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Food finding ability in several molly species (*Poecilia* spp.), including the unisexual Amazon molly*

Die Fähigkeit verschiedener Molly-Arten (*Poecilia* spp.) einschließlich des Amazonen-Mollys, Futter zu finden*

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Summary: Unisexual Amazon mollies (*Poecilia formosa*) and their bisexual host species are remarkably similar in most aspects of their ecology. Here we studied one aspect of feeding ecology, food finding ability, in several clonal lineages of Amazon mollies (including triploids), two of their hosts and a laboratory produced F_1. We found only minor differences between the studied species and lineages; most notably the triploids were able to locate food faster than the sexual *Poecilia mexicana*. Our findings are in agreement with several other studies all showing that niche overlap in this species complex is very high and likely has to do with the unusual reproductive mode of Amazon mollies, gynogenesis.

Key words: Ploidy, diversification, unisexuality, feeding ecology, niche overlap

Zusammenfassung: Die eingeschlechtlichen Amazonenkärpflinge (*Poecilia formosa*) und ihre Wirtsarten sind sich in ihrer Ökologie erstaunlich ähnlich. In der vorliegenden Arbeit haben wir einen Aspekt der Nahrungsökologie, nämlich die Fähigkeit, eine Futterquelle zu finden, bei verschiedenen klonalen Linien (einschließlich triploider Tiere), einem Laborstamm von F_1-Fischen und den Elternarten untersucht. Wir fanden nur geringe Unterschiede zwischen den Arten und Linien; lediglich die triploiden Tieren fanden die Futterquelle deutlich schneller als die sexuellen *Poecilia mexicana*. Unsere Daten unterstützen die Annahme, dass sich die ökologischen Nischen in diesem Artenkomplex hochgradig überlappen.

Schlüsselwörter: Ploidie, Diversifikation, Eingeschlechtlichkeit, Nahrungsökologie, Nischenüberlappung

1. Introduction

One important principle in ecology is that it is very difficult for two species to occupy the same ecological niche (Hutchinson 1959, Pianka 1974). If two species are ecologically very similar to each other selection should generally favor niche separation and evolution of differences between the taxa involved. This principle has a key role in our current thinking about the origin and maintenance of biodiversity (Leibold 1995, Sahney et al. 2010, Broennimann et al. 2012).

Interestingly, there are several groups of organisms in which selection favoring niche separation may be countered by other selective forces that lead to very tight niche overlap. One such group is sperm-dependent, unisexual organisms (Lamatsch & Stöck 2010). These species are often of hybrid origin and always need to co-exist with another species that provide sperm for their peculiar modes of reproduction. Consequently,

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* We dedicate this paper to Dr. Ralf Wanker, Biozentrum Grindel, who died much too early.

these species may be under conflicting selection pressures: they need to be spatially and physically near their hosts to obtain sperm, and might benefit from looking and behaving very similar to the host (Lima et al. 1996, Poschadel et al. 2009), but at the same time they should also be as dissimilar as possible to reduce competition. Given the physiological dependency for sperm it is difficult for the unisexual hybrids to evolve away from the sperm host in terms of sexual behavior (Poschadel et al. 2009), but other areas might allow for differentiation, such as feeding behavior. An additional layer of complexity in these systems is that the unisexuals are thought to have a limited ability to evolve because they lack full meiosis and recombination (Schlupp & Riesch 2011). In addition, as hybrids, they might already have an intermediate niche to begin with (Hubbs 1964). The resolution of these conflicting selection processes has important implications for the population dynamics and coexistence in these multi-species complexes (Schlupp & Riesch 2011). The more similar the niches of the unisexual and their sexual host remain, the more likely it is that the unisexuals can replace the sexual species based on the two fold advantage of not producing males. Obviously, in sperm-dependent unisexuals out-competing the host is not evolutionary stable because after displacing the host species, the sperm-dependent unisexual would also go extinct (Heubel et al. 2009). Consequently, this should be an important factor in regulating coexistence.

We studied these questions using the feeding ecology of a unisexual fish species, the Amazon molly (*Poecilia formosa*) (Schlupp et al. 2007, Lamatsch et al. 2010, Schlupp & Riesch 2011). Feeding ecology is of great importance for all animals and has recently been studied in Amazon mollies in the field and experimentally (Heubel & Plath 2008, Fischer & Schlupp 2010, Fischer & Schlupp 2011, Scharnweber et al. 2011b, Scharnweber et al. 2011c). Amazon mollies occur mainly as diploids, but triploid (Schultz 1968), tetraploid (Lampert et al. 2008), mosaic individuals (Lamatsch et al. 2002, Lampert et al. 2007) and microchromosome-bearing lineages (Schartl et al. 1995, Lamatsch et al. 2002, Lamatsch et al. 2004) have been reported.

In the present study we provide an additional approach to this problem by experimentally exploring aspects of the feeding ecology of Amazon mollies and their hosts in lineages with known ploidy. This allowed us to test the hypothesis that lineages with additional genetic material show incipient divergence and behavior different from the ancestral diploid lineages (Poschadel et al. 2009).

We studied feeding behavior because in general it seems to be a good proxy for overall fitness: Mollies are omnivores that actively explore their habitat in groups searching for food. As in most fishes, fecundity is correlated with female body size (Wootton 1990): Larger females produce more offspring, which links feeding behavior to individual fitness (Magurran & Seghers 1994, Plath et al. 2007, Tobler et al. 2011). As one aspect of feeding, we focus on the time a fish needs to find a food source comparing lineages that differ in ploidy. This trait may reflect several different aspects of a fish's feeding behavior, including boldness (Smith & Blumstein 2008, Riesch et al. 2009, Scharnweber et al. 2011a) or physiological differences, e.g. in the acuity of their sense of smell (Benfey 2011).

In our experiments we used a food tablet to mimic a patchy food source, reflecting a situation often found in nature. Especially high quality food items such as carotenoid containing fruit (Rodd et al. 2002) can be very localized in nature. Being able to find such items quickly may provide a significant benefit to individuals, even though arriving at a new food source first may expose that particular individual to predators. We hypothesized that studying this aspect of feeding behavior would provide a sensitive assay to detect differences between chromosomal lineages.

In the present study, we investigate differences in the ability to find a food source among several chromosomal lineages of the Amazon molly, its two sexual host species, and artificially bred sexual F_1. We predicted that there would be differences between the sexual species and the Amazon molly, and that within Amazon mollies the triploid lineages would be more similar to *P. mexicana*, because they have more genes in common [(*P. mexicana* x *P. latipinna*) x *P. mexicana*].

2. Materials and methods

2.1. Origin of stocks

We used triploid (3N [(*Poecilia mexicana* x *Poecilia latipinna*) x *Poecilia mexicana*]), microchromosome-bearing (2N+), and diploid (2N) individuals of the Amazon molly from the same locality in Northeast Mexico (Río Purificación, Tamaulipas), as well as *P. mexicana* from that site. We further tested one additional population of the Amazon molly, as well as *P. mexicana*, *P. latipinna* and laboratory produced F_1 hybrids of *P. mexicana* and *P. latipinna* (tabl. 1). The different chromosomal lineages of the Amazon molly were kept in large community tanks with Black molly males as sperm-donors. The F_1 were generated by a single cross of a *P. mexicana* (IV/5) female and a *P. latipinna* (Olmito) male.

Ploidy was established at the starting point of the lineages and later independently and blindly confirmed for four randomly selected individuals each during the experiments. To detect presence of absence of microchromosomes in diploids (Schartl et al. 1995) we conducted metaphase spreads (Nanda et al. 1995, 2007). Diploid and triploid lineages were confirmed using flow cytometry (Lamatsch et al. 2000).

2.2. General procedures

All fishes used in this study were laboratory born offspring from wild-caught individuals and kept at a density of about one fish per liter (tab. 1). Tank size varied between 25 and 200 liters. The temperature was maintained at approximately 25 °C, the light cycle was 12:12 h. The fish were fed daily *ad libitum* with commercially available flake food, supplemented by live food like *Daphnia* and *Artemia* nauplii. Our progress with the experiments was determined by availability of adult females. The sequence of experiments was not randomized to minimize the risk of mixing of the clonal stocks, which are indistinguishable visually.

Prior to testing, the fishes were fed food tablets for two days in their stock tanks to familiarize them with this type of food. They were then moved to a holding tank and not fed for 48 hours, to ensure they were motivated to search for food.

For the actual test, we used a 100 l tank. To initiate a test we placed two females of roughly equal size (+/- 5 mm) from the same lineage into the test tank. Females were tested in pairs, because a single fish in a large tank often does not swim freely. A food tablet identical to the ones used in the stock tanks was attached to

Tab. 1: Origin of *Poecilia* spp. populations (more details available on request).
Tab. 1: Herkunft der getesteten *Poecilia* spp.-Populationen (Details auf Anfrage).

Species	Population	ploidy	n tested
P. formosa	III/9 (Mexico)	2N+	17
P. formosa	VI/17 (Mexico)	3N	11
P. formosa	VI/17 (Mexico)	2N	9
P. formosa	Ditch1 (Texas)	2N	21
P. mexicana	III/14 (Mexico)	2N	6
P. mexicana	VI/17 (Mexico)	2N	7
F1	*P. mexicana* (IV/5) female X *P. latipinna* (Olmito) male	2N	9
P. latipinna	Olmito (Texas)	2N	7
P. latipinna	San Marcos (Texas)	2N	5
P. latipinna	Comal Spring (Texas)	2N	2

the aquarium front. We then introduced the test fishes and measured the time the first fish of the pair needed to locate the food source and began feeding. We used this somewhat artificial procedure because it is successfully used also in studying effects of sexual harassment on females. The food source was considered found when at least one fish started feeding directly from the tablet or from the slowly sinking food flaking of the food tablet. If the food was not located within ten minutes, the trial was terminated. This happened 30 times out of 124 trials resulting in a sample size of 94. For technical reasons water was changed after every third trial; the food tablets were still distinctly visible to the observer during all three trials. We tested as many pairs of females as were available in our stock tanks. After being tested in an experiment, both fish were moved to new stock tanks and allowed to breed. Therefore, each individual fish was tested only once. Data were analyzed using IBM SPSS Statistics 19.

3. Results

The different types of fishes studied here differed significantly in the time needed to locate a food source (ANOVA, n = 94, $F_{5,93} = 3.104$, p = 0.013; fig.1, tab. 1). Triploid Amazon mollies were fastest to find and exploit the food source provided. In posthoc Tukey HSD comparisons only triploid Amazon mollies were significantly different from *P. mexicana* (p = 0.012); all other comparisons were not significant.

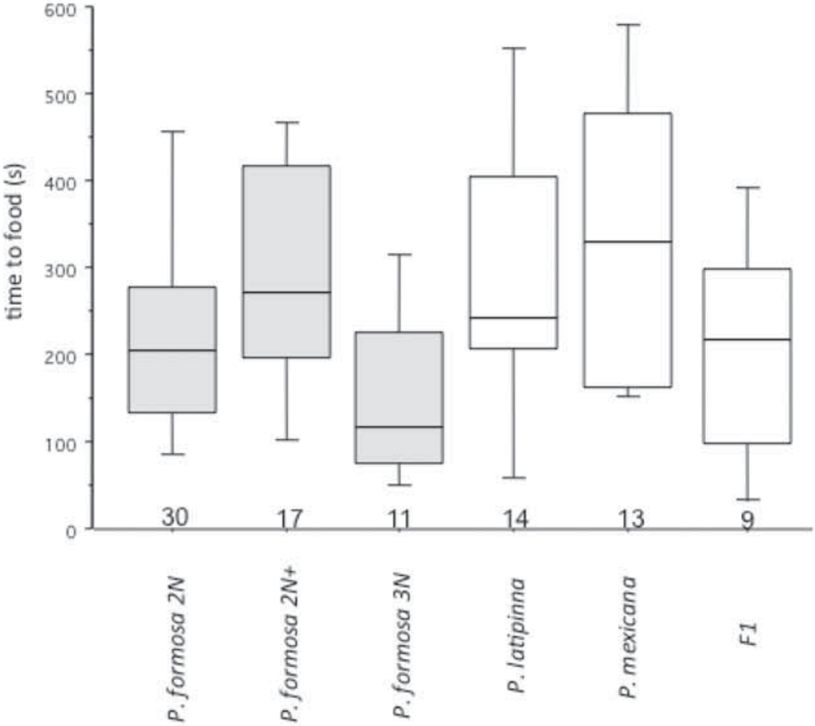

Fig. 1: Box plots comparing all tested fish species and *Poecilia formosa* of different (chromosomal) lineages (numbers refer to sample sizes, see text for additional details).
Abb. 1: Box plots für alle getesteten Fischarten und *Poecilia formosa*-Zuchtlinien (Zahlen geben die Stichprobengrößen an, weitere Details im Text).

4. Discussion

Our main finding was that triploid Amazon mollies find food faster than one other group in our sample (*Poecilia mexicana*). Overall, however, and in agreement with previous studies, we were unable to find clear signs of niche separation (Heubel & Plath 2008, Padur et al. 2009, Fischer & Schlupp 2010, Fischer & Schlupp 2011, Scharnweber et al. 2011b, Scharnweber et al. 2011c). Our study was the first to include several asexual lineages differing in ploidy, as well as laboratory bred sexual F_1 hybrids. The interpretation of our data is limited by a small sample size, a limited number of clonal lineages or species we could test, and by a somewhat artificial task we presented to our test fish, but nonetheless, we were able to detect differences between lineages. The one significant finding we did obtain is interesting to interpret. On the one hand it makes sense that individuals with a whole added genome would differ in some respect from diploid individuals, but one has to keep in mind that triploids in nature are found in mixed groups with diploids and the sexual host *P. mexicana* (Balsano & Rasch 1975, 1989, Rasch & Balsano 1989). In another complex of diploid/triploid hybridogenetic fishes, *Squalius alburnoides*, triploid females were scoring better for measures that might be associated with the food finding ability we measured in our present experiment (Gomes-Ferreira et al. 2005). Triploid *S. alburnoides* were slightly more active in feeding as compared to diploid females (Gomes-Ferreira et al. 2005). This might point to a role for triploidy *per se* in feeding ecology of these complexes and would be in agreement with the thought that adding a genome to a slowly decaying clonal genome might provide a "rescue" effect (Schartl et al. 1995, Schlupp & Riesch 2011). A recent study showed that in triploids indeed all three genomes can be expressed (Schories et al., personal communication).

The differences in feeding ecology detected in this and other studies seem minor in two respects. First, feeding behavior in most species is generally subject to large fluctuations over space and time (Wootton 1990), including the Amazon molly (Scharnweber et al. 2011c). Second, based on their reproductive biology, Amazon mollies and their sexual hosts are locked into a rather close spatial relationship, which makes usage of the same food items likely (Scharnweber et al. 2011c). In addition, many traits and preferences unrelated to feeding ecology have also been found to be rather similar between Amazon mollies and their sexual hosts (Schlupp 2009, 2010).

The combined evidence from this, and other studies, indicates that there is almost no signature of ecological niche separation in this mating complex. While this may be in contradiction to general ecological theory, this lack of divergence seems to be a direct consequence of the sperm-dependency of this (and other) mating system.

In light of this, it will be crucial to develop a better understanding of how female competition for resources (including males) is mediated in the absence of niche separation, (a candidate for this is habitat choice (Lamatsch, Geiger & Schlupp, unpublished)), and how stable coexistence of females is possible (Schlupp & Riesch 2011).

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