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Evidence of disassortative mating in a Tanganyikan cichlid fish and its role in the maintenance of intrapopulation dimorphism

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Morphological dimorphism in the mouth-opening direction ('lefty' versus 'righty') has been documented in several fish species. It has been suggested that this deflection is heritable in a Mendelian one-locus, two-allele fashion. Several population models have demonstrated that lateral dimorphism is maintained by negative frequency-dependent selection, resulting from interactions between predator and prey species. However, other mechanisms for the maintenance of lateral dimorphism have not yet been tested. Here, we found that the scale-eating cichlid fish *Perissodus microlepis* exhibited disassortative mating, in which reproductive pairings between lefties and righties occurred at higher than expected frequency ($p<0.001$). A previous study reported that a lefty–righty pairing produces a 1 : 1 ratio of lefty : righty young, suggesting that disassortative mating contributes to the maintenance of lateral dimorphism. A combination of disassortative mating and negative frequency-dependent selection may stabilize lateral dimorphism more than would a single mechanism.

Keywords: phenotypic polymorphism;
scale-eating cichlid; *Perissodus microlepis*;
Lake Tanganyika

1. INTRODUCTION

Many species exhibit phenotypic polymorphism within a single interbreeding population. One factor maintaining such polymorphism is disassortative mating (Roulin 2004; Roulin & Bize 2007), in which reproductive pairings between different phenotypes are more common than expected by chance. Recently, there has been a focus on disassortative mating as a mechanism for increasing the frequency of heterozygote at a certain locus, which has the positive effects over the homozygote, and/or inbreeding avoidance (Jordan & Bruford 1998; Penn & Potts 1999; Tregenza & Wedell 2000; Penn 2002; Mays & Hill 2004). Experimental evidence of disassortative mating has been reported in mice (Isles *et al.* 2001; Carroll *et al.* 2002) and humans (Wedekind & Füri 1997; Santos *et al.* 2004), and in both cases, females preferred males with odours that differed from their own. However, only a few empirical examples have been reported in the wild (Roulin 2004; Schilthuizen *et al.* 2007).

Perissodus microlepis is a scale-eating cichlid fish endemic to Lake Tanganyika. It is dimorphic with respect to the direction of mouth opening: in one morph, the mouth opens to the right, causing the left side of the head to face forward ('lefty'), whereas the other morph has a mouth that opens to the left ('righty'; Hori 1993). Note that Hori's (1993) 'dextral' and 'sinistral' have recently been redefined as lefty and righty, respectively (Nakajima *et al.* 2004, 2005; Hori *et al.* 2007). There are no individuals with an intermediate morph, and this species is a typical example of 'antisymmetry' (Palmer 1996), which has a bimodal distribution of signed differences on both sides. Lefty and righty individuals coexist in a population, and the proportion of the righty individuals is maintained at approximately 0.5 (Hori 1993). Hori *et al.* (2007) compared the laterality between parents and their young, and suggested that this lateral dimorphism was heritable in a Mendelian one-locus, two-allele fashion with lefty dominant over righty. Interestingly, lefty–lefty pairs produce a 2 : 1 ratio of lefty : righty young, although ratios of 3 : 1 or 1 : 0 would be expected under simple Mendelian genetics. Lethality in the dominant (lefty) allele homozygote or incompatibility between gametes that have the dominant allele may explain this anomalous ratio (Hori *et al.* 2007).

Laterality was recently found in other Tanganyikan cichlids (*Telmatochromis temporalis*: Mboko *et al.* 1998; *Neolamprologus moorii*: Hori *et al.* (2007) and 18 other species: M. Hori, unpublished data collected from 1994 to 2007). The lefty and righty individuals of *P. microlepis* usually attack prey of the righty and lefty individuals, respectively (M. Hori, unpublished data collected from 2005 to 2007). This cross-predation may cause negative frequency-dependent selection, which presumably maintains the lateral dimorphism (Hori 1993). Several population models that assume random mating (Takahashi & Hori 1994; Nakajima *et al.* 2005) or asexual reproduction (Takahashi & Hori 1998, 2005; Nakajima *et al.* 2004) have been developed to support this hypothesis; however, the mating pattern of this species has never been observed. In this study, we observe the mating pattern (disassortative mating between the lefty and righty individuals or assortative mating between the individuals of the same laterality) of *P. microlepis* in the wild, and discuss the role of this mating pattern in the maintenance of lateral dimorphism.

2. MATERIAL AND METHODS

(a) *Sampling of reproductive pairs*

Perissodus microlepis is a monogamous fish. The female broods eggs and embryos in her mouth for 9–11 days, and then both parents jointly guard free-swimming young until they are independent (Yanagisawa & Nshombo 1983; Yanagisawa 1985). The reproductive pair is easily identified through observation of the guarding behaviour against brood predators (Yanagisawa 1985). By means of scuba diving and the use of screen nets, we collected all guarding, reproductive pairs that we found in September–October 2004 (11 pairs) and November 2007 (13 pairs) on a rocky coast around Mpulungu, Zambia. Fish were transported to the laboratory at Mpulungu and anaesthetized in FA-100 (Takeda Pharmaceutical Co. Ltd.). We then determined sex from the shape of the genital papilla under a binocular microscope, and confirmed that each pair was composed of a male and a female.

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Table 1. Frequencies of morph pair combinations in *P. microlepis* in 2004 and 2007.

	male (2004)		male (2007)	
	lefty	righty	lefty	righty
female				
lefty	1	6[a]	2	7[a]
righty	4[a]	0	4[a]	0

[a] The number of disassortative mating pairs.

Hori (1993) and Hori *et al.* (2007) collected several reproductive pairs of *P. microlepis*, but we did not include their samples in the present analysis. These studies attempted to gather the pairs of all mating patterns (lefty–lefty, lefty–righty and righty–righty) with their brood in order to determine a genetic pattern of laterality. Thus, these samples would not accurately reflect the ratio of the mating patterns in the wild.

(b) *Identification of laterality*

We visually examined the mouth under a binocular microscope to determine laterality. To avoid errors in judgement, three persons separately examined the specimens in both their fresh and fixed conditions: T.T. and M.H. observed the fresh specimens, and T.T. and Y. Takeuchi, who is an expert in the laterality of cichlid fish, observed the specimens after fixation in 100% ethanol or 10% formalin.

(c) *Test of mating patterns*

Fisher's exact probability test was performed on a matrix of laterality versus sex to test the null hypothesis that laterality was independent of sex. We then also used Fisher's exact probability test on a matrix of male laterality versus female laterality to test the null hypothesis of random mating.

3. RESULTS

Perissodus microlepis has a large degree of mouth deflection (see fig. 1 of Hori 1993), and laterality was easily identified. In all samples, the identification of laterality was the same among observers and between fresh and fixed conditions, suggesting that the identification was correct.

The laterality and the sex of the 24 pairs examined are shown in table 1. In both the 2004 and 2007 samples, there were more righty than lefty males but more lefty than righty females. However, the differences in frequencies of laterality between the sexes were not significant (Fisher's exact probability test: $n=22$, $p=0.670$ in 2004; $n=26$, $p=0.428$ in 2007; $n=48$, $p=0.244$ in pooled samples from 2004 and 2007).

The ratio of disassortative mating pairs to assortative mating pairs was 10 : 1 in 2004 and 11 : 2 in 2007. The disassortative mating pairs were significantly frequent (Fisher's exact probability test: $n=11$, $p=0.030$ in 2004; $n=13$, $p=0.042$ in 2007). In these analyses, the small sample sizes might cause the marginal p values; however, the dominance of disassortative mating pairs was strongly supported when the samples from 2004 and 2007 were pooled (Fisher's exact probability test: $n=24$, $p<0.001$).

4. DISCUSSION

Experimental evidence of disassortative mating has been reported in mice (Isles *et al.* 2001; Carroll *et al.* 2002) and humans (Wedekind & Füri 1997; Santos *et al.* 2004), but empirical evidence in wild populations is rare (Roulin 2004; Schilthuizen *et al.* 2007). In this study, we present a simple, clear example of disassortative mating in the scale-eating cichlid fish *P. microlepis*.

In *P. microlepis*, the proportion of the righty individuals in a population is maintained at approximately 0.5 (Hori 1993). The lefty–righty (disassortative) pair produces a 1 : 1 ratio of lefty : righty young, whereas a lefty–lefty (assortative) pair produces a 2 : 1 ratio of young and a righty–righty (assortative) pair produces only righty young (Hori *et al.* 2007). Therefore, the high rate of disassortative mating probably contributes to the maintenance of lateral dimorphism at a 1 : 1 ratio. On the other hand, another mechanism to maintain lateral dimorphism has been reported in this species. The lefty and righty individuals of *P. microlepis* attack prey of the opposite morph more frequently than prey of the same morph (M. Hori, unpublished data collected between 2005 and 2007). This cross-predation may cause negative frequency-dependent selection (Hori 1993; Nakajima *et al.* 2004). The combination of intra- (disassortative mating) and interpopulational effects (negative frequency-dependent selection) may stabilize lateral dimorphism at a ratio of 1 : 1 more than a single mechanism.

Disassortative mating sometimes functions as an inbreeding-avoidance mechanism. When particular heritable phenotypes are highly polymorphic, e.g. odour correlated with the major histocompatibility complex, individuals that share phenotypes are likely to be related (Brown & Eklund 1994; Tregenza & Wedell 2000; Bernatchez & Landry 2003). Thus, female preferences for males that have dissimilar phenotypes may prevent kin mating (Potts & Wakeland 1990; Tregenza & Wedell 2000; Bernatchez & Landry 2003). However, disassortative mating in *P. microlepis* probably does not function in inbreeding avoidance. A pairing between different morphs produces lefty and righty young at a ratio of 1 : 1 (Hori *et al.* 2007), which is nearly equal to the ratio in the population (Hori 1993). Thus, young individuals are less likely to recognize siblings by their laterality. Furthermore, disassortative mating is generally considered as a mechanism for increasing genetic compatibility through the positive effects of heterozygosity (Jordan & Bruford 1998; Penn & Potts 1999; Tregenza & Wedell 2000; Penn 2002; Mays & Hill 2004). A fitness comparison between genotypes would be required to test whether *P. microlepis* has a heterozygote advantage at the locus controlling laterality, and whether it is associated with the disassortative mating.

In conclusion, we present clear evidence of disassortative mating in a natural scale-eating cichlid fish population. Disassortative mating in *P. microlepis* probably maintains the lateral dimorphism at a ratio of 1 : 1. The evolution of this disassortative mating is probably not associated with inbreeding avoidance.

This study was carried out in accordance with Guidelines for Animal Experimentation, Kyoto University.

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