

Frequency-Dependent Natural Selection in the Handedness of Scale-Eating Cichlid Fish



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detectable taxol at time zero. Chlorocholine chloride (at 1 mg/ml) in the medium (10) abolished taxol production. This compound is also an effective inhibitor of gibberellin production in *G. fujikuroi* (9), although it stimulates petasol (sesquiterpenoid) production in *Drechlera gigantea* (18). We conclude that the taxol isolated from cultures of *T. andreanae* was a product of the metabolism of this organism.

The amounts of taxol and taxanes produced by *T. andreanae* are low, as reflected by the limited incorporation of ^{14}C -labeled precursors into taxol (Table 1). Quantitation by electrospray mass spectrometry and CIEIA indicated that 24 to 50 ng of taxol were produced per liter. However, many plant-associated microbes require one or more plant metabolites to activate the synthesis of secondary natural products (19). Improved culturing techniques and the application of genetic engineering may improve taxol production by *T. andreanae* (20).

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- S-7 medium consists of 1 g of glucose, 3 g of fructose, 6 g of sucrose, 1 g of Na^+ -acetate, 1 g of soytone, 1 mg of thiamine, 1 mg of biotin, 1 mg of pyridoxal, 1 mg of Ca^{2+} -pantothenate, 3.6 mg of MgSO_4 , 6.5 mg of CaNO_3 , 1 mg of $\text{Cu}(\text{NO}_3)_2$, 2.5 mg of ZnSO_4 , 5 mg of MnCl_2 , 2 mg of FeCl_3 , 5 mg of phenylalanine, 100 mg of Na^+ -benzoate, and 1 ml of 1M KH_2PO_4 buffer (pH 6.8) per liter. Sugar ratio is identical to that occurring in the inner bark of Pacific yew. Modified mycological agar consists of 10 g of bacto-soytone, 40 g of glucose, 15 g of bacto-agar, 1 g of Na^+ -acetate, and 50 mg of sodium benzoate per liter.
- Thin-layer chromatography solvent systems, v/v: a, chloroform-acetonitrile 7:3; b, chloroform-methanol 7:1; c, ethylacetate-isopropanol 95:5; d, dichloromethane-tetrahydrofuran 6:2; and e, hexane-acetone 1:1.
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- Cytotoxic properties of fungal taxol were tested as described [T. Mossman, *J. Immunol. Methods* **65**, 55 (1983)]. KB cells (epidermoid carcinoma) were incubated in 96-well plates at 37°C for 24 hours. Test samples of taxol were dissolved in dimethyl sulfoxide (DMSO) and diluted with medium. The samples were added to the plates in triplicate for each concentration, and incubation was contin-

ued at 37°C for 7 days. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (10 μl of MTT in 20 ml of phosphate-buffered saline) was added, and the plates incubated at 37°C for 6 hours. Medium was then removed, 100 μl of DMSO was added, and plates were shaken gently for 10 min to dissolve the formazan crystals. Absorbance at 535 nm was measured and ED_{50} was calculated as the dilution of sample with an effect one-half that of the control (no agent added).

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Frequency-Dependent Natural Selection in the Handedness of Scale-Eating Cichlid Fish

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Frequency-dependent natural selection has been cited as a mechanism for maintaining polymorphisms in biological populations, although the process has not been documented conclusively in field study. Here, it is demonstrated that the direction of mouth-opening (either left-handed or right-handed) in scale-eating cichlid fish of Lake Tanganyika is determined on the basis of simple genetics and that the abundance of individuals with left- or right-handedness depends on frequency-dependent natural selection. Attacking from behind, right-handed individuals snatched scales from the prey's left flank and left-handed ones from the right flank. Within a given population, the frequency of the two phenotypes oscillated around unity. This phenomenon was effected through frequency-dependent selection exerted by the prey's alertness. Thus, individuals of the rare phenotype had more success as predators than those of the more common phenotype.

Mechanisms that maintain balanced polymorphisms in nature have attracted much attention (1). Frequency-dependent selection has been suggested as one such mechanism (2), and evidence has been accumulated to support it (3-5). However, almost all the recent work done on this topic is concentrated around the sex ratio (4) and alternative mating phenotypes (5) in which the agents of selection are conspecifics, and

little work has involved prey-predator interaction. Although some data suggest that selection by enemy or prey maintains polymorphism (6), this process has not been demonstrated conclusively under natural conditions. I report here a simple example of frequency-dependent selection effected through the differential guarding response of prey toward the two phenotypes of a predator.

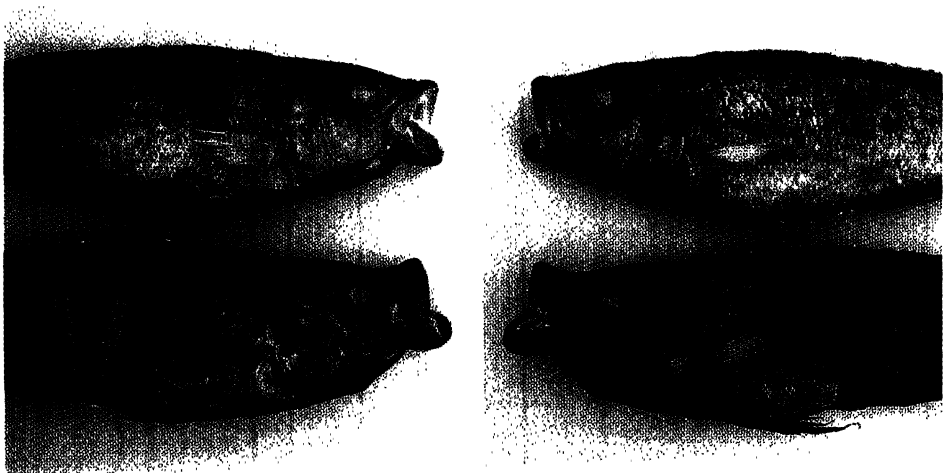


Fig. 1. The handedness of mouth opening of a Lake Tanganyikan scale-eating cichlid, *P. microlepis*. A right-handed (upper) and a left-handed (lower) individual are shown from both sides. [Photo provided by H. Yamasaki]

Some of the most specialized of the African Rift Valley Lake cichlid fishes are the scale-eaters (7), which feed on the scales of other living fish. Seven scale-eating species are known from Lake Tanganyika (8), and all fall within the genus *Perissodus* (9). One of these species, *P. eccentricus*, was originally thought to be unique because an individual's mouth opens either rightward or leftward as a result of an asymmetrical joint of the jaw to the suspensorium (9). This lateral asymmetry of the mouth opening was considered an adaptation for efficiently tearing off prey's scales and the result of specialization among the lineage.

However, further examination revealed that all seven species display asymmetrical mouth opening to some degree, independent of gender (10). A detailed study was made of the dynamics of this dimorphism in *P. microlepis* Boulenger (Fig. 1), the most abundant scale-eater in the littoral area of the lake (11, 12). Habitat conditions in this area are very stable, and most resident fish populations are highly persistent with respect to their density (10, 11). Therefore, in order to consider any change in the frequency of scale-eaters of each handedness, it is sufficient to consider the ratio between the two morphs.

Perissodus microlepis approaches its prey from behind in order to snatch several scales from the flank of the prey (12, 13). A field experiment with the use of common prey species as live lures showed that right-handed (dextral) individuals always attack the victim's left flank, and left-handed (sinistral) ones the right flank (Table 1). Further support for this correspondence was obtained by examination of the handedness of prey's scales found in the stomachs of scale-eaters. This handedness of scales was particularly easy to determine for the pored scales of the lateral lines. Table 2 shows that dextral fish took scales from the left flank of prey and sinistral fish from the right flank. The distorted mouth apparently enlarges the area of teeth in contact with the prey's flank, but this is useful only if the scale-eater attacks one side of the prey. Thus, the correspondence between the handedness and the side of attack should be a functional requisite for the success in feeding of these scale-eaters and may have evolved from the beginning of the lineage.

Handedness is detectable even in fry under parental care (10), a time when they feed exclusively on plankton (12), which indicates that handedness is heritable. The genetics was investigated by examination of sets of parents and their fry collected from natural habitats. *Perissodus microlepis* par-

ents have the unusual habit of farming out their fry to other breeding pairs (14), which makes it difficult to define the exact phenotype frequency in each brood. The results, however, strongly suggest that handedness is determined by a simple Mendelian one

locus-two alleles system, in which dextrality is dominant over sinistrality (Table 3).

As potential prey fish were usually alert to approaching scale-eaters, the hunting success of *P. microlepis* was low; the ratio of the number of successes to the number of

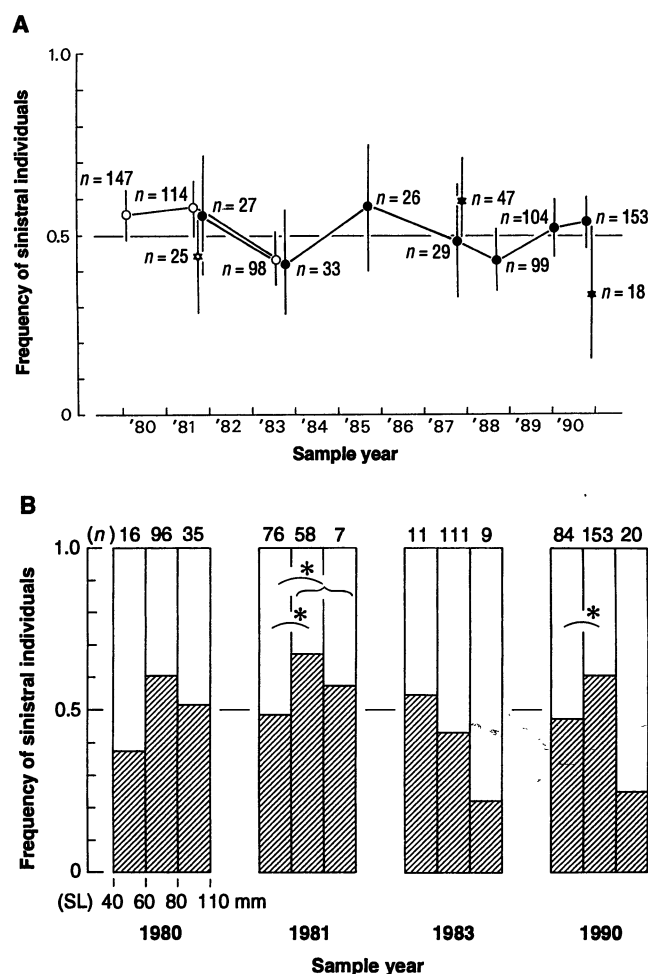
Table 1. Correspondence between the handedness of *P. microlepis* and the flank of prey attacked. This experiment was done under natural conditions with adults of *Cyathopharynx furcifer*, an abundant and common prey species of *P. microlepis* (13), as lures. Each live *C. furcifer* individual was connected by a hook to a fishing line and allowed to swim; each *P. microlepis* that attacked the lure was caught by gill net.

Handedness of <i>P. microlepis</i>	Observations (n)	Attacks on flank (n)	
		Right	Left
Dextral	4	0	4
Sinistral	9	9	0

Table 2. Occurrence of right and left pored scales in the stomach of *P. microlepis*. The handedness of scales was determined under a binocular microscope on the basis of the shape of the exposed granulated portion and the number of basal ridges in the upper and lower parts separated by the mucous tube. Unknown scales are those misshapen as a result of partial digestion and those of abnormal shape.

Handedness of <i>P. microlepis</i>	Fish (n)	Pored scales (n)		
		Right	Left	Unknown
Dextral	32	0	139	31
Sinistral	24	76	0	23

Fig. 2. (A) Oscillation in the ratio of handedness of two adjacent populations (Luhanga coast, open symbols; Bemba coast, closed symbols) of *P. microlepis* at the northwestern Zaire shoreline of Lake Tanganyika over an 11-year period. Sample fish were taken by a gill net of uniform mesh size over a 2- to 3-month period during each sample year, except in 1990, when two sets of samples were taken, one each in January and in September. The ratio is expressed as the frequency of sinistral individuals in each sample with 95% fiducial limits. Star symbols indicate breeding adults, selectively collected with the use of scuba. In both populations, the ratio of handedness remained around unity and oscillated throughout the sample period. A statistical test for combined data of the two populations proves that for a period of 4.3 to 5.3 years, the oscillation is highly probable ($P < 0.001$, analyzed with a Fourier coefficient). (B) The ratio of handedness in each size class of *P. microlepis* in each year. Data are shown only for years when more than 130 fish were taken, and breeding fish are excluded. The ratio in larger individuals [>60 mm standard length (SL)] in any year shows a trend opposite that seen in smaller individuals, which indicates that adults of the rare phenotype show a greater reproductive output than those of the common phenotype. Asterisks show that the difference in ratio between the size classes is significant ($P < 0.05$, normal distribution test).



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attempts was about 0.2 (13). Because handedness is inherited, the ratio between the two phenotypes in a population theoretically should be balanced at the same frequency, as each phenotype will be at an advantage when rare. When one of the two phenotypes—for example, dextral individuals—is more abundant in the population, prey fishes will tend to guard more against attacks to their left side, which results in sinistral individuals gaining greater hunting success and a commensurate greater fitness. Should this simple frequency-dependent selection mechanism operate, then the polymorphism can be regarded as an evolutionary stable state (15).

Table 3. Phenotype frequency in broods of *P. microlepis*. The observed ratio in F_1 is shown with the actual number (n) of dextral (D) and sinistral (S) fry guarded by an adult pair ($D \times D$, $D \times S$, or $S \times S$); expected ratios are theoretical ones that should appear from the parents if the genetics followed a Mendelian one locus-two alleles system with dextrality being dominant over sinistrality. To exclude fry from other parents as much as possible, I omitted from the observed ratio fry whose size conspicuously differed from the majority, although they were included in the total.

$D \times D$ (3)		$D \times S$ (4)		$S \times S$ (5)	
D:S	n	D:S	n	D:S	n
<i>Observed</i>					
23:2	(29)	74:0	(80)	0:79	(80)
35:11	(46)	18:2	(20)	0:29	(30)
39:15	(54)	55:53	(109)	1:19	(21)
		27:29	(59)	15:79	(99)
				12:28	(49)
<i>Expected</i>					
1:0		1:0		0:1	
3:1		1:1			

To verify this prediction, I examined the ratio of handedness each year for samples of *P. microlepis* collected from two adjacent sites (about 7 km apart) along the northwestern shoreline of the lake at 1- to 2-year intervals over an 11-year period. As predicted, the ratio remained at around 0.5 and never deviated from it by more than 0.07 during the period, which provides strong support for the presence of a balancing mechanism (Fig. 2A).

However, closer examination of the temporal changes in the ratio of handedness demonstrates that it oscillated with an amplitude of 0.15 and a period of 5 years. Both populations at the two sites showed this oscillation. The oscillation is also observable upon examination of the ratio of handedness in every size class in each year; the ratio in the larger individuals is opposite that in the smaller ones (Fig. 2B). Provided that the survival rate does not markedly differ between the two phenotypes and assuming that the size classes can be regarded as age classes, evidence for the oscillation can be detected in the difference of ratios among size classes in each year. No previous studies on balancing polymorphism have detected or predicted such an oscillation.

Two hypotheses may be proposed to explain this oscillation: either (i) it is the result of a time lag between the differential hunting success and the resultant increase in progeny or (ii) the prey do not respond in an exact frequency-dependent manner to the ratio of handedness in the scale-eater population. The first possibility appears credible because the time lag between the reproduction of *P. microlepis* and the stage at which the progeny feed mainly on scales (about 60-mm standard length) (12) approaches 2 years (16). At present, however,

it is difficult to accurately evaluate this effect on the oscillation.

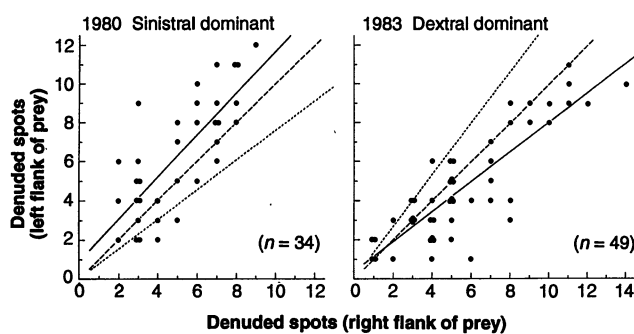
The second possibility can be examined quantitatively by examination of the scars on prey species caused by scale-eating. In this manner, the frequencies of scale-eating on prey by the two phenotypes can be distinguished by counting the characteristic denuded spots on each flank of prey fishes. When sinistral individuals were numerically dominant, the prey suffered scale-eating from dextral individuals more frequently than from sinistral individuals and vice versa (Fig. 3). This result clearly demonstrates that the prey fish focused their guard toward either the right or left flank, depending on which predator phenotype was most abundant. Actually, prey responded to the ratio of handedness of the predator more than predicted by the exact inverse relation to the ratio. This is likely a reflection of prey responding to the number of attacks, including unsuccessful ones, and not to how often they were actually injured.

This differential hunting success should result in a differential reproductive success between the two phenotypes. The ratio of handedness in samples of breeding pairs collected on three occasions were all opposite the ambient ratio in the total population (Fig. 2A). This fact strongly suggests that rare phenotype individuals enjoy some reproductive advantage.

Almost all previous field studies of polymorphism maintained through prey-predator interaction, including the classical example of polymorphism in color and banding pattern of *Cepaea* land snails (2), have shown a combination of frequency-dependent and frequency-independent selection as being necessary to maintain the polymorphism. In marked contrast, the handedness of *P. microlepis* may be maintained solely by frequency-dependent selection. It seems implausible that either dextrality or sinistrality itself has any superiority over the other, and pleiotropic gene effects seem most unlikely. Moreover, habitat heterogeneity should not favor either phenotype. Therefore, the handedness of this scale-eater is a documented example of a polymorphism maintained solely by frequency-dependent selection through prey-predator interaction. It is also the rare example to demonstrate that the minority advantage results in differential reproductive success in a natural population.

The ratio of handedness, however, does not stay at an equilibrium level but oscillates around it, which contradicts an intuitive prediction. Here, this is attributed to a trait of the prey's reaction and the inevitable time lag between the occurrence of the advantage and the resultant change in phenotypic frequency. At first glance, such an oscillation may appear

Fig. 3. Denuded spots on each flank of a prey species during two opposite phases of phenotype abundance of *P. microlepis*. The number of denuded spots on each flank of *C. furcifer* taken from Luhanga during the sampling of *P. microlepis* was measured in 1980, when the sinistral phenotype was dominant, and in 1983, when the dextral phenotype was dominant. An experiment with the use of live fish demonstrated that a successful attack by a scale-eater left a characteristic scar, with several scales missing in two adjacent clusters. As scale-eaters other than *P. microlepis* were very rare at this study site (11), all such scars were regarded as being caused by this species. The solid lines indicate the regression line of the plotted data points; the broken lines represent an equal number of attacks on right and left flanks—that is, an exact inverse relation to the actual frequency of the two phenotypes in the population. The dotted lines represent attacks to flanks proportional to the actual frequency of the two phenotypes—that is, the prey guard both sides equally, irrespective of the frequency of attack. In 1980, prey species suffered more scale-eating from dextral individuals, as indicated by the greater number of scars on left flanks ($P < 0.001$, normal distribution test). Conversely, in 1983 prey suffered more scale-eating on their right flanks ($P < 0.001$).



specific to this system. However, as I cannot expect that both the predator and the prey attain the highest efficiency in their interaction nor that any action of an agent affects the reproduction without a time lag, I speculate that the oscillation is not unique but rather a common process in polymorphic populations under natural conditions.

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Distinct Functions of SR Proteins in Alternative Pre-mRNA Splicing

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Alternative splicing of precursor messenger RNAs (pre-mRNAs) is a common mechanism of regulating gene expression. SR proteins are a family of pre-mRNA splicing factors that are structurally related and evolutionarily conserved. Any member of the SR family can complement a splicing-deficient extract that lacks the entire family of SR proteins. Here it is demonstrated that particular SR proteins have distinct functions in alternative pre-mRNA splicing in vitro. In addition, SR proteins are differentially expressed in a variety of tissues. These results suggest a fundamental role for SR proteins in the regulation of alternative splicing.

SR proteins comprise a family of evolutionarily conserved pre-mRNA splicing factors (1). The primary amino acid sequences of these proteins are highly related, and they share an NH₂-terminal RNA recognition motif (2) and a COOH-terminal domain of variable length that consists almost exclusively of alternating Ser and Arg residues (1, 3). Many animal cells express a set of SR proteins of similar molecular mass: 20,

30, 40, 55, and 70 to 75 kD (1, 3). The 30-kD band contains two distinct polypeptides, SRp30a and SRp30b, which have also been described as SF2/ASF (4, 5) and PR264/SC35 (6), respectively. Furthermore, the sequences of individual SR proteins are highly conserved between species; for example, there is a 58% amino acid identity between nematode and human SRp30a in the non-Ser-Arg region (7).

Individual SR proteins isolated from vertebrates and invertebrates function similarly as essential pre-mRNA splicing factors when tested in depletion and reconstitution assays (1, 4, 5, 8, 9). These tests were possible because SR proteins are absent

from a splicing-deficient extract (S100) of HeLa cells that is prepared by a 100,000g centrifugation of lysate containing 4.5 mM MgCl₂ (10). Under these conditions, SR proteins are insoluble (1, 3). Addition of any one of four different SR proteins purified from calf thymus (1) or any one of five SR proteins purified from human HeLa cells (11) can complement this splicing-deficient extract so that in vitro splicing of pre-mRNAs containing single 5' and 3' splice junctions can then occur. It has also been shown that increasing the concentration of SRp30a (4, 5) or SRp55 (8) results in a shift in the 5' splice site that is used in the case of several pre-mRNAs that contain multiple 5' splice sites. Although all SR proteins exhibit similar activity in these simple biochemical assays, the strict size and sequence conservation between vertebrates and invertebrates of the different SR proteins suggests that each SR protein has a distinct and essential function. Here, we present evidence that individual SR proteins allow the preferential use of different pre-mRNA 5' splice sites.

As a first step toward a quantitative analysis of SR protein function in alternative pre-mRNA splicing in vitro, we determined the relative activity of several different SR proteins in splicing a single intron. SRp30b, SRp40, SRp55, and SRp70 were purified from calf thymus (Fig. 1), and we tested the proteins for their abilities to splice a β -globin pre-mRNA substrate (12) when they were added to the HeLa S100 splicing-deficient extract. The amount of each SR protein added was adjusted such that equivalent amounts of mature mRNA product were generated in each reaction (Fig. 2A). Quantitation of these reactions with the use of a phosphor imager (13) is shown (Fig. 3A). When increasing amounts of SR protein were added to the splicing-deficient extract, the amount of mRNA produced increased. This production was dose-dependent; that is, for each amount of any of the four SR proteins added, the amount of mRNA product appeared to be approximately equal. Splicing approached a maximal rate of 50% conversion to spliced products with the addition of 600 ng of each SR protein preparation. These results show that SR proteins function in a parallel and concentration-dependent manner (Figs. 2A and 3A). The concentration of each SR protein was found to be approximately 150 ng/ μ l (Fig. 1), and therefore the specific activity of each SR protein preparation was nearly identical.

To test the possibility that specific SR proteins confer preferential utilization of different splice sites, we used equivalent amounts of SRp30b, SRp40, SRp55, and SRp70 to splice other pre-mRNAs in a HeLa cell S100 splicing-deficient extract.

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