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Genetic Uniformity Throughout the Range of the Hellbender, *Cryptobranchus alleganiensis*

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Genetic varation was analyzed in populations of the paedogenic salamander *Cryptobranchus alleganiensis* throughout its range. An exceptionally high degree of homozygosity was observed at 24 genetic loci with most populations monomorphic at all loci. No major geographical variation was observed throughout the range of this species despite the fact that it occurs in a number of isolated drainage systems.

THE salamander family Cryptobranchidae is extant in parts of Japan, China and eastern North America. It is comprised of only two genera. *Andrias* is represented by one living species in Asia, but is known from fossils dating from the Oligocene of Europe and the Miocene of North America. *Cryptobranchus* also consists of one living species occurring in a number of isolated drainages in eastern North America. Its fossil history is unknown. Cryptobranchids are considered among the most primitive of salamanders; and appear to have been morphologically conservative throughout their evolutionary history (Meszoely, 1966).

Two subspecies of *Cryptobranchus alleganiensis* are currently recognized: *alleganiensis* occurring in portions of the Susquehanna, Ohio and Missouri river drainages and *bishopi* in portions of the Black River system in southern Missouri and northern Arkansas. Populations referrable to *bishopi* are characterized by rather minor criteria including smaller spiracle size, increased chin blotching, smooth lateral line system in the pectoral region and dorsal blotching as opposed to the spotting usually present in *C. a. alleganiensis* (Nickerson and Mays, 1973). These populations in the past have been assigned specific status, though only subspecific rank is currently accepted (Dundee, 1971). Isolation in a number of drainages has precluded the possibility of natural hybridization, and artificial crosses have not been made.

Several workers have recently attempted to examine differences between the two races of *C. alleganiensis.* Wortham (1970) noted minor differences in a serum protein component of the two races. Jerrett and Mays (1973) compared a number of hematological parameters in both forms. Melton (pers. comm.) observed a difference in DNA content associated with red blood cell size in his comparison of the two taxa.

The present study utilized electrophoretic techniques to examine relationships of the two races of *C. alleganiensis,* as well as to observe the effect, if any, that isolation in a number of drainages has had on the genetic composition of this species.

MATERIALS AND METHODS

A total of 137 specimens of *C. alleganiensis* were used in this study. Specimens were collected from populations in each of the major drainage systems in which this species occurs

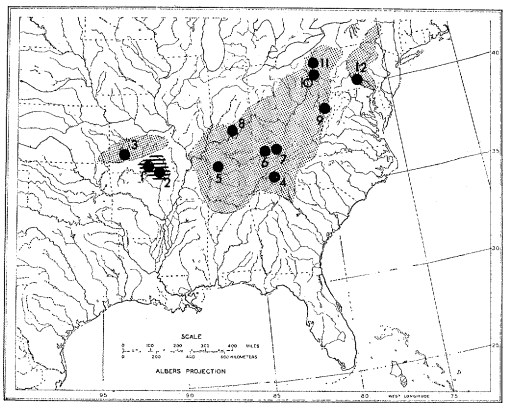

Fig. **1.** Localities sampled in this study. Stippling indicates range of C. *a. alleganiensis,* while horizontal lines indicate range of C. *a. bishopi.* See Table 1 for locality data.

(Fig. 1). Localities, and number of individuals from each locality are presented in Table I.

All individuals were collected in the field, and transported to the laboratory. Specimens were then etherized, and bled by nicking the aorta. Blood was collected in test tubes rinsed with 4% sodium citrate, and centrifuged at 5,130 g for 5 min. Plasma was then decanted and stored at -20 C until use. Hemolysates were prepared by washing the red blood cell fraction 3 times with Ringers solution, then lysing with an equal volume of distilled water. Cell ghosts were removed by centrifugation, and hemolysates stored as above.

Organ homogenates were prepared from the heart and portions of the liver, kidney, spleen and rinsed intestine of each animal. Tissues were homogenized in an equal volume of 2% 2-phenoxyethanol, then centrifuged at 25,000 g for 45 min in a refrigerated centrifuge. Supernatants of soluble proteins were then decanted and stored at -20 C until use. No differences in electrophoretic mobilities were noted between fresh and stored material except for a rapid breakdown of malate dehydrogenase obtained from organ homogenates. Malate dehydrogenases obtained from hemolysates remained stable however. Unlike most vertebrate hemoglobins which rapidly undergo polymerization the hemoglobin of the hellbender is extremely stable (Taketa and Nickerson, 1973).

Twenty-four presumptive genetic loci were examined in this study by electrophoretic techniques coupled with histochemical staining. Starch gel electrophoretic techniques were as described by Selander et al. (1971) with the following modifications: Hemoglobin *(Hb),* and NAO-dependent malate dehydrogenases *(Mdh-I, Mdh-2)* were isolated from hemolysates on the Tris-citrate buffer, **pH** 8.0; glutamate dehydrogenase *(Gdh-I)* and phosphoglucomutases *(Pgm-I,* Pgm-2) were isolated from organ homogenates on the Tris-maleic buffer; while lactate dehydrogenases *(Ldh-I, Ldh-2),* phosphogluco-isomerases *(Pgi-I,* Pgi-2), a-glycerophosphate dehydrogenase *(a-Gpdh-I),* 6-phosphogluconate dehydrogenase (6-Pgdh-l), and indophenol oxidase (lpo-1) were isolated from organ homogenates on the Tris-versene buffer. Glutamate oxaloacetate transaminases *(Got-I,* Got-2), and glyceraldehyde-3-phosphate dehydrogenase *(G-3-Pdh-I)* were isolated from organ homogenates with a boric acid buffer (87 mM Tris, 8.7 mM boric acid, 1 mM EDT A, pH 9.1) (Ayala et al., 1973) in both the gel and electrode compartments. Staining procedures for *Gdh-1* and *G-3-Pdh-l* were as described by Brewer (1970). All gels were 12% starch (Electrostarch Lot #371: Otto Hiller, Madison, Wisconsin).

Three esterase loci *(Est-1, Est-2, Est-3)* and five general proteins *(Alb-I, Pt-I, Pt-2, Pt-3, Pt-4)* were localized from serum on polyacrylamide electrophoresis utilizing an EC Model 490 cell. Seven percent gels were employed using the Tris-glycine buffer described by Guttman and Wilmn (1973) in both gel and electrode compartments. Twenty-five µ.I of a I: I mixture of serum and 5% sucrose were applied to each of the 24 slots in a 6 mm gel. Samples were electrophoresed at 200 ma until a bromphenol blue indicator dye bound to the albumins migrated a minimum of 7 cm from the origin.

Isozymes of various loci are designated numerically in order of decreasing anodal mobility.

RESULTS

A high degree of homozygosity was observed in this species. Most populations were monomorphic at every locus examined. Only 2 loci were polymorphic. These loci were *Mdh-1* in the Sherman Creek sample of C. *a. alleganiensis,* and *Pt-4* in both samples of C. *a. bishopi.* The Sherman Cr. sample possessed a unique allele at the *Mdh-1* locus in a frequency of .27. *Cryptobranchus a. bishopi* populations possessed a unique allele at the *Pt-4* locus in frequencies of .28 and .35 in the Spring River and North Fork of the White River samples respectively (Table I).

TABLE 1. LOCALITIES AND GENETIC PARAMETERS FOR POPULATIONS OF *Cryptobranchus.*

Sample Number	Drainage System	Locality	*N*	Mean Proportion of Loci: Heterozygous per Individual	Mean Proportion of Loci: Polymorphic per Population
1.	Black R.	N Fork White R., above Dawt Mill (Ozark Co., Mo.)	18	.002	.042
2.	Black R.	Spring R., below Mammoth Spring (Fulton Co., Ark.)	12	.003	.042
3.	Missouri R.	Niangua R., below Bennett Springs (Dallas Co., Mo.)	15	.000	,000
4.	Ohio R.	Little R., below Townsend (Blount Co., Tenn.)	28	.000	.000
5.	Ohio R.	Red R., near Clarksville (Montgomery Co., Tenn.)	2	.000	.000
6.	Ohio R.	Rockcastle R., at Lamero (Rockcastle Co., Ky.)	2	.000	.000
7.	Ohio R.	S Fork Kentucky R., below Conkling (Oswley Co., Ky.)	2	.000	.000
8.	Ohio R.	Blue R., above White Cloud (Harrison Co., Ind.)	17	.000	.000
9.	Ohio R.	Shavers Fork of Cheat River (Randoph Co., W. Va.)	2	.000	.000
IO.	Ohio R.	Slippery Rock Creek above Wurtenburg (Lawrence Co., Pa.)	IO	.000	.000
11.	Ohio R.	French Creek below Meadville (Crawford Co., Pa.)	11	.000	.000
12.	Susquehanna R.	Sherman Creek above Dromgold (Perry Co., Pa.)	18	.002	.042

DISCUSSION

Few studies have reported such a lack of genetic variation in a species with a continental distribution. In general, most vertebrates including man, tend to be polymorphic at 10-20% of their loci, with any given individual heterozygous at approximately 6% of its loci (Selander and Johnson, 1973). The data presented for *Cryptobranchus* do not fit this generalization.

Snyder (1974) reported lack of enzyme variation in three species of bees and attributed his findings to the rather limited environmental variability encountered by both immature and adult bees. Numerous workers have provided both theoretical and experimental data to suggest a positive correlation between variability of the environment and the observed genetic variation in an organism (Selander and Kaufman, 1973; McDonald and Ayala, 1974; Powell, 1971). While the habitats typically inhabited by *Cryptobranchus* are generally fairly stable (Nickerson and Mays, 1973), reduced levels of genetic variation have not been reported for fish (Avise and Smith, 1974). Therefore reduced genetic variability is not a prerequisite for existing in freshwater environments. Reduction of variability in *Cryptobranchus* may be related to factors other than or in addition to environmental considerations.

Complete absence of genetic variation has been reported for some populations of the elephant seal *Mirounga angusirostris* along the coast of California and Baja California (Bonnell and Selander, 1974). This lack of variation is most likely the result of almost complete extermination of the species at an earlier time, with the present population descended from a founding population of less than 100 animals. Near complete absence of genetic variation in the wide ranging snail *Ruminia decullata* has been attributed to its breeding system of facultative self-fertilization (Selander and Kaufman, 1973).

Electrophoretic analysis of three other species of paedogenic salamanders has also revealed a

lack of genetic variability in these forms. We have noted complete absence of genetic variation at 20 loci examined **in** a population of the mudpuppy *Necturus maculosus,* and only one polymorphic locus out of 20 examined in a population of dwarf siren *Pseudobranchus striatus.* Coming and Berger (1969) reported absence of genetic variation at 9 loci examined in *Amphiuma means.* Therefore, it appears that reduction or absence of genetic variation may be the rule rather than the exception in paedogenic salamanders.

The paedogenic salamanders also share the distinction of possessing the largest amounts of nuclear DNA of any organisms. The proteid *N. maculosus* possesses 165 picograms per diploid nucleus (pg/n) (Morescalchi and Serra, 1974). The cryptobranchid *Andrias japonicus* possesses 93 pg/n, while the DNA content in *Cryptobranchus* has been shown to vary by as much as 29% between populations, with a range of IOI to 130 pg/n (Melton, pers. comm.). *Amphiuma means* possesses 150 pg/n (Morescalchi and Olmo, 1974). The sirenid *Pseudobranchus striatus* possesses 91 pg/n, while *Siren lacertina* and *S. intermedia* possess 114, and 108 pg/n respectively (Morescalchi and Olmo, 1974). All of these values are substantially above the mammalian mode of 8 pg/n and the 6 pg/n present in man (Bachmann, 1972).

Within the salamanders, it appears that the plethodontids that have been examined appear to have normal levels of genetic variation. This group also has much lower values for DNA content than the paedogenic forms. Webster (1973) reported an average heterozygosity value (H) for a population of *Plethodon cinereus* of .049. Highton and Webster (1976) examined populations of this species throughout its range and reported an average *H* value of .038. The related form *Plethodon richmondi* has a Hof .047. These forms possess DNA at 40 and 41 pg/n respectively (Mizuno and Macgregor, 1974). *Eurycea lucifuga* which possesses 41 pg/n (Morescalchi, Olmo and Serra, 1974) has an *H* value of .071. From these available data, it appears that there may be a relation between DNA content and genetic variability in the salamanders. Other groups such as the birds which generally are reported to have low levels of genetic variability however do not possess large amounts of DNA. There may be a unique relationship in the urodeles as that described between DNA content and degree of terrestrialism within the Amphibia by Goin, Goin and Bachmann (1968). At present not enough data or a plausible mechanism for such a relationship is available and further work will be needed to ascertain whether there is a true relationship or if in fact this is a mere coincidence.

Although populations of C. *a. bishopi* were distinguishable from C. *a. alleganiensis* on the basis of *Pt-4,* the Sherman Cr. sample also differed from most nominal populations by a single locus. Although both differed by a single locus, the Sherman Cr. sample differed at the *Mdh-I* locus, while *bishopi* differed at a general nonspecific protein. The malate dehydrogenase locus is generally less subject to mutational changes than a general protein since it belongs to a class of enzymes involved in complex metabolic pathways. It therefore may represent a greater change in the genome than the general protein polymorphism. By this criteria, the Sherman Cr. sample is more different than are populations of *bishopi* when compared to nominal populations of C. *alleganiensis* by biochemical criteria.

The almost complete genetic similarity between populations of both taxa suggest that this group is in fact represented by a single species. The subspecific status of *bishopi,* while not warranted by biochemical comparisons, should probably be retained in that most individuals can be identified as being from a certain geographic region though some of the characteristics such as blotching patterns occur outside the range of *bishopi.*

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