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Genetic support for random mating between left and right-mouth morphs in the dimorphic scale-eating cichlid fish *Perissodus microlepis* from Lake Tanganyika

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Population genetic analyses were conducted to investigate whether random mating occurs between left and right-mouth morphs of the dimorphic scale-eating cichlid fish *Perissodus microlepis* from two geographical sites in southern Lake Tanganyika. The mitochondrial and nuclear DNA markers (13 microsatellite loci) revealed no genetic differentiation between left and right morphs (*i.e.* widespread interbreeding). The observed lack of genetic divergence between the different morphs allowed for the exclusion of the possibility of assortative mating between same morph types. The microsatellite data showed no significant departures of heterozygosity from Hardy–Weinberg equilibrium, suggesting purely random mating between the morphs. Overall, this study indicated no genetic evidence for either assortative or disassortative mating, but it did provide support for the random mating hypothesis. Highly significant, albeit weak, spatial population structure was also found when samples of different morphs were pooled according to geographical sites. An additional analysis of two microsatellite loci that were recently suggested to be putatively linked to the genetic locus that determines the laterality of these mouth morphs did not show any such association.

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INTRODUCTION

Natural populations of organisms sometimes exhibit pronounced discontinuous phenotypic variation among individuals. Remarkable examples of such morphological polymorphism include left–right asymmetry morphs in which left or right individuals differ asymmetrically (Palmer, 2004). This asymmetry in form is generally classified into two different types: antisymmetry and directional asymmetry (Palmer, 1996). In the case of antisymmetry, the direction of asymmetry is random: left and right

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morphs are equally common within a species [*e.g.* direction of mouth opening in the scale-eating cichlid fish *Perissodus microlepis* Boulenger (Hori, 1993), and the side of the major claw in male fiddler crabs *Uca lactea* (Yamaguchi, 1977)]. On the other hand, under directional asymmetry, the direction of asymmetry is fixed: most individuals are asymmetrical towards the same direction, left or right [*e.g.* direction of shell coiling in the snails, *Euhadra* species (Davison *et al.*, 2005) and *Lymnaea peregra* (Freeman & Lundelius, 1982), and eye side in the polymorphic starry flounder *Platichthys stellatus* (Pallas) (Hashimoto *et al.*, 2002)].

The two types of morphological asymmetry differ in heritability (Palmer, 2004, 2009). In all the 29 cases of antisymmetry known in animals and plants, the direction of asymmetry is not heritable, that is, the trait is not genetically determined (*i.e.* phenotypically plastic), except for the one case of direction of style bend in enantiostylous flowers of one *Heteranthera* species (Jesson & Barrett, 2002). Another exception to this has since been reported for the direction of shell coiling in the south-east Asian camaenid tree snail subgenus *Amphidromus sensu stricto* (Schilthuizen *et al.*, 2005). By contrast, in every case of directional asymmetry, the direction of asymmetry is heritable, mostly *via* Mendelian inheritance (Palmer, 2004).

A well-known example of antisymmetry is lateral dimorphism of mouth-opening direction that is found in several Lake Tanganyikan scale-eating cichlid fish species of the genera *Perissodus* and *Plecodus*. The heads of these fishes are asymmetrical: one morph has a mouth that is turned to the left [left morph; left-handed in Hori (1993)] and the other morph's mouth opens to the right (right morph; right-handed) (Fig. 1). Note that although Hori's (1993) original definition of left-handed (sinistral) and right-handed (dextral) has recently been re-defined as righty and lefty, respectively (Hori *et al.*, 2007), here the initial definitions are used since in the anatomical literature features are described from the perspective of the subject rather than the observer.



FIG. 1. Heads of the right and left morphs of Lake Tanganyikan scale-eating cichlid fish *Perissodus microlepis*.

Perissodus microlepis is one of the nine species of scale-eating cichlids of the tribe Perissodini endemic to Lake Tanganyika (Takahashi *et al.*, 2007). This fish feeds on scales from other fish by ambushing them from behind (Nshombo *et al.*, 1985). Approaching from behind, the asymmetry of their jaws restricts the attack direction of the different morphs to only one side (Hori, 1993). Left morphs attack their prey's right side and right morphs their left side. Interestingly, the number of individuals of each morph fluctuates around an equal 50:50 ratio and is stable over time within a given population (Hori, 1993). This even frequency of both morphs was inferred to be shaped by negative frequency-dependent natural selection, where the rare morph gains a selective advantage over the abundant one (Hori, 1993). Prey are thought to be more alert to being attacked from the preferred side of the more abundant morph. As a consequence, increased prey vigilance would reduce the predation success of the abundant morph, and negative frequency-dependent selection would favour the rarer morph and an equal lateral dimorphism would be maintained.

The direction of mouth opening (*i.e.* laterality) of *P. microlepis* is apparently an unusual exception to the commonly observed pattern that a random, external environmental trigger determines the direction of asymmetry in the case of antisymmetry (Palmer, 2004, 2009), the direction of mouth opening (*i.e.* laterality) of *P. microlepis* is apparently an unusual exception. The laterality of this fish has been suggested to be genetically determined under a single Mendelian locus with two alleles (where right is dominant over left) (Hori, 1993; Hori *et al.*, 2007). Right–right pairs produce a 2:1 ratio of right:left offspring, left–right pairs a 1:1 offspring and left–left pairs only left offspring (Hori *et al.*, 2007). These estimates were based on field-collected offspring from broods guarded by a pair of parents of known mouth morphs. Parents of this species, however, have the unusual habit of farming-out their fry to other conspecific breeding pairs (*i.e.* intraspecific brood mixing; Yanagisawa, 1985) and even to other non-conspecific pairs (*i.e.* interspecific brood mixing; Ochi & Yanagisawa, 1996). Inconsistent with the Mendelian ratio of right:left = 3:1, right–right pairs showed a 2:1 ratio of right:left offspring (right allele is homozygous lethal; Hori *et al.*, 2007). In a recent review, Palmer (2010) has pointed out that the results from the original study (Hori, 1993) are inconsistent with a model where right allele is homozygous lethal and left–left pairs produce only left offspring. The laterality of this fish has recently been suggested to be phenotypically plastic (Van Dooren *et al.*, 2010). The field observation that the laterality of *P. microlepis* is a heritable trait has not yet been confirmed by controlled breeding experiments. Thus, there is no evidence against the laterality of this fish being entirely environmentally determined (*i.e.* phenotypically plastic). However, Stewart & Albertson (2010) suggest that the laterality of *P. microlepis* is genetically determined. They found a putative link between two microsatellite markers and the genes for the laterality of the mouth.

There are three main hypotheses on the mating system associated with the observed laterality in *P. microlepis*: (1) assortative mating, (2) disassortative mating and (3) random mating. The first hypothesis is that in this species mating is assortative according to jaw laterality, so that individuals mate preferentially with some of the same morph. In the case of antisymmetry, Johnson (1982) observed that mating is assortative between individuals of different shell coiling directions in the dimorphic land snails, *Partula suturalis* on the island of Moorea (French Polynesia). Assortative mating causes genetic divergence between left and right morphs by reducing or even inhibiting gene flow between them [*e.g.* the land snail *Cepaea nemoralis* in the

U.K.; (Johnson, 1976)], which allows the evolution of reproductive isolation, driving sympatric speciation (Fitzpatrick *et al.*, 2008). Surprisingly, no previous studies have been performed to test for assortative mating between antisymmetrical morphs, using genetic data.

The second hypothesis is that individual *P. microlepis* fish mate disassortatively, preferentially with individuals of the different morph. Takahashi & Hori (2008) observed that reproductive pairings between different morphs occurred more frequently than pairings between the same morphs in the field. They argued that because left–right pairs produce an approximately equal ratio of left:right offspring (Hori *et al.*, 2007), disassortative mating might be an additional mechanism involved in stabilizing the lateral dimorphism in *P. microlepis*. If the laterality of this fish was determined by non-genetic variation (Van Dooren *et al.*, 2010), however, then it is difficult to conceive what possible benefits this fish could gain by disassortative mating.

The third hypothesis is that this species mates randomly irrespective of lateral jaw morph. If laterality is not heritable (*i.e.* phenotypically plastic) and is therefore an example of antisymmetry (Palmer, 2004, 2009), random mating would be most probable because there would be no selective advantages for this fish to breed either assortatively or disassortatively.

In this study, population genetic analyses were carried out to test whether random mating occurs between left and right mouth morphs of *P. microlepis* from two geographical sites in southern Lake Tanganyika. The predictions of three hypotheses were tested by calculating F_{ST} values and departures of heterozygosity from Hardy–Weinberg equilibrium (HWE) (Table I). The predicted population genetic outcomes from each hypothesis are summarized in Table I. The spatial population genetic structure of this fish was also examined between the sites. In addition, Hori's (1993) two hypotheses were tested: (1) the equal lateral dimorphism hypothesis, where frequencies of left and right morphs are equal and (2) the lateralized foraging behaviour hypothesis, where left morphs only attack the right side of their prey and right morphs the left side.

This study provides the first genetic evidence for widespread interbreeding and most likely, random mating between left and right morphs of *P. microlepis* in a system where the determination of laterality, genetic or environmental, remains unknown.

MATERIALS AND METHODS

SAMPLE COLLECTION

Perissodus microlepis samples were obtained with gillnets in September 2004 from two sites in southern Lake Tanganyika, Zambia (Fig. 2). Specimens were sampled randomly with respect to mouth-opening direction. The two sampling sites were (1) Kalambo Village (KV, 08° 36' 03" S; 31° 11' 03" E) and (2) Toby Veall's Lodge (TL, 08° 37' 21" S; 31° 12' 01" E). The geographical surface distance between KV and TL is *c.* 3 km. This surface distance between the two sampling sites was estimated from the website <http://chemical-ecology.net/java/lat-long.htm>, based on the latitude and longitude of each site.

RATIO OF LEFT AND RIGHT MORPHS IN TWO SITES

The mouth laterality of each sample was determined independently in the field by eye by two different researchers (A.M. and W.S.) (Fig. 1). A few ambiguous individuals with

TABLE I. Predicted outcomes from three mating hypotheses between left and right-mouth morphs in *Perissodus microlepis* where mouth laterality is genetically or environmentally determined. HWE, Hardy–Weinberg equilibrium; f , Weir & Cockerham’s (1984) inbreeding coefficient f [= system of mating of population measured as deviations from HWE genotype frequency expectations (Templeton, 2006)]. The genetic data provide support for the cases highlighted in grey

Hypotheses	If laterality is genetically determined			If laterality is environmentally determined
	Linked*		Unlinked*	
	Predicted F_{ST} values	Predicted HWE	Predicted F_{ST} values	
Assortative mating	Significant F_{ST}	Deviations from HWE because of heterozygote deficiency ($f > 0$)	Significant F_{ST}	HWE ($f = 0$)
Disassortative mating	Non-significant F_{ST}	Deviations from HWE because of heterozygote excess ($f < 0$)	Non-significant F_{ST}	HWE ($f = 0$)
Random mating	Non-significant F_{ST}	HWE ($f = 0$)	Non-significant F_{ST}	HWE ($f = 0$)

*If microsatellites genotyped in this study are linked or unlinked to loci involved in expression of the sexually selected phenotypic trait, mouth laterality, assuming that the laterality is genetically determined.

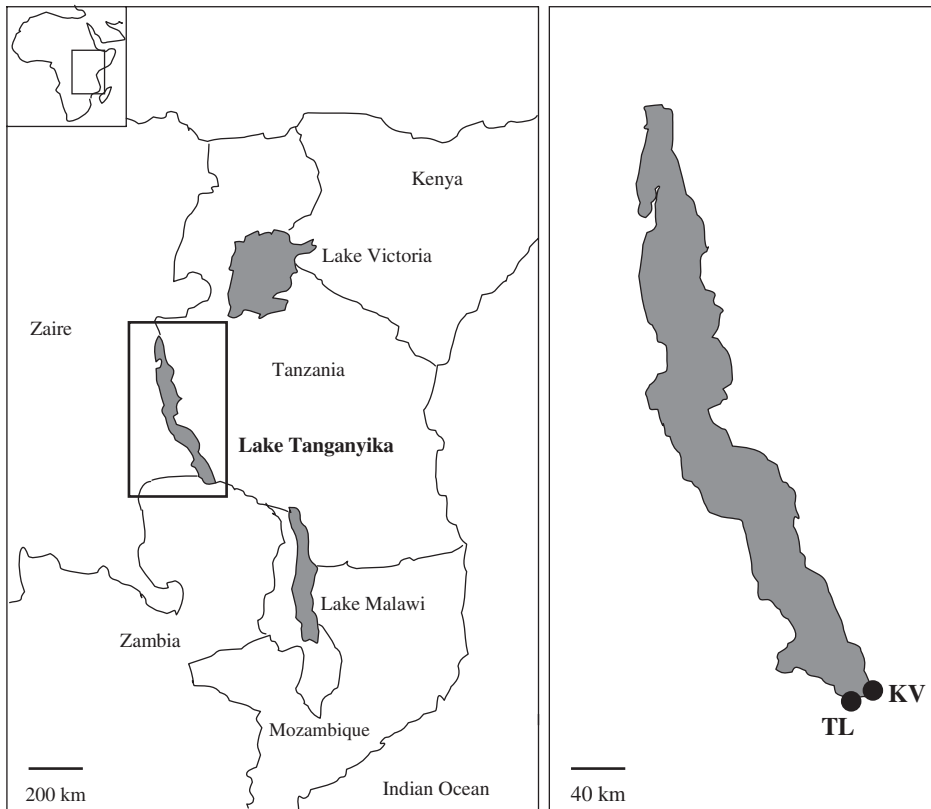


FIG. 2. Sampling sites of *Perissodus microlepis* in southern Lake Tanganyika, Zambia. KV, Kalambo Village (KV, 08° 36' 03" S; 31° 11' 03" E); TL, Toby Veall's Lodge (TL, 08° 37' 21" S; 31° 12' 01" E).

less pronounced laterality were found among the samples (KV: 1 of 52; TL: 10 of 76). The observed frequencies of left and right morphs, excluding the 11 ambiguous individuals, were KV-left: 23, KV-right: 28; TL-left: 31, TL-right: 35. Three different χ^2 tests were used to determine whether the frequencies of left and right morphs observed differed from a 1:1 ratio (equal lateral dimorphism; Hori, 1993). First, a χ^2 test was performed using samples pooled for mouth morph, excluding ambiguous samples. For the next two tests, the 11 ambiguous samples were included; first these fish were assigned to the left morph category, and second to the right-morph category. Only samples with clear differences in laterality were used for genetic analyses.

LATERALIZED FORAGING BEHAVIOUR OF MOUTH MORPHS

Three left morph individuals and five right morph individuals of *P. microlepis* were collected at TL in Lake Tanganyika and immediately shipped to the animal research facility (TFA) at the University of Konstanz. To determine whether left morph individuals only attack the right side of prey fish, and right morph individuals the left side, eight scale-eaters were kept in individual tanks with one prey fish, a Central American cichlid, *Amphilophus citrinellus* (Günther). After 7 days, the prey fish were taken out of each tank and were examined for scars on either side of their bodies. The observed lack of scales was caused by attacks from *P. microlepis*.

MITOCHONDRIAL DNA SEQUENCING AND DATA ANALYSES

Genomic DNA was isolated from a small piece of fin tissue, using a proteinase *K* digestion followed by sodium chloride extraction and ethanol precipitation (Bruford *et al.*, 1998). Mitochondrial DNA (mtDNA) control region was amplified with the published primers, LProF (Meyer *et al.*, 1994) and TDK-DHG (Lee *et al.*, 1995), using standard polymerase chain reaction (PCR) conditions with 50° C for annealing temperature (T_a). Amplified PCR products were purified with QIAquick PCR Purification Kit (Qiagen; www.qiagen.com) following the manufacturer's recommendations. The purified mtDNA fragments were subject to direct sequencing in the forward and reverse directions using the same forward and reverse primers as in the PCR and the BigDye Terminator 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems). All reactions for DNA sequencing were run on a 3130xl DNA Analyser (Applied Biosystems; www.appliedbiosystems.com) and analysed with ABI PRISM DNA Sequencing Analysis Software version 5.3.1. The DNA sequences were edited using Chromas version 2.01 software. Alignment of sequences was conducted using ClustalW2 and then verified by eye. A total of 669 base pairs (bp) of sequence of the mtDNA control region were recovered from 89 samples (KV-left: 20, KV-right: 21, TL-left: 22, and TL-right: 26).

The number of polymorphic sites, mtDNA haplotypes, haplotype diversity (h) and nucleotide diversity (π) were calculated for each left and right sample of *P. microlepis* within two sites (KV and TL) using Arlequin 3.11 (Excoffier *et al.*, 2005). The rarefaction method was employed using CONTRIB 1.02 (Petit *et al.*, 1998) to calculate haplotype richness that was corrected for unequal sample sizes among the four sampling groups.

To explore the phylogenetic relationships among the mtDNA haplotypes, a network approach was employed using TCS 1.21 (Clement *et al.*, 2000), which uses the statistical parsimony method described in Templeton *et al.* (1992). A single deletion mutation was treated as a fifth state as it was retained by five different haplotypes (H13-17). Ambiguous connections in the haplotype network generated by TCS were resolved following the criteria outlined in Templeton & Sing (1993) and Crandall & Templeton (1993).

To estimate inter-morph and spatial genetic structure of *P. microlepis*, a hierarchical analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) was performed using Arlequin. The inter-morph and spatial AMOVA analysis was carried out by grouping samples of left and right morphs according to geographical sites: the KV group comprised KV-left and KV-right and the TL group comprised TL-left and TL-right. This AMOVA analysis partitioned the total molecular variance between geographical sites (F_{CT} = spatial genetic variation), between left and right morphs within sites (F_{SC} = inter-morph genetic variation) and within samples irrespective of group (F_{ST}).

To further examine inter-morph and spatial genetic differentiation between samples, exact tests for population differentiation (Raymond & Rousset, 1995) as well as calculation of pair-wise estimates of F_{ST} (Weir & Cockerham, 1984) were carried out using GENEPOP 4.0 (Rousset, 2008). Another exact test was then performed by pooling samples of both mouth morphs within geographical sites, which could be justified because the AMOVA had shown no significant inter-morph variation within sites. The 95% significance levels for pair-wise exact tests of genetic differentiation were adjusted, using a Bonferroni correction (*i.e.* $P = 0.05/6 \approx 0.0083$).

MICROSATELLITES GENOTYPING AND ANALYSES

Thirteen nuclear microsatellite loci were genotyped for *P. microlepis* samples with the primers that had been developed for the African cichlid fish *Astatotilapia burtoni* (Günther) (Sanetra *et al.*, 2009).

The number of alleles per locus (N_a), expected heterozygosity (H_E), observed heterozygosity (H_O) and allelic richness (AR) within the four samples were calculated using FSTAT 2.9.3.2 (Goudet, 2001). To test for random mating between left and right morphs of *P. microlepis*, HWE deviations were estimated for each sample at each locus by calculating the inbreeding coefficient f (equivalent to Wright's F_{IS}) of Weir & Cockerham (1984), as implemented in GENEPOP. The f values for each sample across the 13 loci were also calculated. The significance levels of every exact test for HWE were adjusted using the Bonferroni correction.

Genotypes at the 13 microsatellite loci were tested for linkage disequilibrium (LD) among pairs of loci for the entire pooled samples of *P. microlepis* using GENEPOP.

The inter-morph and spatial AMOVA analysis was conducted for 13 microsatellite loci, using the same groupings as were used for the mtDNA data (see above). The inter-morph and spatial population differentiation analyses were then performed for 13 microsatellite loci.

Following a quantitative trait-locus (QTL) analysis of an interspecific cross of two Lake Malawi cichlids, Stewart & Albertson (2010) inferred that two microsatellite loci (GM294, UNH2101) are linked to the locus for laterality of the mouth in this species. They used the polymorphic marker (UNH2101) to genotype left and right juveniles from a set of young from one female. This study also tests whether the two microsatellite loci identified by Stewart & Albertson (2010) do segregate left and right morphs of *P. microlepis* at the population level. Sample sizes were 51 left and 59 right.

RESULTS

RATIO OF LEFT AND RIGHT MORPHS IN TWO SITES

Three different χ^2 tests showed no significant deviations from a 1:1 ratio of left:right mouth morphs in *P. microlepis* (Table II), providing support for the hypothesis of equal abundance for both lateral morphs (Hori, 1993).

LATERALIZED FORAGING BEHAVIOUR OF MOUTH MORPHS

In the laboratory test, almost all individuals exhibited the expected foraging behaviour for their morph. All three individuals of the left morph only attacked the right side of their prey fish, *A. citrinellus*. In addition, scars and missing scales were only observed on their right side. Similarly, scars were only found on the left side of the prey fish for four of five right morph individuals. Only one right morph individual attacked both sides of its prey fish in the laboratory test.

MTDNA CONTROL REGION

A total of 17 mtDNA haplotypes were identified in the total of 89 *P. microlepis* samples that were sequenced (Table III; Fig. 3). Sequences of these haplotypes are

TABLE II. Three different χ^2 analyses were performed to test the frequency of lateral dimorphism in *Perissodus microlepis* at two geographical sites in southern Lake Tanganyika. No test showed a significant deviation from a 1:1 ratio of left:right morphs

Samples	Left:right	χ^2 value	d.f.	<i>P</i>
Samples with clear differences in laterality	54:63	0.692	1	0.405
Samples with clear differences in laterality + 11 ambiguous samples assigned to the left morph category	65:63	0.031	1	0.860
Samples with clear differences in laterality + 11 ambiguous samples assigned to the right morph category	54:74	3.125	1	0.077

d.f., degree of freedom.

respectively (Table III). In the complete data set, the transition to transversion ratio was 4.3:1.

All the mtDNA haplotypes that satisfied the probability of parsimony by 95% (as defined in Templeton *et al.*, 1992) were connected to a single network (Fig. 3). The haplotype network was centred on the most common haplotype (H1), which consisted of 39 individuals (44% of total sample) and was detected in all four sampled groups (both localities and jaw morphs) (Fig. 3).

The hierarchical AMOVA of the mtDNA control region data revealed neither significant inter-morph nor spatial genetic structure, although molecular variances partitioned between sites ($F_{CT} = 0.0252$) were greater than between mouth morphs within sites ($F_{SC} = -0.024$) (Table IV). The exact tests for population differentiation showed no significant differentiation between any pairs of left and right samples (*e.g.* left *v.* right comparisons within two sites: KV-left *v.* KV-right: $F_{ST} = -0.0166$, $P > 0.05$; TL-left *v.* TL-right: $F_{ST} = -0.0262$, $P > 0.05$; Table V). The exact test between the pooled KV and TL spatial samples, however, revealed significant differentiation ($F_{ST} = 0.0151$, $P < 0.01$), probably because of increased statistical power owing to the increase in sample size.

MICROSATELLITES

The 13 microsatellite loci varied widely in number of alleles (N_a : 2–13, mean = 6.0), expected heterozygosity (H_E : 0.16–0.89, mean = 0.64), observed heterozygosity (H_O : 0.17–0.90, mean = 0.62) and allelic richness (AR: 1.98–10.79, mean = 5.24) across the four samples (Appendix). The f values of the four samples across the 13 loci were close to zero (KV-left: -0.0008 ; KV-right: 0.0493; TL-left: 0.0195; TL-right: 0.0549). As a consequence, all four samples conformed to HWE expectations at all microsatellite loci except for the TL-right sample at Abur117 locus (Appendix), suggesting random mating at the loci tested. Tests of genotypic LD within the entire pooled samples between the 13 loci showed no significant association of alleles except for between Abur90 and Abur162 ($P < 0.05$), suggesting that all loci but these two can be treated as independent markers.

Similar to the results of mtDNA control region, the hierarchical AMOVA for the 13 microsatellite loci showed neither significant inter-morph nor spatial population structure (Table IV). The genetic variation among samples irrespective of group was significant, although small ($F_{ST} = 0.0094$, $P < 0.01$; Table IV). The exact tests for population differentiation revealed no significant differentiation between left and right samples at either of two sites (KV-left *v.* KV-right: $F_{ST} = -0.0001$, $P > 0.05$; TL-left *v.* TL-right: $F_{ST} = 0.0079$, $P > 0.05$; Table V). The exact tests between two different samples from different sites, however, were always significant (except for KV-left *v.* TL-right; Table V). As a result, the exact test between the pooled KV and TL spatial samples revealed highly significant, albeit weak, genetic differentiation ($F_{ST} = 0.0088$, $P < 0.001$).

The test of the two microsatellites putatively linked by Stewart & Albertson (2010) found no allelic variation in GM294 whereas eight different alleles were identified in UNH2101. [cf. Stewart & Albertson (2010) two alleles: a putative A (right) allele 150bp in length and a B (left) allele 158bp in length.] This study has found no evidence for linkage between UNH2101 and the genes for laterality of the mouth at the population level (Fig. 4).

TABLE IV. Hierarchical AMOVA of inter-morph and spatial genetic structure in *Perissodus microlepis* using the mtDNA control region as well as 13 microsatellite loci. The analyses were performed by grouping left and right samples of *P. microlepis* according to the sampling sites. Permutations (16 000) were carried out separately for each AMOVA

Locus	Source of variation	d.f.	Sum of squares	Variance components	Variation (%)	Fixation indices	<i>P</i>
mtDNA control region	Between sites	1	0.634	0.00965	2.52	$F_{CT} = 0.0252$	>0.05
	Between mouth morphs within sites	2	0.415	-0.00786	-2.05	$F_{SC} = -0.0240$	>0.05
	Within samples	85	32.434	0.38158	99.53	$F_{ST} = 0.0047$	>0.05
	Total	88	33.483	0.38337			
Microsatellites	Between sites	1	6.419	0.02199	0.63	$F_{CT} = 0.0063$	>0.05
	Between mouth morphs within sites	2	8.101	0.01074	0.31	$F_{SC} = 0.0031$	>0.05
	Within samples	214	741.750	3.46612	99.06	$F_{ST} = 0.0094$	<0.01
	Total	217	756.271	3.49886			

TABLE V. Estimates of genetic differentiation (F_{ST}) between left and right samples of *Perissodus microlepis*. Numbers above the diagonal represent F_{ST} values based on mtDNA control region, and numbers below the diagonal represent F_{ST} values based on 13 microsatellite loci. The significant levels were adjusted, using the Bonferroni correction for multiple testing (*i.e.* $P = 0.05/6 \approx 0.0083$). Significant deviations from genetic homogeneity by exact tests are shown in uppercase letters that are given above each F_{ST} value. KV: Kalambo Village; TL: Toby Veall's Lodge

	KV-left	KV-right	TL-left	TL-right
KV-left		-0.0166 ^{NS}	0.0076 ^{NS}	-0.0031 ^{NS}
KV-right	-0.0001 ^{NS}		-0.0011 ^{NS}	0.0153 ^{NS}
TL-left	0.0172 ^{***}	0.0158 ^{**}		-0.0262 ^{NS}
TL-right	0.0045 ^{NS}	0.0017 [*]	0.0079 ^{NS}	

NS, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

DISCUSSION

This study provides the first genetic evidence for random mating between left and right mouth morphs of the dimorphic *P. microlepis*. The observed lack of genetic divergence between the mouth morphs (non-significant F_{ST} values) suggests that there is a high degree of contemporary gene flow between morphs (*i.e.* widespread interbreeding). This lack of divergence would seem to rule out the possibility of assortative mating between individuals of the same mouth morphs in *P. microlepis* (Templeton, 2006; Table I), but so far cannot distinguish between disassortative or random mating as alternative mating systems in this species. No departure of heterozygosity from HWE and f values close to zero at any of the 13 microsatellite loci does, however, provide support for the random mating hypothesis.

Contrary to the result in this study, assortative mating by the direction of asymmetry has been reported in antisymmetrical morphs. Assortative mating occurs according to the direction of shell coiling in the dimorphic [right-handed (dextral) and left-handed (sinistral)] land snail, *Partula suturalis* on the island of Moorea (Johnson, 1982). In no-choice laboratory experiments, Johnson (1982) observed that pairs of *P. suturalis* with same shell coiling morphs mated five times more frequently than did pairs with different morphs. Assortative mating offers an opportunity for genetic divergence between dextral and sinistral subpopulations by suppressing gene flow between them [*e.g.* the land snail *C. nemoralis* in the U.K.; (Johnson, 1976)]. This limitation of gene flow promotes the evolution of reproductive isolation, driving speciation in the absence of geographical barriers (*i.e.* sympatric speciation; Fitzpatrick *et al.*, 2008).

This finding of no genetic structure between left and right morphs of *P. microlepis* cannot reject the disassortative mating hypothesis that was proposed by Takahashi & Hori (2008) in their field-based observational study (Table I). The genetic signature of disassortative mating (*i.e.* significant deviations from HWE because of heterozygote excess), however, could not be detected. One case of a very small negative f value (-0.0008) was found in the KV-left sample. Large negative f values that depart significantly from HWE suggest disassortative mating (Templeton, 2006). Nevertheless, the data to test the prediction (*i.e.* heterozygote excess) of the disassortative mating hypothesis directly could not be exploited. The population genetic consequences of

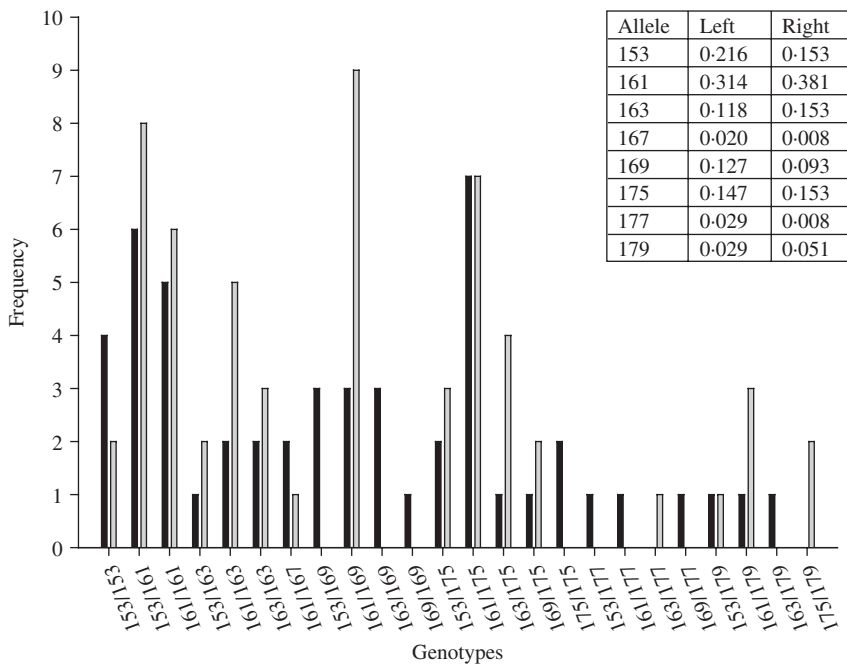


FIG. 4. Genotype frequency distribution between left (■) and right-mouth (□) morphs of *Perissodus microlepis* at UNH2101. The UNH2101 is putatively linked to the gene for mouth laterality according to Stewart & Albertson (2010) who observed only two alleles [A (right allele) – 150 bp and B (left allele) – 158 bp] at this locus. However, eight different alleles were detected in the present study. Alleles, 153 and 161, were presumed to correspond to alleles, A and B. No patterns of segregation of particular alleles were found according to mouth morphs. If this locus is linked to the gene for mouth laterality, left individuals would be expected to be homozygous for a putative left allele, while right individuals would be expected to be heterozygous for left and right alleles (Hori *et al.*, 2007; Stewart & Albertson, 2010), but this is not found. The small table in the figure shows the frequency of each allele for left and right-morphed individuals.

disassortative mating are locus-specific, meaning that this mating regime can only affect loci involved in the expression of the sexually selected phenotypic trait (*i.e.* mouth laterality in this study) and their linked loci (Templeton, 2006; Table I).

If the laterality of *P. microlepis* is a heritable trait with simple Mendelian genetics as suggested by Hori (1993) and Hori *et al.* (2007), the disassortative mating hypothesis remains possible, but perhaps not plausible. Among field-caught offspring from broods guarded by a pair of parents of known asymmetry, Hori *et al.* (2007) observed that a right–right pair yielded a 2:1 ratio of right:left offspring, which is different from the ratio of right:left = 3:1 expected under simple Mendelian genetics. They proposed two hypotheses to explain this abnormal ratio: (1) lethality of homozygotes for the dominant right allele or (2) incompatibility between gametes possessing the dominant right allele. Under either hypothesis, if females can tell the mouth laterality of potential mates, then right females should prefer left mates because mating with left males will result in them gaining higher reproductive success than mating with right males (Hori *et al.*, 2007). The selective advantage of

mating with individuals of the other morph may allow for the evolution of disassortative mating, which in turn stabilizes the lateral dimorphism in *P. microlepis* (Takahashi & Hori, 2008). Schilthuisen *et al.* (2007) showed that disassortative mating occurred between dextral and sinistral morphs of the dimorphic south-east Asian camaenid tree snail *Amphidromus inversus* on Kapas Island, Malaysia. They argued that disassortative mating facilitates the maintenance of phenotypic dimorphism in the snail populations (Schilthuisen *et al.*, 2007).

If the laterality of *P. microlepis* is not a heritable trait (*i.e.* phenotypically plastic) as suggested by Palmer (2004, 2009), random mating would be expected. If the laterality of this fish is determined by phenotypic plasticity, left and right morphs would not encounter any selective pressure facilitating the evolution of either assortative or disassortative mating. The majority of the four sampling groups of *P. microlepis* conformed to HWE expectations at all 13 microsatellite loci examined, which suggests that these loci reflect entirely random mating in this fish, in support of the random mating hypothesis. In their recent controlled laboratory-based foraging experiments, Van Dooren *et al.* (2010) found that laterality in *P. microlepis* has a strong phenotypic component, lateralized foraging behaviour amplifies the extent of morphological asymmetry in adults due to phenotypic plasticity. Although the authors do not know whether genes are also involved in determining laterality, they propose that phenotypic plasticity at least contributes to trait variation in *P. microlepis*. This finding of strong plasticity in mouth-opening direction in *P. microlepis* would tend to lend support to the random mating hypothesis.

This study indicates small but significant amounts of spatial genetic structuring between two geographically adjacent populations of *P. microlepis* (although not for AMOVA). This observed spatial structure suggests that this species has only limited ability for dispersal. This result is consistent with the recent finding of highly significant genetic structure among six populations of *P. microlepis* in southern Lake Tanganyika at *c.* 6–43 km geographic scale, based on variation in a smaller portion of the orthologous mtDNA control region (356 bp) (Koblmüller *et al.*, 2009).

The samples showed a 1:1 ratio of left:right morphs of *P. microlepis* from two geographical sites in southern Lake Tanganyika, as was found before by Hori (1993). The lateralized foraging behaviour hypothesis, where left morphed fish only attack the right sides of their prey and right morphs the left sides, was also supported by the laboratory experiments.

A more complete understanding of the mating system of *P. microlepis* will be aided by additional laboratory-based behavioural experiments to directly test the disassortative–random mating hypotheses. Furthermore, direct estimation of the laterality ratio of broods from different experimental crosses between left and right morphs in the laboratory is required to determine the inheritance pattern of the laterality of *P. microlepis* precisely. A laboratory-based study could also ensure that there is no intraspecific or interspecific brood mixing through farming-out, as has been frequently observed in *P. microlepis* (Yanagisawa, 1985; Ochi & Yanagisawa, 1996), which might have caused complications in the estimates of the genetic basis of laterality. These fish, however, are notoriously difficult to keep and breed in the laboratory and only very few cases of laboratory spawnings have been reported so far. This will continue to make future laboratory and genetic studies on the behavioural and developmental aspects of this interesting morphological and ecological dimorphism difficult.

Lateral dimorphism in mouth-opening direction is not limited to scale-eating Lake Tanganyika cichlids such as *P. microlepis*. Other fishes such as herbivorous cichlids *Telmatochromis temporalis* Boulenger (Mboko *et al.*, 1998) and *Neolamprologus moorii* (Boulenger) (Hori *et al.*, 2007) and a freshwater omnivorous goby *Rhinogobius flumineus* (Mizuno) (Seki *et al.*, 2000) also show this characteristic. Similar to scale-eaters, the laterality of these fishes is also strongly associated with mouth usage in foraging behaviour (*i.e.* lateralized foraging behaviour). In the herbivorous cichlid *T. temporalis*, the right morph tends to use the right side of the jaw more frequently and the left morph the left side (Mboko *et al.*, 1998). Although evidence for ecological effects of laterality is convincing, an understanding of the mechanisms of laterality itself is lacking. Thus, the exact developmental bases of laterality and the mechanisms by which the dimorphism is maintained ecologically would be interesting to explore in these fishes across different habitats.

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APPENDIX. Summary of genetic diversity in left and right samples of *Perissodus microlepis* at 13 microsatellite loci. Sample sizes (N), observed number of alleles per locus (N_a), P values of exact tests for deviation from Hardy–Weinberg equilibrium ($H-W$ test), expected (H_E) and observed heterozygosity (H_O) and allelic richness (AR) within a given sample per locus are provided. P value in boldface denotes significant deviation from H-W test after a Bonferroni correction for multiple testing. KV, Kalambo Village; TL, Toby Veall's Lodge

Sample	Locus	Abur25	Abur28	Abur44	Abur61	Abur90	Abur98	Abur110	Abur111	Abur117	Abur162	Abur163	Abur165	Abur178	Mean
KV-left	N	20	22	22	20	20	21	23	22	19	23	15	22	21	20.8
	N_a	5	2	8	9	6	8	4	4	8	3	5	4	4	5.4
	H-W tests (P)	0.820	1.000	0.984	0.493	0.596	0.423	0.441	0.868	0.687	0.023	0.388	0.668	0.159	—
	H_E	0.717	0.359	0.655	0.823	0.663	0.664	0.437	0.576	0.761	0.465	0.738	0.569	0.765	0.630
	H_O	0.700	0.364	0.773	0.900	0.600	0.667	0.478	0.546	0.842	0.304	0.600	0.546	0.857	0.629
KV-right	AR	4.930	2.000	7.136	7.988	5.440	6.913	3.872	3.680	7.354	2.963	5.000	3.682	4.000	4.997
	N	26	25	25	26	25	22	26	25	24	26	20	26	25	24.7
	N_a	5	2	10	8	5	6	4	6	9	4	5	4	4	5.5
	H-W tests (P)	0.294	0.372	0.878	0.812	0.135	0.524	0.244	0.262	0.642	0.074	0.396	0.187	0.188	—
	H_E	0.612	0.429	0.763	0.807	0.620	0.584	0.610	0.708	0.805	0.280	0.744	0.610	0.758	0.641
TL-left	H_O	0.500	0.520	0.800	0.808	0.600	0.500	0.615	0.640	0.875	0.231	0.650	0.500	0.680	0.609
	AR	4.506	2.000	8.179	7.051	4.521	5.018	3.972	5.521	7.957	3.480	4.750	3.577	4.000	4.964
	N	25	29	29	27	27	27	30	29	29	29	19	30	26	27.2
	N_a	8	2	9	9	5	9	6	5	13	4	5	4	4	6.4
	H-W tests (P)	0.877	1.000	0.121	0.903	0.843	0.184	0.553	0.942	0.329	0.527	0.568	0.293	0.206	—
TL-right	H_E	0.767	0.160	0.717	0.818	0.514	0.703	0.550	0.649	0.886	0.424	0.700	0.605	0.750	0.634
	H_O	0.680	0.172	0.655	0.778	0.593	0.704	0.567	0.621	0.846	0.448	0.579	0.700	0.692	0.618
	AR	6.720	1.979	7.731	7.643	4.098	7.369	4.936	4.033	10.788	3.034	4.579	3.972	4.000	5.453
	N	27	29	29	27	28	29	30	27	27	26	23	30	27	27.5
	N_a	7	2	12	11	4	9	5	5	11	5	7	4	4	6.6
TL-right	H-W tests (P)	0.708	1.000	0.309	0.008	1.000	0.196	0.035	0.681	0.003	1.000	0.056	0.741	0.768	—
	H_E	0.735	0.373	0.725	0.813	0.629	0.666	0.616	0.590	0.860	0.249	0.756	0.626	0.718	0.643
	H_O	0.815	0.414	0.690	0.630	0.643	0.621	0.500	0.556	0.731	0.269	0.565	0.633	0.815	0.606
	AR	5.916	2.000	8.865	8.496	3.536	7.147	4.725	4.111	9.441	3.704	6.187	3.995	3.995	5.548