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Parasites in sexual and asexual mollies (*Poecilia*, Poeciliidae, Teleostei): a case for the Red Queen?

Michael Tobler^{1,2} and Ingo Schlupp^{1,2,3,4,*}¹Zoologisches Institut, Universität Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland²Department of Zoology, University of Oklahoma, 730 Van Vleet Oval, Norman, OK 73019, USA³Biozentrum Grindel, Universität Hamburg, Martin-Luther-King Platz 3, 20146 Hamburg, Germany⁴Section of Integrative Biology, University of Texas, 1 University Station, Austin, TX 78712, USA

*Author for correspondence (schlupp@ou.edu)

The maintenance of sexual reproduction in the face of its supposed costs is a major paradox in evolutionary biology. The Red Queen hypothesis, which states that sex is an adaptation to fast-evolving parasites, is currently one of the most recognized explanations for the ubiquity of sex and predicts that asexual lineages should suffer from a higher parasite load if they coexist with closely related sexuals. We tested this prediction using four populations of the sexual fish species *Poecilia latipinna* and its asexual relative *Poecilia formosa*. Contrary to expectation, no differences in parasite load could be detected between the two species.

Keywords: maintenance of sex; parasites; gynogenesis; parasitic theory of sex

1. INTRODUCTION

The evolution and maintenance of sex and recombination is still a major and unresolved paradox in evolutionary biology. The search for an explanation for the maintenance of sexual reproduction in the face of its supposed costs has resulted in numerous theories (Ladle 1992; Kondrashov 1993; West *et al.* 1999). Currently, one of the most widely accepted hypotheses, the Red Queen hypothesis (Van Valen 1973), states that recombination provides an immediate advantage in biotic interactions. More specifically, the Red Queen hypothesis argues that recombination results in genetically diverse offspring that, contrary to the uniform offspring of asexuals, are difficult targets for parasites and diseases (Hamilton 1980; Bell 1982; Seger & Hamilton 1988; Hamilton *et al.* 1990).

On the basis of the Red Queen hypothesis, empirically testable predictions can be made. First, time-lagged, frequency-dependent selection through parasites is supposed to result in oscillatory gene-frequency dynamics in the host as well as in the parasite (Seger & Hamilton 1988; Dybdahl & Lively 1998). Second, if sexual reproduction is effectively an anti-parasite adaptation of the hosts, and if sexual and asexual lineages coexist, the parasite load should be higher in parthenogenetic lineages (Ladle 1992).

This prediction has been confirmed in field studies on various taxa (Lively 1987; Moritz *et al.* 1991), including fishes (Lively *et al.* 1990; Hakoyama *et al.* 2001). However, empirical data are not unequivocal, since lower mite infestations were found in asexual geckos (Brown *et al.* 1995; Hanley *et al.* 1995) and it was hypothesized that asexual vertebrates may have a higher resistance to parasites owing to their hybrid origin (Brown *et al.* 1995; Hanley *et al.* 1995; Radtkey *et al.* 1996).

The Amazon molly, *Poecilia formosa*, is one of the few clonal vertebrates and always coexists with a closely related sexual species owing to its gynogenetic mode of reproduction (Schlupp *et al.* 1998). All-female, gynogenetic lineages require sperm from closely related species to trigger embryogenesis, but inheritance is clonal. *P. formosa* originated through natural hybridization of a *Poecilia latipinna* male and a *Poecilia mexicana* female (Avisé *et al.* 1991; Schartl *et al.* 1995).

The mating system of *P. formosa* and its parental species is appropriate for investigating the prediction of the Red Queen that asexual lineages suffer a higher parasite load when coexisting with closely related sexual species. Contrary to most other clonal vertebrates, the asexual *P. formosa* has a single origin and has been genetically isolated for a long time (Avisé *et al.* 1991; Schartl *et al.* 1995; Möller *et al.* in preparation). Owing to the formation of mixed groups, the sexual and asexual mollies are likely to face the same parasites. *Poecilia latipinna* and *P. formosa* are known to harbour a diverse parasite community consisting of eukaryotic micro- and macro-parasites (Tobler *et al.* in press).

In this study, we assess the role of parasites in the stability of the gynogenetic/sexual complex around *P. formosa*. We have compared the parasite load of the sexual *P. latipinna* and the asexual *P. formosa* in natural habitats. Contrary to most previous studies that concentrated on only one or few macro-parasites to compare parasite loads, this study included all of the most prevalent eukaryotic macro- and micro-parasites known in these mollies. According to the Red Queen hypothesis, *P. formosa* was expected to have a much higher parasite load than its sexual relative.

2. MATERIAL AND METHODS

Fishes were collected from four populations in Texas (USA) during the spring of 2004. Details about the collection sites as well as information about the collection of the fishes and the parasitological methods can be found in Tobler *et al.* (in press).

Owing to the negative effect of testosterone on immune function (Alexander & Stimson 1988; Folstad & Karter 1992), males were excluded from the analysis to remove any sexual difference. To compare the total parasite load between the two species, an individual parasitization index was calculated for micro- and macro-parasites (Kalbe *et al.* 2002). All of the most prevalent parasite species (prevalence over 5% in the whole dataset), three micro-parasite and five macro-parasite species, were included (Tobler *et al.* in press). In addition, the separate indices were summed up into an overall individual parasitization index. Indices were analysed using ANCOVA, with standard length as covariate, and independent-samples *t*-tests. Datasets did not deviate significantly from the assumptions required by the ANCOVA. Prevalence and mean intensity were calculated for all parasites (Bush *et al.* 1997) and compared among species with Fisher's exact tests and Mann-Whitney *U*-tests, respectively. Parasite infections in individual fishes were highly idiosyncratic. Owing to multiple infections, most

Table 1. Comparison of individual parasitization indices between the asexual *P. formosa* ($n=110$) and the sexual *P. latipinna* ($n=51$) using ANCOVA, where standard length is the covariate. Bold numbers refer to significant p -values.

factor	micro-parasites			macro-parasites			overall		
	d.f.	F -value	p -value	d.f.	F -value	p -value	d.f.	F -value	p -value
population	3	0.810	0.421	3	8.254	<0.001	3	3.784	0.019
species	1	0.623	0.398	1	1.743	0.143	1	2.602	0.109
standard length	1	0.152	0.681	1	5.925	0.024	1	4.259	0.053
lack of fit	74	0.920	0.572	74	0.847	0.772	74	1.101	0.452

Table 2. Pairwise comparison of individual parasitization indices between the asexual *P. formosa* and the sexual *P. latipinna* for each population studied using independent samples t -tests. Bold numbers refer to significant p -values.

population	species	n	mean \pm s.e. index micro- parasites		mean \pm s.e. index macro- parasites		mean \pm s.e. overall index	
			t , p	t , p	t , p	t , p		
CO 101	<i>P. latipinna</i>	14	0.095 \pm 0.083	-1.381,	0.098 \pm 0.072	-2.051,	0.193 \pm 0.104	-2.206,
	<i>P. formosa</i>	20	0.992 \pm 0.538	0.177	0.877 \pm 0.312	0.048	1.869 \pm 0.629	0.035
SMA	<i>P. latipinna</i>	22	0.290 \pm 0.186	-0.508,	0.263 \pm 0.165	-0.827,	0.554 \pm 0.234	-1.000,
	<i>P. formosa</i>	72	0.441 \pm 0.154	0.613	0.525 \pm 0.167	0.410	0.966 \pm 0.216	0.320
LKP	<i>P. latipinna</i>	4	0.207 \pm 0.106	1.248,	2.456 \pm 0.760	0.772,	2.663 \pm 0.730	0.942,
	<i>P. formosa</i>	4	0.068 \pm 0.035	0.258	1.647 \pm 0.720	0.469	1.715 \pm 0.692	0.383
WES	<i>P. latipinna</i>	11	0.292 \pm 0.136	2.056,	0.895 \pm 0.516	-0.864,	1.187 \pm 0.489	-0.451,
	<i>P. formosa</i>	14	0.041 \pm 0.021	0.050	1.400 \pm 0.325	0.397	1.441 \pm 0.322	0.657

individuals provided different numbers of comparisons. It was impossible to track which individual entered which analysis, so we decided to use a conservative approach and not correct alpha-levels.

3. RESULTS

Parasite loads of 110 *P. formosa* and 51 females of *P. latipinna* were defined. We used ANCOVA to analyse individual parasitization indices. The interactions were not significant, so only the main effects were analysed. Here, the factor 'species' had no effect on the individual parasitization (table 1). Comparisons of the parasitization indices within populations showed that Amazon mollies from County Road 101 harboured more parasites, and sailfin mollies from Weslaco had more micro-parasites (table 2). No differences were found in the other comparisons (table 2).

Prevalences of eight parasite species in four molly populations were compared in the two species. Differences were found in 3 out of 27 comparisons (see table 3 in the Electronic Appendix). The monogenean trematode *Dactylogyrus* sp. was more prevalent in *P. formosa* from County Road 101, the digenean trematode *Uvulifer ambloplitis* in *P. formosa* from Weslaco. Contrary to this, *Ambiphyra* sp. was more prevalent in *P. latipinna* from Weslaco. Mean intensities of eight parasites in four molly populations were compared in the two species. Differences were found in 2 out of 20 comparisons (see table 3 in the Electronic Appendix). *Poecilia formosa* from Martindale and *P. latipinna* from Lincoln Park had more *Trichodina* sp.

4. DISCUSSION

We studied multiple parasites in several populations of a sexual/asexual mating complex of mollies. Our approach provides a direct test of one of the predictions of the Red Queen hypothesis, namely higher parasite loads in asexual lineages if closely related sexuals and asexuals coexist. This pattern was clearly absent in the sexual fish *P. latipinna* and its asexual relative *P. formosa*. The parasite load of both species was not consistently different. Our analysis is very conservative because we did not correct alpha-levels for multiple testing and it can thus be assumed that there is no biologically relevant difference in the parasite load at all. The results of this study therefore disagree with this specific prediction of the Red Queen hypothesis.

Especially interesting are the populations in central Texas (County 101 and Martindale). These were introduced in the 1950s and the genetic diversity of *P. formosa* is extremely low (Turner 1982; Turner *et al.* 1983). At the same time, the introduced *P. latipinna* have been reported to have extremely high heterozygosity (Simanek 1978). Again, no consistent pattern was found and, overall, *P. formosa* did not have higher parasite loads.

The lack of differences in the parasite load of the two molly species may be generally explained because some of the assumptions of the Red Queen hypothesis are not fulfilled. First, the genetic diversity in clonal species might be high owing to multiple and continuous origin of new clones via *de novo* hybridization of sexual species. This does not apply to the Amazon molly because of its single origin about 100 000 generations ago (Avise *et al.* 1991; Scharlt *et al.* 1995; Möller *et al.*

in preparation). Second, it is not clear yet if there is negative frequency-dependent selection pressure through fast evolving parasites in this system, since many of the parasite species recorded here have long-lasting, complex life-cycles and can infect a vast variety of fish species.

As a natural hybrid, *P. formosa* has very high levels of heterozygosity (Turner *et al.* 1980), but the parasitization was not lower than in the sexuals as it was found in asexual geckos (Brown *et al.* 1995; Hanley *et al.* 1995). However, the effect of the Red Queen might be blurred owing to heterosis. Asexual mollies possibly have a higher immunity to naive parasites than the sexuals. Through adaptation of parasites to common host genotypes, this initial advantage might be balanced so that differences in parasitization are much lower than expected.

Unfortunately, not enough is known about the actual immunobiology, including MHC-gene variation, of these species. The key for a complete understanding of the parasitization in asexual and sexual mollies thus is not only long-term field studies to find oscillatory gene-frequency dynamics in the host as well as in the parasite, but comparing the susceptibility to parasites in two species inevitably necessitates efforts in understanding and comparing their immunobiology.

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