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Patterns of Geographic Variation in Allozymes of the Olympic Salamander, *Rhyacotriton olympicus* (Caudata: Dicamptodontidae)

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A Contribution in Celebration of the Distinguished Scholarship of Robert F. Inger on the Occasion of His Sixty-Fifth Birthday

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Croat, T. B. 1978. Flora of Barro Colorado Island. Stanford University Press, Stanford, Calif., 943 pp.

Grubb, P. J., J. R. Lloyd, and T. D. Pennington 1963. A comparison of montane and lowland rain forest in Ecuador. I. The forest structure, physiognomy, and floristics. Journal of Ecology, **51:** 567–601.

Langdon, E. J. M. 1979. Yagé among the Siona: Cultural patterns in visions, pp. 63–80. *In* Browman, D. L., and R. A. Schwarz, eds., Spirits, Shamans, and Stars. Mouton Publishers, The Hague, Netherlands.

Murra, J. 1946. The historic tribes of Ecuador, pp. 785–821. *In* Steward, J. H., ed., Handbook of South American Indians. Vol. 2, The Andean Civilizations. Bulletin 143, Bureau of American Ethnology, Smithsonian Institution, Washington, D.C.

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Patterns of Geographic Variation in Allozymes of the Olympic Salamander, *Rhyacotriton olympicus* (Caudata: Dicamptodontidae)

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Patterns of Geographic Variation in Allozymes of the Olympic Salamander, *Rhyacotriton olympicus* (Caudata: Dicamptodontidae)

Abstract

The salamander genus *Rhyacotriton* is a phylogenetically isolated, monotypic genus confined to the Pacific Northwest of the United States. An electrophoretic survey of 29 allozyme loci in 29 populations collected throughout the range of the species revealed great geographic differentiation, with Nei's genetic distances between populations approaching 1.2. Three distinct geographic units are apparent: (1) the Coast Ranges from the Olympic Peninsula of Washington to northwestern Oregon (North Coastal); (2) the Coast Ranges from northwestern Oregon to Mendocino County, California (South Coastal); and (3) the Cascade Mountains of south-central Washington and northern Oregon (Cascade). Nine to 20 fixed allozymic differences exist between populations in the South Coastal and North Coastal groups; nine to 18 between Cascade and North Coastal populations; and nine to 19 between Cascade and South Coastal populations. These levels of genetic differentiation are those expected among congeneric species of salamanders, but because no contact areas or zones of sympatry have been found as yet, we recommend no taxonomic changes. The level of morphological differentiation is not concordant with the genetic patterns observed. The genus is apparently an old one which displays considerable heterogeneity in rates of genic and morphological differentiation.

Introduction

Rhyacotriton olympicus, the Olympic salamander, is the sole member of the dicamptodontid subfamily Rhyacotritoninae (Edwards, 1976; Frost, 1985). It is distributed along the Pacific coast from the northern end of the Olympic Peninsula of Washington to Mendocino County, California, and in the Cascade Mountains from the Cowlitz River between Mts. Rainier and St. Helens, Washington, to Douglas County in south-central Oregon. The species is absent from the Willamette and Cowlitz-Puget Sound lowlands and may exist in two or more isolated units (fig. 1).

Geographic variation in the species was first discussed by Stebbins and Lowe (1951), who recognized two subspecies of *Rhyacotriton olympicus* based on color pattern variation. The northern subspecies *R. o. olympicus* is distributed throughout the Coast Ranges of Washington and northwestern Oregon. Dorsally, it is dark brown, speckled with white guanophores; the ventral surface is yellow with either no or a few scattered large dark blotches. The subspecies *R. o. variegatus,* distributed southward from central Oregon, is dull grayish olive above, with fewer guanophores and a yellow venter well supplied with scattered dark spots and blotches. Between these two subspecies, Stebbins and Lowe envisioned a broad area of intergradation which included all populations in the Cascades and several in central coastal Oregon, indicating that geographically the subspecies were not sharply distinct units.

Wake (1981) reported geographic variation in development of nasal bones during late ontogeny in *Rhyacotriton.* Olympic Mountain and Cascade populations lack nasals at any life stage, while the bones develop during metamorphosis in at least some populations from California and southern Oregon. This lack of nasals is unique among transforming urodeles. Nussbaum and Tait (1977) examined life history features of coastal and Cascade populations of Oregon *Rhyacotriton* and found that Cascade populations take at least a full year longer

TABLE 1. Localities of *Rhyacotriton olympicus* populations surveyed. Voucher specimens are listed in parentheses.

	NORTH COASTAL POPULATIONS
N1.	0.5 mi N Lake Mills, Clallam Co., Wash. (N = 10: MVZ 197375, 197377–385)
N2.	4.2 mi E Hwy. 101 on Rd. to Hoh Rainforest, Jefferson Co., Wash. (N = 10: MVZ 197332–341)
N3.	7.5 mi W Hwy. 101 on Dosewallips Rd., Jefferson Co., Wash. (N = 9: MVZ 197323–331)
N4.	Merriman Creek, upstream from Lake Quinault, Gray's Harbor Co., Wash. (N = 10: MVZ 197386–395)
N5.	N slope N fork Skokomish River, between upper and lower Cushman lakes, ca. 3 mi W Hoodsport, Mason Co., Wash. (N = 6: MVZ 173345–350)
N6.	Fish hatchery, 0.3 mi S Mohrweis at headwaters Weaver Creek, Mason Co., Wash. (N = 10: MVZ 173351–360)
N7.	3.2 mi E Hwy. 107 on road along S side of Chehalis River, Gray's Harbor Co., Wash. (N = 10: MVZ 197347–356)
N8.	Dixon Rd., 1.2 mi W Hwy. 101, Pacific Co., Wash. (N = 10: MVZ 197250–259)
N9.	8.6 mi E jct. Hwy. 409 at Cathlamet on Hwy. 4, Wahkiakum Co., Wash. (N = 10: MVZ 197363–367, 197369–370, 197372–374)
N10.	Jct. Hwy. 26 and Luukinen Rd. (at bridge over Nehalim River), Clatsop Co., Ore. (N = 10: MVZ 197300–305, 197307–310)
N11.	5.3 mi from Kilchis River Rd. on S side of Kilchis River, Tillamook Co., Ore. (N = 10: MVZ 197433–437, 197439, 197442–443, 197446)
N12.	2.9 mi E Hwy. 101 on Little Nestucca River Rd., Tillamook Co., Ore. (N = 10: MVZ 197447–449, 197451–452, 197454–458)
	SOUTH COASTAL POPULATIONS
S1.	5.4 mi NE (by Fall Creek Fish Hatchery Rd.) jct. Hwy. 134, Benton Co., Ore. (N = 20: MVZ 173304–318, 173320–324)
S2.	1.5 mi up Mary's Peak Rd., SW Philomath, Benton Co., Ore. (N = 4: MVZ 158832–835)
S3.	Thompson Rd., 1.2 mi NW Hwy. 36 at Rainrock, Lane Co., Ore. (N = 10; MVZ 197418–427)
S4.	2.7 mi S Hwy. 38 on County Rd. 3 (to Loon Lake), Douglas Co., Ore. (N = 10: MVZ 197261–270)
S5.	Elk River Rd., 0.5 mi E road to MacGribble Campground, Curry Co., Ore. (N = 10: MVZ 197311–320)
S6.	0.0–0.9 mi N on Windy Creek Rd., Douglas Co., Ore. (N = 4: MVZ 173476–479)
S7.	South Fork Rd., N of Steven Memorial Bridge, Del Norte Co., Calif. (N = 10: MVZ 191678–680, 194087, 194344–349)
S8.	2 mi N Ti Creek on Hwy. 96, Siskiyou Co., Calif. (N = 6: MVZ 185813–815, 185876–878)
S9.	Ca. 3.5 mi E Blue Lake on Hwy. 299, then N 0.7 mi, Humboldt Co., Calif. (N = 10: MVZ 197408–417)
S10.	Dark Gulch, 1 mi N Albion on Hwy. 1, Mendocino Co., Calif. (N = 7: MVZ 145074, 158826–831)
	CASCADE POPULATIONS
C1.	0.3 mi S jct. Hwy. 25 on Hwy. 26, N of Mt. St. Helens, Lewis Co., Wash. (N = 10: MVZ 197396–403, 197405–406)
C2.	9.3 mi E Cunningham Rd. jct. at Rose Valley on Coweeman River Rd., Cowlitz Co., Wash. (N = 10: MVZ 197275–277, 197279, 197281–283, 197285, 197287–288)
C3.	Smith-Cripe Rd., at Sneider-Barks Rd., 1 mi N Hwy. 14, ca. 2.5 mi W Skamania, Skamania Co., Wash. (N = 9: MVZ 173361–369)
C4.	Mt. Defiance Trail, near Cabin and Warren creeks, ca. 0.5–1.0 mi W Starvation Creek State Park, Hood River Co., Ore. (N = 20: MVZ 173325–343)
C5.	4.8 mi SE jct. Fish Creek Rd. on Clackamas River Rd., Clackamas Co., Ore. (N = 10: MVZ 197289–298)
C6.	Hammond Camp, 0.4 mi from jct. Snowpack Rd., S side Crabtree Creek, Linn Co., Ore. (N = 16: MVZ 158836–851)
C7.	1.9 mi N of N end of Blue River Lake on Forest Service Rd. 15, Lane Co., Ore. (N = 5: MVZ 197428–432)

to achieve metamorphosis than do South Coastal ones (4.5 years as compared to 3–3.5). Wake interpreted the absence of nasal bones in Cascade populations to be the result of neotenic (Gould, 1977) evolution resulting from the reduced rate of development involved in this delayed maturation. Life history parameters (still unknown) for northern populations were assumed to be similar to those for Cascade populations because of shared environmental conditions (e.g., colder, shorter growing seasons). These similarities were hypothesized to explain the lack of nasals in northern populations.

Wake's (1981) hypothesis relates neoteny in a simple and direct way to ecological factors influencing individual populations of *Rhyacotriton*. It is possible, however, that an underlying historical explanation, perhaps including the presence of cryptic species, is involved. Alternatively, further study might disclose less regularity in the pattern

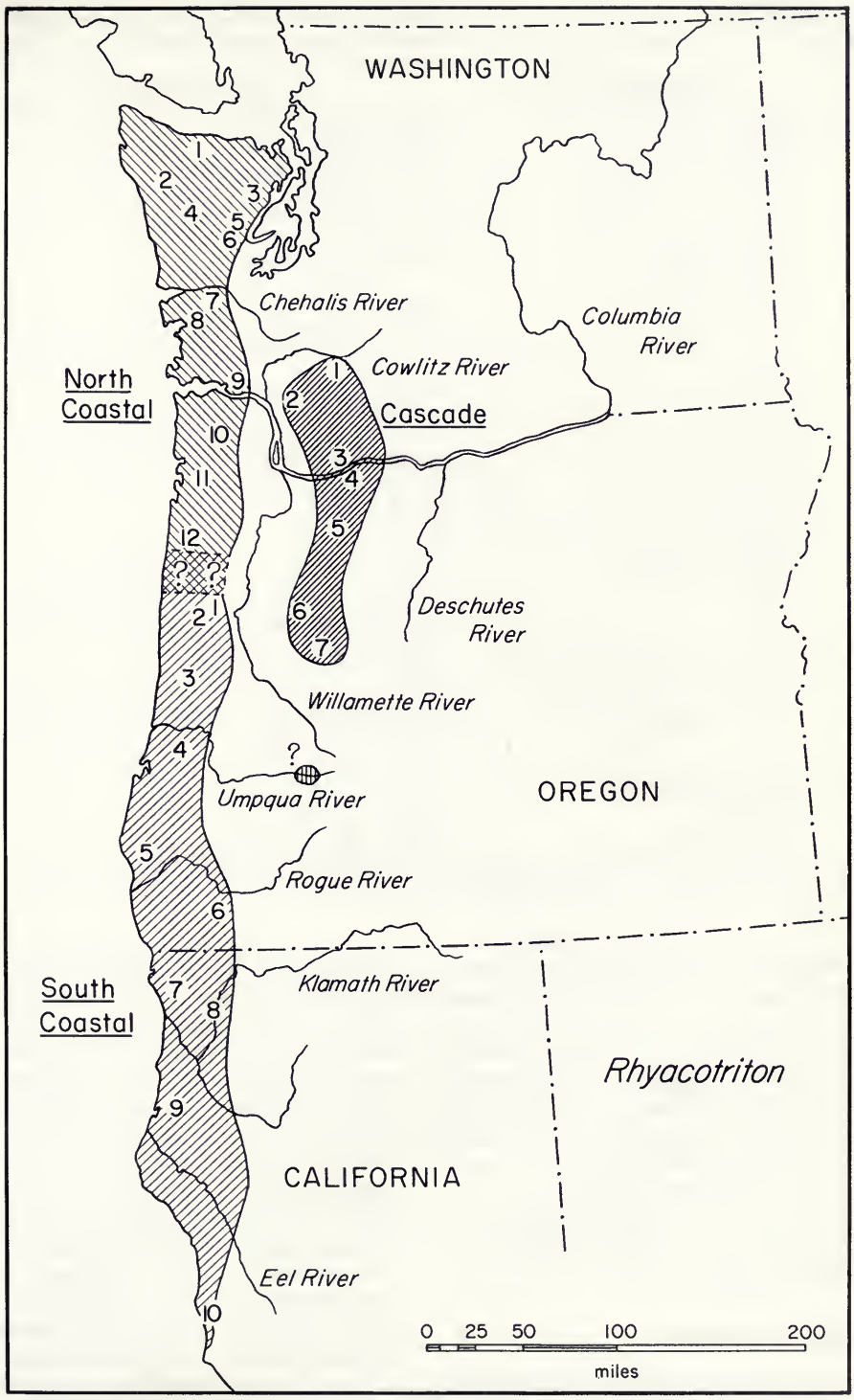

FIG. 1. Distribution of *Rhyacotriton olympicus* in California, Oregon, and Washington. The North Coastal, South Coastal, and Cascade groups identified in this study are indicated by shading. Localities of the populations sampled (see table 1) are numbered for each group.

TABLE 2. Distribution of variants among North Coastal populations for the 29 proteins sampled in *Rhyacotriton olympicus*. The following proteins are monomorphic in these populations for the variant in parentheses: LAP (a), PT (a), SOD (a), ICDH-1 (a), MDH-2 (a), ACP (a), and EST-1 (a). Sample sizes are listed in Table 1.

Protein	Fixed populations	Polymorphic populations
PGM	a (N1–9, 11–12)	N10 (a = .90, d = .10)
6-PGD	a (N2–3, 6–8, 10–12)	N1 (a = .95, b = .05), N4 (a = .95, b = .05), N5 (a = .92, b = .08), N9 (a = .85, c = .15)
LDH-1	a (N2, 8, 10–11), b (N4–6)	N1 (a = .85, b = .15), N3 (a = .06, b = .88, c = .06), N7 (a = .95, d = .05), N9 (a = .45, b = .55), N12 (a = .90, e = .10)
LDH-2	a (N1–8, 10–12)	N9 (a = .60, b = .40)
GPI	a (N1–6), b (N7–12)	...
ICDH-2	a (N1–3, 5–12)	N4 (a = .95, b = .05)
IDDH	a (N3–8), d (N12)	N1 (a = .95, b = .05), N2 (a = .75, c = .25), N9 (a = .17, d = .83), N10 (a = .22, d = .78), N11 (a = .28, d = .72)
MDH-1	a (N1–11)	N12 (a = .90, c = .10)
ME	a (N1, 3–11)	N2 (a = .95, b = .05), N12 (a = .06, f = .72, g = .22)
ACON	a (N1–2, 7–11), b (N3, 5–6)	N4 (a = .80, b = .20), N12 (a = .72, d = .06, e = .22)
ADH-1	a (N1, 3–12)	N2 (a = .75, b = .25)
ADH-2	a (N1, 3–6), c (N7–12)	N2 (a = .70, b = .30)
LA	a (N1, 3–12)	N2 (a = .45, b = .55)
EST-2	a (N1–6), b (N7–12)	...
EST-3	a (N1–10), c (N11–12)	...
LGG	a (N4, 6), e (N10–12)	N1 (a = .25, b = .10, i = .65), N2 (a = .94, b = .06), N3 (a = .79, b = .21), N5 (a = .67, b = .33), N7 (a = .94, b = .06), N8 (a = .85, b = .15), N9 (a = .55, b = .05, c = .10, d = .05, e = .25)
AAT-1	a (N1–2, 4–6), b (N3), c (N8–9, 11)	N7 (c = .95, d = .05), N10 (c = .85, e = .15), N12 (c = .95, e = .05)
AAT-2	a (N5–6, 10–12), c (N7–8)	N1 (a = .85, b = .15), N2 (a = .90, b = .10), N3 (a = .63, b = .37), N4 (a = .95, b = .05), N9 (a = .50, b = .06, c = .44)
HK	a (N1–6), b (N7–12)	...
MPI	a (N1, 3–12)	N2 (a = .65, b = .35)
CK	a (N1–3, 5–12)	N4 (a = .95, b = .05)
PAP	a (N1–3, 5–8, 10–12)	N4 (a = .95, b = .05), N9 (a = .20, c = .80)

of nasal development than was apparent to Wake. In order to address the first of these hypotheses, we studied allozyme variation in *Rhyacotriton* to quantify geographic variation and to help establish a framework on which a large-scale morphological study should be based. We here present the results of this electrophoretic survey. Our genic results demonstrate an unexpectedly high degree of variation in a species considered until now to be relatively uniform, with only weakly differentiated, broadly intergrading northern and southern races (Stebbins & Lowe, 1951).

Materials and Methods

Electrophoretic samples were collected from 29 localities, each listed in Table 1 and illustrated in Figure 1. Preserved voucher specimens are housed in the Museum of Vertebrate Zoology, University of California, Berkeley (MVZ).

Tissues used for electrophoresis included liver and intestine dissected from freshly killed larval and adult specimens (N = 4–20) collected in the field. Both larvae and adults were collected from almost all populations, and no ontogenetic variation was observed in allozyme patterns. Samples were frozen and maintained at −76° C until used. Tissues from each animal were combined and homogenized, then subjected to horizontal starch-gel electrophoresis using standard techniques (Selander et al., 1971; Harris & Hopkinson, 1978).

We were able to score consistently the 29 presumptive allomorphic loci, using the buffer systems listed below. For clarity in scoring certain loci, multiple buffer systems were used.

TRIS-CITRATE II (pH 8.0)—Isocitrate dehydrogenase (ICDH, 2 loci, L-iditol dehydrogenase (IDDH), malate dehydrogenase (MDH, 2 loci), malic enzyme (ME).

TRIS-CITRATE III (pH 7.0)—Phosphoglucomu-

TABLE 3. Distribution of variants among South Coastal populations for the 29 proteins sampled in *Rhyacotriton olympicus*. The following proteins are monomorphic in these populations for the variant in parentheses: LAP (a), PT (a), SOD (b), 6-PGD (a), ACP (b), EST-3 (a), and CK (c). Sample sizes are listed in Table 1.

Protein	Fixed populations	Polymorphic populations
PGM	a (S4–5, 7–8, 10)	S1 (a = .48, b = .48, e = .04), S2 (a = .63, b = .37), S3 (a = .60, b = .40), S6 (a = .13, b = .87), S9 (a = .90, f = .10)
LDH-1	b (S5–10)	S1 (b = .90, f = .10), S2 (b = .63, f = .13, g = .24), S3 (b = .60, f = .40), S4 (b = .75, f = .25)
LDH-2	a (S1–2, 4–5), g (S10)	S3 (a = .80, f = .20), S6 (a = .50, g = .25, h = .25), S7 (a = .50, g = .25, h = .25), S8 (g = .70, h = .30), S9 (g = .95, h = .05)
GPI	b (S1–2), e (S5–6, 8, 10)	S3 (b = .95, d = .05), S4 (b = .85, d = .15), S7 (d = .06, e = .94), S9 (d = .15, e = .85)
ICDH-1	c (S1–4), d (S5–10)	...
ICDH-2	d (S1–5, 7–8), f (S10)	S6 (d = .88, e = .12), S9 (d = .95, e = .05)
IDDH	g (S4–6, 8–10)	S1 (d = .12, g = .88), S2 (d = .38, g = .62), S3 (g = .50, h = .45, i = .05), S7 (g = .95, i = .05)
MDH-1	a (S1–2, 5–6, 8–10)	S3 (a = .95, d = .05), S4 (a = .90, e = .10), S7 (a = .85, d = .05, f = .10)
MDH-2	a (S1–2, 5–10)	S3 (a = .95, b = .05), S4 (a = .85, b = .15)
ME	f (S1–6), h (S7–10)	...
ACON	a (S1–2, 5–10)	S3 (a = .56, e = .44), S4 (a = .65, e = .35)
ADH-1	a (S2–6, 8–10)	S1 (a = .92, d = .08), S7 (a = .65, e = .35)
ADH-2	c (S6, 8, 10), d (S2)	S1 (c = .92, d = .08), S3 (c = .95, d = .05), S4 (c = .80, d = .20), S5 (c = .40, d = .60), S7 (c = .55, d = .45), S9 (c = .65, d = .35)
LA	c (S1–4), e (S8–10)	S5 (c = .95, e = .05), S6 (c = .75, e = .25), S7 (e = .70, f = .30)
EST-1	a (S1–9), b (S10)	...
EST-2	c (S1–2, 4–10)	S3 (c = .85, d = .15)
LGG	b (S4), h (S10)	S1 (a = .75, b = .15, e = .10), S2 (a = .88, e = .12), S3 (a = .45, b = .25, e = .30), S5 (f = .90, g = .10), S6 (b = .25, f = .75), S7 (a = .25, b = .60, f = .15), S8 (b = .92, f = .08), S9 (a = .95, b = .05)
AAT-1	e (S2–8, 10)	S1 (c = .03, e = .93, f = .04), S9 (e = .65, g = .35)
AAT-2	a (S1–2, 5–6), h (S8–10)	S3 (a = .80, f = .20), S4 (a = .95, f = .05), S7 (g = .25, h = .75)
HK	b (S1–2, 6–10)	S3 (b = .95, c = .05), S4 (b = .90, c = .10), S5 (b = .45, c = .55)
MPI	a (S1–3, 5–6, 8–10)	S4 (a = .95, b = .05), S7 (a = .65, f = .35)
PAP	a (S3, 5–9), f (S10)	S1 (a = .85, d = .15), S2 (a = .25, d = .75), S4 (a = .79, e = .21)

TABLE 4. Distribution of variants among Cascade populations for the 29 proteins sampled in *Rhyacotriton olympicus*. The following proteins are monomorphic in these populations for the variant in parentheses: LAP (a), PT (a), MDH-1 (b), MDH-2 (a), ACP (b), ADH-2 (c), EST-1 (a), EST-3 (b), and HK (b). Sample sizes are listed in Table 1.

Protein	Fixed populations	Polymorphic populations
PGM	b (C1, 3–7)	C2 (b = .95, c = .05)
SOD	a (C3, 5–7), b (C2)	C1 (a = .25, b = .75), C4 (a = .55, b = .45)
6-PGD	a (C1–3, 5–7)	C4 (a = .93, d = .07)
LDH-1	b (C1, 3–7)	C2 (a = .25, b = .75)
LDH-2	a (C1–4, 7)	C5 (a = .95, c = .05), C6 (a = .69, d = .09, e = .22)
GPI	b (C1–2, 5, 7)	C3 (b = .89, c = .11), C4 (b = .98, c = .02), C6 (b = .97, c = .03)
ICDH-1	b (C2–7)	C1 (a = .65, b = .35)
ICDH-2	a (C3–4), c (C6–7)	C1 (a = .80, c = .20), C2 (a = .10, c = .90), C5 (a = .05, c = .95)
IDDH	e (C1), f (C4, 7)	C2 (e = .25, f = .75), C3 (e = .22, f = .78), C5 (e = .05, f = .95), C6 (e = .06, f = .94)
ME	c (C1–2), d (C4–7)	C3 (d = .72, e = .28)
ACON	a (C3, 5–7), d (C2)	C1 (a = .70, c = .30), C4 (a = .25, c = .75)
ADH-1	a (C2, 4–7)	C1 (a = .80, c = .20), C3 (a = .78, c = .22)
LA	c (C2, 5, 7)	C1 (a = .45, c = .55), C3 (a = .17, c = .83), C4 (c = .95, d = .05), C6 (c = .91, d = .09)
EST-2	b (C2–7)	C1 (a = .20, b = .80)
LGG	a (C6), b (C1, 4)	C2 (a = .35, b = .65), C3 (a = .89, b = .11), C5 (a = .80, b = .20), C7 (a = .90, b = .10)
AAT-1	c (C5–7), e (C2–3)	C1 (c = .20, e = .80), C4 (c = .75, e = .25)
AAT-2	d (C2–7)	C1 (d = .85, e = .15)
MPI	c (C3–7)	C1 (c = .90, d = .10), C2 (c = .75, d = .25)
CK	c (C2–7)	C1 (a = .30, c = .70)
PAP	a (C1–5, 7)	C6 (a = .19, c = .81)

TABLE 5. Ranges in numbers of fixed variant differences within and between populations in the South Coastal and Cascade population groups and the Olympic and sub-Chehalis subgroups of the North Coastal group.

Populations	North Coastal		South Coastal	Cascade
	Olympic	Sub-Chehalis		
Olympic	0–2			
Sub-Chehalis	5–9	0–3		
South Coastal	9–20 / 13–20	9–18	0–10	
Cascade	9–18 / 13–18	9–13	9–19	0–4

tase (PGM), 6-phosphogluconate dehydrogenase (6-PGD), L-lactate dehydrogenase (LDH, 2 loci), glucose phosphate isomerase (GPI), ICDH (2 loci), acid phosphatase (ACP), aconitate hydratase (ACON), alcohol dehydrogenase (ADH, 2 loci), aminopeptidase, leucine substrate (LAP).

LiOH (pH 8.2)—Superoxide dismutase (SOD), aminopeptidase, L-leucyl-L-alanine substrate (LA), aminopeptidase, L-leucylglycyl-L-glycine substrate (LGG), aspartate aminotransferase (AAT, 2 loci), aminopeptidase, L-phenylalanyl-L-proline substrate (PAP).

POULIK (pH 8.7)—Esterase (EST, 3 loci), AAT (2 loci), mannose-6-phosphate isomerase (MPI), creatine kinase (CK), hexokinase (HK), aminopeptidase, PAP, unidentified protein (PT).

Genetic distance measures were calculated using the methods of Nei (1978) and Rogers (1972). Rogers's distance, which is bounded between 0 and 1, meets the requirements of a metric and is therefore superior for certain types of clustering analyses. Nei's distance, although it has the disadvantage of not being a metric, is useful in that it is more directly comparable to measures which are not bounded, such as time or geographic distances. Various clustering methods, both phenetic and phylogenetic, were employed to examine patterns of variation among *Rhyacotriton* populations. Rogers's and Nei's genetic distance mea-

TABLE 6. Nei's (1978) (above diagonal) and Rogers's (1972) (below diagonal) distances measured among populations of *Rhyacotriton olympicus*.

Populations	North Coastal populations												South Coastal populations	
	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	S1	S2
N1	···	0.038	0.112	0.045	0.075	0.081	0.251	0.250	0.301	0.250	0.297	0.363	0.714	0.739
N2	0.091	···	0.137	0.060	0.101	0.097	0.251	0.253	0.317	0.272	0.321	0.387	0.696	0.712
N3	0.127	0.176	···	0.065	0.040	0.041	0.308	0.311	0.341	0.348	0.399	0.454	0.724	0.761
N4	0.071	0.112	0.091	···	0.026	0.022	0.277	0.282	0.297	0.309	0.358	0.422	0.638	0.667
N5	0.092	0.145	0.058	0.047	···	0.003	0.327	0.329	0.344	0.344	0.394	0.448	0.708	0.749
N6	0.097	0.135	0.058	0.036	0.014	···	0.320	0.235	0.343	0.351	0.401	0.455	0.698	0.732
N7	0.235	0.252	0.273	0.255	0.287	0.276	···	0.000	0.079	0.094	0.129	0.183	0.558	0.566
N8	0.235	0.255	0.271	0.260	0.285	0.281	0.007	···	0.079	0.091	0.125	0.180	0.562	0.572
N9	0.293	0.327	0.318	0.300	0.323	0.327	0.125	0.123	···	0.065	0.104	0.138	0.532	0.508
N10	0.241	0.272	0.311	0.285	0.303	0.304	0.105	0.102	0.115	···	0.036	0.068	0.514	0.514
N11	0.273	0.303	0.342	0.316	0.334	0.335	0.131	0.126	0.143	0.045	···	0.030	0.590	0.592
N12	0.323	0.354	0.378	0.357	0.370	0.371	0.183	0.183	0.187	0.094	0.056	···	0.546	0.542
S1	0.523	0.515	0.523	0.488	0.516	0.515	0.441	0.440	0.442	0.419	0.460	0.447	···	0.014
S2	0.536	0.527	0.546	0.506	0.542	0.533	0.445	0.448	0.427	0.421	0.462	0.448	0.056	···
S3	0.529	0.536	0.527	0.514	0.525	0.530	0.457	0.456	0.452	0.430	0.470	0.438	0.091	0.106
S4	0.531	0.543	0.536	0.514	0.526	0.534	0.486	0.484	0.481	0.453	0.488	0.454	0.103	0.129
S5	0.502	0.516	0.519	0.482	0.504	0.506	0.517	0.518	0.521	0.481	0.516	0.503	0.172	0.204
S6	0.549	0.563	0.565	0.529	0.550	0.554	0.516	0.516	0.504	0.475	0.515	0.502	0.157	0.193
S7	0.578	0.568	0.579	0.561	0.580	0.588	0.535	0.534	0.533	0.540	0.575	0.580	0.285	0.324
S8	0.568	0.586	0.573	0.554	0.572	0.581	0.508	0.507	0.506	0.507	0.543	0.552	0.267	0.297
S9	0.560	0.549	0.548	0.518	0.556	0.545	0.489	0.494	0.504	0.516	0.555	0.563	0.263	0.295
S10	0.644	0.661	0.651	0.626	0.654	0.655	0.584	0.584	0.552	0.581	0.617	0.626	0.372	0.377
C1	0.595	0.594	0.586	0.574	0.586	0.595	0.447	0.475	0.493	0.471	0.477	0.471	0.408	0.442
C2	0.684	0.680	0.661	0.673	0.667	0.675	0.551	0.549	0.569	0.552	0.558	0.544	0.387	0.408
C3	0.595	0.564	0.582	0.559	0.590	0.583	0.448	0.449	0.478	0.470	0.476	0.479	0.388	0.413
C4	0.644	0.648	0.622	0.624	0.619	0.631	0.498	0.497	0.512	0.442	0.498	0.489	0.441	0.472
C5	0.634	0.615	0.619	0.600	0.627	0.627	0.453	0.449	0.475	0.479	0.475	0.482	0.441	0.440
C6	0.673	0.647	0.662	0.626	0.670	0.655	0.487	0.490	0.450	0.519	0.515	0.522	0.448	0.452
C7	0.637	0.612	0.624	0.598	0.632	0.624	0.451	0.450	0.479	0.482	0.478	0.485	0.412	0.438

sures were used in generating UPGMA (Sokal & Michener, 1958) and Fitch-Margoliash (Fitch & Margoliash, 1967) dendrograms. The PAUP version of the Wagner presence/absence phylogenetic method (Kluge & Farris, 1969) was performed with raw allele presence scores using proteins as characters and variant combinations as character states (Buth, 1984).

Estimates of genetic heterozygosity were calculated using the equation

$$H = 1 - \sum_{i=1}^{k} x_i^2$$

averaged over all proteins (x_i is the frequency of the ith allele, and k is the number of alleles at a particular locus). Estimates of genetic subdivision of the species were calculated using the F_{ST} statistic of Wright (1965). Nei's and Rogers's distances, UPGMA phenograms, heterozygosity, and F_{ST} values were all calculated using the BIOSYS computer program; Fitch-Margoliash dendrograms were obtained using EVOLVE.

Results

Of the 29 proteins scored in this survey, two (PT and LAP) were monomorphic in all 29 populations. Some degree of variation was seen in the other 27 proteins (tables 2–4), all of which, except 6-PGD, showed major shifts in variant frequencies and fixed differences between pairs of populations. A total of 139 variants was recorded among the 29 populations sampled, averaging 4.8 per protein and ranging from one (PT and LAP) to nine (LGG and IDDH).

The presence of large amounts of variation among populations in the 29 proteins surveyed suggests significant genetic subdivision within *Rhyacotriton olympicus*. Further, the distribution of protein variants among populations demonstrates the existence of at least three strongly differentiated groups of populations, referred to here as the North Coastal, South Coastal, and Cascade groups (table 1). All populations in each group are differentiated from populations in the other groups

TABLE 6. *Continued.*

South Coastal populations								Cascade populations						
S3	S4	S5	S6	S7	S8	S9	S10	C1	C2	C3	C4	C5	C6	C7
0.724	0.723	0.686	0.774	0.843	0.833	0.810	1.032	0.840	1.167	0.889	1.019	1.000	1.103	1.006
0.722	0.748	0.709	0.803	0.847	0.871	0.788	1.079	0.868	1.149	0.844	1.035	0.956	1.041	0.955
0.725	0.738	0.721	0.810	0.847	0.845	0.788	1.057	0.825	1.095	0.868	0.959	0.980	1.071	0.982
0.675	0.696	0.645	0.730	0.789	0.800	0.716	0.973	0.804	1.110	0.795	0.968	0.899	0.970	0.898
0.712	0.709	0.700	0.783	0.849	0.842	0.802	1.062	0.820	1.100	0.881	0.952	0.992	1.089	0.996
0.712	0.740	0.705	0.795	0.863	0.871	0.783	1.065	0.853	1.113	0.863	0.988	0.977	1.057	0.975
0.565	0.623	0.710	0.712	0.728	0.708	0.661	0.877	0.578	0.785	0.576	0.661	0.596	0.651	0.598
0.567	0.617	0.711	0.711	0.727	0.703	0.667	0.878	0.572	0.783	0.582	0.653	0.597	0.655	0.600
0.557	0.591	0.680	0.667	0.717	0.685	0.664	0.792	0.580	0.832	0.600	0.663	0.613	0.580	0.618
0.508	0.551	0.628	0.624	0.736	0.699	0.715	0.864	0.566	0.792	0.613	0.658	0.644	0.716	0.651
0.586	0.628	0.708	0.711	0.829	0.784	0.800	0.962	0.579	0.811	0.630	0.661	0.641	0.712	0.648
0.528	0.572	0.663	0.665	0.840	0.792	0.811	0.975	0.573	0.788	0.628	0.650	0.646	0.719	0.653
0.021	0.042	0.138	0.113	0.268	0.274	0.261	0.433	0.452	0.456	0.452	0.525	0.505	0.555	0.507
0.039	0.066	0.169	0.151	0.309	0.312	0.292	0.442	0.505	0.494	0.487	0.582	0.547	0.565	0.547
···	0.034	0.155	0.133	0.279	0.275	0.284	0.461	0.450	0.435	0.477	0.513	0.535	0.600	0.539
0.104	···	0.131	0.145	0.249	0.239	0.295	0.439	0.471	0.487	0.563	0.536	0.617	0.694	0.628
0.214	0.171	···	0.059	0.175	0.199	0.208	0.353	0.620	0.632	0.642	0.700	0.712	0.791	0.716
0.197	0.210	0.103	···	0.157	0.150	0.181	0.304	0.495	0.523	0.537	0.576	0.601	0.665	0.609
0.310	0.279	0.207	0.207	···	0.022	0.034	0.172	0.605	0.661	0.668	0.721	0.754	0.819	0.764
0.289	0.252	0.202	0.169	0.082	···	0.040	0.149	0.595	0.653	0.693	0.700	0.766	0.835	0.781
0.304	0.297	0.217	0.207	0.103	0.075	···	0.159	0.683	0.706	0.670	0.780	0.729	0.768	0.730
0.404	0.380	0.312	0.280	0.209	0.147	0.171	···	0.828	0.869	0.868	0.948	0.958	0.949	0.966
0.418	0.424	0.496	0.431	0.483	0.472	0.516	0.576	···	0.114	0.141	0.132	0.211	0.268	0.225
0.378	0.411	0.483	0.427	0.493	0.489	0.521	0.581	0.191	···	0.160	0.144	0.169	0.213	0.173
0.418	0.462	0.489	0.432	0.497	0.505	0.500	0.581	0.201	0.187	···	0.083	0.076	0.110	0.080
0.441	0.449	0.526	0.468	0.539	0.518	0.555	0.623	0.193	0.185	0.133	···	0.086	0.135	0.096
0.441	0.487	0.520	0.464	0.539	0.537	0.522	0.615	0.257	0.183	0.105	0.120	···	0.027	0.000
0.479	0.523	0.560	0.493	0.568	0.567	0.542	0.611	0.301	0.231	0.141	0.164	0.050	···	0.026
0.446	0.490	0.521	0.469	0.545	0.543	0.521	0.619	0.262	0.188	0.104	0.122	0.009	0.048	···

by at least nine fixed protein variant differences, and by as many as 20 in some populations (table 5). The North Coastal populations, distributed in the Coast Ranges from the Olympic Peninsula of Washington south to northwestern Oregon (fig. 1), are characterized by the presence of three unique protein variants (IDDH-a, ME-a, and ACP-a); three other variants are fixed or almost fixed in North Coastal populations but relatively rare elsewhere (ICDH-1-a, also in population C1; IDDH-d, also in populations S1 and S2; LA-a, also in populations C1 and C3). The South Coastal populations, distributed from northwestern Oregon through the Coast Ranges south to Mendocino County, California, are characterized by seven unique variants, or combinations of variants (ICDH-1-c, d; ICDH-2-d, e, f; IDDH-g; EST-2-c), and one found elsewhere only in population N12 (ME-f). The Cascade populations, in the Cascade Mountains of Oregon, share 12 unique variants or combinations (ICDH-1-b; IDDH-e, f; MDH-1-b; ME-c, d, e; AAT-2-d, e; MPI-c, d; EST-3-b).

Among the North Coastal populations, further differentiation is apparent between the Olympic Peninsula populations (N1–N6) and those from south of the Chehalis River (N7–N12, referred to here as the sub-Chehalis populations). The Olympic Peninsula populations share six unique variants or combinations (ADH-2-a, b; GPI-a; AAT-1-a, b; HK-a). Variants CK-a and EST-2-a, common to all Olympic Peninsula populations, are seen elsewhere only in population C1. Populations N7–N12 share no unique variants. A total of 69 protein variants were observed among the North Coastal populations, averaging 2.4 per protein. Among South Coastal populations, 73 variants (2.5 per protein) were seen. In the Cascade population group, the total was 53 (1.9 per protein).

We also analyzed protein differentiation among populations, using the genetic distance measures of Rogers (1972) and Nei (1978) (table 6). North Coastal, South Coastal, and Cascade population groups are recognizable, with the North Coastal group being further subdivided into two less distinct but still well-differentiated subgroups. Nei's distance measures within the three major groups of populations range from 0.000 to 0.455 among North Coastal populations, 0.014 to 0.461 among South Coastal populations, and 0.000 to 0.268 among Cascade populations. In contrast, distances between pairs of populations across groups range from 0.508 to 1.079 between the North and South Coastal groups, 0.566 to 1.167 between the North Coastal and Cascade groups, and 0.435 to 0.966 between the South Coastal and Cascade groups. Nei's distance measures as high as 0.455 among North Coastal populations suggest the relatively strong subdivision of that group. Among Olympic Peninsula populations, distances vary from 0.003 to 0.137. Among the sub-Chehalis North Coastal populations, distances vary from 0.000 to 0.183. Between pairs of populations bridging the gap between these two North Coastal subdivisions, distances range from 0.250 and 0.455. Within the Cascade and South Coastal groups of populations and within each of the two subdivisions of the North Coastal group, matrices of genetic distance do not vary significantly from matrices of geographic distance (Mantel's test; Mantel, 1967), indicating that isolation by distance might be sufficient to explain the patterns observed.

While Nei's genetic distances between members of the major population groups range to 1.079 or more (corresponding to 19 fixed differences out of 29 proteins), large distances are characteristic between populations separated by a great geographic distance (in this case, populations N2 and S10). Genetic distances between populations in different major groups that are geographically closer are somewhat smaller, though still very much greater than those between populations within groups at similar geographic distances. For example, the genetic distance between the adjacent North Coastal and South Coastal populations (N12 and S1) is 0.546, while the genetic distance from N12 to the geographically nearest North Coastal population (N11, at approximately the same distance from N12 as S1, fig. 1) is only 0.030. The genetic distance between the geographically nearest North Coastal and Cascade populations (N9 and C2) is 0.832 (although the genetic distance between N9 and C1 is less, 0.580); between the nearest South Coastal and Cascade populations (S1 and C6), it is 0.555.

The dramatic genetic differentiation between major population groups is exemplified in Figure 2, which illustrates geographic distance and Nei's genetic distance values between pairs of populations, selected so that they lie in a more-or-less straight line from north to south along the Coast Ranges. Among populations in the Olympic (N1–N2, and N4) and sub-Chehalis (N7–N12) population subgroups, genetic distances are invariably less than 0.10, often much less. Among the South Coastal populations (S1–S5, S7, S9–S10), genetic distances are sometimes somewhat higher, although the higher values are correlated with larger geographic distances between populations and do

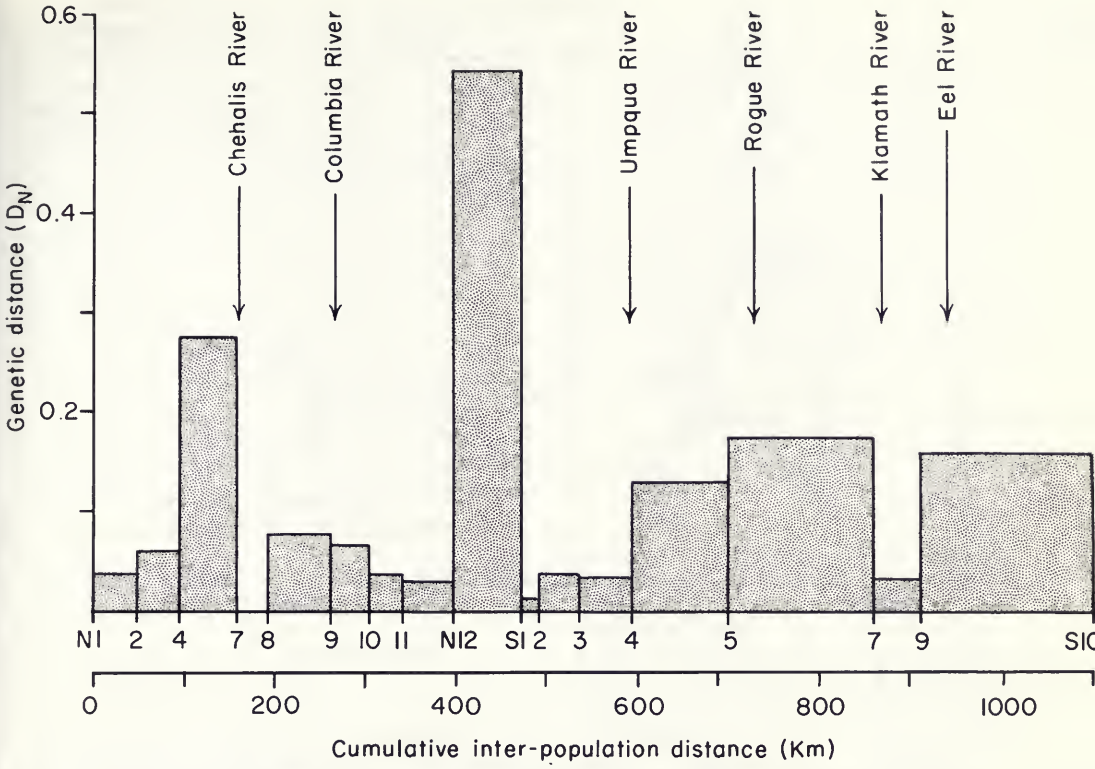

FIG. 2. The relation of genetic distance to geographic distance on a north to south transect along the Pacific coastal region. The straight line distance between adjacent populations is marked on the horizontal axis, the genetic distance (D_N), on the vertical axis. The histograms indicate the amount of genetic change between adjacent populations. For population abbreviations, see Table 1; for genetic distances, see Table 6. Locations of potential major river barriers are indicated.

not exceed 0.20 units. In contrast, two genetic distance peaks stand out clearly in the figure. The most dramatic is that between the North Coastal and South Coastal population groups, populations N12 and S1, with a genetic distance almost eight times that between either populations N11 and N12 or populations S1 and S2. This large genetic distance results from the fact that nine proteins (SOD, LDH-1, ICDH-1, ICDH-2, ACP, LA, EST-2, EST-3, and CK) show fixed alternative variants on either side of the gap between populations N12 and S1. The genetic distance (D_N) between populations N4 and N7 is also surprisingly large (0.277) and corresponds to the boundary between the Olympic and sub-Chehalis subgroups.

Included in Figure 2 are the geographic locations of the six rivers which bisect the Coast Ranges, and therefore might be expected to present potential barriers to gene flow (*Rhyacotriton* occurs mainly in seeps and small creeks). The only one of these six that corresponds to a jump in genetic distance is the Chehalis River, between the Olympic and sub-Chehalis subgroups of the North Coastal population group. None of the other rivers seems to have any effect on population divergence in *Rhyacotriton*.

A representation of overall genic similarity among populations, based on Nei's D, is provided in the UPGMA diagram in Figure 3, the data from which are superimposed on a map of the distribution of collection sites in Figure 4. Again, three major groups of populations can be seen, with the North Coastal group divided into two parts. Some subdivision of the South Coastal and Cascade groups is also suggested, but it is relatively weak and is not supported by the presence of unique protein variant suites, as are the groups discussed above.

Two methods were used to examine variation among *Rhyacotriton* populations from a phylogenetic perspective. Rogers's (1972) genetic distance was analyzed by using the method of Fitch

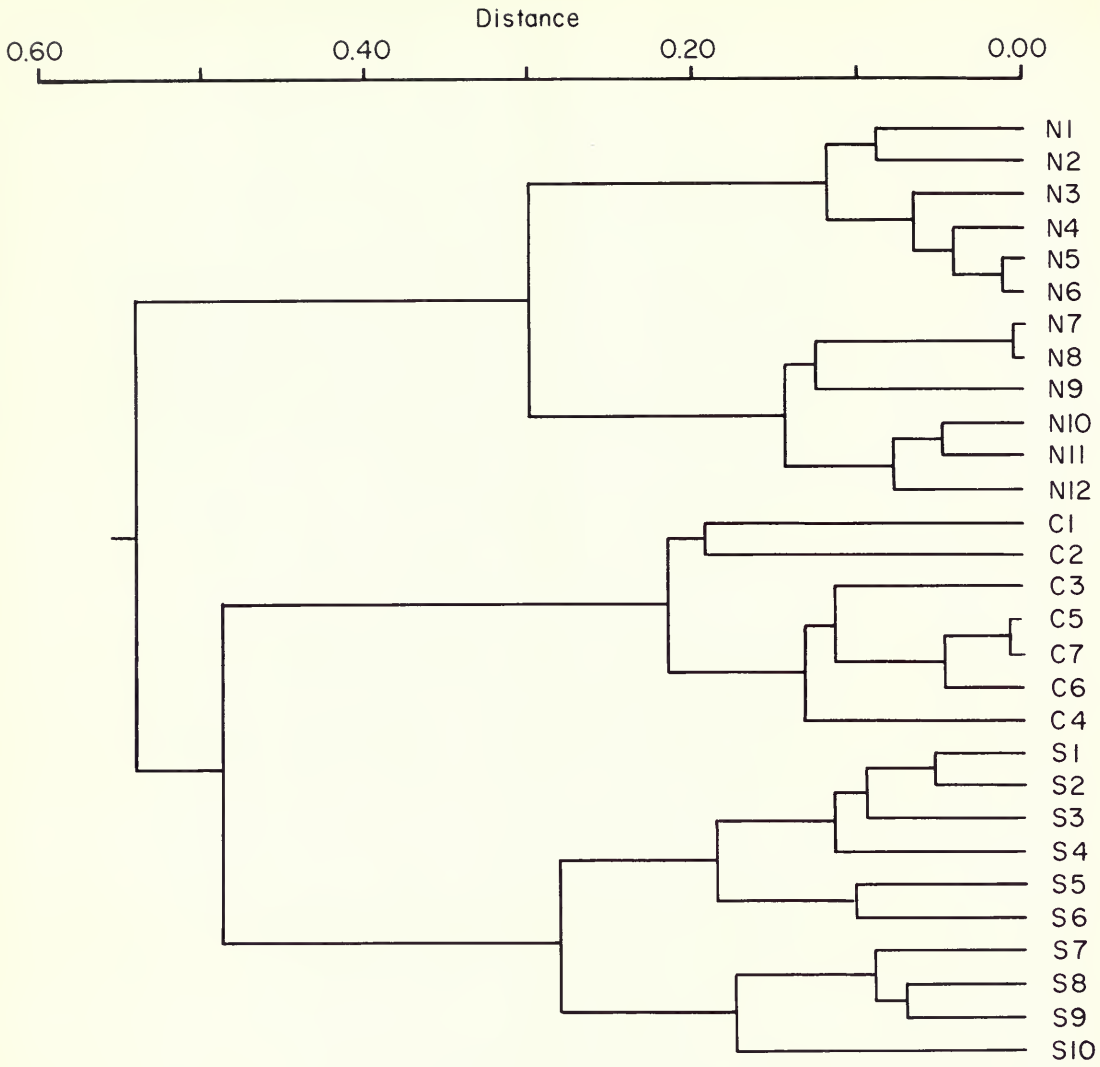

FIG. 3. UPGMA dendrogram based on matrix of Nei's genetic distances shown in Table 6. Cophenetic correlation coefficient = 0.94.

and Margoliash (1967) and protein variant presence/absence among populations by using the unrooted Wagner method (no outgroup to *Rhyacotriton* is available; neither *Dicamptodon* nor *Ambystoma* is similar enough in its allozymes to make comparison with *Rhyacotriton* feasible). These analyses (figs. 5–6) separate populations into major groups matching those discussed above, except that in the Wagner analysis population N9 is grouped with the Olympic Peninsula populations instead of with the sub-Chehalis North Coastal populations. This is due entirely to the sharing of a single variant (LDH-1-b) by population N9 and most Olympic Peninsula populations. However, assuming that the South Coastal and/or Cascade populations are outgroups to the North Coastal group, it becomes apparent that LDH-1-b is the ancestral LDH-1 variant for that group and should not be taken to indicate a relationship of population N9 with the Olympic populations.

All of the above analyses which produce rooted trees agree on grouping the South Coastal and Cascade groups together to the exclusion of the North Coastal group. Despite these groups being phenetically more similar to each other than either is to the North Coastal populations, little phylogenetic significance should be placed on this fact without outgroup comparison.

The standardized variance of gene frequencies (F_{ST}; Wright, 1965; Nei, 1973) is used to quantify the level of genetic subdivision within species. In this analysis, higher values imply greater subdivision into isolated populations than lower values. When calculated for *Rhyacotriton olympicus* as a whole, F_{ST} was 0.880, exceeding values reported for other salamander species (Larson et al., 1984), most of which average much higher than values for almost any other organism (Wright, 1978). When calculated for the South Coastal and Cascade groups of populations separately, F_{ST} values were more in line with those for other groups of salamanders, though still high (0.689 for the South Coastal group and 0.643 for the Cascades). The North Coastal group as a whole showed an extremely high F_{ST} of 0.828. When the Olympic and sub-Chehalis groups of populations were separated, their values were 0.588 and 0.676, respectively.

Average heterozygosities in the *Rhyacotriton* populations surveyed varied from 0 to 0.145 (table 7).

TABLE 7. Estimates of the average number of variants per protein and heterozygosity values for populations of *Rhyacotriton olympicus*.

Population	Alleles	Heterozygosity
N1	1.21	0.031
N2	1.28	0.097
N3	1.14	0.040
N4	1.21	0.031
N5	1.07	0.017
N6	1.00	0
N7	1.10	0.011
N8	1.03	0.010
N9	1.38	0.091
N10	1.10	0.033
N11	1.03	0.011
N12	1.24	0.056
S1	1.38	0.076
S2	1.21	0.069
S3	1.52	0.133
S4	1.34	0.091
S5	1.14	0.048
S6	1.21	0.069
S7	1.45	0.145
S8	1.07	0.026
S9	1.24	0.076
S10	1.00	0
C1	1.38	0.117
C2	1.21	0.052
C3	1.21	0.061
C4	1.21	0.064
C5	1.14	0.017
C6	1.21	0.024
C7	1.03	0.007

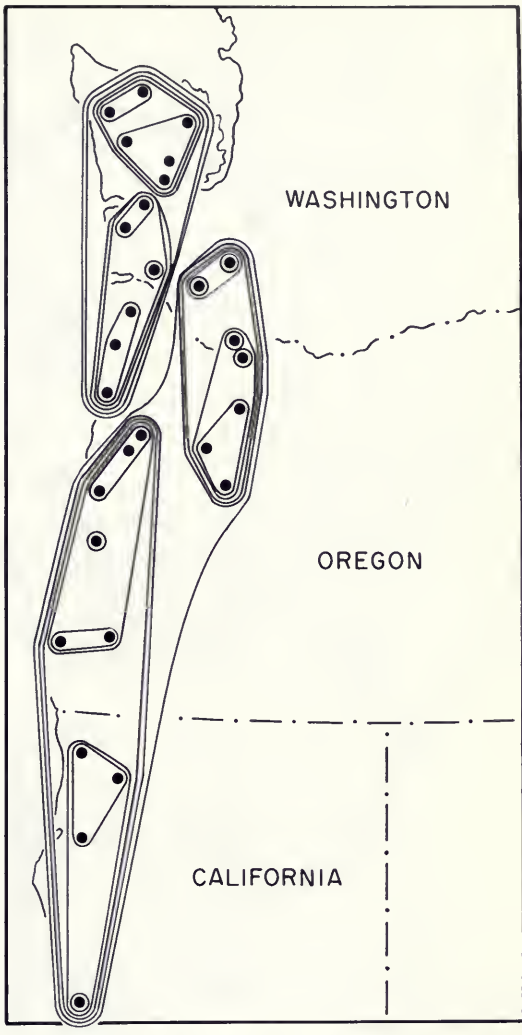

FIG. 4. Map of localities of populations sampled (see fig. 1), illustrating isophenes of genetic distance, based on a UPGMA treatment (fig. 3) of the matrix of Nei's genetic distances shown in Table 6. Interval between levels is $D_N = 0.1$.

Discussion

The hypothesis of Wake (1981) concerning the effect of environment on development in *Rhyacotriton* depends on the species having relatively low intraspecific genetic differentiation, so as to eliminate the possibility that the osteological pattern had a longer term historical explanation. While low genetic differentiation was a reasonable expectation, following from low variability in external morphology, *Rhyacotriton* populations in fact show differentiation in allozymes at a level as great as or greater than that seen among species in many

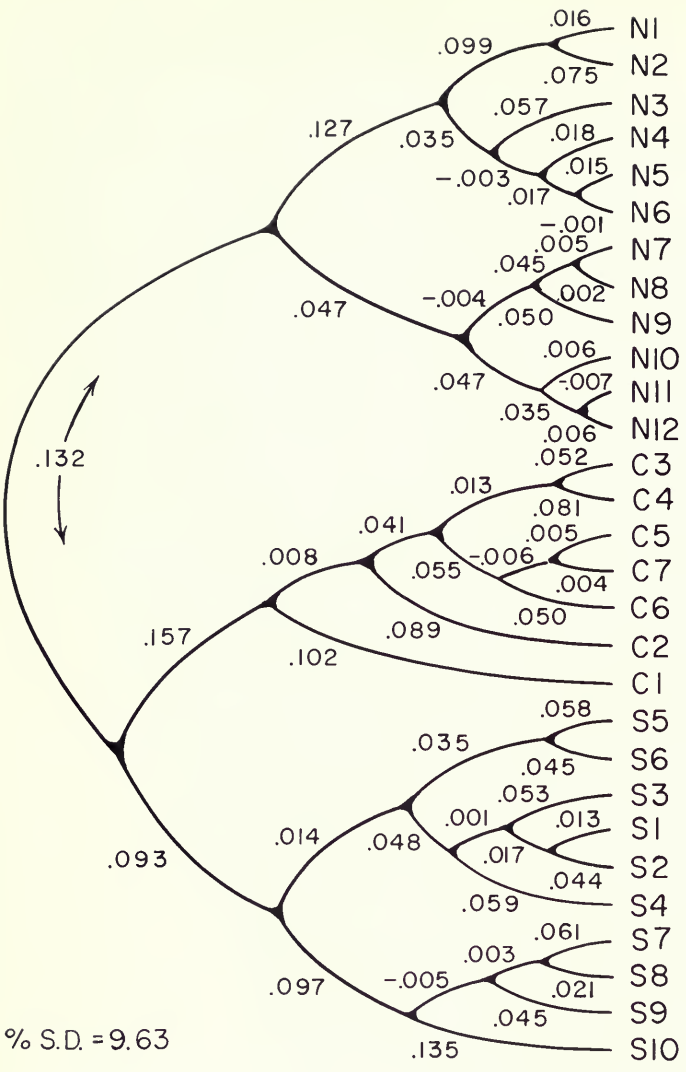

FIG. 5. A Fitch-Margoliash tree of Rogers's genetic distances, from Table 6.

other salamander genera (Larson, 1984). Thus, the possibility that the differential nasal development observed by Wake was due to genetic and perhaps species-level differences rather than to a more direct environmental effect cannot be ruled out. Wake examined material from only four populations; in view of our present results, further populations should be studied.

Reexamining the analysis of geographic variation in coloration by Stebbins and Lowe (1951) in the light of our genetic data is instructive. Color pattern is only partly correlated with geographic variation in allozymes. The distribution of the subspecies *olympicus* exactly matches the distribution of our North Coastal populations. The South Coastal populations coincide with the ranges of the subspecies *variegatus* and the "intergrades" of Stebbins and Lowe, while the Cascade group includes solely "intergrades."

In view of the large genetic distances between groups of *Rhyacotriton* populations, the question of specific status arises. We reserve judgment on this question for the present, pending further analysis of potential contact zones. The Cascade populations are almost certainly geographically isolated from coastal *Rhyacotriton* by the Puget Sound-Cowlitz-Willamette lowlands. A test of sympatry there is impossible, but *Rhyacotriton* is abundant throughout the area in northwestern Oregon where the North Coastal and South Coastal groups approach one another. An analysis of allozymes from populations here may supply the

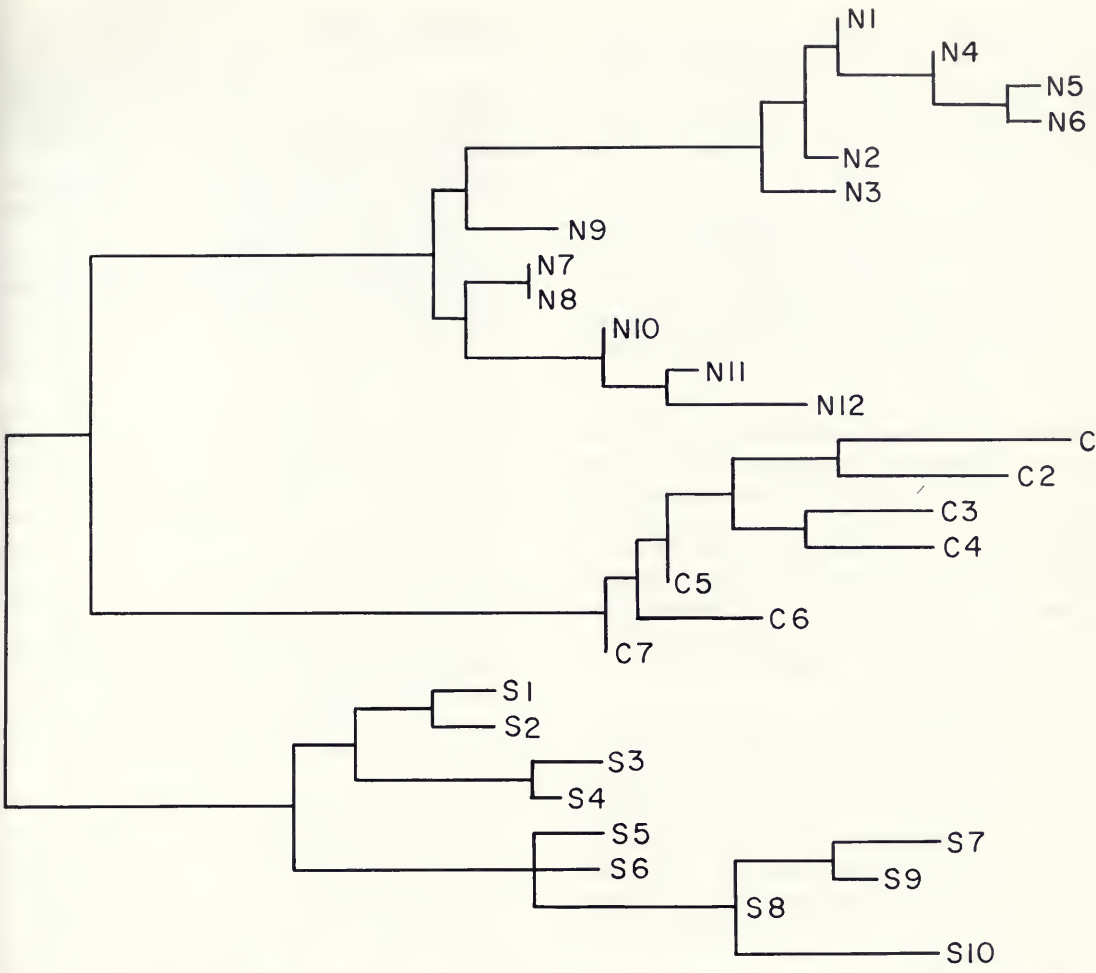

FIG. 6. An unrooted dendrogram constructed according to the Wagner phylogenetic method using presence absence of protein variants (data taken from tables 2–4).

information necessary to resolve the taxonomic status of these groups. Such an analysis is now in progress.

The history of *Rhyacotriton* in the Pacific Northwest, and particularly the explanation for differentiation into such distinct population groups, is obscure. North Coastal forest of an aspect similar to that currently seen in the Pacific Northwest has been present since at least the Eocene (Axelrod, 1977). It is therefore likely that the evolution of *Rhyacotriton* has occurred essentially *in situ*. The great genetic differentiation in the genus suggests that the populations probably have been diverging for a very long time. Since some of the largest genetic distances involve populations in the Coast Ranges, it is possible that *Rhyacotriton* has been occupying the region since shortly after it rose out of the Pacific Ocean, sometime near the Oligocene-Miocene boundary (Nilsen & McKee, 1979; Cole & Armentrout, 1979). Genetic differentiation is great throughout the entire range of the species, suggesting that *Rhyacotriton* spread rapidly throughout its present range and has been gradually differentiating in relatively isolated populations (as suggested by high F_{ST} values) since that time.

McKee (1972) set the date for the uplift of most of the Coast Range province from the sea at 15 million years ago. The beginning of the Miocene, in the analysis of Cole and Armentrout (1979), was 22 million years ago. If we assume that the invasion of the Coast Ranges by *Rhyacotriton* occurred somewhere around 15–22 million years ago, and since the maximum genic differentiation is

1.167 Nei's genetic distance units, an electrophoretic clock can be calibrated at somewhere between 12.8 and 18.9 million years per Nei's distance unit. Clocks calibrated by Maxson and Maxson (1979) and Sarich (1977) using Nei's D suggest 14 and 20 million years, respectively, making this scenario possible.

If this calibration of the electrophoretic clock for *Rhyacotriton* is roughly correct and the clock is relatively constant, the North Coastal, South Coastal, and Cascade groups of populations have been genetically isolated from each other for about 6 to 11 million years (D_N = 0.435–0.566). No readily apparent paleogeographic or paleoclimatological events would account for a physical isolation of populations at that time (Cole & Armentrout, 1979), although such physical isolation still seems to us more likely than a sympatric or parapatric model of differentiation (Endler, 1977). No paleogeographic explanation exists for the differentiation of the Olympic and sub-Chehalis North Coastal groups. Here, a Nei's distance measure of 0.320 would suggest a divergence time of between 4 and 6.5 million years.

Far from being a weakly differentiated, geographically homogeneous species, *Rhyacotriton olympicus* harbors enormous amounts of hidden genic variability. *Rhyacotriton* probably includes three separate species, each of which displays substantial intraspecific differentiation. This paper represents a baseline for conducting further work on allozymic and osteological variation in this unique salamander.

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We dedicate this paper to Robert F. Inger on the occasion of his 65th birthday, in recognition of his outstanding contributions to the ecology, biogeography, and systematics of amphibians and reptiles.

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