicates, however, that masculinized gynogens may occur in nature and play a yet unknown role in the breeding complexes related to *P. formosa*.

In our experiments masculinized gynogens failed to induce embryogenesis in normal gynogens. Testosterone treatment of *P. formosa*, however, was able to change the gonadal sex from female to male (TURNER & STEEVES, 1989; SCHARTL *et al.*, 1991). These findings, together with the findings mentioned above, indicate that there is at least the possibility that functional males might occur, perhaps leading to a new gonochoristic species, as reported in the genus *Poeciliopsis* by VRIJENHOEK (1989).

For several reasons parthenogenesis is thought to be disadvantageous in the long run (see MAYNARD SMITH, 1982; BELL, 1982; TRIVERS, 1985), so that the evolution of functional males might be a promising way out of the ecological constraints a unisexual species has to tackle. This, however, has not yet happened and it remains an open question whether the reported pseudomale behaviour is adaptive or not and what the underlying mechanisms might be.

Pseudosexual behaviour (*sensu* CREWS, 1982, 1987, 1988, 1989) is a rare phenomenon associated with unisexuality in vertebrates. Its evolutionary origin and adaptive significance has been demonstrated in the parthenogenetic reptiles *Cnemidophorus uniparens* (see CREWS, 1989) and *Lep-*

*idodactylus lugubris* (WERNER, 1980; ZAWORSKI, 1987). Masculinized gynogens of *P. formosa* not only exhibit male-like behaviour but also male secondary sexual characteristics in body shape, colouration and, most important, the complicated copulation apparatus, the gonopodium (Figs. 1, 2). As far as we know, *P. formosa* is the first unisexual fish species showing this type of behaviour. *P. formosa* also is the first gynogenetic species showing pseudosexual behaviour. Up to now, however, neither evolutionary origin nor adaptive significance is clear in *P. formosa*.

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### Zusammenfassung

Pseudosexualverhalten ist ein seltenes Phänomen. Es kommt bei unisexuellen Vertebraten vor. Bei der gynogenetischen Weibchenart *Poecilia formosa* treten gelegentlich Individuen auf, deren Habitus und Verhalten demjenigen von Männchen nahe verwandter gonochoristischer Arten entspricht. Mittels genetischem Fingerabdruck läßt sich zeigen, daß diese Pseudomännchen aus demselben Klon wie normale *P. formosa* stammen. Das Verhalten dieser Pseudomännchen wird beschrieben und mit dem von Männchen der Arten *P. mexicana*, *P. latipinna*, deren Hybriden und durch Androgenbehandlung vermännlichter Individuen von *P. formosa* verglichen. Zwischen den Pseudomännchen und den androgenbehandelten Tieren bestanden keine statistisch signifikanten Unterschiede. Auch zwischen den gonochoristischen *P. mexicana* und *P. latipinna* bestanden keine statistisch signifikanten Unterschiede, während zwischen unisexuellen und gonochoristischen Arten Unterschiede bestehen. Mögliche Ursachen und Effekte der Vermännlichung werden diskutiert.

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