

## Coercive mating and gonopodium length in two populations of a livebearing toothcarp (*Poecilia mexicana*): do cave molly males have shorter gonopodia?

Erzwungene Kopulationen und Gonopodienlänge bei einem Lebendgebärenden Zahnkarpfen (*Poecilia mexicana*): haben Höhlenmolly-Männchen kürzere Gonopodien?

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**Zusammenfassung:** Wir haben die kürzlich aufgestellte Frage verfolgt, ob durch die Paarungsunwilligkeit der Weibchen und durch das daraus resultierende erzwungene Paarungsverhalten der Männchen bei Lebendgebärenden Zahnkarpfen (Poeciliidae) Selektion in Hinblick auf längere Gonopodien erfolgt. Höhlenbewohnende *Poecilia mexicana* zeigen eine genetische Reduktion von (erzwungenem) männlichem Paarungsverhalten und könnten daher kürzere Gonopodien als oberirdische *P. mexicana* haben; es wurde jedoch kein statistisch signifikanter Unterschied entdeckt.

Male livebearing toothcarps (Poeciliidae) use their gonopodium, a modified anal fin, to transfer sperm bundles (spermatozeugmata) into the female's gonoduct. There is a wide range of variation in the patterns of male behavior preceding and accompanying copulations in poeciliid fishes. Males of some species, such as the guppy (*Poecilia reticulata*) may court females before they attempt to insert their gonopodium (HOUDE 1997), whereas males of other species never court, and the latter completely rely on coercive mating attempts ("sneak copulations", BISAZZA 1993; *Poecilia mexicana*: PLATH et al. 2003). Poeciliid females litter their broods in an approximately monthly cycle and females can store sperm for several months (CONSTANTZ 1989). Therefore, poeciliid females require only few copulations every few months. However, in many species males almost constantly attempt to mate, indicating a strong inter-sexual conflict over the optimal number of copulations (MAGURRAN 2001). This conflict is assumed to be costly for females. For example, *P. mexicana* females have less time available for feeding in

the presence of a male than in the presence of a female because they need to dedicate attention to avoiding male sexual harassment (PLATH et al. 2003).

The length of the gonopodium is highly variable across species ranging from less than 20% to more than 70% of a male's body length (ROSEN & GORDON 1953, GHEDOTTI 2000). Several evolutionary mechanisms have been hypothesized to affect gonopodium lengths in poeciliid fishes. (i) Across species comparisons revealed that males of species with a coercive mating system typically have relatively longer gonopodia than males of species with a less coercive system (ROSEN & TUCKER 1961). The most plausible explanation is that mating with a female that does not cooperate requires that the male follows the escaping female while trying to insert his gonopodium. Thus, sexual selection may favor longer gonopodia that are more efficient to transfer sperm. (ii) Female *Gambusia affinis* have been shown to prefer males with a larger relative gonopodium length (LANGERHANS et al. 2005). Sexual selection through fe-

male mate choice may thus select for longer gonopodia in males. (iii) Natural selection through predation by fish can counteract sexual selection and select for shorter gonopodia. Relatively large gonopodia have been shown to reduce the burst-swimming speed in *G. affinis* (LANGERHANS et al. 2005) imposing a direct cost on the character state favored by sexual selection.

The different selection pressures lead to apparently contradicting patterns observed in natural populations. In *G. affinis* and *G. hubbsi*, both of which rely completely on coercive mating strategies, males exhibited longer gonopodia in predator free environments (LANGERHANS et al. 2005). In the guppy (*Poecilia reticulata*) and *Brachyrhaphis episcopi*, both of which exhibit courtship displays as well as coercive mating, populations from low-predation sites have relatively shorter gonopodia compared to populations from habitats harboring more piscine predators (KELLY et al. 2000; JENNIONS & KELLY 2002). In the guppy, it was shown that males use more coercive mating under high predation, probably because courtship is conspicuous to predators (ENDLER 1987).

In the present study, we examined gonopodium lengths in the Atlantic molly, *Poecilia mexicana*. This species is widely distributed in freshwaters along the Central American Atlantic coast (MILLER 2005). A unique population of *P. mexicana* has colonized a sulfidic subterranean ecosystem, the Cueva del Azufre in Tabasco, southern Mexico (the cave molly, PARZEFALL 2001, PLATH 2003). Unlike males of typical surface populations, cave molly males have genetically reduced aggressive behavior (PARZEFALL 2001) as well as coercive mating behavior (PLATH 2003). When cave molly females were allowed to feed in the presence of a male or another female, no reduction in feeding times by male harassment was detected (PLATH 2003). These reduction processes are likely adaptations to the extreme environmental conditions in the Cueva del Azufre, namely darkness, the presence of toxic hydrogen sulfide and hypoxia, in order to save energy (TOBLER et al. 2006).

We asked whether differences in mating systems relate to different gonopodium lengths

across populations. We predicted males from the surface to have longer gonopodia, because coercive mating is much more frequent. We also predicted the absolute gonopodium length to increase with increasing body size (KELLY et al. 2000, JENNIONS & KELLY 2002); hence, male standard length was included as a covariate in our analyses.

Another question we pursued was how the relative length of male gonopodia correlates with male body size. As is typical for poeciliids, small males are rejected as mating partners by females in this species (PLATH et al. 2004). The same is true for cave mollies, where females use non-visual cues to assess male size and prefer to associate with large males (PLATH et al. 2004). Small surface-dwelling *P. mexicana* males attempt to compensate this disadvantage by showing considerably higher numbers of coercive mating attempts than large ones (PLATH et al. 2003). Again, this pattern breaks down in the cave form in which large males are sexually more active than small males (PLATH et al. 2003). We predicted small (surface-dwelling) males to invest *relatively* more in gonopodium development, leading to a hypo-allometric relation between body size and gonopodium length. In summary, the *relative* gonopodium length should be longer in small males in the surface form, whereas this correlation might be less pronounced in cave molly males.

The surface-dwelling mollies used in this study originated from randomly outbred large stocks that were derived from animals collected in 1998 in the Río Oxolotan, near Tapijulapa, Tabasco, Mexico. For the cave form we used a stock originating from the innermost chamber of the nearby Cueva del Azufre (chamber XIII after GORDON & ROSEN 1962), first collected in 1970 and repeatedly refreshed in 1975, 1982 and 1996. Data were collected in 2004. Stocks were maintained in tanks of 50-200 l at the Zoological Institute and Museum of the University of Hamburg. The surface form was reared under artificial daylight with a 16 : 8 h light/dark cycle; cave mollies were kept in a dark room. Temperature ranged between 25-30 °C. Fish were fed *ad libitum* twice a day with

commercially available flake food, *Artemia* naupliae, water fleas and *Tubifex* worms. Males were anesthetized using MS222 and were then stored in 70% Alcohol until data collection. We measured the standard length (SL) and the gonopodium length (from the base on the abdomen to the distal tip of the hook, KELLY et al. 2000) of  $n=67$  males from the surface form, and  $n=34$  cave molly males to the nearest 0.1 mm using a caliper gage.

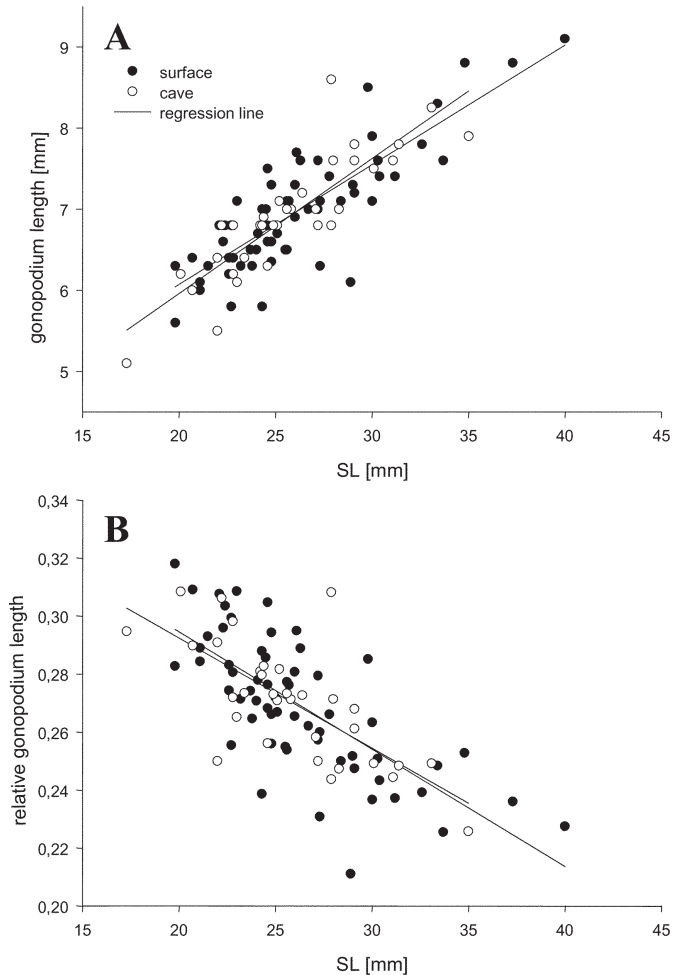
We employed one-way-ANCOVA to analyze the data, where 'standard length' [mm] was the covariate, and 'population' was a separating factor. In one analysis, absolute gonopodium length was the dependent variable; in another analysis, the relative gonopodium length (gonopo-

dium length/standard length) was analyzed. The interaction terms were not significant in both cases (absolute length:  $F_{1,97}=0.79$ ,  $P=0.38$ ; relative length:  $F_{1,97}=0.084$ ,  $P=0.77$ ), indicating that the linear regressions for the two populations did not show slope heterogeneity. Hence, only the main effects were analyzed.

The covariate (body size) had a significant effect on the absolute gonopodium length, which increased with increasing standard length ( $F_{1,98}=262.73$ ,  $P<0.0001$ ; fig. 1A). The two populations did not differ in gonopodium lengths ( $F_{1,98}=0.00028$ ,  $P=0.99$ ). Likewise, standard length had a significant effect on the relative gonopodium length ( $F_{1,98}=117.40$ ,  $P<0.0001$ ; fig. 1B), which decreased with in-

**Figs. 1 A and B:** **A** Absolute gonopodium length and **B** relative gonopodium length (gonopodium length/SL) of surface-dwelling (black circles) and cave-dwelling *P. mexicana* males (white circles). Lines represent linear regression lines (absolute length, surface:  $y = 3.12 + 0.15 x$ , cave:  $y = 2.63 + 0.17 x$ ; relative length, surface:  $y = 0.38 - 0.0041 x$ , cave:  $y = 0.37 - 0.0038 x$ ).

**Abb. 1 A und B:** **A** Absolute Gonopodienlänge und **B** relative Gonopodienlänge (Gonopodienlänge/Standardlänge) von oberirdischen (schwarze Symbole) und höhlenbewohnenden *P. mexicana*-Männchen (weiße Symbole). Linien stellen lineare Regressionen dar (absolute Länge, oberirdisch:  $y = 3.12 + 0.15 x$ , Höhle:  $y = 2.63 + 0.17 x$ ; relative Länge, oberirdisch:  $y = 0.38 - 0.0041 x$ , Höhle:  $y = 0.37 - 0.0038 x$ ).



creasing body size. Again, the two populations did not differ significantly ( $F_{1,98}=0.043, P=0.84$ ).

We did not find the predicted pattern of longer gonopodia in the surface form of *P. mexicana* compared to the cave form. This contrasts with previous results from other poeciliid species. Longer gonopodia have been found in habitats with high predation pressure in Trinidadian guppies (*Poecilia reticulata*, KELLY et al. 2000) and Panamanian *Brachyrhaphis episcopi* (JENNIONS & KELLY 2002). High-predation regimes are known to favor coercive (sneak-) mating in guppies (ENDLER 1987, MAGURRAN & SEGHERS 1994), and differences in gonopodia lengths have been interpreted as a consequence of more coercive mating in this species (KELLY et al. 2000). However, the opposite pattern was found in two species of mosquitofish (*Gambusia affinis*, *G. hubbsi*), where male genitalia were even shorter in high-predation populations (LANGERHANS et al. 2005). In the latter two species, female mate choice for large gonopodia appears to promote elongated gonopodia, whereas a reduced escape ability (i.e. a worse swimming performance) of males with long gonopodia may select against elongated genitalia under predation (LANGERHANS et al. 2005).

Several interacting selective forces might explain the strikingly equal gonopodium sizes in surface- and cave-dwelling *P. mexicana*. (i) coercive mating is common in the surface form, but is clearly reduced in the cave form (PLATH et al. 2003), which should favor longer gonopodia in surface populations. (ii) Potentially, sexual selection for longer gonopodia by female choice (LANGERHANS et al. 2005) might act in the cave and might balance selection for shorter gonopodia arising from the absence of coercive mating. However, female mate choice for large genitalia in darkness seems unlikely, because the gonopodium is attached to the body most of the time, and is unlikely to be detected by females via mechanosensory (water-pressure-wave) cues. Nevertheless, this topic needs to be examined in the future. (iii) Selection through predation of males with long gonopodia may counteract the evolution of longer gonopodia in surface habitats. Contrary to the

cave, in which piscine predators are absent, surface habitats harbor various predatory fish species (TOBLER et al. 2006). (iv) Alternatively, not enough time may have elapsed since the colonization of the cave for the evolution of shorter gonopodia.

On the basis of the present data, it is impossible to decide which evolutionary scenario has led to unchanged gonopodium lengths in the cave molly, but our data clearly indicate that the mere absence of coercive mating does not necessarily promote shorter gonopodia in poeciliid fishes.

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