BRIEF COMMUNICATIONS

Limia vittata as host species for the Amazon molly: no evidence for sexual reproduction

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DNA fingerprinting demonstrated no sexual reproduction of the gynogenetic Amazon molly Poecilia formosa with Limia vittata as host species, in contrast with a single report that claims to have found sexual reproduction with such matings.

Key words: Poecilia formosa; Limia vittata; DNA fingerprinting; clonal reproduction.

The Amazon molly Poecilia formosa (Girard, 1859), became widely known because of its asexual, gynogenetic reproductive mode (Turner, 1982). Only a single case of a seemingly sexual reproduction was reported (Haskins et al., 1960). In nature P. formosa forms breeding complexes with the closely related species P. latipinna (Lesueur, 1821) or P. mexicana (Steindachner, 1863). Males of these species serve as sperm donors for the unisexual Amazon mollies (Hubbs & Hubbs, 1932). In the laboratory P. formosa may be bred with more distantly related species, like Limia vittata (Guichenot, 1853) (Kallman, 1964; Schultz & Kallman, 1968).

In 1960 Haskins et al., described a single case of sexual reproduction of P. formosa. The authors reported that crosses of males of a certain, heavily spotted stock of L. vittata with P. formosa led to L. vittata-like F₁ offspring. This F₁ was fully fertile and the F₂ was also L. vittata-like. Crosses of F₁ males with P. formosa led to mixed broods, including both P. formosa-like and L. vittata-like offspring, the latter also of both sexes. In contrast to the predictions from mendelian genetics and experimentally produced F₁ hybrids of other Poecilia species (Schlupp et al., 1992) offspring were not of intermediate phenotype. Ploidy was not determined. Backcrosses of L. vittata-like F₁ males with P. formosa resulted in mixed broods, including both L. vittata-like and P. formosa-like phenotypes. This report on a seemingly sexual—and definitely not gynogenetic—reproduction, however, remained a remarkable exception. All other published data confirm the genetic view that reproduction of P. formosa is never truly sexual, although the incorporation of minute, subgenomic amounts of host DNA into the P. formosa genome has been demonstrated recently (Schartl et al., 1995).

The possible importance of these results with L. vittata is obvious: if P. formosa were able to switch to sexual reproduction and/or control the conditions under which such a shift would happen, this would be a unique case among vertebrates. If this would be some form of cyclical event, both the advantages of clonal and recombinational reproduction would be combined. Alternatively it could lead to the creation of a sexual form with unisexual ancestry. If confirmed as a general phenomenon in the reproductive biology of P. formosa, these results would mean an extraordinary expansion of the
DNA-fingerprints from Hinf I digested DNA of two different P. formosa strains (P. formosa I and II) and two siblings (1 and 2) from a cross of P. formosa II with L. vittata, probed with (GGAT)$_4$. Overall identity of the pattern of P. formosa II and the offspring demonstrates the clonality of the fish. Differences in lane intensities are due to differences in the amount of loaded DNA. DNA was extracted from pooled organs (liver, gonad, brain and gills) of single fish and further processed essentially as described (Nanda et al., 1990).
evolutionary potential of *P. formosa*, being able to shift from asexual to sexual reproduction. To the best of our knowledge, this puzzling result has never been reinvestigated systematically.

We have repeated the original experiment of Haskins et al. (1960) and employed DNA fingerprinting to detect non-clonal reproduction readily. We used a genetically and molecularly well defined clone of *P. formosa* (Schartl et al., 1991). This clone originates from a collection by the Drs Haskins in 1953. The *L. vittata* stock we used was heavily spotted as was the stock used by Haskins et al. (1960).

A total of seven replicates with one *L. vittata* male and up to five virgin *P. formosa* each were conducted. In four experiments after 4 weeks without obtaining offspring the male was exchanged and after 4 more weeks without breeding success the trial was terminated. Three replicates were set up as long-term experiments. In these experiments, fish that died during the trial were replaced immediately. A total of 11 males was used. Finally after more than 8 months a single brood of four fish was obtained in one of the long-term experiments.

None of the offspring resembled *L. vittata*; all of them were clearly of the *P. formosa* phenotype. DNA-fingerprinting of two offspring confirmed the clonal mode of inheritance. The banding pattern was identical to that of the maternal *P. formosa* clone (Fig. 1). Additionally we bred the remaining two offspring with genetically marked (black molly: a commercially bred *Poecilia* hybrid) males to detect any long term alterations that would lead to deviations from clonality or male contribution to the offspring. The young resulting from this experiment were also of the *P. formosa* type, as was their DNA-fingerprint pattern. As with their aunt’s genomes, we could find no deviation from the typical clonal transmission of genetic traits known from *P. formosa* and definitely no evidence for sexual reproduction.

In contrast to Kallman (1964) and Schultz & Kallman (1968) who bred large numbers of *P. formosa* with *L. vittata*, we found it difficult to propagate *P. formosa* with *L. vittata* as host species. One reason for that might have been that they used a different strain of *L. vittata*.

Neither Kallman (1964), nor Schultz & Kallman (1968) reported truly sexual reproduction of *P. formosa* with *L. vittata*. Haskins et al. (1960) also failed to repeat their experiment with a different strain of *L. vittata*. All this is in agreement with our findings. In summary this leads to the conclusion that the original results of Haskins et al. (1960) are not unequivocal.

Due to the limited sample size available to use, our findings do not completely rule out occasional sexual reproduction. It is possible that Haskins et al. (1960) used a unique combination of stocks and the experiment cannot be repeated for that reason. Testing this with more stocks and more individuals is open to future investigation. Whatever the explanation for their unusual result may be, it appears to be not significant for the reproductive biology and evolution of *P. formosa*.

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**References**


