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Mitochondrial DNA Variation in *Cryptobranchus alleganiensis*, a Salamander with Extremely Low Allozyme Diversity

ERIC ROUTMAN

The distribution of mtDNA variation was measured in **the hellbender, *Cryptobranehus alleganiensis*, a salamander species with extremely low allozyme variation.** Fifty-seven restriction sites were **mapped** for the 226 individuals sampled. Thirty-three of these sites were polymorphic and together determined 15 different mtDNA haplotypes. Within-population mitochondrial variation is somewhat lower in hellbenders than for other vertebrates. Between-population variation **for hellbenders is extremely high; F,. for the entire species is 0.865. A past bottleneck followed by rapid mtDNA evolution and slow allozyme evolution is probably responsible for the different levels of variation in mtDNA and allozymes in this species.**

THE hellbender, *Cryptobranchus alleganiensis*, is a large, permanently aquatic salamander found in clear, spring-fed streams in mountainous areas of the eastern United States. An electrophoretic study of 24 loci in hellbenders from 12 populations sampled from throughout the range yielded the surprising result that this species has very little allozyme variation (Merkle et al., 1977). Twenty-two of the loci were monoallelic in all populations, whereas Mdh-1 was polyallelic in one population, and Pt-4 showed multiple alleles in two populations. Shaffer and Breden (1989) also found extremely low levels of allozyme variation in two large samples ofhellbenders fromthe Gasconade **River** drainage in Missouri. Shaffer and Breden (1989) calculated average heterozygosity (H) and proportion of loci polyallelic **(P)** for the Merkle et al. (1977) data **(H** = 0.007 and P = 0.026) and for their own sample (H = 0.019 and P = 0.095). Low levels of genetic variation in these and other nontransforming salamanders have been attributed to population bottlenecks caused by temporal instability of aquatic habitats (Shaffer and Breden, 1989).

Because of its rapid rate of evolutionary change in mammals and other vertebrates (Brown et al., 1979; Avise, 1986) and its maternal, haploid mode of inheritance, mtDNA often displays greater variation and population substructure than do nuclear markers such as allozymes (reviewed by Avise, 1986). Comparison of mtDNA variation with that of nuclear markers often yields more insight than analysis of either one alone (Templeton, 1985; DeSalle et al., 1987). In this paper, I will present the results of a study of mtDNA variation in *Cryptobranchus alleganiensis* and compare that variation to that reported in previous allozyme studies.

METHODS

Collection of specimens.-Collection sites are shown in Figure 1, and sample sizes and collection site details are given in Table 1. Many of these sites are the same as those sampled by Merkle et al. (1977) and by Shaffer and Breden (1989). Within Missouri, some rivers were sampled at several sites, and these subsamples are listed in Table 1. Hellbenders were collected by lifting rocks and grabbing exposed salamanders by hand. Captured individuals were anesthetized in aqueous solution of ethyl m- aminobenzoate methansulfonate (MS-222), and blood samples were taken from an incision in the tail. Blood was diluted in STE buffer (Maniatis et al., 1982) and stored in liquid nitrogen until returned to the laboratory where it was transferred to a -80 C freezer until use. Voucher specimens (one or two per river) were deposited in the Museum of Vertebrate Zoology at the University of California, Berkeley **(MVZ** 205728-205739). All others were released at the site of capture.

DNA extraction and restriction mapping.-Total genomic **DNA** was obtained by digesting blood samples with Proteinase **K** followed by phenol and chloroform extractions and ethanol precipitation, as described in Maniatis et al. (1982). The DNA was pelleted by centrifugation and resuspended in distilled water. Aliquots of DNA were digested overnight according to manufacturer's instructions with the following restriction enzymes: *BamHI, Bell, Bglll, BstEII, Eco*RI, *EcoRV, Ncol, Pstl, Pvull, Sacl, Stul, Xbal,* and *Xmnl.* Each enzyme has a unique six base-pair recognition sequence. Restriction digests were electrophoresed on 0.8% agarose gels and transferred to nylon filters by "wickless"

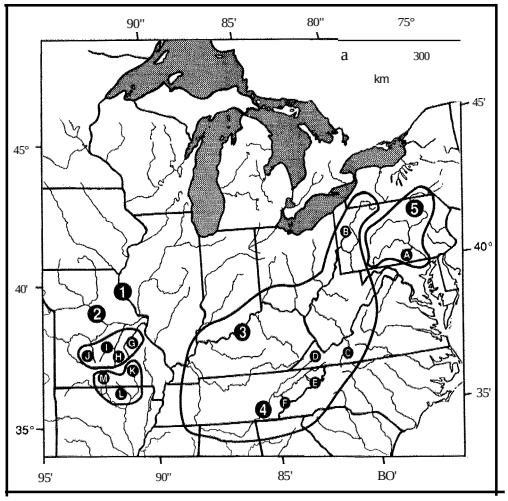

Fig. 1. Range map of *Cryptobranchus alleganiensis.* Areas outlined in black are the four major disjunct regions of the range. Letters indicate rivers sampled for this study as follows: A. Sherman Creek, B. Slippery Rock Creek, C. New River, D. Copper Creek, E. Beaverdam Creek, F. Little River, G. Meramec River, H. Big Piney River, I. Gasconade River, J. Niangua River, K. Current River, L. Spring River, M. North Fork of the White River. Numbers indicate major river drainages as follows: 1. Mississippi River, 2. Missouri River, 3. Ohio River, 4. Tennessee River, 5. Susquehanna River.

Southern blotting (no buffer reservoir was used). Filters were probed with radiolabeled DNA from one or more of the following clones: pX131-theentire mtDNA molecule of *Xenopus laevis* in pBR322; pCa6 and pCal8-two *Bglll* fragments (6.3 and 3.0 Kb, respectively) ofmtDNA of C. *alleganiensis* each cloned into plasmid Pucl8; pCal4 and pCa36-two *BamHI* fragments (11.1 and 5.3 Kb, representing the entire molecule) of the mitochondrial genome of C. *alleganiensis* each cloned into Pucl 8. (The hellbend r clones and the technique used to isolate mtDNA from salamander ova are available from the author.) Hybridization was done at 65 C in SSPE buffer containing 1% SDS and nonfat dry milk as a blocking agent (Reed and Mann, 1985). Mitochondrial DNA restriction fragments were visualized by autoradiography (Fig. 2). Restriction sites were mapped with the appropriate combination of double digestion and partial probing. A preliminary subset of these data has appeared in Templeton et al. (1990).

Estimation of genetic diversity.-Genetic diversity at the haplotype level, or nucleon diversity, is defined as the probability of drawing two identical haplotypes from a population (Nei, 1987). The unbiased estimator given by Nei and Tajima (1981) was used to estimate nucleon diversity within hellbender populations. At the nucleotide level, diversity was estimated with the method described in Ewens et al. (1981). At this level, genetic diversity is the probability that two alleles chosen at random from a sample of alleles are different at a randomly chosen nucleotide site (also known as the probability of heterozygosity or 0). One estimate of 0 is

$$0 = k/[2rm(\log_e(n))]$$

where k = the number of polymorphic sites in the sample, r = the length of the restriction enzyme recognition sequence, m = the total number of sites in the sample, and n = the number of individuals sampled. This method corrects for the fact that polymorphism in restriction sites does not represent the variation for randomly chosen potential sites but rather is conditional on the presence of each site in at least one of the molecules sampled. The parameter 0 obtained from this equation is the same parameter that forms the basis of coalescent theory (Hudson, 1990).

Measures of population subdivision.-Wright's F,. (Wright, 1951) was used to measure population subdivision. The sample-reuse technique (Davis et al., 1990) makes use of the probability of identity-by-descent definition ofF,.(Cockerham and Weir, 1987). Full details of the algorithm are given in Davis et al. (1990) and is summarized as follows: for every individual in the sample, two additional individuals are chosen at random-one from the same population and one from a different population. The identities of the mtDNA haplotypes between the purposely chosen and each of the randomly drawn individuals are recorded (1 if identical and O if different), and the procedure is repeated over all individuals in the sample. The identity scores are summed over individuals and divided by the sample size to calculate the total identity within and between populations. To convert total identity to identity by descent, correction for identity by state was made by estimating F,. with the equation

$$F_{st} = (ID_w - ID_b)/(1 - ID_b)$$

where IDw is total identity within populations and IDh is total identity between populations. This procedure is then repeated 1000 times to generate 95% confidence intervals for the statistics. The final estimate of F,. is the average of these 1000 replicates. It should be noted that this estimator of F,. is unbiased and, therefore,

TABLE 1. COLLECTING LOCALITIES, SAMPLE SIZES (n), DISTANCE FROM MOST UPSTREAM SITE FOR WITHIN-RIVER SUBSAMPLES, AND MAJOR DRAINAGE INTO WHICH THE COLLECTED RIVERS FLOW. Distances are given in kilometers.

River	n	Distance	Major Drainage (State)
Big Piney River:			Gasconade River (Missouri)
Barton Branch		0	
Boiling Spring	24	14.3	
Slabtown Spring	11	38.5	
Spring Creek	14	84.4	
Devil's Elbow	12	105.5	
Gasconade River	13	151.7	Missouri River (Missouri)
Niangua River:			Missouri River (Missouri)
Bennett Spring	6	0	
Ho Hum Camp	20	13.0	
Meramec River:			Mississippi River (Missouri)
Meramec Springs	3	0	
Drda Bluffs	25	5.0	
Archie Hole	14	36.25	
Current River	10		Black River (Missouri)
Spring River	7		Black River (Arkansas)
North Fork of the White River:			White River (Missouri)
Blair's Ford	14	0	
Patrick's Bridge	3	4.9	
Little River	12		Tennessee River (Tennessee)
Beaverdam Creek	8		Tennessee River (Tennessee)
Copper Creek	16		Tennessee River (Virginia)
New River	2		Ohio River (Virginia)
Slippery Rock Creek	1		Ohio River (Pennsylvania)
Sherman Creek	7		Susquehanna River (Pennsylvania)

can take on negative values when the actual F., is near zero (Davis et al., 1990).

Gene flow among populations was also calculated. The average (per generation) number of individuals migrating between demes is equal to Nm, where N is effective population size and m is migration rate among demes. The value of Nm determines the evolutionary independence of the subdivisions of a population. Nm was calculated from F,, using the equation

$$F_{,.} = 1/(Nm + 1)$$

(modified from Wright, 1951). Because of the haploid nature and maternal inheritance of the mitochondrial genome, equations based on autosomal genes need to be adjusted (Birky et al., 1983, 1989) to account for the smaller effective population size of mtDNA in the migrant pool. In this case, I divided Nm in Wright's equation by 4. This assumes the number and reproductive success after migration to be equal for males and females.

Although this equation was derived using finite island model assumptions, Slatkin and Barton (1989) have shown that it holds approximately for some types of selection and for two-dimensional stepping stone models as well. Slatkin and Barton did not discuss one-dimensional stepping-stone models, which more closely reflect the geographic structure of riverine organisms such as hellbenders. However, Kimura and Weiss (1964, discussed by Crow and Kimura, 1970) developed some theory for one-dimensional models. They derived an equation identical in form to the above formula if m is replaced by the square root of ($2m_1m$;), where m_1 is the migration rate between subpopulations one step apart and m; is the long-distance migration rate, which carries the same assumptions as the standard island model and is assumed to be much lower in magnitude than m_1. Using two-dimensional stepping-stone models, Kimura and Maruyama (1971) have shown that, if $Nm < 4$ geographically structured, populations will diverge due to genetic drift of neutral alleles and that, if $Nm < 1$, major local divergence will occur.

Larson et al. (1984) discussed the problems of estimating gene flow from F" when populations are not in equilibrium between gene flow and drift. Sharing of allele frequencies caused by historical association (rather than gene flow)

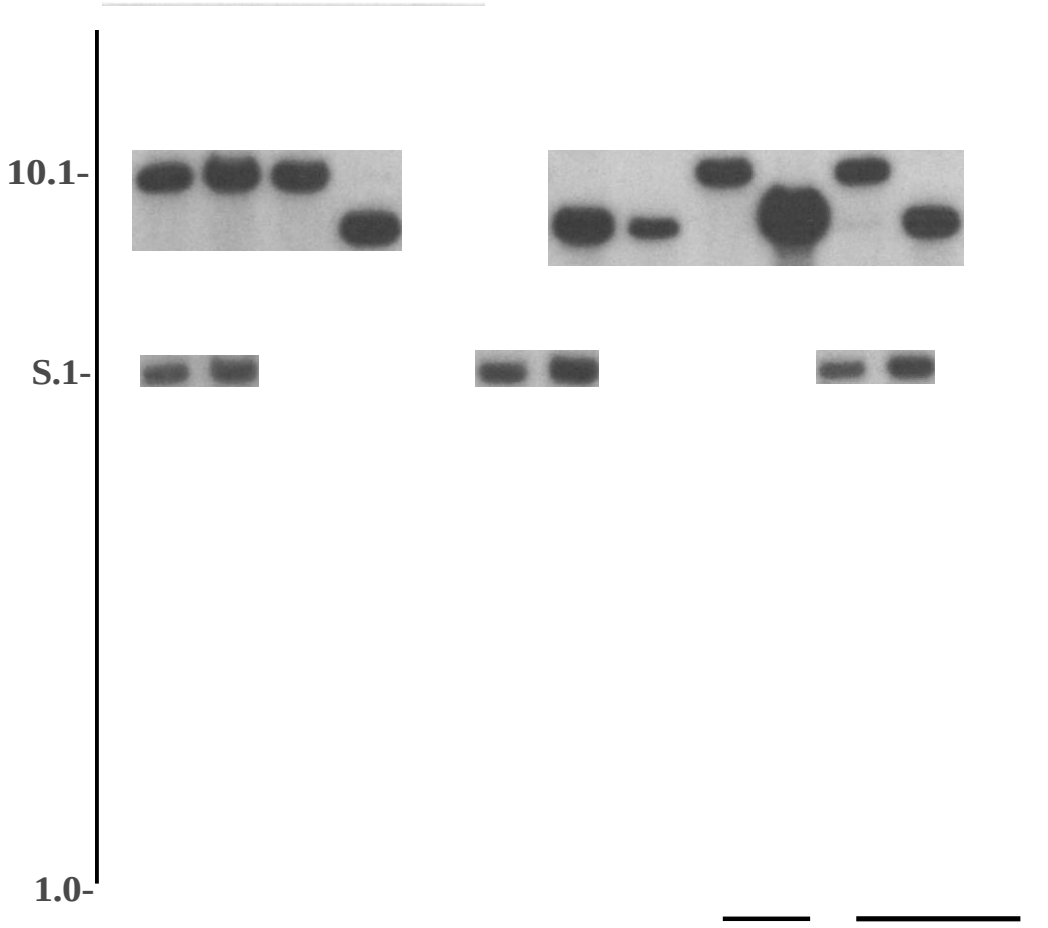

Fig. 2. Autoradiograph of EcoRV digests of 13 *Cryptobranchus alleganiensis,* showing variation of mtDNA. Lanes, from left to right, **l.** Beaverdam Creek, 2. Little River, 3. Little River, 4. Slippery Rock Creek, 5. New River, 6. Copper Creek, 7. Big Piney River, 8. Gasconade River, 9. North Fork of the White River, 10. Meramec River, 11. Spring River, 12. Niangua River, 13. Current River. Numbers along left border indicate sizes in kilobase pairs of selected bands.

tends to inflate Nm estimates. I will draw conclusions about the magnitude of this effect by comparing the value of F., for the entire species to values from geographically restricted population subsets.

RESULTS

Restriction map.-Figure 3 is a restriction site map of the hellbender mitochondrial genome. Fifty-seven sites were mapped for the 226 individuals sampled. Thirty-three of these sites were polymorphic and together determined 15 unique haplotypes (Table 2). No length variants were detected, nor were any definitely heteroplasmic individuals found (although low levels of heteroplasmy could be mistaken for partial digestion with the methods used for this study). Discussion of the phylogenetic relationships among the haplotypes and biogeographic implications are presented elsewhere.

Genetic variation.-Table 3 lists the within-population values of genetic diversity for the hellbender populations surveyed. Average within-population nucleon diversity is 0.0956. Average within-population 0 is 0.0005. Mean within-population values of0 calculated from mtDNA for the salamanders *Ambystorna tigrinum* and *A. rnaculaturn* are provided for comparison. The range of mean 8 values from other salamanders (0.001-0.004) is similar to the average value for mtDNA from humans (0.001-0.005, Excoffier and Langaney, 1989), whereas hellbenders average much lower nucleotide diversity. Thus, hellbenders have somewhat lower mtDNA vari-

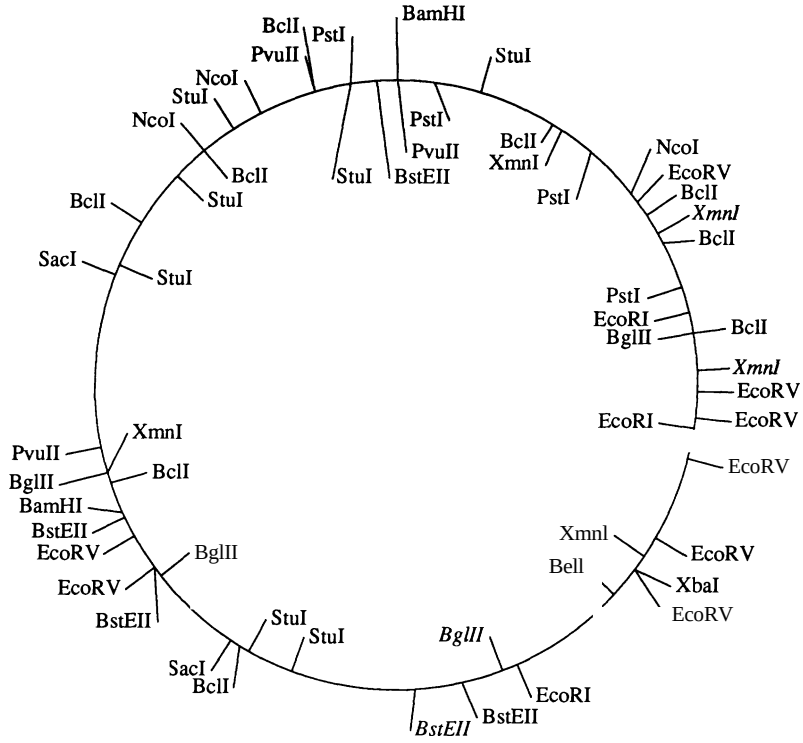

Fig. 3. Restriction site map of *Cryptobranchus* mtDNA. Total length is 16,200 base pairs. Sites outside the circle are variable among haplotypes. Sites inside the circle are invariant in the sample. Italicized sites indicate that the exact location of the site was not determined, although presence or absence is known with certainty.

ation as well as lower allozyme diversity. However, restriction analysis of hellbender mtDNA reveals much more variation than was found in studies of allozyme markers and is, therefore, useful for drawing some conclusions about population structure.

Population structure.-Table 4 shows the distribution of the 15 mtDNA haplotypes among the 13 rivers sampled. Multiple samples were taken from the Big Piney (plus the Gasconade into which it flows), Niangua, Meramec, and North Fork of the White rivers. In two of these rivers, hellbenders were monomorphic for mtDNA haplotype (Table 4), and, therefore, the samples were not informative regarding within-river gene flow. The Big Piney and Meramec populations, however, do possess mtDNA variation, and analysis of the between subsample haplotype frequencies reveals no substructuring within rivers [95% confidence intervals for F,. overlap O (unpubl.)]. It is likely that hellbenders are panmictic within rivers.

In contrast to the within-river panmixia, most hellbender populations found in different rivers are apparently separate evolutionary lineages. F,. for the species as a whole is 0.86, with a 95% confidence interval of 0.R 17-0.909 (Table 5). Most of the restricted geographic subsets possess F,. values nearly as high as that for the entire species (Table 5). The only exception is the subset of populations found in Tennessee. The 95% confidence interval of **F,.** for these two populations overlaps zero. Comparison to values from some ambystomatids (Table 5) reveals much greater population subdivision in *Cryptobranchus alleganiensis.* Values of Nm calculated from these F,. values are much less than **1,** indicating little gene flow among populations.

DISCUSSION

Most of the streams sampled for this study drain into the Mississippi, Missouri, and Ohio rivers. These rivers are apparently unable to support sufficient levels of hellbender migration to allow spread of different mtDNA haplotypes. Despite the relatively high levels of gene flow within rivers, the between river gene flow is extremely low. Assuming a 1:1 sex ratio in the migrant pool, this conclusion extends to the nuclear genome as well, because the values of Nm reported in Table 5 have been adjusted to make them comparable to values that would be cal-

TABLE 2. RESTRICTION SITES FOR HELLBENDER mtDNA HAPLOTYPES. Haplotype designations consist of the first initial of the state (M = Missouri, P = Pennsylvania, T = Tennessee, V = Virginia) in which the collection was made followed by a separate number for each haplotype. The variable sites only (1 = present, 0 = absent) are listed in clockwise order within enzyme on the map in Figure 3. For restriction enzymes, B = *BamHI,* C = *Bell,* G = *Bglll,* Bs = *BstEll,* E = EcoRI, Ev= *EcoRV,* N = *Ncol,* P = *Pstl,* V = *Pvull,* S = *Sacl,* T = *Stul,* X = *Xbal,* M = *Xmnl.*

	Restriction sites															
	B		C					G	Bs		E	Ev				
Haplotype		2		2	3	4	5	6	I		2	I		2	3	4
Ml		0			0	0			0		0	0		0	0	0
M2					0	0			0		0	0		0	0	0
M3					0	0			0		I	0		0	0	0
M5		I			0	0			0		0	0		0	0	0
M6		0		I	0	0	I		0	I	0	0	I	0	0	0
M7		0		0			0		0	0	0	0	0	0	0	
MS	I	0	I	0	I		0		I	0	0	0	0	0	0	I
M9	0	I	0	0	0	I			0	0	0	0	0	0		0
Pl		0	I	I	0	0	I		0	I	0	I	I	I	I	0
Tl		0	0	0	0		0		0	0	0	0	0	0	0	
T2		0	0	0	0		0		0	0	0	0	0	0	0	
T3		0	0	0	0		0		0	0	0	0	0	0	0	
T4		0	0	0	0		0		0	0	0	0	0	0	0	
VI	I	0		0	0		0	I	0	0	0	0	0	0	0	
V2	0	0		0	0			0	0	0	0	0	0	0	0	

culated from autosomal markers. In fact, because F., values among the populations in Missouri alone range from 90-100.1% of the value for the species as a whole, the estimates of **Nm** from F,. are probably grossly inflated. Given that the population structure of C. *alleganiensis* is best described by a stepping-stone model, one would expect that gene flow would decrease as the geographic scope of analysis decreases. Because F,. does not decrease, I conclude that the magnitude of F,. reflects something other than gene flow. In other words, the nonequilibrium scenario of Larson et al. (1984) probably applies, and F,. overestimates the amount of gene flow.

A possible exception to this pattern of isolation involves populations in the Little River and Beaverdam Creek in Tennessee, both tributaries of the Tennessee River. These hellbender populations have an F., value that is effectively zero (Table 5) and could currently be exchanging migrants at a high rate. It is possible, however, that the Tl haplotype (the only shared haplotype) is ancestral and has been retained from the original dispersal of hellbenders into these rivers. If Nm is estimated from the Tennessee data with the private alleles method of Slatkin (1985), a method that is less sensitive to the retention of primitive allele frequencies, Nm equals 0.368 (Routman, 1990). Support for the possibility of retention of primitive haplotypes is found by comparing Slippery Rock Creek and Sherman Creek in Pennsylvania. There is no extant river connection between these two drainages; therefore the presence of the P1 haplotype in both rivers cannot be due to current gene flow. The fact that no unique alleles are found in either of these two Pennsylvania populations is due to either small sample size or recency of colonization.

It is not surprising that a high degree of subdivision exists among populations of *Cryptobranchus.* By being restricted to clean, fast moving water, hellbenders have two major impediments to gene flow. One is the obvious problem of unsuitable habitat acting as a barrier. Rivers with high silt load would effectively prevent migration, because cover and breeding sites are unavailable. The second impediment is isolation by distance. Linear systems such as rivers are the most likely to exhibit such isolation because of the extreme restriction on migrant source for each population (Kimura and Weiss, 1964; Kimura and Maruyama, 1971; Wright, 1951), as well as the additional migratory distance between populations.

The low allozyme diversity in hellbenders is difficult to reconcile with the high overall mtDNA diversity. One potential explanation is that increased resolution of restriction mapping allows the discovery of more alleles per locus than does starch gel electrophoresis. However,

TABLE 2. CONTINUED.

Restriction sites																
	Ev				N		P	V		S		T		X	M	
5	6	7	8		2	3	I		2		2		2	I		2
0	0	0			0		0							0	0	
0	0	0			0		0							0	0	
	0				0		0								0	
	0				0		0								0	
I	0	I		I	0	I	0		I	I	I	I			0	
0		0		0		0	0		0	0	0	0			0	
0	I	0		I		0	0	I	0	0	0	0	I			I
	0	0		0	I	0	0	0		0		0	0		I	0
I	0	I			0		0			I	I	I			0	I
0	I	0			0		0			0	0	0			0	0
0	0	0			0		0			0	0	0	I		0	0
0	I	0			0		0			0	0	0	0		0	0
0	0	0	I		0	I	0		I	0	0	0	0		0	I
0	0	0	0		0	0	I		0			0			0	0
0		0			0		0		0			0			0	0

TABLE 3. VALUES OF NUCLEON DIVERSITY (h) AND PER NUCLEOTIDE PROBABILITY OF HETEROZYGOSITY (8) AVERAGED OVER POPULATIONS FOR THREE SPECIES OF SALAMANDERS. All data were collected by mtDNA restriction mapping. The adjusted values for *Ambystoma tigrinum tigrinum* represent the omission of populations in a hybrid zone, which would tend to spuriously inflate **8.** The adjusted values for *A. maculatum* involve the combination of 11 populations fixed for a single haplotype in Missouri, which are probably due to a recent range expansion. Data for *A. tigrinum* are from Routman (1990). Data for *A. maculatum* are from Phillips (1989). n = number of populations. No population with a sample size less than five was used. NA= not available.

Species	h	e	Range	n
Cryptobranchus alleganiensis				
Big Piney River	0.2263	0.0008		
Gasconade River	0	0		
Niangua River	0	0		
Meramec River	0.1359	0.0005		
N. Fork White River	0	0		
Spring River	0	0		
Current River	0	0		
Little River	0.4394	0.0027		
Beaverdam Creek	0.2500	0.0011		
Copper Creek	0	0		
Sherman Creek	0	0		
Average	0.0956	0.0005	0-0.0027	II
Ambystoma t. tigrinum	**NA**	0.0040	0-0.0137	14
A. t. tigrinum (adjusted)	**NA**	0.0017	0-0.0112	11
A. t. mavortium	**NA**	0.0019	0-0.0078	39
Ambystoma maculatum	**NA**	0.0010	0-0.0089	23
A. maculatum (adjusted)	**NA**	0.0020	0-0.0089	13

TABLE 4. DISTRIBUTION OF HAPLOTYPES AMONG GEOGRAPHIC LOCATIONS SAMPLED. Names listed immediately below a river are locations where subsamples were taken, in linear order starting from the most upstream site (see Table 1 for distances between sites).

Location	Haplotype														
	Ml	M2	M3	M5	M6	M7	MS	M9	Tl	T2	T3	T4	VI	V2	Pl
Northern Missouri															
Big Piney River															
Barton Branch															
Boiling Spring				23											
Slabtown Spring			3	11											
Spring Creek				11	3										
Devil's Elbow				12											
Gasconade River				13											
Niangua River															
Bennett Spring					6										
Ho Hum Camp					20										
Meramec River															
Meramec Springs	3														
Drda Bluffs	23	2													
Archie Hole	13														
Southern Missouri															
North Fork of the White River															
Blair's Ford							14								
Althea Springs							3								
Spring River						7									
Current River								10							
Tennessee															
Little River									9	2					
Beaverdam Creek									7						
Virginia															
New River													2		
Copper Creek														16	
Pennsylvania															
Slippery Rock Creek															1
Sherman Creek															7

TABLE 5. VALUES OFF" AND Nm BASED ON F" CALCULATED FROM THE DISTRIBUTION OF HELLBENDER mtDNA HAPLOTYPES. The subsets of the data are groups of populations for which gene flow is geographically reasonable. Within-river subsamples were combined for this analysis. undf = undefined for negative F.,. Numbers in parentheses are 95% confidence intervals for the estimates. Values calculated from mtDNA of other species are shown for comparison. Sources are (1) Phillips (1989), (2) Routman (1990).

Population		Nm
All rivers	0.865 (0.817-0.909)	0.156 (0.100-0.224)
Missouri rivers	0.866 (0.795-0.925)	0.155 (0.081-0.258)
Northern Ozarks (Big Piney, Gasconade, Niangua, Meramec rivers)	0.831 (0.744-0.901)	0.203 (0.110-0.344)
Northern Ozarks (Meramec excluded)	0.779 (0.613-0.890)	0.284 (0.124-0.631)
Tennessee rivers	-0.122 (-0.999-0.429)	undf(<0.799)
Other species:	F,.	**Nm** Source
Ambystoma maculatum	0.401	1.14 1
A. t. tigrinum	0.460	1.17 2
A. t. mavortium	0.404	1.47 2

the values of heterozygosity and polymorphism for the hellbender allozyme diversity are much lower than those calculated from allozymes of most other salamanders (Shaffer and Breden, 1989), suggesting that some reduction of variation has occurred.

If migration among populations is strongly male biased, it is possible that the between-population component of variation will be high for mtDN A and low for nuclear markers. However, a five-year mark-recapture study has not detected sex differences in migration (unpubl.). In any event, sex-biased migration would not explain the relative magnitudes of overall variation.

Another possibility is that hellbenders underwent a severe or prolonged reduction in population size. Because the effective population size of mtDNA is less than that of nuclear markers (Birky et al., 1983, 1989), this population bottleneck could have reduced genetic variation in both allozymes and mtDNA. The rapid evolution for which mtDNA is famous (Brown et al., 1979) could have reconstituted variation in the mtDNA while the slower evolving allozymes remained relatively monomorphic. This would suggest that the bottleneck occurred relatively recently; otherwise variation in allozymes would have also been regained. However, the pairwise differences among haplotypes range from one restriction site (0.2% sequence divergence) to 21 sites (4.6% sequence divergence). The average pairwise sequence divergence is 2.6%. This large amount of difference suggests that either mtDNA evolution has been extremely rapid, allozyme evolution unusually slow, or the amount of nuclear genetic variation has been underestimated. Preliminary analysis of nuclear rDNA in some hellbender populations has shown some variation (unpubl.), so future analysis at the DNA level may yield insights into the processes responsible for the low allozyme variation in hellbenders.

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LITERATURE CITED

AVISE, j. C. 1986. Mitochondrial DNA and the evolutionary genetics of higher animals. Phil. Trans. R. Soc. London. B. 312:325-342.

BIRKY, C. W. JR., P. FUERST, AND T. MARUYAMA. 1989. Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. Genetics 121:613-627.

———, T. MARUYAMA, AND P. FUERST. 1983. An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. *Ibid.* 103:513-527.

BROWN, W. M., M. GEORGE, JR., AND A. C. WILSON. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA 76:1967-1972.

COCKERHAM, C. C., AND B. S. WEIR. 1987. Correlations, descent measures: drift with migration and mutation. *Ibid.* 84:8512-8514.

CROW, J. F., AND M. KIMURA. 1970. An introduction to population genetics theory. Harper and Row, New York, New York.

DAVIS, S. K., J. E. STRASSMAN, C. HUGHES, L. S. PLETSCHER, AND A. R. TEMPLETON. 1990. Population structure and kinship in *Polistes* (Hymenoptera, Vespidae): an analysis using ribosomal DNA and protein electrophoresis. Evolution 44:1242-1253.

DESALLE, R., A. R. TEMPLETON, I. MORI, S. PLETSCHER, AND J. S. JOHNSTON. 1987. Temporal and spatial heterogeneity of mtDN A polymorphisms in natural populations of *Drosophila mercatorum.* Genetics 116: 215-223.

EWENS, W. J., R. S. SPIELMAN, AND H. HARRIS. 1981. Estimation of genetic variation at the DNA level from restriction endonuclease data. Proc. Natl. Acad. Sci. USA 78:3748-3750.

EXCOFFIER, L., AND A. LANGANEY. 1989. Origin and differentiation of human mitochondrial DNA. Am. J. Hum. Genet. 44:73-85.

HUDSON, R. R. 1990. Gene genealogies and the coalescent process. Oxford Surveys in Evol. Biol. 7:1-44.

KIMURA, M., AND T. MARUYAMA. 1971. Pattern of neutral polymorphism in a geographically structured population. Gen. Res. Camb. 18:125-131.

———, AND G. H. WEISS. 1964. The stepping-stone model of population structure and the decrease of genetic correlation with distance. Genetics 49:561-576.

LARSON, A., D. B. WAKE, AND K. YANEV. 1984. Measuring gene flow in populations having high levels of genetic fragmentation. *Ibid.* 106:293-308.

MANIATIS, T., E. F. FRITSCH, AND J. SAMBROOK. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

MERKLE, D. A., S. I. GUTTMAN, AND M. A. NICKERSON. 1977. Genetic uniformity throughout the range of the hellbender, *Cryptobranchus alleganiensis.* Copeia 1977:1016-1023.

Nu, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York, New York.

———, AND F. TAJIMA. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97:145-163.

PHILLIPS, C. A. 1989. Population structure, breeding pond fidelity, and phylogeography in the spotted salamander, *Ambystoma maculatum.* Unpubl. Ph.D. diss., Washington Univ., St. Louis, Missouri.

REED, K. C., AND D. A. MANN. 1985. Rapid transfer of DNA in agarose gels to nylon membranes. Nucl. Acids Res. 13:7207-7221.

ROUTMAN, E. J. 1990. Paedomorphosis and population structure in the salamanders *Cryptobranchus alleganiensis* and *Ambystoma tigrinum.* Unpubl. Ph.D. diss., Washington Univ., St. Louis, Missouri.

SHAFFER, H. B., AND F. BREDEN. 1989. The relationship between allozyme variation and life history: nontransforming salamanders are less variable. Copeia 1989:1016-1023.

SLATKIN, M. 1985. Rare alleles as indicators of gene flow. Evolution 39:53-65.

———, AND R. TAYLOR. 1989. A comparison of three indirect methods for estimating average levels of gene flow. *Ibid.* 43:1349-1368.

TEMPLETON, A. R. 1985. Genetic systems and evolutionary rates, p. 218-234. *In:* Rates of evolution. K. Campbell and M. Day (eds.). Australian Acad. Sci. Press, Canberra, Australia.

———, K. SHAW, E. ROUTMAN, AND S. DAVIS. 1990. The genetic consequences of habitat fragmentation. Ann. Missouri Bot. Gard. 77:13-27.

WRIGHT, S. 1951. The genetical structure of populations. Ann. Eugenics 15:323-354.

DEPARTMENT OF BIOLOGY, WASHINGTON UNIVERSITY, ST. LOUIS, MISSOURI 63130. PRESENT ADDRESS: DEPARTMENT OF ANATOMY AND NEUROBIOLOGY, WASHINGTON UNIVERSITY SCHOOL OF MEDICINE, ST. LOUIS, MISSOURI 63110. Submitted 6 Jan. 1992. Accepted 4 April 1992. Section editor: D. G. Buth.

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Absorption Efficiency of the Lemon Shark *Negaprion brevirostris* at Varying Rates of Energy Intake

BRADLEY M. WETHERBEE AND SAMUEL H. GRUBER

The efficiency with which the lemon shark, *Negaprion brevirostris,* is able to absorb energy, organic matter, and dry matter was measured at five levels of energy intake. An indirect method was used by incorporating celite, an inert reference substance, into food. Absorption efficiencies ranged from 62-83% for energy, 76-88% for organic matter, and 76-87% for dry matter. Absorption efficiencies increased as energy intake increased but declined at the highest level of intake. Fecal composition varied little throughout the digestive process, and absorption efficiency did not depend on time of fecal collection. Absorption efficiency was overestimated based upon a total fecal collection method. To our knowledge, these are the first measurements of absorption efficiency reported for any species of elasmobranch and demonstrate that the lemon shark is as capable as carnivorous teleosts of efficiently absorbing nutrients from food.

SHARKS are abundant, wide-ranging predators and may play an important role in the transfer of energy between trophic levels (Gruber, 1982). Knowledge of the efficiency with which sharks are able to extract energy from food will aid in understanding the energy requirements of these animals.

The ability of an animal to digest and absorb the nutrient component of a diet can be expressed as an absorption efficiency. Determination of absorption efficiency of energy requires knowledge of the energy content of a representative sample of food and feces, as well as the total amount of food consumed and feces produced, or the concentration of an inert indicator in feed and feces (Koth and Luckey, 1972; Talbot, 1985). The difficulty of collecting feces in an aquatic environment has necessitated use of different methods of fecal collection from those used for terrestrial organisms (Atherton and Aitken, 1970; Cho et al., 1985).

The surface area for absorption of nutrients in the digestive tracts of elasmobranchs is increased in the form of a spiral valve intestine