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GENETIC VARIATION IN WESTERN SALAMANDERS OF THE GENUS *PLETHODON*, AND THE STATUS OF *PLETHODON GORDONI*

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ABSTRACT: Samples of *Plethodon gordoni*, *Plethodon dunni* and *Plethodon vehiculum* from an area of sympatry in western Oregon were examined by methods of starch-gel electrophoresis involving 24 presumptive genetic loci. Although *P. dunni* and *P. vehiculum* are very distinct from one another ($D = 1.208$) and from *Plethodon elongatus* ($D = 1.320$ and 1.708, respectively), *P. gordoni* is nearly identical ($D = 0.001$) to *P. dunni*. Attempts to verify reported morphological distinctions between *P. dunni* and *P. gordoni* were unsuccessful, and thus available evidence suggests strongly that *P. gordoni* is only a somewhat localized color morph of *P. dunni*.

GEOGRAPHIC variation in morphology within and between species of the salamander genus *Plethodon* which occur in northwestern United States was studied by Brodie (1970). The Vehiculum species group is of special interest. In contrast to the usual situation in this genus in eastern North America, where closely related species are typically allopatric or parapatric (Highton, 1972; Highton and Webster, 1976), the 3 members of the Vehiculum group are sympatric. *Plethodon dunni* and *Plethodon vehiculum* have long been known to have extensive overlap in their geographic distribution, but they are ecologically segregated to a large degree (Dumas, 1956). Brodie described a new species, *Plethodon gordoni*, said to be a close relative of *P. dunni*. Not only is the range of *P. gordoni* entirely included within that of *P. vehiculum* and *P. dunni*, but it is also said that there are ". . . no obvious differences between the habitat preferences of *gordoni* and *dunni*" (Brodie, 1970). While it was noted that *P. gordoni* and *P. dunni* are very similar in most features of their morphology, differing principally in coloration, Brodie argued that the 2 species were most distinct where they occurred in sympatry, and attributed this to character displacement.

Because of the several unusual aspects of the situation relating to these 3 species, we analyzed their genetic relationships. We also studied morphological features. Our limited samples of the 3 species are from an area of sympatry near the type locality of *P. gordoni* in Benton County, Oregon. A sample of *Plethodon elongatus* (sympatric with *P. dunni*) from southwestern Oregon was included in the genetic study as a representative of another species group. We view the study as a test of the validity of *P. gordoni* as a separate taxon.

TABLE 1.—Buffer systems, running conditions and enzyme assays. Enzyme designations according to Harris and Hopkinson (1976); old designations (from Brewer, 1970; Selander et al., 1971; Ayala et al., 1972) in parentheses.

Buffer type	Voltage (V)	Time (h)	Stain
Continuous Tris citrate (pH 8.0)	100	4	Isocitrate dehydrogenase, ICD [=IDH] 1 & 2 Glycerol-3-phosphate dehydrogenase, GPD [=αGPD] Sorbitol dehydrogenase, SORDH [=SDH] Mannose phosphate isomerase, MPI Malic enzyme, ME
Continuous Tris citrate (pH 7.0)	180	3	Malate dehydrogenase, MDH 1 & 2 Leucine aminopeptidase, LAP Phosphoglucomutase, PGM
Tris maleic EDTA (pH 7.4)	100	4	Fumarate hydratase, FUM Phosphogluconate dehydrogenase, PGD [=6-PGD] Adenosine deaminase, ADA
Phosphate (pH 6.7)	130	3	Glucose phosphate isomerase, GPI [=PGI] Glutamate oxaloacetate transaminase, GOT 1 & 2
Discontinuous Tris citrate (Poulik)	250	3	Lactate dehydrogenase, LDH 1 & 2 Esterase, ES (=Est) Glucose-6-phosphate dehydrogenase, Gd [=G-6-P], H-6-P
Discontinuous lithium hydroxide	300	4	Peptidase, PEP [=Pept] Amido black, general proteins, PT-1

MATERIALS AND METHODS

Samples of 4 nominal species of *Plethodon* were analyzed for allozyme variation. These are as follows: (A). 11.3 km SE Philomath (by road), Benton County, Oregon: *P. dunni* (6 specimens), *P. gordoni* (2), *P. vehiculum* (22), (B). Corvallis Watershed, 16.9 km up Road 1208 and 10.5 km up Road 121, Benton County, Oregon: *P. dunni* (10), *P. gordoni* (12), (C). Sixes River Road, 3.2 km E junction with Highway 101, Curry County, Oregon: *P. dunni* (2), *P. elongatus* (6). *Plethodon gordoni* was identified solely on the basis of coloration (solid black, with no yellow or greenish chromatophores and no yellow or greenish dorsal stripe). All specimens are preserved in the Museum of Vertebrate Zoology.

Horizontal starch gel electrophoresis was carried out according to the methods of Brewer (1970), Selander et al. (1971), and Ayala et al. (1972). Extracts of water soluble proteins were obtained by homogenizing liver, heart, spleen, stomach, and body wall, together with an equal volume of grinding buffer. Buffer systems and stains for proteins and enzymatic activity appear in Table 1.

Morphological measurements were made on animals from sympatric populations of *Plethodon gordoni* and *Plethodon dunni*. Head width (HW), at the angle of the jaw; head length (HL), from the tip of the snout to the posterior margin of the maxilla; and snout-vent length (SVL), from the tip of the snout to the posterior margin of the vent, were measured to the nearest 0.1 mm. We measured head length from the tip of the snout to posterior border of the maxilla, rather than to the gular fold as Brodie (1970) had done. This was necessary because the throat region had been dissected to obtain blood. Number of trunk vertebrae was determined by direct count from radiographs. Costal grooves were counted from behind the forelimbs to directly in front of the hindlimbs, and number of vomerine teeth (VT) was counted directly.

TABLE 2.—Allele frequencies in 4 nominal species of *Plethodon*. All frequencies 1.0 except where indicated.

Locus	*P. vehiculum* N = 30	*P. elongatus* N = 2	*P. dunni* N = 18	*P. gordoni* N = 14
ICD-1	a	b	b	b
ICD-2	b	a	b	b
MPI	b	a	c	c
SORDH	b	c	a	a
GPD	a(0.75), b(0.25)	c	b	b
MDH-1	c	a(0.25), b(0.75)	c	c
MDH-2	a(0.07), b(0.93)	b	b	b
PGM	a	b	c	c
GPI	b	c	a	a
LAP	c(0.64), d(0.36)	a	b	b
PGD	a	b	c	c
ADA	a	a	a	a
FUM	a	a	a	a
XDH	b	a	a	a
PEP	c	a	a(0.20), b(0.80)	a(0.33), b(0.67)
GOT-1	a	b	c	c
GOT-2	b	a	c	c
PT-1	b	a	b	b
ES-1	a(0.39), b(0.61)	b	b	b
ES-2	c(0.77), d(0.23)	a	b	b(0.96), c(0.04)
LDH-1	b(0.25), c(0.75)	d	a	a
LDH-2	c	a	b	b
Gd	b	c(0.75), d(0.25)	a	a
H-6-P	b	b(0.50), c(0.50)	a	a
Polymorphism	0.250	0.125	0.041	0.083
Mean heterozygosity	0.089	0.040	0.009	0.018

RESULTS

A total of 19 proteins encoded by 24 presumptive genetic loci was examined in all individuals (Table 2). No variation among the 3 species was noted for 5 loci (ICD-2, MDH-1, ADA, FUM, PT-1). *Plethodon gordoni* and *P. dunni* share the same alleles at all loci, except one (ES-2), where a single heterozygotic individual of *P. gordoni* has an allele not present in our sample of *P. dunni*. This is an insignificant difference, for the frequency of the allele found in *P. gordoni* is <.05. In contrast, *P. vehiculum* shares no alleles with *P. dunni—P. gordoni* at 15 of the 19 variable loci. The allele in highest frequency in *P. vehiculum* at 2 additional loci (GPD, ES-2) is either not present, or present in only 1 individual of the combined *P. dunni—P. gordoni* sample. At the 2 remaining variable loci (MDH-2, ES-1) the allele in highest frequency in *P. vehiculum* is fixed in the *P. dunni—P. gordoni* sample.

Values of Nei's (1971) *I* and *D* are found in Table 3. The *P. gordoni* and *P. dunni* samples are virtually identical ($I = 0.999$), while *P. vehiculum* is about equidistant from the 2 other nominal species, *I* averaging 0.300.

The proportion of polymorphic loci in *P. dunni—P. gordoni* is extremely low (0.041, Table 2). Only a single locus fulfills a criterion commonly applied for recognition of polymorphic loci, e.g., the frequency of the most common allele is less than or equal to 0.95. By the same criterion, 25% of the loci in *P. vehiculum* are polymorphic. Polymorphism reflects trends visible in heterozygosity (heterozygous individuals per locus per population), with *P. dunni—P. gordoni* exhibiting very low

TABLE 3.—Genetic distances (D, above diagonal) and genetic identities (I, below diagonal) between 4 nominal species of *Plethodon* (based on Nei, 1971).

	(1)	(2)	(3)	(4)
P. vehiculum (1)	.....	1.708	1.208	1.200
P. elongatus (2)	0.181	.....	1.320	1.295
P. dunni (3)	0.299	0.267	.....	0.001
P. gordoni (4)	0.301	0.247	0.999	.....

levels ($\overline{H} = 0.012$) as compared with *P. vehiculum* ($\overline{H} = 0.089$).

On the basis of our limited sample *P. elongatus* is remote from the other 3 nominal species ($\overline{I} = 0.232$), but at this level of genetic similarity the number of codon differences is underestimated (see Nei, 1971).

In view of the results of the genetic analysis, we examined those features of morphology which were stressed by Brodie (1970) as being different in *P. gordoni* as compared with *P. dunni*. We were unable to analyze all features studied by Brodie, and we were forced to modify procedure slightly for 1 measurement (HL, **see above**). However, we did examine the features stressed most strongly by Brodie. Although various statistical procedures are superior to those used by Brodie (see Lynch and Wake, 1975), we tried to use his techniques to make our results more directly comparable with his. Further, we limited our study to the sample used for genetic analysis, and did not attempt a reanalysis of type materials. Because individuals of the 2 color morphs were initially grouped on the basis of pigmentation, no further analysis was made of that feature.

Brodie (1970) reported that *P. dunni* has slightly more costal grooves, on average ("80% level"), than *P. gordoni*. We obtained values of mean costal groove numbers (counted by the method used by Brodie) of $15.29 \pm .47$ for *P. gordoni* and $15.39 \pm .50$ for *P. dunni*. Modal numbers are 15 in both nominal species, and both have a range of 15 to 16. The difference between the two means is not statistically significant ($t = .592$, 30 df, $.5 < P < .6$).

Mean numbers of trunk vertebrae (from radiographs) are $16.29 \pm .47$ for *P. gordoni* and $16.53 \pm .50$ for *P. dunni*. While the range is 16 to 17 for both samples, the modes are 16 for the *P. gordoni* and 17 for the *P. dunni* samples. The difference between the two means again is not statistically significant ($t = 1.397$, 30 df, $.1 < P < .2$).

TABLE 4.—Slopes (b) and Y-intercept (a) of regressions ($\pm$ 95% confidence intervals) of morphological data. In the first 3 cases, SVL is independent variable; in fourth case, HW is independent variable. Results of t-tests listed below.

Species and dimensions	N	r	b	a
P. dunni (range of SVL 21.4–57.9, $\bar{x} = 37.7$)				
HW vs. SVL	16	.994	0.110 ± 0.007	1.778 ± 0.283
HL vs. SVL	16	.990	0.107 ± 0.009	1.638 ± 0.351
VT vs. SVL	16	.932	0.336 ± 0.075	-1.170 ± 3.000
HL vs. HW	16	.987	0.960 ± 0.089	0.034 ± 0.542
P. gordoni (range of SVL 22.4–69.0, $\bar{x} = 39.5$)				
HW vs. SVL	14	.994	0.111 ± 0.008	1.715 ± 0.325
HL vs. SVL	14	.994	0.114 ± 0.008	1.427 ± 0.321
VT vs. SVL	14	.808	0.241 ± 0.111	1.819 ± 4.640
HL vs. HW	14	.996	1.020 ± 0.058	-0.302 ± 0.367

HW vs. SVL $t = 0.0774$ ($P < .9$)
HL vs. SVL $t = 1.2169$ ($.2 < P < .4$)
VT vs. SVL $t = 1.537$ ($.1 < P < .2$)
HL vs. HW $t = 1.216$ ($.2 < P < .4$)

Vertebral number and costal groove counts are highly correlated in *Plethodon*; we present both counts because certain difficulties are encountered in either determination. Radiographs are not useful in identifying sacral vertebrae in some of our very small specimens. In other individuals, it is impossible to decide whether to count a groove just past the groin or not. In our total sample there is a 9% difference between our estimates of trunk vertebral number from costal groove counts and from direct count on radiographs. This compares with a 6% difference reported by Highton (1957) for 11 species of *Plethodon*. We are but 1 specimen away from the 6% error level.

We also examined the relationship of numbers of vomerine teeth, width of head, and length of head in relation to snout-vent length by least squares regression analysis. In every case there was a significant, high ($r > .800$) correlation between the variables (Table 4). However, we found no significant differences in slopes of these regressions, and the elevations were very similar. Brodie (1970) reported that the head width to head length slope differed at the .01 level in *P. dunni* and *P. gordoni*, and it is unfortunate that we were unable to repeat exactly his measurements on our sample. However, neither the slopes nor the elevation of the regressions of our similar measurements differ significantly in the two samples.

Discussion

Our goals were to determine the level of genetic differentiation among the 3 nominal members of the Vehiculum group of *Plethodon*, and to test the hypothesis of Brodie (1970) that *P. dunni* and *P. gordoni* are reproductively isolated. Genetic differentiation between *P. vehiculum* and *P. dunni—P. gordoni* is great, and in fact higher than the values reported for the majority of congeneric comparisons in vertebrates (see summary of Ayala, 1975). In contrast, *P. dunni* and *P. gordoni* are genetically identical, as far as the loci examined are concerned, and apparently represent a freely interbreeding population.

Previous studies concerning genetic differentiation among closely related, congeneric species of salamanders provide a basis for comparison with our results. Hedgecock and Ayala (1974) report a mean value of I of 0.633 for the three species of *Taricha*. Highton and Webster (1976) show that some differentiation has taken place among populations of *Plethodon cinereus* that are continuously distributed ($\bar{I} = 0.90$). To the south of the main range of this species a series of discontinuous populations of morphologically similar salamanders occurs, and these are well differentiated genetically from the more northern *P. cinereus* ($\bar{I} = 0.66$). On this basis, as well as on the relatively high genetic similarity of the discontinuously distributed populations to each other ($\bar{I} = 0.93$), these southern populations were recognized as a distinct species, *P. serratus*. Lynch et al. (1977) examined 2 morphologically similar, sympatric species of *Pseudoeurycea* from Oaxaca, Mexico, and reported a genetic similarity (Roger's coefficient) of 0.43.

Congeneric, sympatric species are typically rather well differentiated genetically (Ayala, 1975). This is so much the case that workers such as Highton and Webster (1976) feel justified in making taxonomic decisions concerning allopatric populations on the basis of allozymic differentiation alone. To our knowledge the sympatric species of vertebrates which are most similar genetically are the two California minnows reported by Avise et al. (1975). These species are estimated to differ by 1 electrophoretically detectable codon substitution for every 20 loci ($I = 0.948$). However, these species show considerable differentiation in morphological and ecological attributes, and they have been placed in separate genera.

Brodie (1970) reported some differences in morphology between *P. gordoni* and *P. dunni*, at least one of which (HW:HL ratio) was reported to be significant. In

contrast, our study of the specimens used in the genetic analysis reveals no significant differences in morphology between the two nominal species.

In view of the great amount of genetic differentiation shown by species of western *Plethodon* known to be distinct, and the absence of either genetic or morphological differentiation between *P. gordoni* and *P. dunni*, we suggest that *P. gordoni* is a color morph of *P. dunni*, and propose that the name be considered a junior synonym of *P. dunni*. Similar color morphs in *P. cinereus* have been shown to result from a relatively simple genetic mechanism (Highton, 1959, 1975). Sage and Selander (1975) used reasoning similar to ours from genetic information in proposing that polymorphism in a single species rather than speciation was responsible for morphological diversification in a group of cichlid fishes. Our data strongly suggest that *P. gordoni* is not a separate taxon. Conclusive proof would require demonstration that a single clutch of eggs includes both morphs.

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