Mating success of subordinate males in a poeciliid fish species, *Limia perugiae*

Manfred Schartl[1], Claudia Erbelding-Denk[2], Indrajit Nanda[3], Michael Schmid[3], Johannes Horst Schröder[2], Jörg T. Epplen[4].

[1] Genzentrum, Max-Planck-Institut für Biochemie, Am Klopferspitz 18 a, W-8033 Martinsried, F.R.G.
[2] Institut für Strahlenbiologie, Gesellschaft für Strahlen- und Umweltforschung, Ingolstädter Landstraße 1, W-8042 Neuherberg, F.R.G.
[3] Institut für Humangenetik der Universität, Koellikerstraße 2, W-8700 Würzburg, F.R.G.
[4] Max-Planck-Institut für Psychiatrie, Am Klopferspitz 18 a, W-8033 Martinsried, F.R.G.

Hierarchical structures among male individuals in a population are frequently reflected in differences in aggressive and reproductive behaviour and access to the females. In general social dominance requires large investments which in turn then may have to be compensated for by high reproductive success. However this hypothesis has so far only been tested in small mating groups (1-2 males with 1-2 females) due to the difficulties of determining paternity by conventional methods. DNA fingerprinting overcomes these problems offering the possibility to determine genetic relationships and mating patterns within larger groups. This approach allows a broad spectrum of ecological and evolutionary biological questions to be examined (Burke 1989). We show here that in the poeciliid fish *Limia perugiae* in small mating groups the dominant male has a mating success of 100%, while in larger groups its contribution to the offspring unexpectedly drops to zero.

In the livebearing poeciliid fish many species are polymorphic for male body size (Hughes 1985). Large males outcompete smaller ones and become dominant in the social structure of a given group (see Farr 1989). Using phenotypic markers in progeny tests of two females with one large and one small male the dominant large male was found to be rewarded by a greater reproductive success (Zimmerer and Kallman 1989). This is in agreement with considerations that in the natural environment alternative mating tactics exist as an evolutionarily stable strategy (Maynard Smith 1981).

Here, we use *Limia perugiae*, a poeciliid fish endemic to the southeast of the Caribbean island Hispaniola, to study mating patterns in relation to mating group size by DNA fingerprinting. Simple repeat oligonucleotides represent useful tools to study genetic relationships within nearly all species tested at all levels of eukaryotic organismic evolution (Epplen et al. 1991). Evaluating different restriction enzyme/probe combinations on Hinf I digested DNA the $(GGAT)_4$ probe was found to be the most informative with respect to individualization in *L. perugiae* even in closely inbred stocks. Additional information was obtained by rehybridization of the same gel with $(GACA)_4$, $(GAA)_8$ and $(CA)_8$ probes (see figure).

In a first series of experiments one large and one small male were tested with two females. Offspring from two different broods in two independent experiments were tested for paternity. In the first case of 14 animals, 13 were attributable to the large dominant male and one was of uncertain paternity. In the second case all 12 fish tested were unequivocally offspring of the α-male.

In the second set of experiments four males ranging in size from 25 mm to 45 mm were assembled with four or five juvenile, virgin females. Within two days the male fish established a size-dependent ranking that remained stable throughout the duration of the experiment. Neonates were taken out of the aquarium immediately after birth and raised separately. DNA fingerprinting of a representative number of offspring revealed that all females had contributed to the offspring generation. In the first mating group all offspring tested were from one of the subordinate males. In the second group paternity was assigned to the β and γ male. In no case the

smallest and most subordinate male had offspring. This is in agreement with its exclusively defensive behaviour. Surprisingly, we could not identify progeny of the dominant male (Table 1). This observation is in direct contradiction to the expected results and the behavioural data reported so far. However, the only component of fitness that has been possible to monitor is the number of observed matings or mating attempts by each male, but even this may be misleading as the number of successful fertilizations may be substantially different. After analyzing the genetic relationships of the progeny with the males it became apparent that intermediate size males, that exhibit only little courtship behaviour are more effective reproductively than the more extreme social and size classes (represented by the α and δ males), who are practically excluded from reproduction. As population size increases the dominant male must spend more time fighting and less time pursuing females. Furthermore, attacks on non-aggressive subordinates decrease as aggressive males devote proportionally more time fighting each other allowing lower rank males a greater opportunity to successfully mate.

Table 1: Paternity of F_1 - offspring (n=106) of large mating groups of *Limia perugiae*

♀ \ ♂	α	β	γ	δ	uncertain
Experiment 1					
1	0	6	0	0	0
2	0	10	0	0	0
3	0	4	0	0	1 *
4	0	8	0	0	0
uncertain	0	1	0	0	0
Total	0	29	0	0	1
Experiment 2					
1	0	0	1	0	0
2	0	7	1	0	4 **
3	0	15	0	0	1 ***
4	0	0	1	0	0
5	0	25	6	0	3 **
n. d.	0	6	6	0	0
Total	0	53	15	0	8

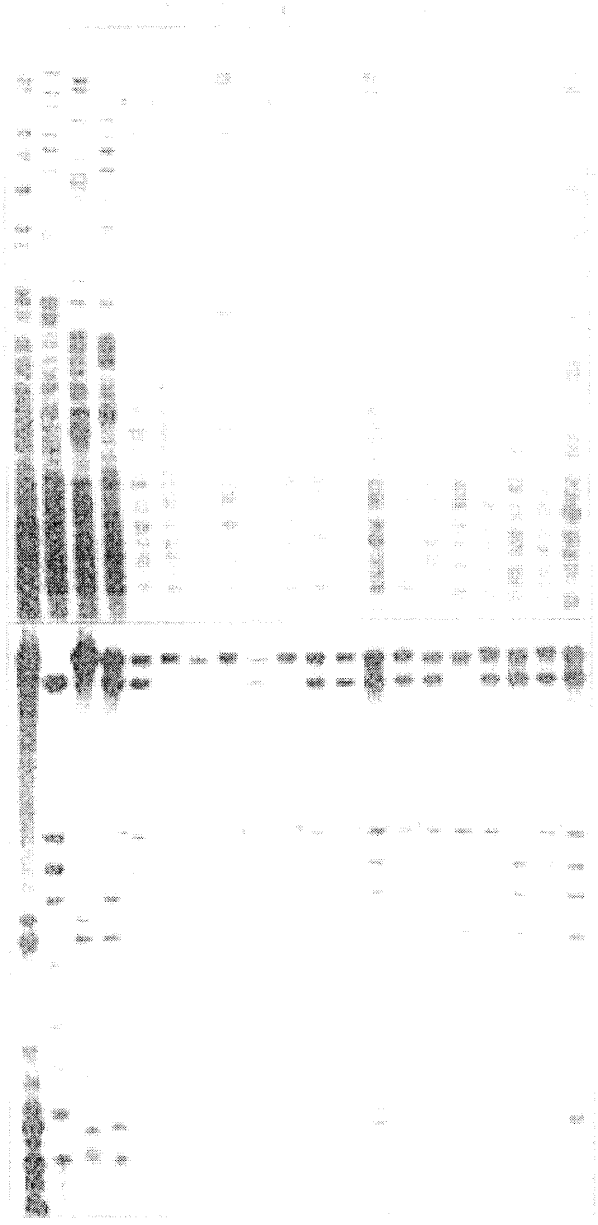
* not unequivocally ascribable to one of the four possible fathers

** attributable either to β or γ

*** by assumption of one mutation attributable to β male

n. d. mother not determined. Because of the similarity of the fingerprint pattern of females (high inbreeding coefficient) in the second experiment this precluded in some cases unequivocal determination of maternity.

Our findings in *L. perugiae* are not compatible with the current understanding that social dominance leads to reproductive success and the model of alternative mating behaviour as an evolutionary stable strategy. The difference observed in mating success of the dominant *L. perugiae* male in small and large groups documents the need to apply molecular biology methods to the study of mating success in other species in groups larger than the 3-4 individuals that can be studied by conventional methods. Finally this approach can also be extended to field studies which will yield information on mating success within the natural environment.



Determination of paternity in large mating groups of *L. perugiae* by DNA fingerprints from one female, three of the possible fathers and their offspring as obtained with the probes $(GGAT)_4$ (top) and $(CA)_8$ (bottom). Representative diagnostic bands marked by the arrows could only be transmitted by one of the putative fathers (in this case β-male). Methods: DNA was prepared from pooled organs of individual fish and proceeded for restriction enzyme digest and agarose gel electrophoresis essentially as described (see Schartl 1988). For DNA fingerprinting the gel was dried and hybridized to ^{32}P-end labelled oligonucleotides. For details on the hybridization and washing conditions see Nanda et al. 1990. Fragment lengths markers are indicated in kilobase pairs on the right.

References

Burke, T. *Trends Ecol. Evol.* **4**, 139-144 (1989).

Epplen et al. In: Jeffreys, A. J., Burke, T., Wolfe, J., Dolf, G., (eds.) (Birkhäuser Verlag, Bern), In press (1991).

Farr, J. A. In: Ecology and Evolution of Livebearing Fishes (Poeciliidae), Meffe, G. K. and Snelson, F. F. Jr. (eds.), (Prentice Hall, Englewood Cliffs, New Jersey), pp. 91-123 (1989).

Hughes, A. L. *Behav. Ecol Sociobiol.* **17**, 271-278 (1985).

Maynard Smith, J. *Amer. Natur.* **117**, 1015-1018 (1981).

Nanda, I., Feichtinger, W., Schmid, M., Schröder, J. H., Zischler, H. and Epplen, J. T. *J. Mol. Evol.* **30**, 456-462 (1990).

Schartl, M. *Genetics* **119**, 679-685 (1988).

Zimmerer, E. J. and Kallman, K. D. *Evolution* **43(6)**, 1298-1307 (1989).

Acknowledgements: We thank B. Wilde, and S. Hölter for technical assistance. Supported by grants to Ma. S. and J. T. E. (VW-Stiftung) and Mi. S. (DFG).