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in the Torrent Salamanders of the Genus
Rhyacotriton (Caudata: Rhyacotritonidae)

David A. Good and David B. Wake

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(CAUDATA: RHYACOTRITONIDAE)

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A Contribution from the Museum of Vertebrate Zoology
of the University of California at Berkeley

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Abstract

Salamanders of the genus *Rhyacotriton* are restricted to the Pacific Northwest region of the United States. The genus long has been perceived as phylogenetically isolated, but a new evaluation of data from morphology and proteins leads to the conclusion that a monotypic, monophyletic family Rhyacotritonidae is warranted. The single species that is currently recognized was shown in a recent study of protein variation to display extreme and unexpectedly high levels of genetic differentiation. A more detailed examination of protein data is presented here, with special emphasis on regions where large amounts of change were detected earlier. We report on a contact zone in north coastal Oregon, where 9 (of 29) allozyme loci show fixed differences between nearby populations. No merging of the two populational units was found, even at distances on the order of 100 m, nor was any sympatry discovered. An analysis of this contact zone together with a detailed consideration of the pattern of buildup of genetic distance with geography leads to the conclusion that four species should be recognized in what previously was a single polytypic species (two subspecies): *R. olympicus* (the generotype), from the Olympic Peninsula of Washington; *R. variegatus* (raised from subspecific rank), from the Coast Ranges of central and southern Oregon and northern California, with an isolated population in the southern Cascade Mountains of Oregon; *R. cascadae* (a new taxon), from the Cascade Mountains of southern Washington and northern and central Oregon; and *R. kezeri* (a new taxon), from the Coast Ranges of northern Oregon and southern Washington. The four species resemble each other closely in morphology, as is demonstrated by a detailed treatment of comparative osteology and morphometrics. While osteological variation is present, species-specific variation is weak. Morphometric analyses (principal component, discriminant function, and bivariate analyses) disclose substantial variation, but only weak species-specific patterns. There are geographic trends, but these rarely correspond to species borders. Variation in color patterns is evident, and species-specific patterns are described for the three coastal species; *R. cascadae* is more variable in coloration than the other three species. There is substantial variation in life history traits, and while it is difficult to sort phylogenetic from ecological factors, the species differ in larval and adult size, length of larval life, and egg number. When all information is taken

into account, a picture emerges of an ancient and very distinct lineage that is represented by four genetically distinct species, each itself geographically variable. These species diverged from each other during a period estimated (from molecular clock calibrations) to be between 9.5 and 3.2 million years ago, and they are considered to be much younger than the family as a whole. These relatively ancient and genetically diverse species show only subtle morphological differentiation, as is frequently the case in salamander phylogenesis, where morphological stasis often predominates within genera. A biogeographic scenario based on the patterns of genetic differentiation envisions two vicariant events in the evolutionary history of the genus, the first involving the isolation of the three most distinct groups (one in the inland mountains, two along the coast) and the second involving the later separation of a north coastal unit into two taxa.

INTRODUCTION

With few exceptions, in-depth analysis of biochemical variation in salamanders (e.g., Highton et al., 1989, and papers summarized by Larson, 1984) has led to the discovery of unexpected levels of differentiation within species that had been considered to be relatively uniform on the basis of morphology. In an earlier paper (Good et al., 1987), we presented an example of this phenomenon in the genus *Rhyacotriton* (Caudata: Rhyacotritonidae), a characteristic and locally very common salamander in cool, rapidly flowing streams in the coniferous forests of the Pacific Northwest of the United States (Figure 1). This monograph, stimulated by our earlier observations, is a comprehensive treatment of biochemical and morphological variation in the genus.

Although *Rhyacotriton* has some uniquely derived characteristics, it is generalized in many other respects, and its phylogenetic relationships to other salamander groups remain unclear. When first described (Gauge, 1917), it was considered to be a member of the Asian hynobiid genus *Ranodon*. Since the work of Dunn (1920), it has been considered to belong among the "advanced" (i.e., non-cryptobranchoid) salamanders, and until the work of Edwards (1976) it usually was thought to be a member of the family Ambystomatidae, although some workers (e.g., Noble, 1927; Regal, 1966) saw it as an "intermediate" between various other groups. Tihen (1958) recognized that *Rhyacotriton* differs strongly from other ambystomatids (*sensu lato*) in retaining numerous primitive characters otherwise lacking in the family; he isolated the genus into the separate subfamily Rhyacotritoninae. Regal (1966) placed *Rhyacotriton* in the ambystomatid subfamily Dicamptodontinae, together with the Pacific Giant Salamander *Dicamptodon*, but he offered no synapomorphies for this grouping. Edwards (1976) went a step further and suggested that *Rhyacotriton* and *Dicamptodon* are neither ambystomatids nor particularly closely related to the Ambystomatidae, and he raised Regal's Dicamptodontinae to family status as the Dicamptodontidae. This is the generally accepted taxonomy at present (Frost, 1985). However, examination of the characters used by the above authors to reconstruct the phylogenetic relationships of *Rhyacotriton*, as well as recent biochemical information (Larson and Wilson, 1989; Larson, 1991), suggests that *Rhyacotriton* and *Dicamptodon* are not sister taxa, and in this monograph we recognize a new family, the Rhyacotritonidae.

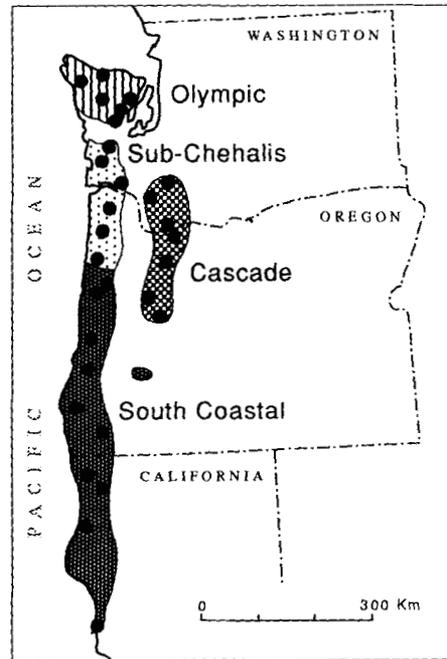


Figure 1. The distribution of the 29 populations of *Rhyacotriton*, arranged into the four groups based on patterns of protein variation discovered by Good et al. (1987).

Until the work of Good et al. (1987), *Rhyacotriton* was thought to contain only the single species *R. olympicus*, the Olympic salamander. Although some geographic differentiation was recognized by Stebbins and Lowe (1951), Nussbaum and Tait (1977), and Wake (1980), most workers gave it little consideration and apparently assumed *Rhyacotriton* to be relatively uniform across its range. Good et al. examined allozyme variation in the genus and found that, far from being geographically uniform and invariant, *Rhyacotriton* shows enormous amounts of genetic variability. In the following chapters, we extend the biochemical work of Good et al. (1987). We demonstrate that *Rhyacotriton* consists of a radiation of four species (Chapter 2), and provide formal taxonomic diagnoses, raising two taxa to specific status and providing new names for the other two (Chapter 3). Because the common name in present use ("Olympic salamander") is appropriate only for one of these species, a new common name, "torrent salamander," is proposed for the genus. The habitat (Chapter 4) and distribution (Chapter 5) of the species are summarized. Patterns of variation are analyzed for osteology (Chapter 6), morphometrics (Chapter 7), color pattern (Chapter 8), and life-history characteristics (Chapter 9). The evolutionary history (Chapter 10) and phylogenetic position (Chapter 11) of *Rhyacotriton* are discussed. This monograph is intended as an overview of our current knowledge of *Rhyacotriton* and as a baseline for future work; Chapter 12 is a brief discussion of what we see as potentially productive and important directions for such work.

BIOCHEMICAL VARIATION IN *RHYACOTRITON*

Rhyacotriton is presently considered to be a single species (*R. olympicus*) that displays geographic variation in color pattern (Stebbins and Lowe, 1951), life history (Nussbaum and Tait, 1977), and osteology (Wake, 1980). Good et al. (1987) examined 29 proteins in 29 populations from throughout the range of *Rhyacotriton* (Figure 1), and found unexpectedly high levels of variation. Nei (1978) distances (Table 1) ranged as high as 1.17 between populations (19 fixed differences among the 29 proteins examined), a level equivalent to that seen between some subfamilies of lizards (Good, 1987) or families of birds (Marten and Johnson, 1986); yet *Rhyacotriton olympicus* had been supposed by most workers to be a single species that is fairly undifferentiated in morphology.

The analysis of Good et al. (1987) demonstrated that *Rhyacotriton* is divisible into three major sets of populations on the basis of protein distribution (Figure 1): the "North Coastal" group (in the Coast Ranges from the Olympic Mountains of Washington south to northwestern Oregon); the "South Coastal" group (in the Coast Ranges from northwestern Oregon south to central California); and the "Cascade" group (in the Cascade Mountains). The North Coastal group was further divisible into two well-differentiated subsets: the "Olympic" and "Sub-Chehalis" groups in the Olympic Mountains and south of the Chehalis River, Washington, respectively. Good et al. also showed that even within these groups, levels of genetic differentiation among populations are among the highest seen in any salamander (Larson, 1984). F_{ST} values (Wright, 1965; Nei, 1973) among populations within the four groups range from 0.588 to 0.689.

The Cascade group is isolated from the North Coastal and South Coastal groups by the Willamette Valley, an alluvial plain with low relief that is unsuitable for *Rhyacotriton* (see Chapter 4). This contradicts the assertion by Nussbaum (1976) that the Willamette Valley is not an important barrier to gene flow in amphibians and reptiles. Similarly, the Olympic and Sub-Chehalis groups may be isolated from each other by an area of relatively flat terrain between the Olympic Mountains and the Chehalis River. The only obvious contact between any two of the major groups identified by protein variants is

Table 1. Summary of Nei (1978) genetic distances among the 29 populations of *Rhyacotriton* illustrated in Figure 1, from the analysis of Good et al. (1987).

Population	Olympic	Sub-Chehalis	South Coastal	Cascade
Olympic	0.003-0.137	0.235-0.455	0.638-1.079	0.795-1.167
Sub-Chehalis		0.000-0.183	0.508-0.975	0.566-0.832
South Coastal			0.014-0.461	0.435-0.966
Cascade				0.000-0.268

therefore in northwestern Oregon, between the North Coastal (Sub-Chehalis) and South Coastal groups (Figure 1). There are nine fixed differences ($D_N=0.546$) between these groups in this area.

In this chapter, we analyze protein variation in this contact zone and consider its implications for the intrageneric systematics of *Rhyacotriton*.

MATERIALS AND METHODS

Tissue samples for investigation of the contact zone in *Rhyacotriton* were collected from the 54 populations listed in Appendix 1 and mapped in Figure 2. The term "population" in this study corresponds to single seepages or less than 10 m lengths of single streams. Preserved voucher specimens are housed in the Museum of Vertebrate Zoology, University of California, Berkeley (MVZ).

Tissues used for electrophoresis include liver and intestine dissected from freshly killed larval and adult specimens collected in the field. 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 and Hopkinson, 1978). Most populations contained both larval and adult specimens; no ontogenetic differences in allele frequencies were observed, so all age classes were combined for this analysis. Fixed differences for 9 of the 29 proteins studied (following Good et al., 1987) segregate the northernmost population of the South Coastal group and the southernmost population of the North Coastal group. These 9 proteins were scored using the buffer systems listed in Table 2.

Genetic distance measures were calculated using the methods of Nei (1978) and Rogers (1972).

ANALYSIS OF THE NORTHWESTERN OREGON CONTACT ZONE

The southernmost North Coastal (Sub-Chehalis subunit) population (in Tillamook County, Oregon) and the northernmost South Coastal population (in Benton County, Oregon) discussed by Good et al. (1987) are separated by approximately 75 km (Figure 2). No obvious barriers to the dispersal of these salamanders occur in the area; no large rivers cut completely through the Coast Range as do the Eel, Klamath, Rogue, and Umpqua Rivers to the south and the Columbia and Chehalis rivers to the north, and the terrain has sufficient relief to be generally suitable for *Rhyacotriton* (see Chapter 4). The

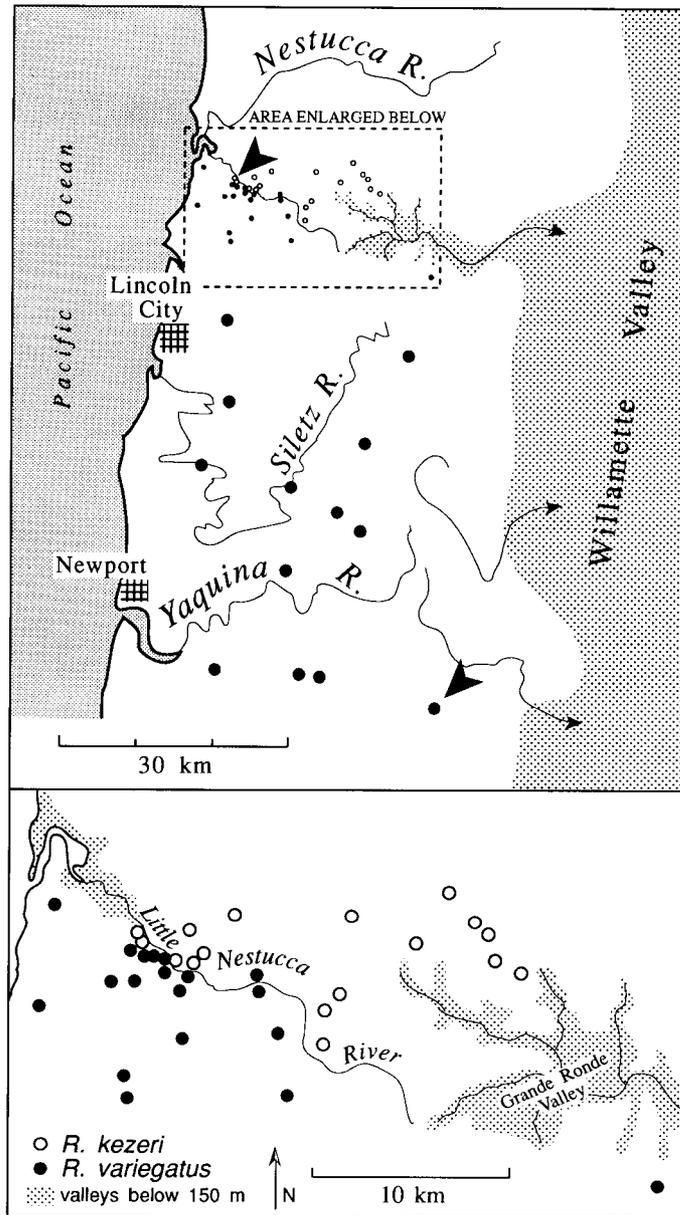


Figure 2. Upper map: The distribution of populations of *Rhyacotriton* analyzed for the 9 fixed protein differences between the Sub-Chehalis (open circles) and South Coastal (closed circles) population groups. The arrows indicate the northernmost South Coastal and southernmost Sub-Chehalis populations included in the analysis of Good et al. (1987). Lower map: Zone of contact between the Sub-Chehalis (open circles) and South Coastal (closed circles) genotypes. Stipling indicates areas of low relief that are unsuitable for *Rhyacotriton*.

Table 2. Buffer systems and enzymes scored in the analysis of the contact zone between North Coastal and South Coastal *Rhyacotriton*.^a

Enzyme	Enzyme Commission Number	Locus	Buffer System
Acid phosphatase	3.1.3.2	Acp	A
Creatine kinase	2.7.3.2	Ck	B
Dipeptidase	3.4.13.11	LA	C
Esterase (2 loci)	3.1.1.1	Est-2, 3	B
Isocitrate dehydrogenase (2 loci)	1.1.1.42	Icdh-1, 2	A
L-lactate dehydrogenase	1.1.1.27	Ldh-1	A
Superoxide dismutase	1.15.1.1	Sod	C

^aBuffer systems as in Selander et al. (1971). Abbreviations: A=Tris-citrate III (pH 7.0), B=Poulik (pH 8.7), C=LiOH (pH 8.2).

only relatively flat terrain in the region is found in the Grande Ronde Valley, which extends into the eastern edge of the Coast Range to the west of Salem, Oregon. In general, the populations north of the Grande Ronde Valley and the Little Nestucca River in Tillamook and Yamhill counties are of the North Coastal type, while populations found to the south are of the South Coastal type.

The distribution of populations near the Little Nestucca River is also illustrated in Figure 2. Sample sizes ranged from 1 to 58 (see Appendix 1). Although populations of the two alternative protein types approach to within less than 100 m of each other, we found no evidence of any genetic influence of one type on the other, despite sample sizes as high as 58 for these populations, and despite the fact that *Rhyacotriton* is known to travel, at least rarely, over distances of at least 500 m from appropriate stream habitats (Nussbaum et al., 1983; Mierzwa, 1988; personal observation). All individuals in all populations studied exhibited either a pure North Coastal or a pure South Coastal protein profile. No population contained individuals with both protein patterns, and no individual showed any sign of introgression of alleles from the alternative population type.

This pattern of protein variants in *Rhyacotriton* in the area of contact can be explained by some combination of the following: (1) a severe selection gradient is present in this region for the loci sampled; (2) some geographic or ecological feature is keeping the two forms separated; (3) the populations only recently have come into proximity, and will "momentarily" come into contact; or (4) a zone of parapatry has been present for some finite length of time, and no hybridization occurs. These alternatives will be considered below.

Although it is possible that selection is responsible for the patterns observed, any selection gradient would have to be restricted to the less than 100 m between the populations of alternative protein type. It seems reasonable to assume that any factors yielding a gradient of the magnitude necessary to cause such a sharp "cline" in allele frequency at so many loci (some of which at least must be relatively independent) would

affect the distribution patterns of other species as well. Since the vegetation appears to be uniform across the *Rhyacotriton* contact zone, and since there are no obvious breaks in the distributions of other animal species, this possibility seems remote.

On the eastern edge of the Coast Range, the inhospitable Grande Ronde Valley almost certainly maintains the isolation of the two protein classes. Farther to the west, the path of the Little Nestucca River correlates well with the zone of proximity, and it might be hypothesized that this river functions as a physical barrier isolating the two groups. *Rhyacotriton* is restricted to relatively small watercourses and is unknown in larger streams (see Chapter 4), so these relatively large streams may be effective barriers to dispersal. However, the Little Nestucca River is much smaller than many other rivers in the area: the Nestucca River is less than 3 km to the north (at its closest point) and has 4.6 times the drainage area of the Little Nestucca; similarly, the Siletz River comes to within 9 km to the south and has 6.2 times the drainage area. Why the relatively minor Little Nestucca River should have any influence on the distribution of *Rhyacotriton*, when these much larger rivers apparently do not, is unclear. A further complication is the occurrence of one population of the South Coastal type on the north side of the river, and two populations of the North Coastal type on the south side (see Figure 2); apparently *Rhyacotriton* is capable of crossing the stream, yet the populations remain "pure." There are no obvious differences in vegetation or stream microhabitat on the two sides of the Little Nestucca River. A geographic or ecological feature causing isolation therefore seems unlikely.

The possibility that the North Coastal and South Coastal types have only recently come into close proximity and will soon come into contact also seems unlikely. The chances of our catching this process at exactly that stage are slight, especially in view of the stability of the vegetation (and presumably climatic conditions) in the area; the vegetation has retained its present form at least since the early Pleistocene, 1.5 million years ago (Waring and Franklin, 1979).

It is also possible that the patterns seen result in part from our chance failure to find individuals of the alternative protein type that were in fact present at relatively low frequency. However, the probability that alleles from the alternative protein type were missed through sampling error is low. *Rhyacotriton* is known to travel over distances of at least 500 m (see above), and they probably occasionally move even farther. A total of 248 of the specimens used in this analysis were collected within 1 km of the Little Nestucca River, and 141 of these were collected within 100 m. Yet no "inappropriate" alleles were found. There is a 0.95 probability that individuals with one or more such alleles would appear in this sample of 248 if they were present in the sampled population at a frequency as low as 1.2% (3.0% for the 141 specimens within 100 m of the river). If sympatry occurs, it is very rare. If this rare sympatry (assuming it occurs) has ever resulted in hybridization, any progeny resulting from it, either F_1 or backcross, also are rare. No evidence was seen in this analysis for either sympatry or hybridization.

If it is accepted that no sympatry or hybridization occurs, and that the contact zone has been in existence for a finite length of time, an explanation for the observed parapatry is required. It is possible that the salamanders are so nonvagile or so philopatric that individuals from one population essentially never emigrate to another. This seems unlikely, in view of the demonstrated ability of *Rhyacotriton* to move substantial distances. We therefore assume that *Rhyacotriton* at least occasionally moves across the

narrow contact zone, so in the absence of any detected gene flow we conclude that members of the alternative types either do not mate or that they produce inviable offspring. Further, the fact that the two types were never found in sympatry in the present analysis suggests some sort of active exclusion of one type by the other. There may be a pre-emptive occupancy of space by the relatively dense populations of the alternative types.

Contact zones between biochemically divergent but morphologically more or less cryptic groups in salamanders have been studied in the plethodontid genera *Batrachoseps* (Yanev, 1978), *Bolitoglossa* (Wake et al., 1980), *Desmognathus* (Karlin and Guttman, 1981), *Ensatina* (Wake et al., 1986, 1989), *Eurycea* (Guttman and Karlin, 1986), *Plethodon* (Duncan and Highton, 1979; Highton, 1985; Highton and Henry, 1970; Highton et al., 1989; Peabody, 1978; Wynn, 1986), and *Thorius* (Hanken, 1983); the ambystomatid genus *Ambystoma* (Pierce and Mitton, 1980); the dicamptodontid genus *Dicamptodon* (Good, 1989); and the salamandrid genus *Triturus* (Arntzen, 1986). In most of these cases, some degree of hybridization occurs. In some of these and in many other cases, morphologically similar forms have turned out to be genetically isolated when found in sympatry (see Larson, 1984, and Highton et al., 1989, for reviews). The close parapatry observed in our investigation of *Rhyacotriton* is unique among studies that have included electrophoretic analyses, although Highton (1972) suggested without biochemical evidence that *Plethodon hoffmani* and *P. richmondi* occupy exclusive ranges that are separated only by the New River in western Virginia and southern West Virginia.

THE SPECIES OF *RHYACOTRITON*

Because species concepts are controversial and vary greatly among systematists (see, e.g., Frost and Hillis, 1990, and Highton, 1990), we will specify our concept and its application. In theory, we see species as genetically cohesive units that are evolutionarily independent of each other. In naming species, however, we are forced to take a somewhat more operational approach. Although these independent sets of populations are the basic units of evolution, nomenclatural anarchy would result if each allopatric (and hence by definition evolutionarily independent at the point in time of our study) group of populations were given a species name. We therefore restrict the use of specific names to those genetically cohesive units for which there is evidence for genetic independence on some other grounds than simply geographic isolation. Such evidence might be through a test of sympatry, or through the presence of obviously incompatible reproductive characteristics in allopatric populations. It also might be interpreted from patterns of genetic differentiation over geography, as discussed in the following paragraphs.

Although the North Coastal and South Coastal genotypes of *Rhyacotriton* contact each other, there is no evidence that they hybridize. Accordingly, they are distinct species by the above (or indeed virtually any) criteria. However, tests of sympatry are not possible among the other allozyme groups of *Rhyacotriton* discussed by Good et al. (1987), and their possible species status is not as straightforward.

Figure 3 is a plot of Nei (1978) genetic distance against geographic distance for all pairs of the 29 populations of *Rhyacotriton* analyzed by Good et al. The regression in such a plot for a set of points representing pairs of populations within a genetically

Table 3. Allele frequencies in geographically intermediate populations for the two loci showing fixed differences between the major subsets of *Rhyacotriton variegatus*, as observed by Good et al. (1987).^a

Population	n	Aat-2			Me	
		a	g	h	f	h
1. Elk River	10	1.00	0	0	1.00	0
2. Rogue River	10	1.00	0	0	1.00	0
3. Pistol River	8	0.69	0	0.31	0.63	0.37
4. Chetco River	10	0.60	0	0.40	1.00	0
5. Winchuck River	10	0.35	0	0.65	0.30	0.70
6. Gasquet	3	0	0	1.00	1.00	0
7. Smith River	10	0	0.25	0.75	0	1.00

^aPopulations 1 and 7 are equivalent to populations N5 and N7 in Good et al. Allele designations are also as in Good et al. Lists of voucher specimens can be found in Appendix 2.

cohesive unit should pass through the origin, because there is increased genetic influence of one population on another with greater proximity (resulting from gene flow). The regression for a set of populations that are genetically isolated will not pass through the origin (unless gene flow has only recently ceased), because no population has any genetic influence on any other, regardless of geographic proximity. We assume that an "isolation by distance" model is the most appropriate for an analysis of this kind.

For *Rhyacotriton*, the regressions for comparisons within each of the four allozyme groups identified by Good et al. pass essentially through the origin (not statistically different from it at $p < 0.05$; see regression statistics in legend to Figure 3), so that there is no evidence for a lack of genetic cohesion within each of these groups, despite the high values of F_{ST} recorded by Good et al. The steepness of the slope is a reflection of the scale on which gene flow occurs; the steeper the slope, the smaller the geographic scale of interaction, assuming equilibrium conditions of gene flow and population differentiation. In contrast to the patterns seen within groups, none of the regressions for between-group comparisons pass through the origin (all statistically different from zero at $p < 0.05$). This pattern is a strong indication that the groups are genetically isolated from each other, and have been for some time. A standard measure of genetic divergence between sets of populations is the mean genetic distance for all between-group pairs of populations. However, we believe that a more appropriate measure for *Rhyacotriton* is the y-intercept in Figure 3B, the level of genetic differentiation corresponding to zero geographic distance. The positive slope of the between-group comparisons in *Rhyacotriton* is the result of geographic differentiation that was present in the widespread ancestor before the vicariant divergence of the modern population groups. The differentiation that has occurred since that divergence is represented only by the intercept of the between-group comparison line. This model will be discussed at length elsewhere (Good, in prep.). For the four groups of *Rhyacotriton*, these genetic distances range from approximately 0.25 to 0.90, corresponding to divergence times of 3.2-4.7 and 11.5-17.0 m.y. using the electrophoretic clock calibrated by Good et al.

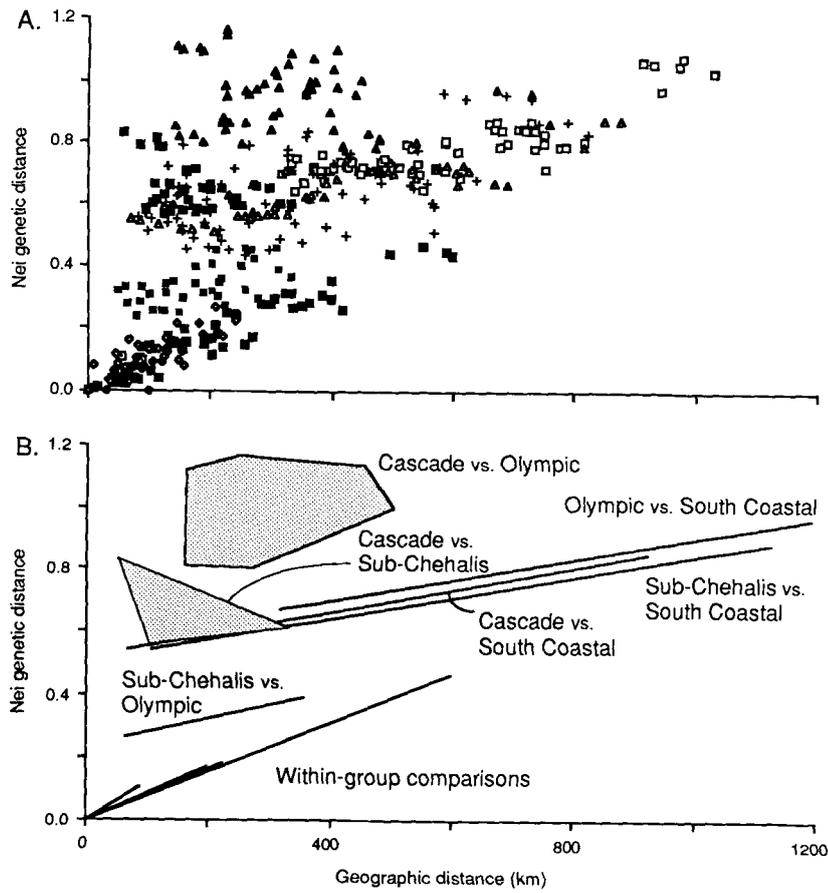


Figure 3. The relationship of Nei (1978) genetic distance to geographic distance in *Rhyacotriton*. Figure 3A illustrates all of the pairwise comparisons of populations within and among the four species in the genus; Figure 3B illustrates the regression lines and polygons summarizing the distribution of those points. Summary statistics for the regression lines in Figure 3B are:

	Slope	Intercept	r^2	df
within Olympic	0.001	-0.019	0.710	4
within Sub-Chehalis	0.001	0.002	0.908	4
within South Coastal	0.001	0.01	0.902	8
within Cascade	0.001	0.04	0.456	5
Olympic vs. Sub-Chehalis	0.00046	0.25	0.317	34
Olympic vs. South Coastal	0.00047	0.51	0.711	58
Sub-Chehalis vs. South Coastal	0.00046	0.48	0.653	58
South Coastal vs. Cascade	0.00042	0.50	0.357	68

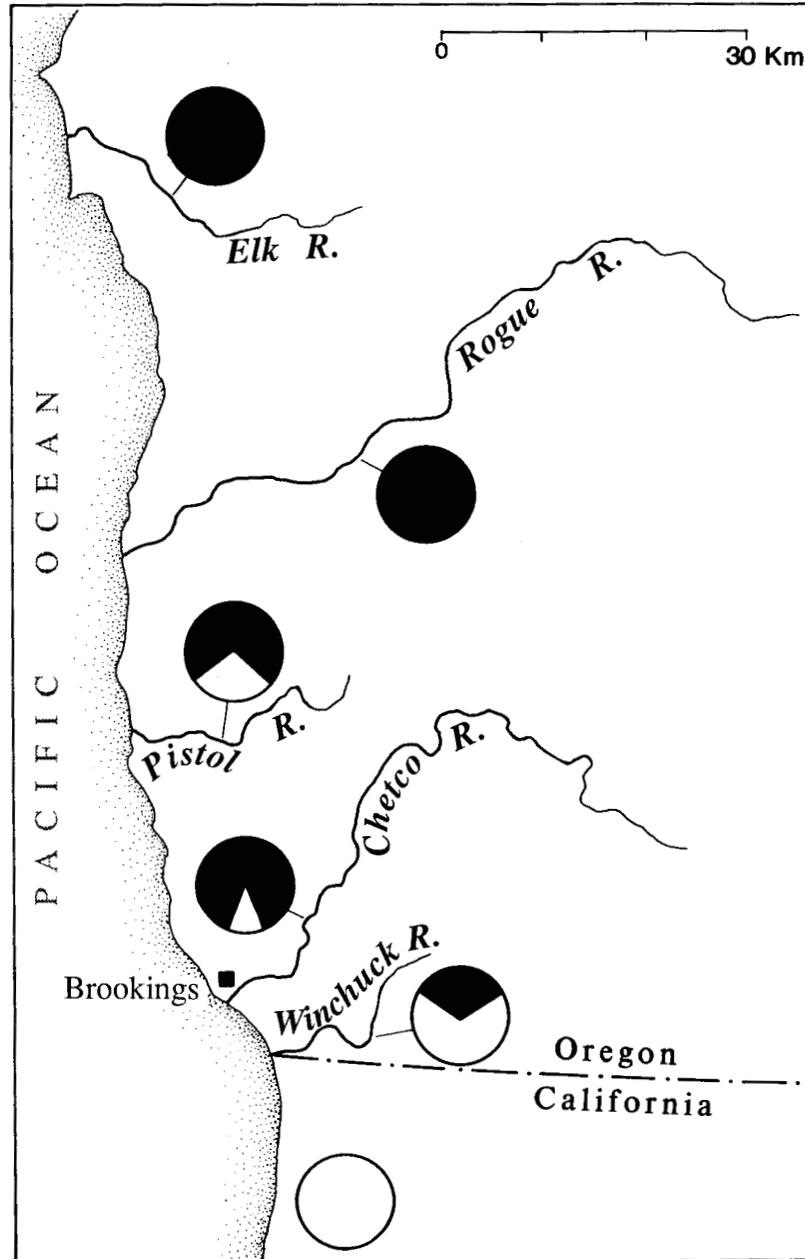


Figure 4. Allele composition in populations distributed between the two major subdivisions of *Rhyacotriton variegatus* suggested by the UPGMA analysis of Good et al. (1987). Black shading indicates the frequency of northern alleles at the two loci showing fixed differences between these groups; white shading indicates southern alleles. The northernmost population in the figure is the Elk River Road population (S5) of Good et al., and the southernmost is the South Fork Road population (S7).

Rhyacotriton includes four groups of populations within which there appears to be genetic cohesion (or at least there was until very recently), and between which there is clearly no genetic continuity (Figure 3). In each of the between-group comparisons, we infer that genetic contact was interrupted a long time ago (at least 3 m.y. for the most similar pair of groups, if the electrophoretic clock is accepted). Accordingly, we think that the most appropriate action is to recognize all four population groups as distinct species. Names exist for two of the units: for the Olympic group, *R. olympicus*, and for the South Coastal group, *R. variegatus* (Stebbins and Lowe, 1951). The remaining two groups are herein described as new species, *R. cascadae* (the Cascade group) and *R. kezeri* (the Sub-Chehalis group) (see Chapter 3). Furthermore, we show that all four of these species are subtly distinct in morphology as well as being strongly divergent in biochemical traits.

The possibility that one of these species is further divisible into even smaller genetically independent units was also investigated. In Figure 3, each of the within-species regressions passes through the origin, so no evidence is provided for further subdivision. However, the variances about the regressions in the figure are such that the recent loss of genetic cohesion between two subsets in one of these "species" would not be detectable. To test this possibility, five additional populations (Appendix 2) were sampled between the two sets of populations that showed the greatest level of divergence in the analysis of Good et al., other than those already identified as species in this paper. These two sets are the Oregon and California samples of *R. variegatus* (Figure 1). The five populations were analyzed for the two loci showing fixed differences between these subsets (Aat-2 and Me), and both loci showed intergradation between the groups (Table 3 and Figure 4). No evidence of genetic independence was observed, despite the relatively high genetic distances recorded among populations of *R. variegatus* (up to $D_N=0.461$; Good et al., 1987). Once again, this provides a vivid demonstration of the small scale on which genetic differentiation has taken place in this genus.

SYSTEMATIC ACCOUNTS

We recognize four species of *Rhyacotriton*. In this chapter we provide diagnoses for all of them, and propose two new names. A third newly recognized species takes the name of one of the two currently recognized subspecies. We also propose a new family, Rhyacotritonidae, to include these four species. Biochemical evidence for the specific status of these species is presented in the preceding chapter, while morphological characters of the species and family are presented in the following chapters. The names are proposed here to facilitate discussion in the following sections.

Family Rhyacotritonidae, fam. nov.

Rhyacotritoninae Tihen, 1958, Bull. Florida State Mus. 3: 25.

Type genus. *Rhyacotriton* Dunn.

Content. The single genus *Rhyacotriton*.

Etymology. As for the genus (below).

Diagnosis. Rhyacotritonids are distinguished from all other families of salamanders by the presence of unique, characteristically expanded and "squared-off" glands lateral and posterior to the vent in adult males (see Sever, 1988, for discussion, and Stebbins, 1985, for illustration). Rhyacotritonids are further distinguished from all other salamanders (except some *Ambystoma*) by the presence of an epihyal, and from all except a few derived hynobiids by lacking an operculum and *opercularis* muscle. Adults are distinguished from cryptobranchids, sirenids, amphiumids, and proteiids by the absence of a suite of paedomorphic characters seen in those taxa (gills, lidless eyes, various skin characters, dentition arrangement, etc.). Among transforming salamanders, some populations of *Rhyacotriton* differ from all other species in the absence of nasal bones (see Chapter 6), and no species in the genus have anything other than very small, erratically formed nasals that are bilaterally asymmetrical. Rhyacotritonids are distinguishable from all other salamanders except dicamptodontids in having the caudal

spinal nerves penetrating the vertebrae through foramina, but most presacral spinal nerves pass between the vertebrae. They are distinguishable from all other transforming salamanders except plethodontids and a few derived hynobiids and salamandrids by their greatly reduced lungs and various characters correlated with lung loss (e.g., no larynx and no ypsiloid cartilage attached to the pubes). They differ from the Hynobiidae and Cryptobranchidae in sharing a series of synapomorphies with dicamptodontids, ambystomatids, salamandrids, and plethodontids (the presence of elongate frontal processes of the premaxillae that lie between the nares, bicapitate ribs, separate *pubotibialis* and *puboischiotibialis* muscles in the thigh, internal fertilization, and the absence of distinct microchromosomes and an angular bone). Rhyacotritonids differ from hynobiids and dicamptodontids by lacking a hypohyal in adults. The presence of a separate lacrimal bone in adults of the Rhyacotritonidae further distinguishes them from all other families except the Dicamptodontidae and Hynobiidae, and the presence of a *subhyoideus* distinguishes them from ambystomatids, plethodontids, and dicamptodontids. In addition to the characters listed above, Rhyacotritonids are distinguishable from single families by the following characters: from the Plethodontidae by any of the great array of characters unique to plethodontids (nasolabial groove, fused premaxillae at the outset of ossification, pterygoids lacking, various ear characters, etc.); from the Salamandridae by having more chromosomes (26 rather than 22-24), and posterior rather than medial replacement of vomerine teeth; and from the Dicamptodontidae by smaller size, absence of an otoglossal plate in the hyobranchial apparatus, no coronoid bones in either larvae or adults, and no mineralized hyobranchial or mesopodial elements.

Comments. The generally accepted familial placement of *Rhyacotriton* is in the Dicamptodontidae, which also includes the genus *Dicamptodon* (Frost, 1985; Stebbins, 1985; Duellman and Trueb, 1985). This classification was proposed by Edwards (1976), who recognized that there was no morphological evidence in the form of shared derived characters for the inclusion of *Rhyacotriton* and *Dicamptodon* in the family Ambystomatidae, the classification generally accepted before his analysis. Edwards found that *Rhyacotriton* and *Dicamptodon* are unique in having all of the postsacral and the anteriormost (atlas) presacral vertebral nerves exiting through foramina in the vertebrae, but all other presacral nerves exiting intervertebrally. Other salamanders either have most of the presacral nerves exiting through foramina or have intervertebral postsacral nerves. Edwards used the similarity of *Rhyacotriton* and *Dicamptodon* in this character as the basis for recognition of the family Dicamptodontidae. However, the condition of the nerve foramina seen in *Rhyacotriton* and *Dicamptodon* is probably not a synapomorphy, but rather an intermediate stage in the transformation series from having all spinal nerves intervertebral (cryptobranchids, hynobiids, and proteiids) to having the posterior postsacral nerves passing through foramina (amphiumids), to having all postsacral nerves passing through foramina (*Dicamptodon* and *Rhyacotriton*), to having all but the first two or three trunk nerves passing through foramina (ambystomatids, salamandrids, sirenids, and plethodontids). Hence *Rhyacotriton* and *Dicamptodon* share no derived states in this character not also seen in ambystomatids, salamandrids, sirenids, and plethodontids. In addition, analysis of variation in ribosomal RNA sequences among salamander families strongly suggests that *Rhyacotriton* and *Dicamptodon* are phylogenetically independent, and that a single family containing both genera is

polyphyletic (Larson, 1991). Accordingly, taking all available evidence into account, we separate *Rhyacotriton* and *Dicamptodon* into the two families Rhyacotritonidae and Dicamptodontidae.

Rhyacotriton Dunn
"Torrent Salamanders"

Rhyacotriton Dunn, 1920, Proc. New England Zool. Club 7: 56.

Type species. *Rhyacotriton olympicus* (Gäike).

Etymology. *Rhyacotriton* is from the Greek *rhyaco*, meaning "stream" or "torrent," and *Triton*, the name of a Greek sea god commonly used with reference to salamanders.

Diagnosis. As for the family (above).

Comments. The common name "Olympic salamander" was appropriate for this genus when it was thought to consist of a single species originally discovered in the Olympic Mountains of Washington. However, the name is appropriate only for one of the four species we recognize (*R. olympicus* sensu stricto). We therefore propose the common name "torrent salamander," in reference to the occurrence of these animals along cascading streams and around waterfalls.

Rhyacotriton cascadae, sp. nov.
"Cascade Torrent Salamander"

Holotype. Museum of Vertebrate Zoology (MVZ) 90795, an adult female from the base of Wahkeena Falls, Multnomah County, Oregon, collected by D. B. Wake, T. H. Wake, and J. Everett on December 22, 1965.

Paratypes. MVZ 90793, 90794, 90798, 90799, 90815, adult females; MVZ 90796, 90813, 90819, 90824, 90825, adult males; and MVZ 90808, 90812, larvae. Same data as holotype.

Etymology. The specific epithet *cascadae* refers to the distribution of this species in the Cascade Mountains of Oregon and Washington.

Diagnosis. A highly variable *Rhyacotriton* with no unique diagnostic characteristics of color pattern, although most *R. cascadae* can be distinguished from other species in the genus as follows: from *R. olympicus* and *R. kezeri* by heavy spotting and blotching of the dorsal surfaces, especially along the sides. These spots also are usually denser than those seen in *R. variegatus*, although there is considerable variation in both species. The heavy spotting on the sides in *R. cascadae* usually makes the line of demarcation between the dorsal and ventral ground color more distinct than in either *R. kezeri* or *R. variegatus*, regardless of the degree of spotting in the latter species. This line of demarcation is also distinct in *R. olympicus* (which lacks dorsal spots), but it is wavy in *R. olympicus* and relatively straight in *R. cascadae*. A dark preorbital stripe found in heavily spotted *R. variegatus* is usually weak or absent in *R. cascadae*. Variation in ventral coloration is greater than in any other species of *Rhyacotriton*, and unique patterns are encountered in some populations. Many populations of *R. cascadae* have ventral spotting in the form of fine grayish flecking that is especially abundant on the throat and chest. Others have a

dark band across the ventral surface just posterior to the cloaca. See Chapter 8 for further discussion of color pattern variation.

Description of holotype. The holotype is an adult female measuring 49.5 mm snout-vent length. Other measurements (in mm) are as follows: head width 7.5, snout-gular fold (head) length 9.5, tail length 37.0, forelimb length 11.0, hindlimb length 12.5, foot length 6.5, and axilla-groin (trunk) length 27.0. The dorsal ground color (on the dorsal aspects of the head, trunk, limbs, and tail) is dark brown, heavily supplied with black mottling on all surfaces except the legs, where mottling is lighter. The ventral ground color (seen on all ventral surfaces) in life was bright yellow; in alcohol, it is faded to a yellow-tan. Three distinct large dark spots are present fairly evenly spaced along the midline between the pectoral and pelvic girdles. The throat and chin are prominently speckled with smaller spots. The line of demarcation between the dorsal and ventral ground colors on the flank is straight, distinct, and very sharp because of the presence of the heavy mottling on the dorsal surface. A few guanophores are apparent on the head, but they are very indistinct due to the heavy dark mottling in that area.

Distribution. Restricted to the west slope of the Cascade Mountains from just north of Mount Saint Helens, Skamania County, Washington, south to northeastern Lane County, Oregon (Figure 5).

Rhyacotriton kezeri, sp. nov.
"Columbia Torrent Salamander"

Holotype. Museum of Vertebrate Zoology (MVZ) 197300, an adult female from the junction of Highway 26 and Luukinen Road (at the Nehalem River Bridge), Clatsop County, Oregon, collected by D. A. Good on May 3, 1985.

Paratypes. MVZ 197301-303, 197305, adult females; MVZ 197304, adult male; MVZ 197307, 197310, subadult females; and MVZ 197306, 197308-309, larvae. Same data as holotype.

Etymology. The specific epithet honors Dr. James Kezer of the University of Oregon, who has conducted pioneering work on the cytogenetics of salamanders and has long been a prominent student of the amphibians of the Pacific Northwest. Dr. Kezer's enthusiasm and generosity have encouraged many young biologists to pursue careers in herpetology.

Diagnosis. A *Rhyacotriton* distinguished from *R. variegatus* and *R. cascadae* by the absence of dorsal spotting or blotching. It is also distinguished from *R. olympicus* by the line of demarcation between the dorsal and ventral ground color being less distinct and being straight, without the characteristic wavy pattern seen in the latter species. *R. kezeri* lacks the large, well defined ventral spots seen in *R. olympicus*. *R. kezeri* is difficult to differentiate from the immediately adjacent populations of *R. variegatus*, but is distinguished from them by one or more of the following characteristics: dorsal spots absent (weak but usually present in northern populations of *R. variegatus*, see below), a few small ventral speckles usually present in the gular and chest regions (these are lacking from *R. kezeri* away from the vicinity of the *R. kezeri*-*R. variegatus* contact zone), lighter limb insertions, and less extensive dark pigmentation of the soles of the feet. See Chapter 8 for further discussion of color pattern variation.

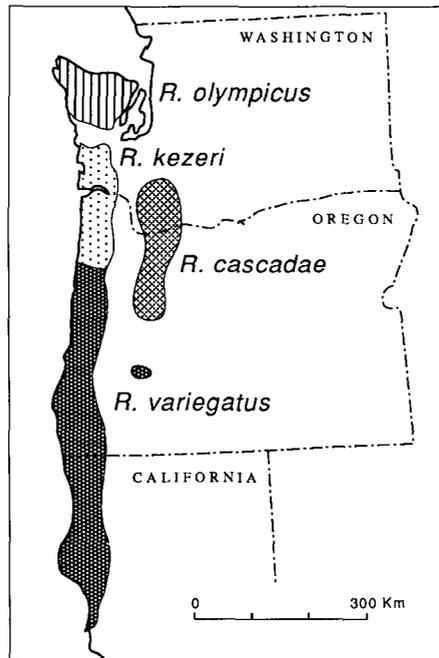


Figure 5. Distribution of the species of *Rhyacotriton*.

Description of holotype. The holotype is an adult female measuring 59 mm snout-vent length. Other measurements (in mm) are as follows: head width 9.0, snout-gular fold (head) length 10.5, tail length 31.0, forelimb length 12.0, hindlimb length 14.5, foot length 8.0, and axilla-groin (trunk) length 32.0. The dorsal ground color (on the dorsal aspects of the head, trunk, limbs, and tail) is dark brown; there are no dark spots. The dorsal ground color extends onto the ventral aspect of the hind feet from the postaxial direction. The ventral ground color was bright yellow in life, but it has faded to a yellow-tan in alcohol. This vivid pigmentation was present on all ventral surfaces and extends onto the dorsal aspect of the anterior half of the hind feet. Ventral dark spotting is restricted to some subdued mottling in the pectoral region and two faint spots at midbody and one just anterior to the pelvis. The line of demarcation between the dorsal and ventral ground colors on the flank is straight and distinct, but not sharp. Guanophores are limited primarily to the snout and temporal regions, with a few scattered along the flank.

Distribution. In the Coast Ranges from the vicinity of the Chehalis River in Grays Harbor County, Washington, south to the zone of contact with *R. variegatus* along the Little Nestucca River and the Grande Ronde Valley in Polk, Tillamook, and Yamhill counties, Oregon (Figure 5).

Rhyacotriton olympicus (Gaige)
"Olympic Torrent Salamander"

Ranodon olympicus Gaige, 1917, Occ. Pap. Mus. Zool. Univ. Michigan 40: 2.

Rhyacotriton olympicus Dunn, 1920, Proc. New England Zool. Club 7: 56.

Rhyacotriton olympicus olympicus Stebbins and Lowe, 1951, Univ. Calif. Publ. Zool. 50: 465.

Holotype. Museum of Zoology, University of Michigan (UMMZ) 48607, an adult female from Lake Cushman, Mason County, Washington, collected by P. Putnam on April 20, 1916.

Paratypes. MCZ 4103 (formerly UMMZ 48608), UMMZ 49945-48, larvae; UMMZ 49949, adult male. Same data as holotype, except that UMMZ 49946-47 were collected on April 30, 1916.

Etymology. The specific epithet is from the Latin *olympicus*, with reference to the occurrence of the species in the Olympic Mountains of Washington.

Diagnosis. A *Rhyacotriton* distinguished from all other species by the presence in adults and larger larvae of a distinct wavy line of demarcation between the dorsal and ventral ground color and large, well-defined dark spots scattered over the ventral surface. *R. olympicus* is further distinguished from *R. variegatus* and *R. cascadae* by the absence of dorsal spotting or blotching. See Chapter 8 for further discussion of color pattern variation.

Description of holotype. See Gaige (1917).

Distribution. Restricted to the Olympic Peninsula in Clallam, Grays Harbor, Jefferson, and Mason counties, Washington (Figure 5).

Rhyacotriton variegatus Stebbins and Lowe
"Southern Torrent Salamander"

Rhyacotriton olympicus variegatus Stebbins and Lowe, 1951, Univ. Calif. Publ. Zool. 50: 471.

Holotype. Museum of Vertebrate Zoology (MVZ) 45868, an adult female from 1.3 mi west of Burnt Ranch Post Office, Trinity County, California, collected by R. C. Stebbins on November 16, 1947.

Etymology. The specific epithet is from the Latin *variegatus*, meaning "variegated," with reference to the heavily spotted and mottled pattern of many populations, exemplified by the type specimen.

Diagnosis. A *Rhyacotriton* distinguished from *R. kezeri* and *R. olympicus* by the presence of dorsal dark spotting. This spotting is weak in northern populations in geographic proximity to *R. kezeri*. Further distinguished from *R. olympicus* by lacking the wavy line of demarcation between the dorsal and ventral ground colors and the large, well-defined ventral spots in that species. *Rhyacotriton variegatus* varies geographically in the extent of dorsal and ventral spotting, with southern populations being heavily spotted on both surfaces and northern populations lacking ventral spots and being only

weakly speckled above. Heavily spotted populations resemble many populations of *R. cascadae*, but in the latter the concentrations of dorsal and ventral spotting usually are not as equally balanced as in *R. variegatus*. Furthermore, ventral spotting in *R. cascadae* is often lacking or consists only of a few very fine speckles. In addition, heavy spotting along the sides of most *R. cascadae* produces a much more sharply defined line of demarcation between the dorsal and ventral surfaces than is true in *R. variegatus*. Heavily spotted *R. variegatus* usually have a strong preorbital stripe anterior to the eye; this stripe is weak or absent in most *R. cascadae*. Northern, weakly spotted populations of *R. variegatus* are very similar to geographically contiguous populations of *R. kezeri*. The distinguishing characteristics are discussed in the diagnosis of *R. kezeri* above. See Chapter 8 for further discussion of color pattern variation.

Description of holotype. See Stebbins and Lowe (1951).

Distribution. *R. variegatus* ranges from southern Mendocino County, California, north through the Coast Ranges to the Little Nestucca River and the Grande Ronde Valley in Polk, Tillamook, and Yamhill counties, Oregon, where its range abuts that of *R. kezeri*. An apparently isolated population also exists on the west slope of the Cascade Mountains in the vicinity of Steamboat, Douglas County, Oregon (south of the range of *R. cascadae*). It has both color and allozyme patterns similar to the *R. variegatus* in Curry and Josephine counties, Oregon (biochemical voucher specimens from a tributary of the north fork of the east fork of Rock Creek, North Umpqua River drainage, 9.3 miles northeast of Oregon Highway 138 on County Road 78 and BLM roads, Douglas County, Oregon = MVZ 214935-940) (Figure 5).

THE HABITAT OF *RHYACOTRITON*

Rhyacotriton is a characteristic inhabitant of the humid coniferous forests of the Pacific Northwest, ranging from sea level to about 1200 m elevation (Nussbaum et al., 1983). Within this habitat, both larvae and adults occur in well shaded areas in seepages and along the margins of small cascading streams where the water is clear, well aerated, and cold (5-12°C: Brattstrom, 1963; Stebbins, 1951; Stebbins and Lowe, 1951; Nussbaum et al., 1983). Larvae are abundant in gravel through which water is percolating. Although there is considerable overlap in microhabitat, adults tend to prefer larger rocks; they are particularly abundant in the splash zones of waterfalls and under loose sheets of rock on rock faces down which a film of water is running. Stebbins and Lowe (1951) suggested that adult *Rhyacotriton* prefer fairly small rocks ("five to six inches"), but the senior author has found them to be abundant under very large sheets of rock in this latter microhabitat. Neither adults nor larvae are often found submerged in more than a few millimeters of water unless they have been disturbed; in such cases they often use deep, rapidly flowing water as a refuge (but see Myers, 1943). Adults are occasionally found at some distance from stream or spring habitats, especially after heavy rains (Nussbaum et al., 1983). The junior author has encountered adults more than 50 m from water in Douglas fir forests in northern California. Published habitat descriptions include Noble and Richards (1932), Fitch (1936), Wood (1939), Myers (1943), Stebbins (1951, 1954, 1955, 1985), Stebbins and Lowe (1951), Anderson (1968), Nussbaum (1969), Bury (1970), Nussbaum and Tait (1977), Nussbaum et al. (1983), Bury and Corn (1988b), and Welsh (1990).

Because *Rhyacotriton* prefers rapidly flowing, well aerated streams and springs, it is restricted to the vicinity of areas with considerable physical relief. These salamanders are absent from flat areas and areas with only low, gently sloping hills where such streams are lacking. In those cases in which specimens are found on flat ground there is invariably steeper terrain nearby. In addition, they are closely tied to old-growth forest. Their density in younger forests is only a fraction of that in old-growth (Bury, 1983; Bury and Corn, 1988a, b; Corn and Bury, 1989; Welsh, 1990; personal observation). Brattstrom (1963) showed that *Rhyacotriton* requires lower temperatures than most other salamanders, and Ray (1958) found them to be exceptionally poor at resisting desiccation. Probably because

of increased temperatures and decreased humidity, they are virtually absent from recently logged areas, and almost all of the few specimens seen in such areas are adults. Like all salamanders, *Rhyacotriton* live for several years, and it seems likely that the animals encountered in disturbed areas are simply surviving in areas which were forested when they were larvae. We have no evidence of breeding in areas denuded of forest.

THE DISTRIBUTION OF *RHYACOTRITON*

Through much of its range (Figure 1), *Rhyacotriton* is one of the commonest members of the salamander fauna. However, probably because of its limited microhabitat, the genus was unknown to science until the early 20th century (Gaige, 1917).

The first population of *Rhyacotriton* discovered was found in the Olympic Mountains near Lake Cushman, Mason County, Washington, in 1916 (Gaige, 1917). For 16 years after its discovery, the genus was thought to be restricted to the Olympic Mountains (e.g., Slevin, 1928), but in 1932 a population was discovered near Spirit Lake, at the base of Mount Saint Helens, Skamania County, Washington (Slater, 1934). This was the first report of what is here called *R. cascadae*. The range of *R. cascadae* was extended into Oregon by a collection at Alder Creek, seven miles above Sandy, Clackamas County, in 1936 (Slater, 1938), and Gordon (1939) and Graf et al. (1939) recorded a population from the Middle Santiam River near Foster, Linn County, nearly at the southern limit of the species. Also in 1936, a population of *Rhyacotriton* was found at Yale, Cowlitz County, Washington (Slater, 1938); this is the first record of what is here called *R. kezeri*. Slater made several other collections of *R. kezeri* in southwestern Washington later in 1936. Fitch (1936) collected the first specimens of what is here called *R. variegatus* in 1935 along the Rogue River, 11 miles upstream from its mouth, in Curry County, Oregon, and between then and 1939, several more collections were made in Benton, Lincoln, Tillamook, and Polk counties, Oregon, near the northern limit of the species (Gordon, 1939; Graf et al., 1939). In 1939 the known range of *R. variegatus* was pushed south by the discovery of the first California population at Wilson Creek, seven miles north of Klamath, Del Norte County (Wood, 1939). The southern limit was extended further south, to Willow Creek, Humboldt County, by Myers (1943), to 10.6 miles west of Dyerville, Humboldt County, by Stebbins and Lowe (1951), and to Dark Gulch, Mendocino County, by Stebbins (1955). Dark Gulch, about 2 kilometers north of Albion, remains the southernmost known locality for *Rhyacotriton*, although rumors persist of its existence as far south as Sonoma County. Bury et al. (1969) extended the known distribution of *R. variegatus* inland by collecting a sample in Josephine County, southern Oregon, in 1969. The disjunct population of *R. variegatus* in the Cascade Mountains near

Steamboat, Douglas County, Oregon, was discovered in 1968 (specimens in the University of Michigan Museum of Zoology), but was unknown to Bury et al.

The factors limiting the distribution of *Rhyacotriton* are largely the habitat requirements discussed in the last chapter. The southern limit of *R. olympicus*, the northern and eastern limits of *R. kezeri*, the eastern limit of *R. variegatus*, and the western limit of *R. cascadae* are determined by the unsuitably flat terrain in the Willamette Valley and in the area just south of the Olympic Mountains. *R. cascadae* is restricted to the western side of the Cascade Mountains: the conditions on the drier and colder eastern slopes are apparently unsuitable for these salamanders. The absence of *R. olympicus* on Vancouver Island, only a few kilometers north of the Olympic Peninsula, is not unique. Among amphibians, the Straits of Juan de Fuca also form a barrier at the northern limit of *Dicamptodon copei*, *Plethodon vandykei*, *Ascaphus truei*, *Rana pretiosa*, and *Rana cascadae*. *Aneides ferreus* is unique in being restricted to the north of the straits (it occurs farther south in Oregon and California, but is absent from Washington).

In contrast to the factors limiting the distribution of *Rhyacotriton* in all other parts of the species' ranges, the southern limit of *R. kezeri* and the northern limit of *R. variegatus* appear to be determined by some form of interspecific exclusion (see Chapter 2).

The factors resulting in the northern and southern limits of the distribution of *R. cascadae* and the southern limit of *R. variegatus* are not apparent. *R. cascadae* ranges north only to approximately the Cowlitz River Valley, just north of Mount Saint Helens in southern Washington, although apparently suitable habitat is present in the Cascades to the north. Similarly, *R. cascadae* ranges south in the Cascades only to central Oregon (northern Lane County), although apparently suitable habitat occurs farther south (as demonstrated by the Steamboat population of *R. variegatus*). The northern limit of *R. cascadae* corresponds approximately to the northern limit of *Dicamptodon copei*, but no other amphibian shows similar distribution limits, although *Plethodon larselli* is restricted to the Columbia River Gorge not far to the south.

The southern limit of *R. variegatus* in Mendocino County, California, is also difficult to explain, because apparently suitable habitat is present to the south. Coast redwood (*Sequoia sempervirens*), Bishop pine (*Pinus muricata*), and Douglas-fir (*Pseudotsuga menziesii*), which dominate the forests in which *Rhyacotriton* is common to the north, extend southward for approximately 100 km, and it is unclear why *Rhyacotriton* does not inhabit this area. However, as pointed out by Good (1989), the southern limit of the distribution of *R. variegatus* corresponds approximately to the southern limit for *Ambystoma gracile*, *Aneides ferreus*, and *Ascaphus truei*, as well as to zones of contact between *Dicamptodon ensatus* and *D. tenebrosus* (Good, 1989), *Ensatina eschscholtzii oregonensis* and *E. e. xanthoptica* (D. B. Wake, unpublished data), *Rana aurora aurora* and *R. a. draytoni* (Hayes and Miyamoto, 1984), *Zonatrachia leucophrys nutalli* and *Z. l. pugetensis* (Corbin, 1981), and two electrophoretic and morphological morphs of *Pinus muricata* (Millar, 1983).

OSTEOLOGICAL VARIATION IN *RHYACOTRITON*

Because of the prevailing impression prior to publication of Good et al. (1987) that *Rhyacotriton* consisted of a single geographically uniform species (despite the work of Stebbins and Lowe, 1951), workers analyzing osteological or dentitional characters in the genus (Dunn, 1920, 1922; Eaton, 1933, 1934; de Villiers, 1938; Tihen, 1958; Cloete, 1961; Srinivasachar, 1962; Larsen, 1963; Regal, 1966; Worthington and Wake, 1971; Krogh and Tanner, 1972; Edwards, 1976; Kraus, 1987, 1988; Beneski and Larsen, 1989) usually found it unnecessary to provide exact locality data for the specimens they examined or, when such information was provided, the specimens were limited to single populations, usually from the Olympic Mountains of Washington. The specimens examined by Worthington and Wake were from the Columbia River Gorge in the Cascade Mountains. The only report of geographic variation in osteological characters was that of Wake (1980), who studied nasal bone variation in three populations of *Rhyacotriton*. By the classification in the present paper, these are assigned to the three species *R. olympicus*, *R. cascadae*, and *R. variegatus*. In this chapter we present detailed information on intra- and interspecific variation in certain osteological characters in *Rhyacotriton*.

MATERIALS AND METHODS

For all of the analyses discussed below, we had available from 10 to 46 cleared-and-stained adult specimens of each of the four species of *Rhyacotriton* (listed in Appendix 3). We re-examined all specimens studied by Worthington and Wake (1971) and Wake (1980). In addition to the specimens used for determining numbers of teeth, we had available a few dried skeletal preparations (also listed in Appendix 3). Specimens were examined using a dissecting microscope.

DENTITION

The maxillary, premaxillary, dentary, and vomerine teeth of *Rhyacotriton* are generally similar to those of other species of salamanders—small, pedicillate, and bicuspid.

However, the teeth have a somewhat unusual shape. Those of adults are cylindrical and distinctly recurved, with a small, disc-like, vertically oriented labial cusp and a large, conical lingual cusp (Beneski and Larsen, 1989). While we have perceived no sexual, geographic, or interspecific variation in shape, there are some differences of possible systematic significance in numbers of maxillary-premaxillary and vomerine teeth.

As a general rule, the numbers of maxillary-premaxillary teeth increase with increasing body size in salamanders, while the numbers of vomerine teeth have a less regular relationship to body size. Our samples are inadequate to permit detailed analysis of variation, but some differences have been detected, both within and between species. Numbers of maxillary-premaxillary teeth are apparently greatest in *R. variegatus*. Mean numbers of teeth in this species range from 62 (s.d. 8.3) in the Siskiyou County sample (in which the size of individuals averages 47.3 mm SVL, s.d. 2.9 mm) to 70.7 (s.d. 5.4) in the Benton County sample (mean size 43.0 mm SVL, s.d. 3.5). The Lincoln County sample also has a large number of teeth (mean 70.2, s.d. 6.2; mean size 50.0 mm SVL, s.d. 4.9 mm). Our *R. cascadae* sample has the fewest teeth: 51.7 (s.d. 6.5), with a mean size of 44.9 mm SVL (s.d. 4.6). In our sample of *R. olympicus*, the mean tooth number is 57.5 (s.d. 8.9), and the mean size 47.4 mm SVL (s.d. 4.6). The *R. kezeri* sample has a mean of 62.6 teeth (s.d. 4.3), with a mean size of 47.4 mm SVL (s.d. 4.6 mm).

Numbers of vomerine teeth also are highest in *R. variegatus*. The Siskiyou County sample has 20.9 (s.d. 3.0); the Curry County sample has 18.5 (s.d. 5.1—this sample has a mean size of 43.3 mm SVL, s.d. 6.3 mm), that of Benton County 20.9 (s.d. 2.9), and of Lincoln County 20.7 (s.d. 2.3). A similar number of vomerine teeth, 19.1 (s.d. 2.8), are found in *R. kezeri*. The other two species appear to have fewer vomerine teeth: our *R. cascadae* sample has 17.5 (s.d. 1.7), and the *R. olympicus* sample has only 14.1 (s.d. 1.1).

CRANIAL OSTEOLOGY

Cranial structure in the genus has been relatively well studied, and descriptions of the skull of *R. olympicus* (sensu stricto) have been presented by Dunn (1920), Noble (1921), Tihen (1958), Cloete (1961), Larsen (1963), and Srinavasachar (1962). Worthington and Wake (1971) described larval skulls and late ontogeny in *R. cascadae*, and Wake (1980) added some information and figured the anterior part of the skull of *R. variegatus*. No detailed descriptions will be presented here; comments will be restricted to features that show interspecific variation. The skulls of adult *R. kezeri* and *R. variegatus* are illustrated in Figure 6.

The four species differ primarily in the degree to which the nasal bones are developed. We have never seen the slightest sign of a nasal bone in any specimen of *R. olympicus*, and none has been reported in the literature. Our largest specimen of this species is an approximately 57 mm SVL adult from near the type locality at Lake Cushman, Mason County, Washington (MVZ 27285). However, nasal bones are present in at least some specimens of all the other species. We note here that the figure of the skull of *Rhyacotriton* in Duellman and Trueb (1985, fig. 13-3c) erroneously shows the prefrontal labeled as the nasal; no nasal is drawn in the specimen illustrated.

In *R. kezeri*, nasal bones are absent on both sides in 8 of our sample of 14 metamorphosed adults (42.7-52.2 mm SVL, 4 males and 4 females). In two females,

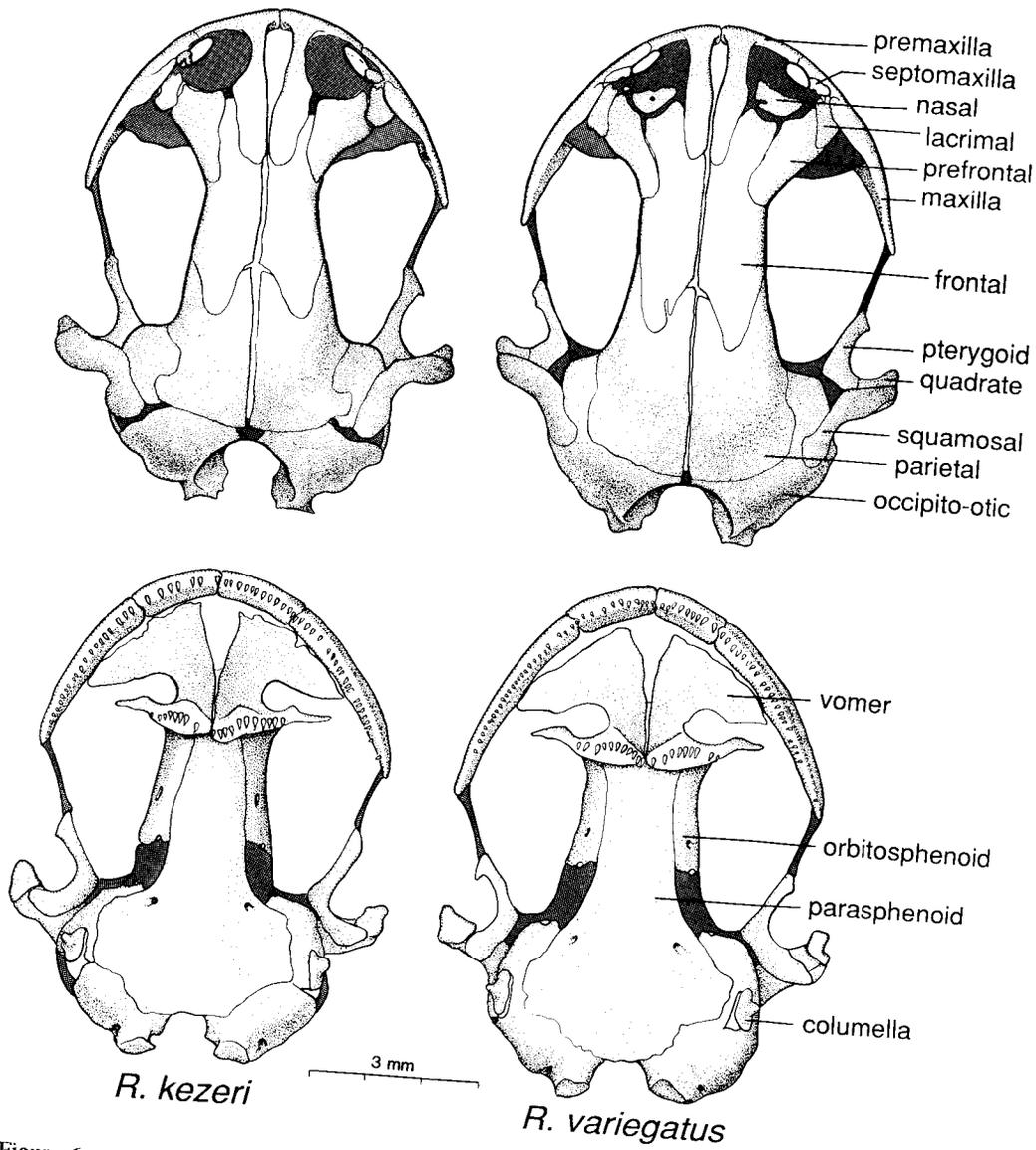


Figure 6. Representative skulls of southern *Rhyacotriton kezeri* (left; MVZ 205937) and northern *R. variegatus* (right; MVZ 206020). These specimens are from populations within 10 km of the contact zone discussed in Chapter 2. The upper pair are in dorsal view, the lower ventral. The dark stippling is cartilage.

nasal bones approximately 0.2 to 0.5 times the area of the adjacent lacrimal bone are present on one side only. We compare the size of the nasals to the lacrimals in this discussion because the latter bones appear to be relatively invariant in size once they are fully formed during metamorphosis, and because we have found no significant interspecific differences. In two other females (47.5 and 48.5 mm SVL), bones that are from 0.6 to 0.8 times the area of the lacrimals are present on both sides. Tiny fragments are present on both sides in one male (45.6 mm SVL), and slightly larger bones (ca. 0.2 times the area of the lacrimal) are present on both sides in another male (51.1 mm SVL). Thus, the trait appears to be intraspecifically polymorphic in this species, with no clear relationship to body size.

The nasal bones are also polymorphic in their degree of development in *R. cascadae*. In a sample of 10 individuals from Hood River County, Oregon, the bones are absent in 4 individuals, including the largest specimen (52.4 mm SVL). In 5 individuals, a nasal is represented by a tiny dot of mineralized tissue on one side only. The remaining specimen (the second-smallest, at 39.6 mm SVL) has a very small but discrete crescent, less than 0.5 times the size of the lacrimal, on one side only. Nasals were absent in all specimens studied by Worthington and Wake (1971), but most of these were larvae, and nasals are usually absent in larvae of small species of salamanders.

The nasal bones are larger and more consistently present in *R. variegatus* than in the three other species in the genus. They are present on both sides in 19 of 20 specimens examined from the Lincoln County sample (which was collected only a few kilometers from the sample locality of *R. kezeri*), and in 15 of these they were approximately as large or larger than (to a maximum of 1.5 times in two specimens) the lacrimal bones (Figure 6). They are absent in a single moderately large specimen (50.1 mm SVL). The bones are present in all of the metamorphosed specimens from the Benton County sample (6 specimens). Nasal bones are present on both sides of one of the 6 specimens from Curry County, and they are present on one side only in 3 other specimens (ranging from a bone larger than the lacrimal in one specimen to a tiny fragment in another). The bones are absent from a particularly small metamorphosed specimen (33.4 mm SVL), but they are also absent in a 41.5 mm SVL specimen. In our northern California samples, we have found the nasal bone on both sides of the skull in 9 of 17 specimens, ranging from about the same size as the lacrimal to very tiny dots of mineralized tissue. The bones are present on one side only in 7 specimens, and they are lacking completely only in one moderately large (48.2 mm SVL) specimen.

No other osteological elements display as much variation as the nasal bones, and in general the species are very similar in osteological structure. However, there are some differences among the species in degree of ossification of the elements. In general, *R. variegatus* has a skull that is more fully differentiated from the larval state, and is more nearly comparable to that of metamorphosed individuals in such outgroups as *Dicamptodon*, *Hynobius*, or *Ambystoma*. The bones in the skull of *R. variegatus* are relatively larger and more extensively articulated to neighboring elements than those of *R. kezeri*. This is especially true in respect to the relation of the frontals and parietals to each other and to their bilateral counterparts, and there is a larger fontanelle in *R. kezeri* than in *R. variegatus*.

There is also substantial variation in the degree to which the prootic and opisthotic fuse during ontogeny in the genus. In larvae the two bones are always well separated by a zone

of cartilage, but in metamorphosed animals they fuse to varying degrees (Figure 6). Our largest samples were for *R. variegatus* and *R. kezeri* from localities very near their mutual zone of contact. In only 2 of the 14 specimens of *R. kezeri* examined is there any fusion of the two bones: a slight fusion in one 45.6 mm SVL, and about one-half fusion in one 48.1 mm SVL; the largest specimen lacking any degree of fusion is 52.2 mm SVL. In contrast, in 10 of 20 *R. variegatus* the bones are partially fused, and in 3 individuals (between 52 and 56 mm SVL) the bones are completely fused; the largest specimen lacking any degree of fusion is 52.5 mm SVL, and the smallest showing any fusion is 45.6 mm SVL. Some individuals showing partial to complete fusion are found in other populations of *R. variegatus* as well, but never in all individuals, and in the Curry County sample no fusion is found. In *R. olympicus*, partial fusion is found in 6 of 10 specimens, all larger than 45 mm SVL, but the bones are unfused in one specimen of 53.1 mm SVL. In the Hood River County sample of *R. cascadae*, 9 of 10 specimens have partly to completely fused bones, and they are unfused only in one small specimen (37.6 mm SVL). The bones are fully fused in two specimens, the smallest 41.6 mm SVL.

VERTEBRAE

The numbers of vertebrae vary within and between the species of *Rhyacotriton*. General features of the vertebrae were described by Worthington and Wake (1971). There is a single atlas, from 14 to 17 trunk vertebrae, a single sacral vertebra (there are two sacral vertebrae in one specimen of *R. cascadae*, MVZ 173338), two to four (strong mode of three) caudosacral vertebrae (with many sacral and caudosacral anomalies), and as many as 28 caudal vertebrae. So many tails were broken in our samples that numbers of caudal vertebrae could not be determined accurately.

Variation in number of trunk vertebrae in *Rhyacotriton* is illustrated in Table 4. The northern (*R. olympicus*) and inland (*R. cascadae*) species have more vertebrae than the other two species. This is expected for salamanders that develop in colder environments, and it may be a plastic response to environmental temperature (cf. Lindsay, 1966).

HANDS AND FEET

The standard phalangeal formula for the genus is 2-2-3-2 for the forelimb and 2-2-3-3-2 for the hindlimb. We encountered virtually no variation, except for two specimens with 1-2-3-2 in one hand each, and one specimen with 2-3-3-3-2 in one foot.

Mesopodial elements remain cartilaginous throughout life. The standard arrangement in the manus is six elements (Figure 7B). There are three basal units: a radiale-centrale 1, an intermedium-centrale, and an ulnare. Apparently there has been a failure in this genus of the basic segmentation process which in most other salamanders leads to the separation of the first two units (see Shubin and Alberch, 1986). The distal elements are distal carpals 1-2 (unseparated, as normal in salamanders), 3, and 4. The commonest variant condition (less than 10% of the total sample, and a minority of every sample) observed was the separation of the radiale and centrale 1, producing 7 mesopodials (Figure 7A). Our material was not suitable to determine any systematically significant variation. In the pes, the standard arrangement includes 9 mesopodials (Figure 7C): 3 basal units (tibiale, intermedium, fibulare), a centrale, a centrale 1, and distal tarsals 1-2, 3, 4, and 5. There is

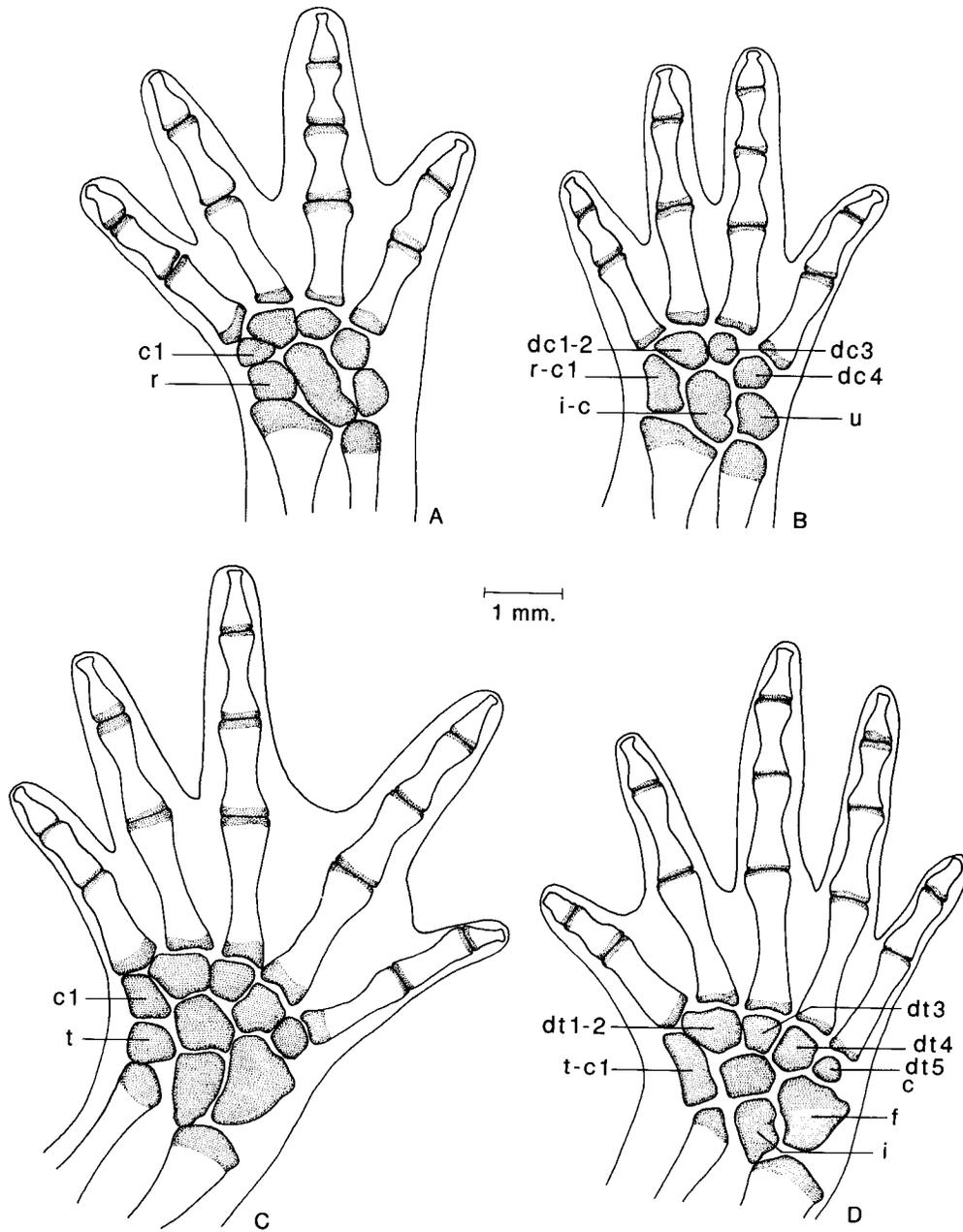


Figure 7. Dorsal views of the right manus and pes of *Rhyacotriton olympicus* (A and C based on MVZ 173353) and *R. cascadae* (B and D based on MVZ 173333). Bone is outlined and cartilage is stippled. The patterns in B and C are the commonest patterns in all four species of the genus, and the alternative patterns are the most frequently encountered variants. Abbreviations: c, centrale; c 1, centrale one; dc 1-2, distal carpals one and two; dc 3, distal carpal three; dc 4, distal carpal four; dt 1-2, distal tarsals one and two; dt 3, distal tarsal three; dt 4, distal tarsal four; dt 5, distal tarsal five; i, intermedium; i-c, intermedium and centrale; r, radiale; r-c 1, radiale and centrale one; t, tibiale; t-c, tibiale and centrale one; u, ulnare.

Table 4. Variation in trunk vertebrae among populations of *Rhyacotriton*.^a

Population	Specimens with trunk vertebrae numbering:								Mean	
	14	14 ^{1/2}	15	15 ^{1/2}	16	16 ^{1/2}	17	17 ^{1/2}		
<i>R. cascadae</i>										
Hood River County, Oregon				1	9					16.0
Multnomah County, Oregon			6		35	1	2	1		16.0
Totals			6	1	44	1	2	1		16.0
<i>R. kezeri</i>										
Lincoln County, Oregon			11	1	2					15.1
<i>R. olympicus</i>										
Mason County, Washington			1	1	7	2				16.0
<i>R. variegatus</i>										
Northern California	3		35	1	4					15.0
Curry County, Oregon	1		5		1					15.0
Benton County, Oregon			11							15.0
Lincoln County, Oregon			12	1	7					15.4
Totals	4		63	2	12					15.1

^aValues between columns are asymmetric at the sacrum.

substantial variation, but again our samples are inadequate to determine whether or not it has systematic significance. A frequent variant observed was 8 mesopodials, resulting from the failure of the intermedium and the centrale to separate, in some instances, of the distal tarsals 4 and 5 to separate in others, and of the tibiale and centrale 1 to separate in still others (Figure 7D). In one instance of 7 elements, a coincidence of the first two segmentation failures mentioned above was encountered. Several bizarre limbs (one with 13 mesopodials), apparently resulting from partial regeneration, also were seen.

MORPHOMETRIC VARIATION IN *RHYACOTRITON*

Stebbins and Lowe (1951) briefly discussed some differences between populations of *R. olympicus* and *R. variegatus*, but no morphometric analyses of body proportions were undertaken. However, with four species that have extensive intraspecific biochemical variation and have had independent evolutionary histories extending back for several million years, the question of morphometric differentiation among the populations becomes potentially interesting.

MATERIALS AND METHODS

A total of 246 specimens of *Rhyacotriton* from the following 11 populations were sampled for morphometric analysis (Appendix 4 and Figure 8); at least two populations from each of the four species were included: *R. olympicus*: Port Angeles and Quinault Valley, Washington; *R. kezeri*: Chehalis River, Washington, and Yoncalla Creek, Oregon; *R. variegatus*: Siletz River, Reedsport, and Chetco River, Oregon, and Blue Lake and Dark Gulch, California; *R. cascadae*: Mount Saint Helens, Washington, and Wahkeena Falls, Oregon. Sample sizes ranged from 10 to 56, and only transformed individuals were used. Both males and females were represented in each sample. Although Nussbaum and Tait (1977) found that females were significantly larger than males in the two populations they examined, little sexual dimorphism was observed in the present analysis when size was eliminated, warranting combination of the sexes for most analyses (see below). Nine measurements (Figure 9) were taken on each specimen, using digital calipers: snout-vent length (SVL), tail length (TL), snout-gular (head) length (SG), head width (HW), axilla-groin (trunk) length (AG), trunk width (TW), forelimb length (FLL), hindlimb length (HLL), and foot length (FL).

Multivariate and univariate statistical analyses were conducted, using the University of California (Berkeley) version of SAS (SAS Institute, Inc., 1985) with \log_{10} -transformed measurements. Principle-components analyses on covariance matrices were done using the PRINCOMP procedure, and canonical-discriminant analyses using CANDISC. DISCRIM

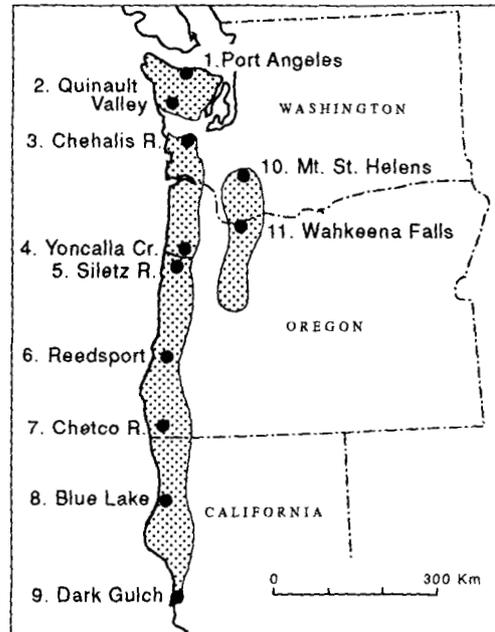


Figure 8. Populations of *Rhyacotriton* sampled for morphometric analysis.

analyses were used to calculate probabilities of misidentification of specimens. These procedures were conducted both on raw data and on the residuals of each of the other eight measurements regressed on SVL.

RESULTS AND DISCUSSION

Each character was examined first for variation within populations due to sex and to age (estimated by SVL). Although there is considerable overlap, it has always been the impression of the senior author that male *Rhyacotriton* in all species tend to have larger feet than females (for a given snout-vent length). However, this trend was only apparent in FL in the samples from Mount Saint Helens, Chetco River, and Blue Lake. No other sexual dimorphism was observed among the non-size characters examined here, and males and females were combined for the analyses discussed below. All measurements showed significant ($p < 0.05$) regressions with SVL.

In order to test whether the populations form discernible clusters of points in multivariate space, a principle-components analysis was conducted. As might be expected from the significant relationship of all characters with snout-vent length, the first principle component (PC-1) of an analysis of the raw data is essentially a size axis (cf. Bookstein et al., 1985). This is apparent from the similarity in both sign and magnitude of the loadings on this axis for all characters. Over 84% of the variation among the *Rhyacotriton* sampled is explained by this component, largely because of the presence in most samples of specimens ranging from 36 mm to as high as 60 mm SVL. Because of this, we conducted

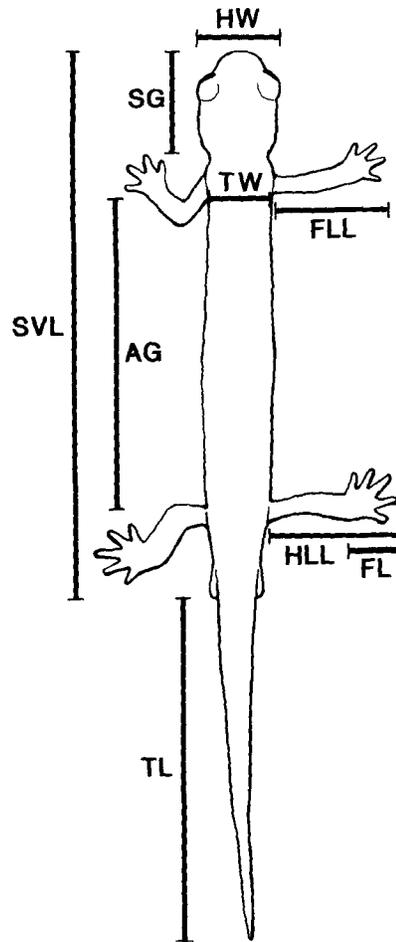


Figure 9. Nine morphometric characters measured in *Rhyacotriton*: SVL=snout-vent length, TL=tail length, SG=snout-gular (head) length, HW=head width, AG=axilla-groin (trunk) length, TW=trunk width, FLL=forelimb length, HLL=hindlimb length, FL=foot length.

Table 5. Character loadings for a principle-components analysis on the covariance matrix of the residuals of 8 log₁₀-transformed raw measurements among 11 populations of *Rhyacotriton* regressed against snout-vent length.^a

Character	PC-1	PC-2	PC-3	PC-4	PC-5	PC-6	PC-7	PC-8
TL	0.71	-0.51	0.48	0.02	-0.09	0.03	0.02	-0.02
SG	0.35	0.15	-0.20	0.16	0.65	-0.14	-0.06	0.59
HW	0.35	0.24	-0.23	0.01	0.35	-0.06	0.38	-0.71
AG	0.51	0.44	-0.37	-0.05	-0.57	0.14	-0.22	0.11
TW	-0.04	-0.12	-0.14	0.37	-0.04	0.75	0.49	0.17
FLL	-0.03	0.54	0.59	0.54	0.03	0.08	-0.19	-0.11
HLL	0.03	0.22	0.26	-0.67	0.29	0.56	-0.20	-0.02
FL	0.01	0.33	0.31	-0.31	-0.19	-0.27	0.70	0.31
Variance resolved	39.4%	20.2%	13.8%	8.6%	6.7%	4.5%	3.7%	3.2%

^aSee Figure 9 for character abbreviations.

all further analyses on the residuals of the regressions of each character against snout-vent length.

The first and second principle components on residual data account for 60% of the variation in "shape" (see Figure 10A). Characters loading particularly heavily (>0.4) on PC-1 are tail length and foot width. These same two characters, as well as trunk width (Table 4), load heavily on PC-2. Tail length and trunk length are also important on PC-3 (which accounts for a further 14% of the total variation). The 11 populations fail to form obvious clusters: the only populations that do not overlap in Figure 10A are Mount Saint Helens (*R. cascadae*) with Chehalis River (*R. kezeri*), Yoncalla Creek (*R. kezeri*), and Reedsport (*R. variegatus*). Plots of the other 5 principle components also show extensive overlap among populations. However, the population centroids cluster more or less by species (Figure 10B). The only exception to this is the Reedsport population of *R. variegatus* (population 6), which differs from other *R. variegatus* primarily along PC-1. The Reedsport population is unique among *R. variegatus* in tail length (see below), and this character loads heavily on PC-1 (Table 5).

Principle-components analysis examines variation among specimens without reference to a priori groupings. The 11 populations of *Rhyacotriton* are, however, real groups and it is of interest to see how well these groups can be distinguished from one another. For this reason, a canonical discriminant-function analysis was conducted. In this case, removal of the confounding effect of size variation was approximated by running the analysis on the residuals of each of the other 8 characters against snout-vent length. Figure 11 is a plot of the first two canonical axes based on these residuals. Again, although discrimination of groups is more complete than in the principle-components analysis above, there is still considerable overlap. Figure 11, however, represents only 2 of the 8 canonical axes. If all axes are taken together, the populations can be discriminated with greater accuracy. Discriminant-function analysis is capable of providing a posteriori probabilities of classifying individual specimens into defined groups. In the analysis performed on our samples of *Rhyacotriton*, 115 of the 246 specimens studied (46.7%) were misclassified. This again demonstrates that the species of *Rhyacotriton* are very similar morphometrically.

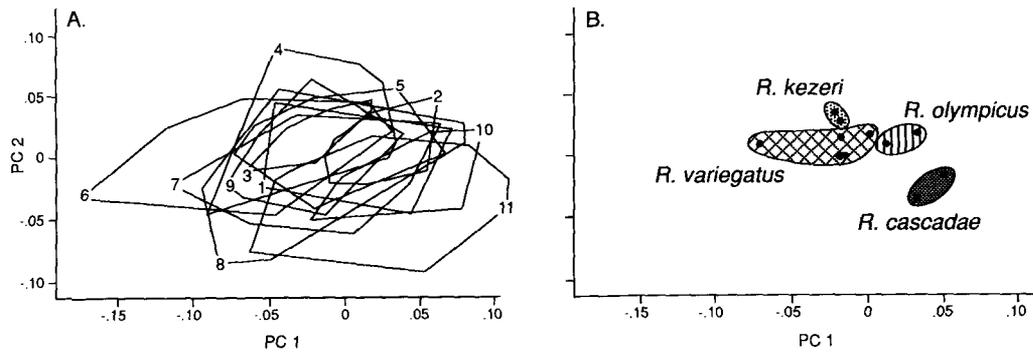


Figure 10. Figure 10A represents the distribution of individuals from the 11 populations of *Rhyacotriton* (see Figure 8) on the first and second principle-component axes, based on the residuals of the other 8 characters (see Figure 9) regressed on snout-vent length. The polygons represent the populations. Figure 10B represents the distribution of population centroids from Figure 10A.

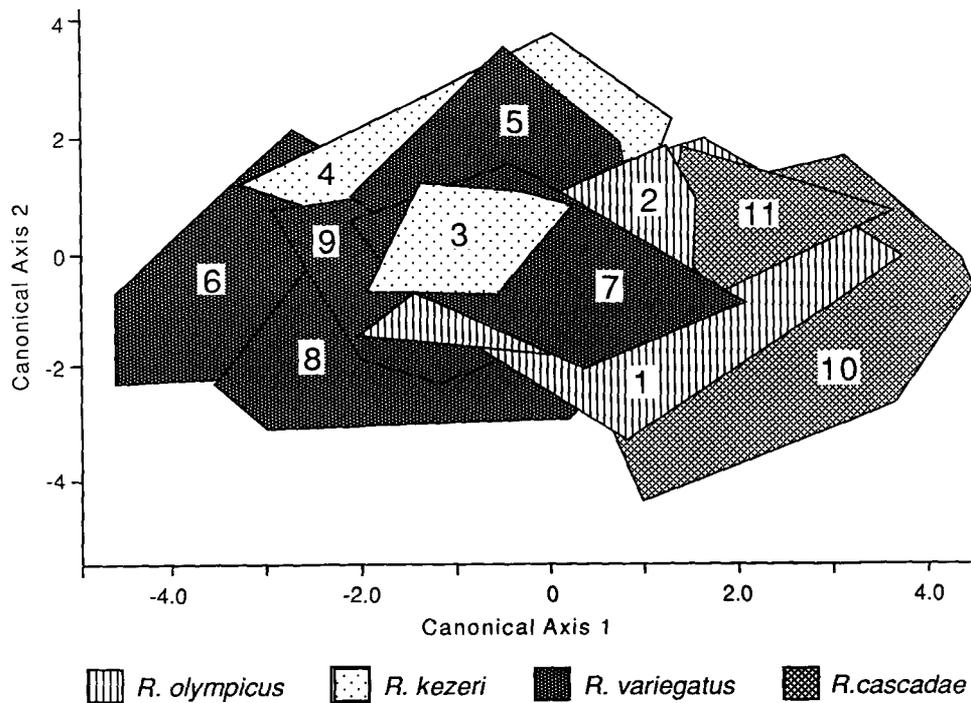


Figure 11. The distribution of individuals from the 11 populations of *Rhyacotriton* (see Figure 8) on the first and second canonical axes, derived from a discriminant-function analysis of the 9 characters illustrated in Figure 9.

Table 6. Probabilities of classifying specimens of *Rhyacotriton* into the 11 populations listed in Appendix 4, using residuals of the other 8 morphometric characters regressed on snout-vent length.

	From population:		Into population:						
	<i>R.olympicus</i>		<i>R.kezeri</i>		<i>R.variegatus</i>				
	1	2	3	4	5	6	7	8	9
1 Port Angeles	62.5	25.0	6.3	0.0	0.0	0.0	0.0	6.3	0.0
2 Quinalt Valley	21.7	39.1	21.7	0.0	8.7	4.4	4.4	0.0	0.0
3 Chehalis River	0.0	18.2	54.6	9.1	0.0	0.0	0.0	9.1	9.1
4 Yoncalla Creek	4.0	0.0	24.0	40.0	20.0	4.0	8.0	0.0	0.0
5 Siletz River	0.0	3.7	0.0	11.1	70.4	0.0	7.4	3.7	3.7
6 Reedsport	0.0	7.1	14.3	0.0	0.0	64.3	7.1	7.1	0.0
7 Chetco River	5.9	5.9	5.9	11.8	5.9	5.9	41.2	5.9	11.6
8 Blue Lake	5.6	7.6	1.9	5.7	1.9	17.0	15.1	26.4	18.8
9 Dark Gulch	0.0	5.6	0.0	11.1	5.6	5.6	0.0	5.6	66.7

Although variation in the morphometric characters measured is such that almost half of the specimens in the analysis were misclassified by the discriminant-function analysis, there is nonetheless a geographic component to this variation. Misclassifications among the 9 populations forming a north-south series along the Coast Ranges (Port Angeles, Quinalt Valley, Chehalis River, Yoncalla Creek, Siletz River, Reedsport, Chetco River, Blue Lake, and Dark Gulch; see Figure 8) were examined in detail. The probabilities of placement of any specimen from any one of these populations are listed in Table 6 and illustrated in Figure 12. For all populations, more specimens were correctly classified than were misclassified. Particularly noteworthy, however, is the fact that in most cases a specimen is most likely to be misclassified to a geographically adjacent population. This was true in all but four cases. Two of these involve comparisons of the Siletz River and Reedsport populations, and are the result of the distinct differences between these two populations in tail length and trunk width discussed below. The other two cases involve the Yoncalla Creek's population being more similar to the Chetco River and Dark Gulch populations than is either to its geographically adjacent populations. In the case of Chetco River, however, one of the adjacent populations is again Reedsport, which differs significantly in tail length.

Despite these exceptions, in most cases geographically close populations are more similar than geographically distant populations. This is true regardless of the species boundaries recognized through biochemical and color-pattern analysis. For example, specimens from the Siletz River population (*R. variegatus*) were more likely to be misclassified by the discriminant-function analysis as belonging to the Yoncalla Creek population, which is very close geographically but assigned to a different species (*R. kezeri*), than they are to geographically more distant population of *R. variegatus*. The same is true of specimens from the Chehalis River population of *R. kezeri* (which are most likely to be misclassified as *R. olympicus* from Quinalt Valley).

Species discrimination was only slightly better when populations were pooled by species. The percentages of correctly identified specimens of *R. olympicus*, *R. kezeri*, and *R. variegatus* were 66.7%, 64.4%, and 59.7%, respectively. By comparison, Shaffer (1983) found only a 12-25% misclassification rate in *Ambystoma rosaceum*.

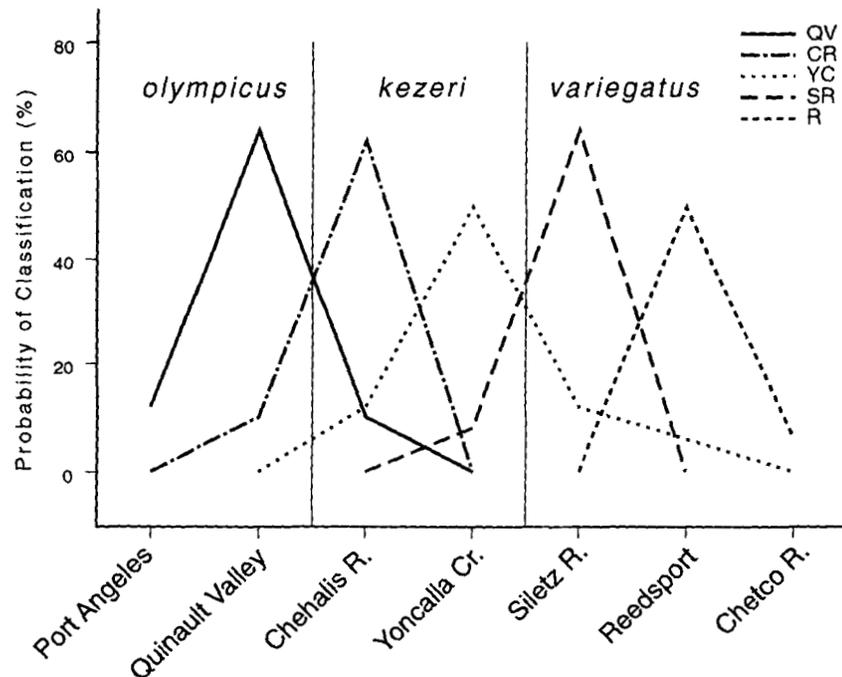


Figure 12. The probability of classifying specimens from 5 populations of *Rhyacotriton* in the Coast Ranges (see Figure 8) into predetermined units, based on discriminant-function analysis. Vertical lines separate species.

This lack of correspondence of morphometric variation with species boundaries or with color-pattern variation is also apparent in univariate analyses of the characters. Figure 13 illustrates the means and 95% confidence limits for each population for the 8 characters other than snout-vent length. These were calculated by regressing each of the characters against snout-vent length for each population and calculating the mean and confidence limits for a standard snout-vent length of 46 mm (the overall mean for all specimens in the survey). This technique is discussed at length by Lynch and Wake (1975).

All of the characters show geographic trends of variation along the transect (see Figure 8) from Port Angeles to Dark Gulch, although in some cases the differences among individual pairs of populations may not be significant at $p < 0.05$. The *R. cascadae* populations, although not part of this transect, are included in Figure 13 for the sake of completeness.

Hindlimb length, forelimb length, and foot length are all measures of limb proportions and vary similarly with geography. All show a trend from longer limbs in the north to shorter limbs in the south (although the Reedsport population has shorter feet than expected from its position in this cline). The opposite trend is apparent in head width, which is

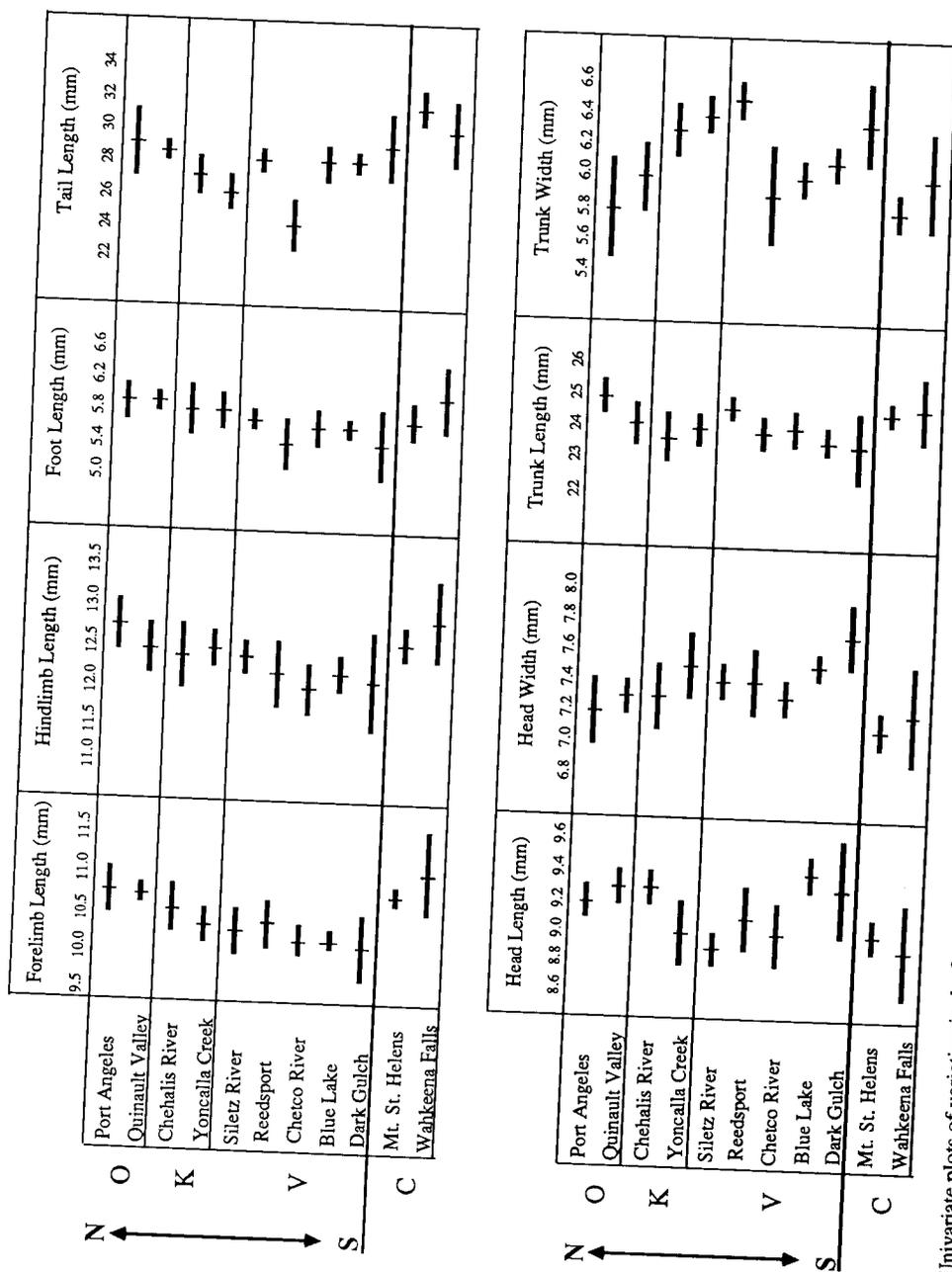


Figure 13. Univariate plots of variation in the 9 characters illustrated in Figure 9 among populations of *Rhyacotriton*. Horizontal lines separate species.

trunk width is seen between Reedsport and Dark Gulch. Between Siletz River and Reedsport, on the other hand, there is a discontinuity between these two clines. Similarly, between Port Angeles and Yoncalla Creek there is a cline in tail length from short to long, but between Siletz River and Dark Gulch two interpretations are possible for the patterns seen. One is that tail length varies little among these populations, except that the Reedsport population has distinctly short tails, and the other is that there are two discontinuous clines similar to those seen in trunk width. Unlike all of the other characters, geographic variation in head length does not appear to show any north-south trends, but rather the populations seem to fall into two more or less distinct groups: those from Port Angeles, Quinault Valley, Chehalis River, Blue Lake, and Dark Gulch have longer heads than those from Yoncalla Creek, Siletz River, Reedsport, and Chetco River.

The relatively simple north-south clines in forelimb length, hindlimb length, foot length, and head width show no discontinuities at the species boundaries. In the case of trunk width, the sharp break in the north-south cline occurs between Siletz River and Reedsport, both of which are populations of *R. variegatus*. The *R. olympicus*-*R. kezeri* and *R. kezeri*-*R. variegatus* species boundaries show no effect on the cline between Port Angeles and Siletz River. In addition, the geographic discontinuity in this character within *R. variegatus* correlates with neither the color-pattern variation (Chapter 8) nor variation in size at transformation (Chapter 9): discontinuities in both of the latter characters occur south of Reedsport. The two population groups defined by head length similarly do not correspond to species in *Rhyacotriton*. Among the longer-headed populations, Port Angeles and Quinault Valley are *R. olympicus*, Chehalis River is *R. kezeri*, and Blue Lake and Dark Gulch are *R. variegatus*. Among the shorter-headed populations, Yoncalla Creek is *R. kezeri* and Siletz River, Reedsport, and Chetco River are *R. variegatus*. The only character that shows a discontinuity at a species boundary is tail length, which shows a distinct difference between Yoncalla Creek (*R. kezeri*) and Siletz River (*R. variegatus*). The cline to the north of Yoncalla Creek in the same character, however, appears to be unaffected by the *R. olympicus*-*R. kezeri* species boundary.

The possible factors resulting in this uncoupling of morphometric variation from species and color-pattern variation are discussed in Chapter 10.

COLOR-PATTERN VARIATION IN *RHYACOTRITON*

MATERIALS AND METHODS

The discussion of color-pattern variation below is the result of an examination of approximately 1400 *Rhyacotriton* specimens in the herpetology collection in the Museum of Vertebrate Zoology (list available from the junior author on request). The analysis of spotting density in *R. variegatus* from southern Oregon and northern California (Table 7) was conducted by establishing a subjective coding system in which the least heavily spotted specimens observed were scored as 0 and the most heavily spotted as 4.

ADULT COLOR PATTERN

Stebbins and Lowe (1951) analyzed color-pattern variation among populations of *Rhyacotriton* from throughout its range and recognized two basic color patterns which were geographically cohesive. On the basis of these, they recognized the two subspecies *R. olympicus olympicus* and *R. o. variegatus* (Figure 14). In their view, adult *R. o. olympicus*, ranging from northwestern Oregon northward, were characterized by a uniformly dark chocolate brown coloration above, with the dark dorsal pigmentation ending abruptly along the sides. The ventral surface was characterized as being unmarked orange-yellow, or with mottlings of brownish in the gular area and a few well defined dark spots on the underside of the body and tail. Stebbins and Lowe also noted that some individuals have dark markings only in the gular area. In *R. o. variegatus*, distributed from southwestern Oregon southward, the adults were characterized by an olive or pale olive ground color dorsally, largely obscured by numerous spots, blotches, and reticulations of black to blackish brown. The dorsal ground spotting/mottling grades into a similar pattern on the venter, where the ground color is greenish yellow. Stebbins and Lowe suggested that the guanophores present in both forms are more conspicuous in *R. o. olympicus*, probably because of the lighter ground color and heavy spotting in *R. o. variegatus*.

Table 7. The degree of dorsal and ventral spotting in *Rhyacotriton variegatus* in southern Oregon and northern California.^a

Locality	Distance south of Reedsport (km)	N	Average score
1. Reedsport, Douglas Co., Oregon	0	14	0.0
2. Coquille, Coos Co., Oregon	60	7	0.1
3. Rogue River, Curry Co., Oregon	135	17	2.6
4. Pistol River, Curry Co., Oregon	160	8	3.0
5. Chetco River, Curry Co., Oregon	180	18	3.2
6. Del Norte Co., California	230	17	3.6
7. Humboldt Co., California	310	53	4.0

^aPopulations are listed from north to south. Individuals in each population were scored from 0 to 4, and these scores were averaged over all individuals in each population.

While Stebbins and Lowe recognized two subspecies, they did not perceive any sharp distributional boundaries within the range of the genus (Figure 14). All of the populations in the Coast Range between Tillamook and Coos counties, Oregon, and all of the populations in the Cascade Mountains, were considered by them to represent intergrades in color pattern.

When viewed from the standpoint of the specific differentiation in *Rhyacotriton* outlined in the present paper, the analysis of Stebbins and Lowe (1951) can be clarified and extended. Comparison of the maps in Figures 5 and 14 shows that the distribution of *R. o. olympicus* corresponds closely to the combined ranges of *R. olympicus* and *R. kezeri*, and that all of the populations designated by Stebbins and Lowe as *R. o. variegatus* are *R. variegatus*. The "intergrades" identified by Stebbins and Lowe are here recognized as northern *R. variegatus* (in the Coast Range) and *R. cascadae* (in the Cascades). The "contact" between *R. o. olympicus* and the intergrades in Figure 14 lies approximately on the contact zone between *R. kezeri* and *R. variegatus*.

Figures 15-17 illustrate adult color-pattern variation in *Rhyacotriton*. In their description of *R. o. olympicus*, Stebbins and Lowe for the most part were describing *R. olympicus* as designated in the present paper. *R. olympicus* is perhaps the most distinctive species in color pattern in the genus, with a dark, unspotted dorsal ground color, sharply demarcated from the yellow of the venter by a distinct wavy line unique among *Rhyacotriton*. Also unique are the large dark blotches on the ventral surface; dark ventral markings in the other species, when present, usually take the form of speckling and do not occur in the form of large blotches (Figures 15 and 17).

Stebbins and Lowe mentioned that some individuals of "*R. o. olympicus*" have dark ventral markings only on the gular area. This is true of some populations of *R. kezeri*, a species which otherwise is separated only with difficulty from the populations of northern *R. variegatus* that it contacts in Tillamook County, Oregon (see Chapter 2). Apart from a few small, dark, often faint speckles usually present in the gular and chest regions (and rarely elsewhere on the venter) in these populations of *R. kezeri*, which almost always are lacking from northern populations of *R. variegatus*, the two usually can be distinguished by the presence in *R. variegatus* of some very light dorsal speckling (lacking in *R. kezeri*), more dark pigmentation on the undersurface of the foot, and darker limb insertions. The

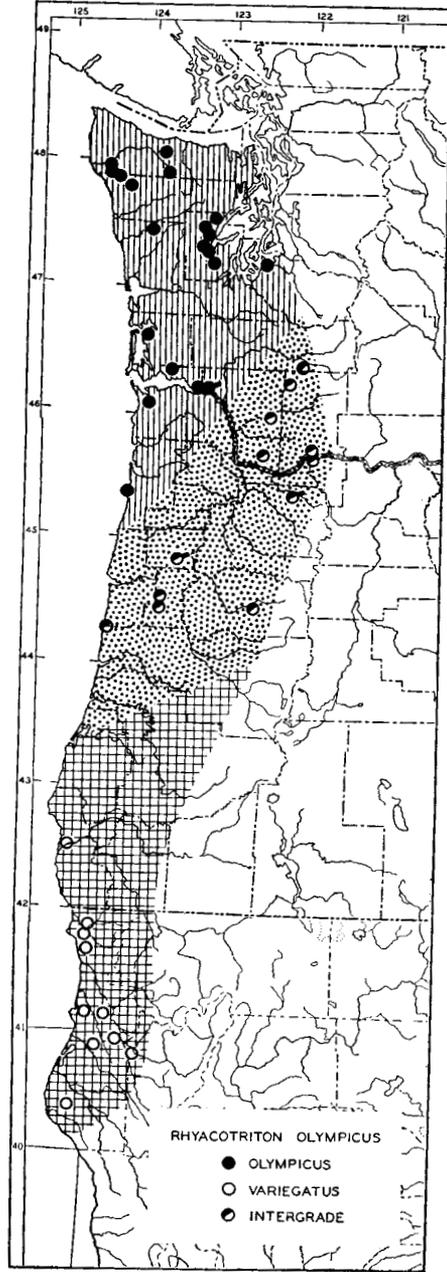


Figure 14. The distribution of color morphs ("subspecies") in *Rhyacotriton*, as outlined by Stebbins and Lowe (1951).

darker dorsal ground color used by Stebbins and Lowe to distinguish *R. o. olympicus* from *R. o. variegatus* is of little use in distinguishing *R. kezeri* from northern *R. variegatus*.

Although *R. kezeri* tends to have a somewhat darker dorsum, there is considerable individual variation in both species. Ventral spotting, present in the populations of *R. kezeri* in the vicinity of the contact zone with *R. variegatus*, is lacking in populations of *R. kezeri* farther north (in Washington and in Clatsop County, Oregon).

Within *R. variegatus*, both dorsal and ventral spotting (which are strongly correlated) are highly variable. Otherwise, color pattern in this species varies little geographically. Populations at the southern end of the range in California are strongly spotted, while those from central Oregon are at most very faintly spotted (Figures 15 and 16). There is a stepped cline in this character in *R. variegatus*, as represented in Table 7. Stebbins and Lowe may have been influenced by the degree of spotting in delimiting their subspecies, because the step we observe in this cline occurs in Curry County or Coos County, Oregon, more or less where the "contact" between *R. o. variegatus* and the "intergrades" was perceived by Stebbins and Lowe (Figure 14).

Thus, while complex, there is a geographically comprehensible pattern of variation in color pattern in populations of *R. olympicus*, *R. kezeri*, and *R. variegatus* in the Coast Ranges of the Pacific Northwest. *R. cascadae*, on the other hand, presents a different picture. Almost every population of this species seems to differ from every other population, and the range of variation essentially encompasses that among all three species in the Coast Ranges. This is exemplified by the ventral spotting patterns illustrated in Figure 17. This lack of a geographically comprehensible pattern of variation in color pattern in *R. cascadae* (among the populations available for this analysis) may account for the placement of all Cascade populations in the "intergrade" zone by Stebbins and Lowe (Figure 14).

Some of the color patterns encountered in *R. cascadae* are found nowhere else. For instance, at Wahkeena Falls, Multnomah County, Oregon, the ventral spotting tends to occur as a zone of heavy black blotches along the midventral line. In other populations, ventral spotting takes the form of very tiny grayish speckles concentrated on (but not necessarily limited to) the gular region. Several populations have a tendency for the darker dorsal pigmentation to extend in a band across the venter, posterior to the cloaca. However, none of these characters are diagnostic of *R. cascadae* as a whole. When taken together, *R. cascadae* tends to have a dark dorsum which is usually supplied with strong spotting and blotching, especially along the sides. These spots usually are larger than those seen in spotted *R. variegatus*, and are present even when ventral spotting is weak or lacking (a condition never seen in *R. variegatus*, where dorsal and ventral spotting are always similar in degree). This spotting tends to be responsible for a sharp (much more evident than in *R. variegatus*), straight line of demarcation between the dorsal and ventral ground colors. Unlike the spotting pattern in *R. variegatus*, spotting in *R. cascadae* (when present) is often distinctly different on the dorsum and venter of the same individual. The distinct preocular stripe always seen in spotted and blotched *R. variegatus* is usually lacking in spotted *R. cascadae*.

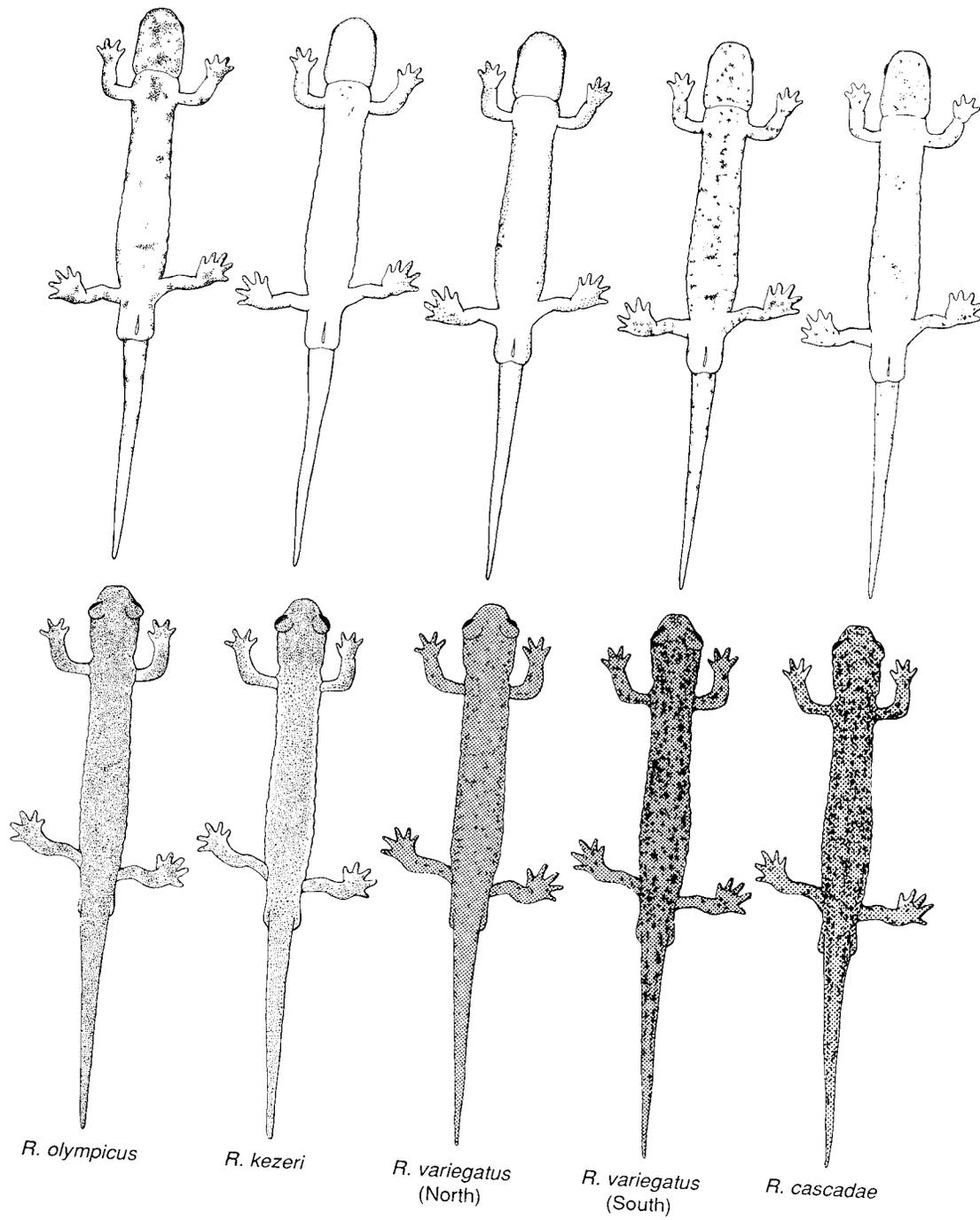


Figure 15. Color-pattern variation in the four species of *Rhyacotriton* (dorsal and ventral views).

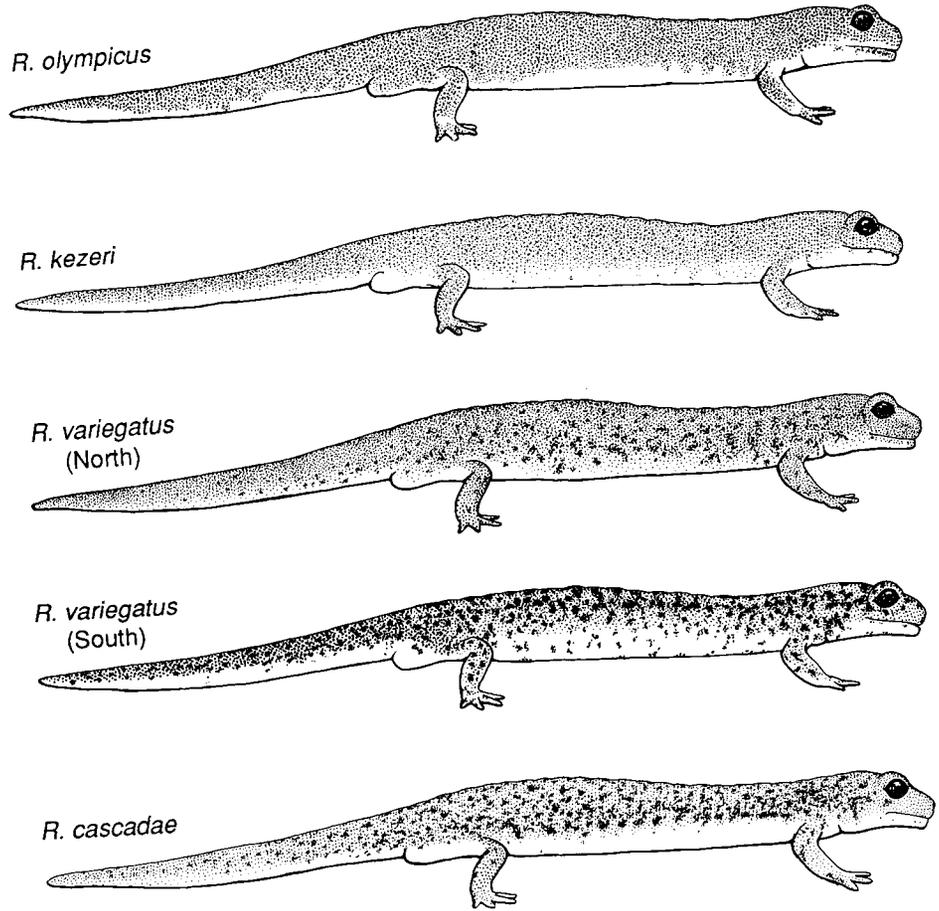


Figure 16. Color-pattern variation in the four species of *Rhyacotriton* (lateral views).

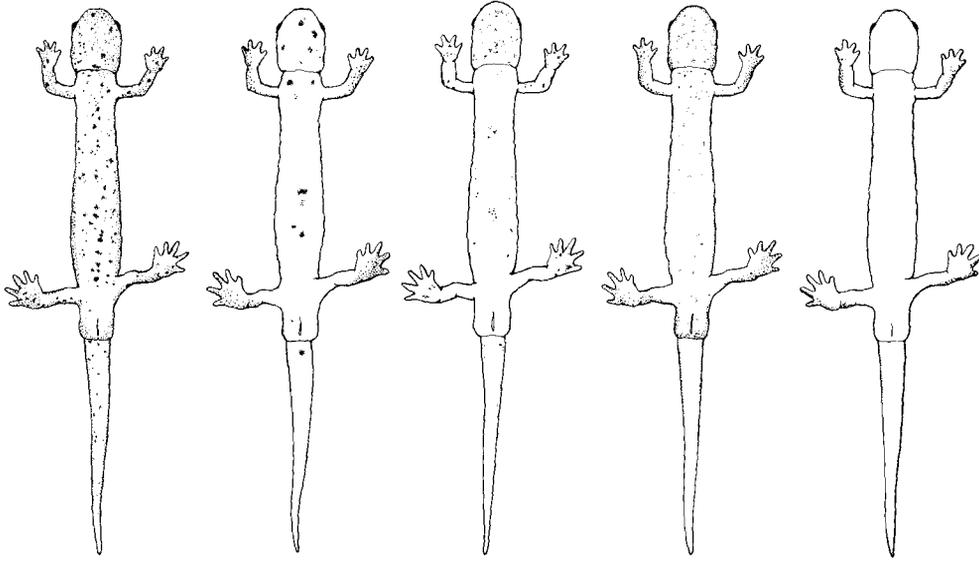


Figure 17. The range of variation in ventral color pattern in *R. cascadae*.

LARVAL COLOR PATTERN

Stebbins and Lowe observed little geographic differentiation in color pattern in larval *Rhyacotriton*, although they recognized a tendency toward somewhat more ventral spotting in larvae from California (*R. variegatus*) than in larvae from the Olympic Mountains (*R. olympicus*), mirroring the pattern seen in adults. More extensive examination of larvae from throughout the range of *Rhyacotriton* suggests that larval color pattern tends to show patterns of variation similar to those seen in adults (also noted by Gaige, 1917), with the largest larvae being the most similar to the adults (the smallest larvae tend to be similar throughout the range of *Rhyacotriton*, with very little color pattern yet developed). Southern *R. variegatus* larvae tend to have considerable spotting on the dorsum and venter; *R. kezeri* larvae tend to have no spotting, except for occasional light gular flecking in southern populations; and *R. olympicus* larvae tend to have a wavy demarcation between the dorsal and ventral ground color and a few large distinctive blotches on the venter. Larvae were available from too few *R. cascadae* populations to determine whether the variability seen in adults extends to them.

LIFE-HISTORY VARIATION IN *RHYACOTRITON*

Aspects of life history in *Rhyacotriton* have been discussed by various workers (Noble, 1931; Noble and Richards, 1932; Stebbins, 1951, 1954; Stebbins and Lowe, 1951; Bury, 1970; Arnold, 1977; Nussbaum, 1985). In particular, Nussbaum (1985) found that larval *Rhyacotriton* display a rather "terrestrial" feeding strategy, taking mollusks, insects, and arachnids rather than zooplankters.

Variation in life history among populations has been examined in detail only by Nussbaum and Tait (1977), who compared the life-history characteristics of a population of *R. cascadae* from Wahkeena Falls, Multnomah County, Oregon, with those of a population of *R. variegatus* from Fall Creek, Lincoln County, Oregon (both were called *R. olympicus* at the time). They observed the following differences:

1. Days to hatching appear to be fewer in *R. variegatus*. A clutch of *R. variegatus* in the laboratory took 210 days to hatch at 8°C (from early tail-bud stage) while a clutch of *R. cascadae* took 290 days under the same laboratory conditions.

2. Hatchling size is smaller in *R. variegatus*. The minimum hatchling size observed in the *R. variegatus* population was 13.5 mm SVL; the smallest hatchling *R. cascadae* was 15.8 mm SVL.

3. Size at transformation is smaller in *R. variegatus*. The smallest transformed individual from the *R. variegatus* population was 30.2 mm SVL and the largest larva was 38.6 mm SVL. In the *R. cascadae* population, the corresponding measurements were 37.7 mm and 44.7 mm.

4. The larval period is shorter in *R. variegatus* (3.0-3.5 years) than in *R. cascadae* (4-5 years).

5. Age at maturity is lower for *R. variegatus*. Nussbaum and Tait hypothesized that maturity is reached at ages of 4.5-5.0 years in *R. variegatus* and 5.5-6.0 years in *R. cascadae*.

6. Mean adult size is smaller in *R. variegatus*. The mean sizes of transformed *Rhyacotriton* in the *R. variegatus* sample were 42.5 mm SVL for males and 44.9 mm SVL for females. In the *R. cascadae* population, the means were 46.5 mm for males and 49.5 mm for females.

7. Maximum adult size is smaller in *R. variegatus*. The maximum sizes observed in the *R. variegatus* population were 51.4 mm SVL for males and 50.8 mm for females. In the *R. cascadae* population, these values were 52.7 mm and 56.3 mm.

8. Fecundity is higher in *R. variegatus*. The average number of yolked ovarian eggs calculated for a 50 mm SVL *R. variegatus* was about 12.9. The average number for a similar-sized *R. cascadae* was approximately 8.0. Nussbaum and Tait thought that this was probably due to smaller egg size in *R. variegatus*.

Although they demonstrated that their population of *R. variegatus* contains smaller, more rapidly developing, and more fecund individuals than their population of *R. cascadae*, Nussbaum and Tait were cautious about hypothesizing reasons for these differences because they had compared only two populations. They postulated that local environmental conditions might be a primary factor. Wake (1980) suggested that a more pervasive regional variation in environment might be important, with the *R. cascadae* population requiring more time to develop in the relatively harsher inland climate. Wake also suggested that coastal populations farther north, where conditions are also presumably harsher, might share certain life-history characteristics with inland populations.

Our discovery that gene flow between coastal and Cascade populations is nonexistent, and that the populations have been isolated for a very long time raises the possibility of an alternative hypothesis: that life-history traits are species-specific.

MATERIALS AND METHODS

In order to test among the hypotheses discussed above, some of the characters analyzed by Nussbaum and Tait (1977) were reexamined over a wider range of populations. The populations sampled for this analysis were (Table 8): *R. olympicus* (Olympic Mountains, Washington), *R. kezeri* (Tillamook County, Oregon), *R. cascadae* (Columbia River Gorge, Oregon and Washington), and *R. variegatus* (3 populations: Tillamook County, Oregon; Curry County, Oregon; and Humboldt County, California). The Tillamook County populations of *R. kezeri* and *R. variegatus* are within a few kilometers of each other on either side of the contact zone between the two species, and the Tillamook County *R. variegatus* population is very close to the Lincoln County population analyzed by Nussbaum and Tait. Many of the specimens in the *R. cascadae* sample above are from the same locality (Wahkeena Falls) as the *R. cascadae* population of Nussbaum and Tait.

Observations were made using vernier calipers and a dissecting microscope where appropriate.

RESULTS AND DISCUSSION

All data for the present analysis were obtained from museum specimens, so no direct information was available on hatching time, length of larval period, or age at maturity. In addition, there are few collections of very small larvae, so no useful data were available concerning hatchling size. However, pertinent data were available concerning the other characters discussed by Nussbaum and Tait.

Table 8. Selected life-history parameters for species and populations of *Rhyacotriton*.

Population	N (trans- formed)	Maxi- mum larval size	Minimum transformed size	Trans- formed <38 mm SVL	Mean largest 10% males	Mean largest 10% females	Yolked ovarian eggs (N)
<i>R. olympicus</i>							
Olympic Mountains	113	40 mm	36 mm	1.8%	59.0 mm	57.3 mm	7.9 (11)
<i>R. kezeri</i>							
Tillamook County	42	42 mm	36 mm	7.1%	52.0 mm	55.0 mm	11.0 (8)
<i>R. variegatus</i>							
Tillamook County	59	40 mm	31 mm	36.8%	50.0 mm	51.6 mm	10.0 (5)
Curry County	55	39 mm	36 mm	10.9%	48.5 mm	50.8 mm	8.7 (6)
Humboldt County	102	41 mm	35 mm	6.9%	55.8 mm	56.2 mm	8.4 (10)
<i>R. cascadae</i>							
Columbia River	85	42 mm	37 mm	1.2%	50.5 mm	51.3 mm	7.9 (7)

Size at Transformation

The smallest transformed individual from the Tillamook County *R. variegatus* population was 31 mm SVL, similar to the 30.2 mm SVL measured by Nussbaum and Tait for their nearby population of the same species. The largest Tillamook County *R. variegatus* larva observed was 39 mm, again similar to the 38.6 mm observed by Nussbaum and Tait. Nussbaum and Tait found the smallest transformed salamander and largest larva in their *R. cascadae* population to be 37.7 mm and 44.7 mm SVL, respectively; in the present survey, these measurements were 37 mm and 42 mm (Worthington and Wake, 1971, reported 37.5 and 45 mm for this population).

Table 8 lists the maximum larval size and minimum transformed size for each of the populations sampled in the present analysis. The largest larvae in all populations were similar in size, ranging from 39 to 42 mm SVL. The smallest transformed specimens in each population were also similar (35-37 mm SVL), except in the Tillamook County population of *R. variegatus*, where specimens were substantially smaller (31 mm SVL). The small size at transformation in this population of *R. variegatus* also is evident in the percentage of transformed individuals at the smallest sizes (<38 mm SVL). The Tillamook County *R. variegatus* population had by far the highest percentage of such specimens (36.8%), while the other two *R. variegatus* populations and *R. kezeri* had only 6.0-10.9%. The fact that the *R. olympicus* and *R. cascadae* populations had a lower percentage (1.8% and 1.2%) suggests that average size at transformation is larger for these two populations than for other *Rhyacotriton*, even though the single smallest transformed specimens in these populations were not appreciably different in size.

Adult Size

Unlike Nussbaum and Tait's survey, in which two populations were examined in detail, the present analysis has involved only the smaller samples that have been preserved in the collection of the Museum of Vertebrate Zoology. Because these museum specimens were not collected with such an analysis in mind, and because the bias in general collecting (at least in relatively common species) is toward larger individuals, estimates of mean adult

size may be inaccurate. Adult size variation across geography, however, can be examined by considering only larger specimens. Table 8 lists the mean snout-vent length for the largest 10% of males and females in each of the populations of *Rhyacotriton* listed above. As shown by Nussbaum and Tait, females appear to be larger than males (except in *R. olympicus*, though this may be due to sampling error). *R. olympicus* is the largest species, followed by *R. kezeri* and the Humboldt County population of *R. variegatus*. The remaining *R. variegatus* and *R. cascadae* populations are smaller.

Fecundity

Nussbaum and Tait found that the average number of yolked ovarian eggs was 12.9 in *R. variegatus* and 8.0 in *R. cascadae*. Mean numbers of yolked eggs observed in the present survey are listed in Table 8. *R. variegatus* and *R. kezeri* in Tillamook County have the highest counts (10.0 and 11.0), while all other populations of *Rhyacotriton* average 7.9-8.7. This trend corresponds to that seen by Nussbaum and Tait. Noble and Richards (1932) cited an anecdotal observation of *R. olympicus* laying about 12 eggs in the field; this number is higher than the number of yolked ovarian eggs observed in the present analysis for that species. However, Noble and Richards also cited five other female *R. olympicus* as laying 3-8 eggs in the laboratory.

Although the detailed size-class data used by Nussbaum and Tait to hypothesize age at metamorphosis and age at sexual maturity were not available for other populations, some information concerning the possible factors involved in geographic variation in life-history parameters in *Rhyacotriton* can be gleaned from the discussion above. *R. olympicus* and *R. cascadae* appear to transform at the largest sizes. If size is a good predictor of age, this might support the hypothesis of Wake (1980) that northern and inland populations take longer to reach transformation because of harsher conditions in those areas. These species have more trunk vertebrae on average than *R. kezeri* and *R. variegatus* (Table 4). However, if this hypothesis is valid, one might expect a cline toward decreasing size at transformation from north to south throughout the range of *Rhyacotriton*. The fact that the population with by far the smallest size at transformation is the northernmost population of *R. variegatus* suggests that a simple north-south, harsh-mild cline of this sort is not sufficient to explain all of the variation seen. However, north-south climatic differences along the coast are relatively slight.

Examination of other, smaller samples of *R. variegatus* between the Tillamook County and Curry County populations suggested a geographic pattern in size at metamorphosis, although only very weakly. Table 9 lists the smallest specimen observed and the percentage of transformed specimens smaller than 38 mm SVL for the Tillamook County and Curry County samples from Table 8 as well as for four populations between them. Small size at metamorphosis appears to be characteristic of populations at least as far south as Douglas County, Oregon, within 80 km of the Curry County sample with a larger transformation size. However, populations with a larger size at metamorphosis appear to range north to at least the vicinity of Valsetz, Polk County. In this area the populations with small size at transformation appear to occur on the west slope of the Coast Ranges and those with a larger transformation size on the east slope (in the Willamette River drainage). It is possible that a broad-scale pattern of variation in size at metamorphosis is present in

Table 9. Selected life-history parameters for populations of *Rhyacotriton variegatus* in central and southern Oregon.^a

Population	N (transformed)	Minimum transformed size	Transformed <38 mm SVL
Tillamook County (west slope)	59	31 mm	36.8%
Polk County (east slope)	19	38 mm	0.0%
Benton County (east slope)	21	37 mm	9.5%
Lane County (west slope)	12	33 mm	16.7%
Douglas County (west slope)	14	31 mm	42.8%
Curry County (west slope)	55	36 mm	10.9%

^a"Tillamook County (west slope)" and "Curry County" are the same as in Table 8. "East slope" and "west slope" refer to the east and west slopes of the Coast Ranges.

Rhyacotriton, but that it is superimposed by local environmental conditions, combining the regional hypothesis of Wake (1980) and the local hypothesis of Nussbaum and Tait (1977). Alternatively, historical constraints may be important. This possibility will be discussed in Chapter 10.

Although the northernmost species, *R. olympicus*, attains the largest body size, there does not appear to be any kind of north-south cline in this character. Overall body size is not clearly correlated with any sort of harsh-mild continuum as proposed by Wake (1980). While specimens from the northernmost populations are the largest in the genus, specimens from inland populations (*R. cascadae*) are among the smallest. Either local environmental factors or historical constraints (see Chapter 10) could be of importance to adult size in *Rhyacotriton*.

Nussbaum and Tait found that fecundity was higher in *R. variegatus* than in *R. cascadae*. In the present analysis, we found that fecundity (as measured by the number of yolked ovarian eggs) is higher in *R. kezeri* and northern *R. variegatus* than in any other populations in the genus. It is particularly noteworthy that these populations are in geographic proximity. This might suggest that environmental factors in this area of northwestern Oregon/ southwestern Washington select for increased fecundity. Although there may be a correlation with body size (cf. Kaplan and Salthe, 1979) within populations, there apparently is none among populations: very small females in the populations with high fecundity have more ovarian eggs than the largest females in populations with lower fecundity.

THE EVOLUTIONARY HISTORY OF *RHYACOTRITON*

In the preceding chapters we have demonstrated that *Rhyacotriton* consists of four apparently cohesive units that are genetically independent of each other: the four species *R. cascadae*, *R. kezeri*, *R. olympicus*, and *R. variegatus*. The genetic cohesion within each of these four species is suggested by the approximate intersection of the within-group comparison lines in Figure 3 with the origin, suggesting that the closer two populations are geographically, the more genetic influence they have on one another and the more similar they are in allozymes. There are, however, two possible mechanisms for this genetic cohesion. One is gene flow in the classical sense: the interchange of genetic material among populations through migration. The other, discussed by Slatkin (1985, 1987) and Wade and McCauley (1988), among others, involves extinction and recolonization of populations. If populations periodically go extinct at approximately the same rate throughout the range of a species, and if nearby populations are more likely to invade the area opened by an extinction event than are distant populations, the regression of all pairwise comparisons among populations will also pass through the origin, as in Figure 3.

These two processes are not mutually exclusive, and Slatkin (1985, 1987) considered extinction/recolonization to be a form of gene flow. However, the question of whether extinction/recolonization or gene flow through migration among populations predominates in *Rhyacotriton* warrants examination. Through extinction and recolonization, the patterns in Figure 3 could result even if all of the populations sampled were genetically isolated from all others (i.e., if migration among populations were nonexistent even within the four species).

Wright's F_{ST} (Wright, 1965) is a measure of the degree of subdivision of a set of populations into subunits, and therefore an indirect measure of the level of gene flow (Nm) among these subunits. The F_{ST} values seen in *Rhyacotriton* are among the highest recorded among salamanders (Larson, 1984), and indeed among the highest recorded in any organism. F_{ST} values for the species are: *R. cascadae*, 0.643; *R. kezeri*, 0.676; *R. olympicus*, 0.588; and *R. variegatus*, 0.689 (Good et al., 1987). The magnitudes of these values suggest that gene flow is minimal within the species, and it may be that gene flow is relatively unimportant to the patterns of genetic connectedness seen in *Rhyacotriton*.

Extinction and recolonization instead might be the major factors producing the patterns in Figure 3. However, it is hard to imagine that an area opened through extinction could be recolonized by animals from another population, when those same animals had been incapable of migrating the same distance to the population before it went extinct (unless there was some sort of active exclusion going on, possibly arising from preemptive occupancy of space, as discussed earlier). Additionally, if extensive extinction and recolonization (presumably by small subsets of nearby populations) were occurring, one would expect to see low levels of intrapopulational polymorphism, because polymorphism would be decreased at each recolonization event (by founder effects). Our data permit various interpretations. Some populations show no genetic variability in the loci studied, while others have heterozygosities ranging as high as 14.5% (Good et al., 1987). Populations near the *R. kezeri*-*R. variegatus* border have about average heterozygosities (6-8%).

Accordingly, we suggest that gene flow through migration plays an important role in determining the relationship of genetic distance to geographic distance in *Rhyacotriton*. We also think, however, that gene-flow rates are extremely low (with a strong correlation of F_{ST} with geographic distance), so that there may be essentially no exchange of alleles between populations at the extreme ends of the range of, for instance, *R. variegatus*: we envision a situation in which it might take longer than the lifetime of the species for an allele to move that far. Gene flow among adjacent populations, however, holds the species together as a cohesive unit.

Rhyacotriton therefore consists of four species held together internally by low rates of gene flow, and between which genetic contact is nonexistent. Figure 3 represents the patterns of genetic divergence within and among these populations. The patterns in a graph of this sort differ, depending on the evolutionary history of the groups involved (Good, in prep.). In a situation in which independent groups were formed through vicariance, within-group comparisons should show similar slopes and between-group comparisons should show a linear relationship of genetic distance to geographic distance. Most of the patterns seen in *Rhyacotriton* are indicative of vicariance: all of the within-group comparisons are similar and the between-group comparisons (except the comparisons of *R. olympicus* and *R. kezeri* with *R. cascadae*) form straight lines. Ignoring the *R. olympicus*-*R. kezeri* and *R. olympicus*-*R. cascadae* comparisons for the moment, the patterns seen in *Rhyacotriton* are best explained by two episodes of vicariance: an earlier one isolating the three taxa *R. variegatus*, *R. cascadae*, and the common ancestor of *R. olympicus* and *R. kezeri* from each other, and a later one isolating *R. olympicus* from *R. kezeri*. The genetic-distance comparisons relative to geographic distance for *R. cascadae*-*R. kezeri* and *R. cascadae*-*R. olympicus* do not meet the predictions from this scenario, because their comparison lines should fall in the vicinity of the lines representing the comparisons of each species with *R. variegatus*. However, the geographic distances measured for Figure 3 were straight-line distances, which may have been inappropriate. If *Rhyacotriton* had been distributed in the past as in Figure 18, the appropriate geographic distances from *R. olympicus* and *R. kezeri* to *R. cascadae* would have been much greater (around the curve in the distribution), pushing the comparison points to the right in Figure 3 to an appropriate position on top of the lines of comparison involving *R. variegatus*.

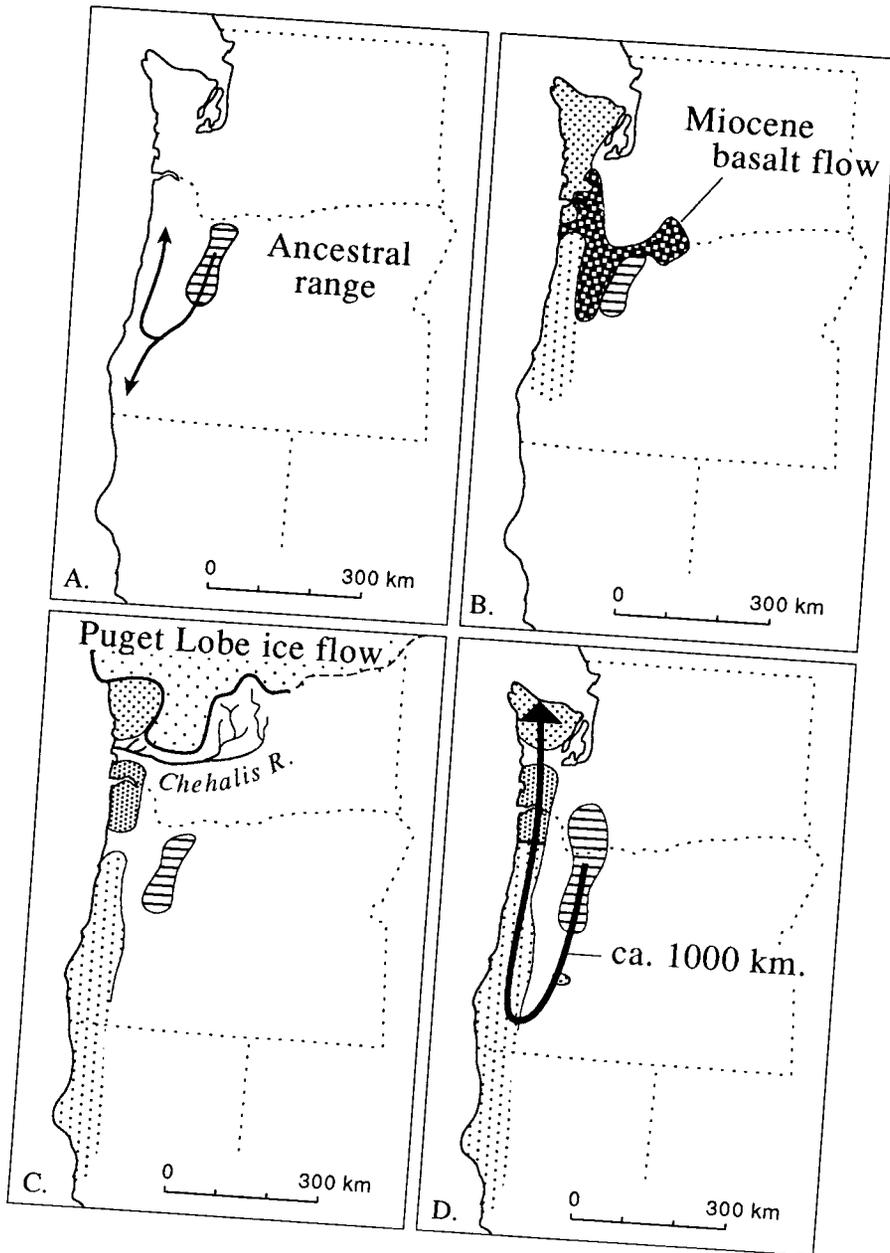


Figure 18. Hypothesized stages in the evolution of *Rhyacotriton*, as suggested by patterns of genetic distance in relation to geography (Figure 3). Present-day geography is used as a background outline, although details certainly differed through time. A. Hypothesized range of the species ancestral to the modern four species, in the northern Cascades of central Oregon, and dispersal into the Coast Ranges as they rose between 15-22 m.y. ago. B. Disruption of the continuous range by volcanic activity of Miocene age, with separation into three main segments. C. Glacial expansion into the Puget Sound region created a massive river that divided the northern coastal segment into two isolates. D. The distribution of the four extant species, showing the appropriate geographic distance measure for comparisons between *R. cascadae* and *R. olympicus*. Compare with Figure 1.

Good et al. (1987) proposed a preliminary historical scenario for the evolution of *Rhyacotriton*, suggesting that the ancestral *Rhyacotriton* inhabited the area now occupied by *R. cascadae* before the Cascade Mountains were uplifted, and that this ancestral form spread into the area of the Coast Ranges almost immediately after this land rose from the sea 15-22 million years ago (McKee, 1972; Cole and Armentrout, 1979). Calibrating an electrophoretic clock on these dates, they suggested that *R. cascadae*, *R. variegatus*, and the ancestor of *R. olympicus* and *R. kezeri* became isolated from each other 6.0-11.0 m.y. ago, and that *R. olympicus* and *R. kezeri* separated 4.0-6.5 m.y. ago. These values were based on the range of Nei (1978) distances among pairs of populations in these four groups. More appropriate times of divergence are based on the y-intercepts in Figure 3 (see Chapter 2 for discussion). This would place the former divergence at 6.4-9.5 m.y. and the latter at 3.2-4.7 m.y.

Present knowledge of the paleogeography of the Pacific Northwest suggests possible vicariant events involved in the diversification of *Rhyacotriton*. These events probably took place following the rise of the Coast Ranges, and they may have been associated with major disruptions of the Coast Ranges by ancient rivers and by the volcanism involved in the rise of the Cascades and more interior ranges. Columbia River Basalt, of Miocene age, is found in the Coast Ranges in the general vicinity of the contact between *R. kezeri* and *R. variegatus*, and this same formation fills the Willamette Valley and the Columbia River Gorge (McKee, 1972, fig. 11-9). The distribution of this extensive volcanic formation as well as its age suggests that it might have been a factor in the separation of a continuously distributed ancestral population (as in Figure 18) into units in the northern and southern Coast Ranges and in the southern Cascades. The second vicariant event may have been associated with the formation of the broad valley of the Chehalis River. During the last glacial period, the Puget Lobe of ice extended as far south as the northern front of the Olympic Mountains, and it completely blocked the connection of Puget Sound to the Pacific Ocean. It is thought that the Chehalis Valley was formed at this time, as dammed water from the Cascade and Olympic drainages into Puget Sound found access to the sea via the Chehalis River (McKee, 1972). However, genetic distances suggest an earlier divergence of *R. olympicus* from *R. kezeri* (see above). It is possible that similar, earlier events might have taken place, and one of these might have been the vicariant event responsible for the separation of these two forms. The populations of *R. cascadae* to the north of the Columbia River and of *R. variegatus* in the southern Cascades may be relatively recent incursions.

Morphological characters can be either environmentally or genetically controlled, and any single character will be affected by both factors (Shaffer, 1986). Most of the morphological characters discussed in the preceding chapters vary within species, but seem to show distinct breaks at the species boundaries—suggesting that, although environmental conditions may play an important role in geographic diversification, there is also a genetic component. If the characters studied in *Rhyacotriton* were dependent solely on environment, clines in character state would be unaffected by species boundaries. This is in fact the case with several morphometric characters (Chapter 7) and ovarian egg number (Chapter 9), and one might hypothesize that environmental trends override genetic interactions among populations in determining patterns of variation in these characters. These environmental trends may be quite complex, as witnessed by the pattern of ovarian egg number in *R. variegatus*.

Alternatively, similarity between two populations might be the result of neither similar environmental conditions nor genetic contact. Rather, phylogenetic constraint might be paramount. In the case of ovarian egg number, it is possible that northern *R. variegatus* and southern *R. kezeri* have particularly high numbers of eggs not because of some environmental factor in northwestern Oregon leading to high fecundity, but rather because the ancestral condition was to have many eggs. The relative importance of genetic, environmental, and historical factors in determining patterns of geographic variation in *Rhyacotriton* is not possible on the basis of available evidence.

TAXONOMIC HISTORY AND PHYLOGENETIC RELATIONSHIPS OF *RHYACOTRITON*

Rhyacotriton currently is considered to be a member of the Dicamptodontidae, otherwise containing only the genus *Dicamptodon* (Frost, 1985). Until recently (Edwards, 1976), these genera were considered to be members of the Ambystomatidae, and the two families still are often thought to be closely allied. Here we examine the evidence for the relationships of *Rhyacotriton* that have been discussed in past analyses of taxonomy and phylogenetics, in order to test the validity of these views.

Ranodon olympicus was described by Helen Thompson Gaige in 1917 from a series of specimens collected in 1916 at Lake Cushman in the Olympic Mountains of northwestern Washington. *Ranodon*, a member of the Hynobiidae, otherwise is known only from central Asia, and Gaige not surprisingly found the occurrence of the genus in western North America to be "noteworthy."

Although Gaige considered *R. olympicus* to "undoubtedly represent a new species of the genus *Ranodon*," she did not discuss the evidence which led her to this conclusion. Dunn (1920), on the other hand, recognized that *R. olympicus* is more similar in morphology to *Ambystoma* and *Dicamptodon* than to *Ranodon* and the other hynobiids in having an otoglossal and lacking a second epibranchial in the hyoid, and in the presence of long, broad premaxillary nasal processes that meet at the midline. He therefore removed *R. olympicus* from *Ranodon* and erected the genus *Rhyacotriton* to contain this single distinctive species which he allied with the ambystomatids. Dunn also stated that:

Ambystoma, in a broad sense, and *Rhyacotriton*, have similar prevomers, and dentition that varies from that of *Rhyacotriton olympicus* and *Ambystoma ensatum*, both of which rather resemble *Ranodon*, to the different condition observed in *Ambystoma tigrinum*. In this species there is a continuous, nearly straight, row of teeth across the roof of the mouth.

This is rather poorly worded, but we take it to mean, first, that *Rhyacotriton* and *Ambystoma* (including *Dicamptodon*, which Dunn called *A. ensatum*) have similar vomers (=prevomers) and, second, that *Rhyacotriton* and *Dicamptodon* have vomerine teeth that are more similar to those of *Ranodon* than to those of *Ambystoma*.

Dunn (1926) listed the following as features of the Ambystomatidae (including *Dicamptodon* and *Rhyacotriton*): No anterior projection of the pubis; an ypsiloid cartilage; body muscles primitive (a rectus abdominus superficialis and no rectus abdominus profundus); lungs present; no naso-labial groove; a well developed otoglossal; columella and operculum variable; no posterior projections of the prevomers; no trace of a postfrontosquamosal arch; a bony pterygoid; and no neural ridge on the amphicoelous vertebrae.

In 1927, Noble agreed with a conclusion he attributed to Dunn that *Rhyacotriton* and *Dicamptodon* are closely allied and differ from *Ambystoma* in "certain skull features." It is possible that Noble was referring to the statement by Dunn (1920) quoted above, but, if so, it seems that Noble misinterpreted it; Dunn did not suggest this relationship in either of the papers he wrote discussing these taxa (Dunn, 1920, 1922). However, it is unclear from Noble's work whether he was citing a paper by Dunn or quoting something Dunn had said in person. In either case, it appears that Noble (1927) was the first to suggest in print that *Dicamptodon* and *Rhyacotriton* are closely related relative to all other salamanders (he had referred to them in 1925 as "closely related," but he did not say with respect to what other taxa). No specific characters were provided to support this hypothesis in his 1927 work, but in 1931 Noble went on to state that "*Rhyacotriton* is apparently a dwarf derivative of *Dicamptodon*" and pointed out that both differ from *Ambystoma* in possessing lacrimals and having stream-type larvae.

Noble (1927) questioned Dunn's (1920, 1922, 1926) hypothesis that *Rhyacotriton* and *Dicamptodon* are allied with *Ambystoma*, although he continued to include them in the Ambystomatidae. To him the morphological characters listed by Dunn did not appear to be so "fundamental" that *Dicamptodon* and *Rhyacotriton* could not have evolved separately from the Hynobiidae (i.e., from what Noble viewed as a basal salamander stock).

The observation of "streptostyly" (pivoting of the squamosal, pterygoid, and quadrate as a unit on the side of the prootic) in *Rhyacotriton* and some *Ambystoma* led Eaton (1933, 1934) to suggest that *Rhyacotriton* is related to *Ambystoma* and not to *Dicamptodon*. He suggested that the presence of the lacrimal in *Rhyacotriton* is the result of "an arrest of development preventing the loss of the lacrimal bone at metamorphosis," while in *Dicamptodon*, the presence of the element is a retention of a primitive character. Based on this hypothesis of relationships, he further suggested that *Dicamptodon* had stream-type larvae long before *Rhyacotriton* evolved from *Ambystoma* and re-invaded stream habitats. De Villiers (1938), however, disagreed with the interpretation of the jaw suspension as streptostylic in these salamanders.

Tihen (1958) agreed with the by-then generally accepted placement of *Rhyacotriton* and *Dicamptodon* in the Ambystomatidae, and defined the resulting family as containing "salamanders having the angular fused with the articular; second epibranchial lost; premaxillary spines elongated; prevomers without extensive processes extending over the parasphenoid region; vertebrae amphicoelous; skull without a frontosquamosal arch; [and] fertilization internal." He divided this family into the three subfamilies Dicamptodontinae (containing *Dicamptodon*), Rhyacotritoninae (containing *Rhyacotriton*), and Ambystomatinae (containing the remaining forms). Tihen pointed out that *Rhyacotriton* and *Dicamptodon* share several features that are unique among ambystomatids (failure of the prootic and exoccipital to fuse into a single periotic bone, presence of a free lacrimal, resemblance of the hyoid to that of hynobiids, an M-shaped pattern of vomerine teeth, three

phalanges on the fourth toe, usual lack of an enclosed procoracoid foramen in the pectoral girdle [Stokely and Holle, 1954], and larvae with the dorsal fin not extending onto the trunk), but he went on to say that

despite these many resemblances each form, particularly *Rhyacotriton*, has so many unique characteristics that the two are no more similar to each other than to the remainder of the family. All of these resemblances represent apparently primitive features; none of the specialized characteristics of either form are found in the other. The points of similarity do not, then, denote any close relationship between the two, but simply indicate that the lines leading to these two forms were derived from the ancestral proto-Ambystomatidae at an early stage, prior to the development of the specializations characteristic of the remainder of the family.

Tihen (1958) also suggested that, in a number of characters, *Rhyacotriton* more closely resembles *Ambystoma* than *Dicamptodon*. These characters were: conical teeth which may be slightly hooked at the tip; the loss of palatine teeth; the cartilaginous condition of the hyobranchial apparatus; an annular otoglossal; and a premaxillary fontanelle.

Regal (1966) viewed *Dicamptodon* and, particularly, *Rhyacotriton* as intermediate between the Ambystomatidae and the Plethodontidae:

Rhyacotriton and *Dicamptodon* in many respects resemble hynobiids and, for that matter, plethodontids, more than they resemble species of the genus *Ambystoma*. The tendency toward an "M" shaped palatal dentition in *Rhyacotriton* and *Dicamptodon*, in hynobiids, and its extreme in plethodontids, is in contrast to the condition in *Ambystoma* and all other salamanders (see also Tihen, 1958). In reduction of lungs and hydrostatic mechanisms, association with mountain-brook habitat, as well as in certain cardiovascular modifications (Noble, 1925), *Rhyacotriton* resembles the hypothetical ancestral plethodontid (Wilder and Dunn, 1920; Noble, 1925; Dunn, 1928). The premaxillaries of *Rhyacotriton*, with long slender nasal processes forming a prominent fontanelle, recall the plethodontid condition rather than the terrestrial ambystomatid condition.

Although Regal stated that "it is necessary to conclude that plethodontids are probably more closely related to the Dicamptodontinae than to the more 'typical' ambystomatids," it seems clear that he was not referring to a strict genealogical relationship (as suggested by Estes, 1981) because he later stated that "it similarly appears that the hynobiids are also more closely related to *Rhyacotriton* stock than to *Ambystoma* stock." Regal retained *Rhyacotriton* and *Dicamptodon* as the single subfamily Dicamptodontinae in the Ambystomatidae, and it therefore seems that these comments were made simply to emphasize the point made by Tihen (1958) that *Rhyacotriton* and *Dicamptodon* represent an early offshoot of the Ambystomatidae which in many respects resembles the presumed ancestral stock that gave rise to the plethodontids and hynobiids.

Salthe and Kaplan (1966) found that both *Dicamptodon* and *Rhyacotriton* are immunologically more similar to *Amphiuma* in lactic dehydrogenase than is *Ambystoma*. This, however, provides no evidence concerning the relationships of these genera to each other.

Krogh and Tanner (1972) analyzed various characters of the hyobranchium and throat musculature of *Dicamptodon*, *Rhyacotriton*, and *Ambystoma*, which they assumed to form a monophyletic group because they were all considered at the time to be members of the single family Ambystomatidae. They concluded that Tihen (1958) was correct in considering *Rhyacotriton* and *Dicamptodon* as offshoots of an early ambystomatid stock.

However, because they provided no diagnostic characters for their "Ambystomatidae," and because they did not compare these genera to any other salamander groups, they provided only an argument for the monophyly of *Ambystoma* with respect to *Dicamptodon* and *Rhyacotriton*; no evidence was presented either for or against a closer phylogenetic relationship of any of these genera with other salamanders.

Edwards (1976) analyzed the pattern of spinal nerve roots in relation to vertebrae and found that *Rhyacotriton* and *Dicamptodon* are unique in having all of the postsacral and the anteriormost presacral (atlas) nerves exiting through vertebral foramina, but all other presacral nerves exiting between vertebrae. Other salamanders either have more of the presacral nerves exiting through foramina, or have postsacral nerves that exit between vertebrae. Edwards used this similarity as the basis for erecting the separate family Dicamptodontidae, which he considered to be the sister group to the Ambystomatidae (excluding *Rhyacotriton* and *Dicamptodon*) plus Plethodontidae. The relationship of the Dicamptodontidae to this clade was hypothesized on the basis of a similar vomerine-tooth replacement pattern.

Hecht and Edwards (1977) reanalyzed the characters of Edwards (1976) and initially came to the same conclusions concerning the relationships of *Rhyacotriton* and *Dicamptodon*. They then weighted their characters according to a variety of criteria and hypothesized, on the basis of this new analysis, that the Dicamptodontidae may be the outgroup for the clade including the Ambystomatidae, Plethodontidae, Salamandridae, and perhaps Sirenidae. The relationship of these families was based on a hypothesized transformation series in spinal-nerve morphology from the dicamptodontid type to an ambystomatid-plethodontid type to a salamandrid-sirenid type.

Arnold (1977) compared courtship behavior among salamander groups including *Rhyacotriton*, and found a "peculiar tail-wagging display" prior to spermatophore deposition in the genus. It was not observed in any other salamander.

Milner (1983) pointed out that Edwards' (1976) diagnosis of the Dicamptodontidae, using spinal-nerve morphology, essentially was based on primitive salamander characters and did not include any unique features. Milner reanalyzed the phylogenetic relationships among the salamander families and hypothesized that the Dicamptodontidae is polyphyletic, in that *Rhyacotriton* is more closely related to the Plethodontidae and Amphiumidae while *Dicamptodon* is closer to the Ambystomatidae and Salamandridae. The characters listed allying *Rhyacotriton* with the Plethodontidae and Amphiumidae were loss of a true operculum, loss of the ypsiloid cartilage on the pelvis, and reduction or loss of the pterygoids. *Dicamptodon* was allied with the Ambystomatidae and Salamandridae by enlargement of the levator scapulae, lateral vomerine tooth replacement, and loss or fusion of the angular.

Duellman and Trueb (1985) accepted the placement of *Rhyacotriton* and *Dicamptodon* in the family Dicamptodontidae on the basis of Estes' (1981) opinion that the spinal-nerve similarities outlined by Edwards (1976) are sufficient to warrant familial status. They then conducted a cladistic analysis of relationships among the salamander families and concluded that the Dicamptodontidae is the sister group to a clade containing the Amphiumidae, Salamandridae, Ambystomatidae, and Plethodontidae. The Dicamptodontidae was allied with these taxa on the basis of maxillary presence, formation of the nasal from only the lateral ossification center, a diploid chromosome number of 22-28 with a gradual reduction from macrochromosomes to microchromosomes, and the

spinal-nerve transformation series discussed by Hecht and Edwards (1977). A character provided by Duellman and Trueb on the basis of their analysis diagnosing (but not restricted to) the Dicamptodontidae was the presence of 26-28 chromosomes without microchromosomes.

Nussbaum (1985) analyzed the distribution of parental care among salamander groups. He found that, unlike *Dicamptodon*, *Rhyacotriton* adults do not guard their eggs. Parental care of eggs is usually present among terrestrial direct-developing salamanders and usually absent from aquatic forms. Nussbaum also pointed out that *Rhyacotriton* have fewer larval gill rakers than most other salamanders (see also Valentine, 1964, and Valentine and Dennis, 1964).

Despite familiarity with the work of Edwards (1976), Laurent (1986) chose to return to the classification of Tihen (1958) in recognizing three subfamilies (Rhyacotritoninae containing only *Rhyacotriton*) in the Family Ambystomatidae. He added no new characters or new interpretations, but emphasized that the ambystomatids together with the Plethodontidae, Amphiumidae, and the extinct Scapherpetontidae should be united in a suborder Ambystomatoidea.

Kraus (1987, 1988), in his analysis of phylogenetic relationships among the species of *Ambystoma*, presented a cladogram of salamander family relationships based on work in progress. He placed *Rhyacotriton* and *Dicamptodon* together as the sister group to the Ambystomatidae, but no specific characters were discussed.

Sever (1991) conducted an analysis of phylogenetic relationships among the salamander families, using cloacal anatomy characters. Although he found that *Dicamptodon*, *Rhyacotriton*, and *Ambystoma* are unique among salamanders in having derived folds from the dorsolateral walls of the cloaca of the male, he considered this synapomorphy to be outweighed by two characters *Rhyacotriton* shares with plethodontids and proteids: (1) Extending of the epidermis into the lining of the female cloacal chamber. This is seen also in some amphiumids, some hynobiids, some salamandrids, and sirenids, but Sever considered it diagnostic only of hynobiids, sirenids, proteiids, plethodontids, and *Rhyacotriton*. (2) Presence of ciliated epithelium in the cloacal tube and/or cloacal chamber of females. This is seen also in sirenids and some salamandrids, but Sever did not consider it diagnostic of the Salamandridae.

The most recent evaluation of familial relationships among salamanders is that of Larson (1991), who presented phylogenetic hypotheses based on a comprehensive comparative study of aligned sequences of 233 nucleotides encoding 28s ribosomal RNA as a point of departure. Among a number of surprising results, he found that *Rhyacotriton* appears not to be closely related to any of the other taxa tested. *Dicamptodon* was found to be the sister taxon of the Ambystomatidae, but this clade was remote from *Rhyacotriton*. The relationships inferred by Larson view *Rhyacotriton* as one of the most basal among salamander families (together with the Amphiumidae and Plethodontidae, which form a weak sister group). Larson undertook a re-evaluation of the 29 characters used by Duellman and Trueb (1985) in order to determine how divergent his molecular results were in relation to the morphological data. Following this re-evaluation, Larson found that the ribosomal tree is somewhat less parsimonious in relation to the morphological data than the tree presented by Duellman and Trueb, but the difference was not found to be statistically significant. Larson argued that homoplasy is so common in both trees as to make rejection of either impossible on the basis of currently available data. No new insights into

characters of significance in determining relationships of *Rhyacotriton* to other families were obtained, but Larson again showed that morphological evidence for determining phylogenetic relationships of the taxon are ambiguous.

As discussed above, a variety of morphological characters have been used by different workers for generating hypotheses concerning the phylogenetic relationships of *Rhyacotriton*. We here re-examine these characters individually to assess their applicability to the analysis of the relationships of the genus. Character polarity in this discussion is based on comparison with other living and fossil amphibians as outgroups. Several genera of potential significance (particularly some of those currently assigned to the Hynobiidae) have been unavailable for study. Accordingly, we leave a full analysis of the cladistic relationships of the families and genera of salamanders to a later time, and here concentrate on the immediate question of whether any character evidence allies *Rhyacotriton* to *Dicamptodon* or *Ambystoma*, the most often-cited potential relatives, or to any other genera or families.

The following is an annotated list of all the characters included by previous workers in discussions of the systematic placement of *Rhyacotriton*:

1. Long, broad premaxillary nasal processes. Dunn (1920) listed this as a character allying *Rhyacotriton* with *Dicamptodon* and *Ambystoma* rather than with the Hynobiidae, and Tihen (1958) listed it as a character of the Ambystomatidae (sensu lato, including *Rhyacotriton* and *Dicamptodon*). The presence of long processes on the premaxilla is a derived condition among salamanders, and all salamanders except hynobiids and cryptobranchids have such processes. Regal's assertion (1966) that the prominent fontanelle between the premaxillae in *Rhyacotriton* approximates the plethodontid condition more than that of *Ambystoma*, is obscured by variability in the Plethodontidae. Some plethodontids approximate *Ambystoma* considerably more closely than they resemble *Rhyacotriton*, and in fact this is true of the ancestral plethodontid morphology hypothesized by Wake and Larson (1987).

2. Maxilla presence. The cladogram of Duellman and Trueb (1985) requires that a reversal to the ancestral condition of maxillary presence constitutes a synapomorphy that unites *Dicamptodon* and *Rhyacotriton* with the Amphiumidae, Salamandridae, Ambystomatidae, and Plethodontidae, all of which possess maxillae. However, unless the relationships outlined in their analysis are accepted a priori, this character must be considered to be ancestral. Maxillae are absent only in larvae and in adults of a variety of perennibranchiate forms.

3. Tooth shape. Tihen (1958) suggested that *Rhyacotriton* more closely approximates *Ambystoma* in tooth shape than it does *Dicamptodon*. Tooth shape is variable in many salamander families, and Tihen's observation was not corroborated by the detailed analysis of Beneski and Larsen (1989).

4. Nasal formation. Duellman and Trueb (1985) used formation of the nasal from only the lateral of the two ossification centers (Jurgens, 1971) as a synapomorphy of the Amphiumidae, Salamandridae, Ambystomatidae, Plethodontidae, *Dicamptodon*, and *Rhyacotriton*. The ancestral condition, in which the nasals form from both ossification centers, is seen in hynobiids and cryptobranchids. In sirenids the nasals form from only

the medial center. Nasals are absent from proteids (i.e., ossification is from neither center). In all other salamanders, only the lateral center ossifies.

5. Lacrimal presence. Both Noble (1931) and Tihen (1958) cited the presence of lacrimals in the adult as differentiating *Rhyacotriton* and *Dicamptodon* from *Ambystoma*. Lacrimals are absent from all larval salamanders and perennibranchiates. Among transforming salamanders other than the above three taxa, lacrimals are present in hynobiids and lacking in salamandrids and plethodontids. Lacrimal presence is ancestral for the Caudata.

6. Frontosquamosal arch absence. The absence of a frontosquamosal arch was listed as an ambystomatid (sensu lato) character by Dunn (1926) and Tihen (1958). The presence of a frontosquamosal arch is seen only in some salamandrids and apparently is derived within that family.

7. Streptostyly. A capacity for some movement in the back of the skull in *Rhyacotriton* and some *Ambystoma* led Eaton (1933, 1934) to suggest a relationship of these genera to the exclusion of *Dicamptodon*. However, Eaton observed streptostyly in some *Ambystoma* only, and it is therefore a synapomorphy of *Rhyacotriton* with only those species. If it is assumed that *Ambystoma* is monophyletic, the usefulness of this character, even if it is present (see de Villiers, 1938), is questionable.

8. Vomer shape. Dunn (1920) made obscure reference to a similarity of the vomers of *Rhyacotriton* to those of *Dicamptodon* and *Ambystoma*, but the only explicit vomer character that has been discussed is the lack of a posterior process overlying the parasphenoid mentioned by Dunn (1926), Tihen (1958), and Regal (1966). This is primitive among salamanders, with separately derived conditions occurring in plethodontids, salamandrids, and to some degree in *Amphiuma*.

9. Vomerine teeth. Both Tihen (1958) and Regal (1966) made reference to an M-shaped vomerine tooth pattern, with regard to a similarity of *Rhyacotriton*, *Dicamptodon*, the Plethodontidae, and the Hynobiidae as opposed to *Ambystoma*. As pointed out by Tihen, this is a primitive salamander character. Edwards (1976) suggested that his Dicamptodontidae (*Dicamptodon* and *Rhyacotriton*) is related to the Ambystomatidae and Plethodontidae on the basis of lateral to medial vomerine tooth replacement, and Milner (1983) suggested that *Dicamptodon* (but not *Rhyacotriton*) is related to the Salamandridae and Ambystomatidae on the basis of a different interpretation of the same character. According to Regal (1966), tooth replacement is primitively from posterior to anterior, and it diverges from this significantly only in those families in which there is a pronounced extension of the vomers posteriorly over the parasphenoid (see above). In these families, replacement is lateral to medial in plethodontids and medial to lateral in salamandrids. Both *Rhyacotriton* and *Dicamptodon* retain the primitive condition. An alternative interpretation of this character was presented by Larson (1991), who considered "parallel replacement" (i.e., that found in species in which the vomerine teeth lie in a position parallel to the maxillary teeth) to be ancestral, and "posterior replacement" (found in *Rhyacotriton*, among other genera) to be derived. This interpretation provides a potential synapomorphy of the Hynobiidae, *Rhyacotriton*, *Dicamptodon*, and *Ambystoma*. Based on his analysis of RNA sequences, Larson argued that the character has arisen three times (recognizing the last two genera as sister taxa) among salamanders.

10. Palatine teeth. Tihen (1958) listed loss of the palatine teeth as a similarity between *Ambystoma* and *Rhyacotriton* as opposed to *Dicamptodon*. No transformed salamander

has palatine bones, and most larvae, including *Rhyacotriton* (Worthington and Wake, 1971) and at least some *Ambystoma* (Larsen, 1963), have teeth on the palatopterygoids.

11. Pterygoid reduction or loss. Milner (1983) listed the reduction of the pterygoids in *Rhyacotriton* and *Amphiuma* and their loss in plethodontids as a synapomorphy. Milner was assuming a transformation series from unreduced to reduced to absent pterygoids. Since there is no particular reason to assume that pterygoids had to be reduced in an ancestral plethodontid before they were lost in the modern Plethodontidae, reduction in *Rhyacotriton* is not a compelling synapomorphy. Dunn (1926) listed pterygoid presence as a character of the Ambystomatidae (sensu lato), but he did not view this as evidence for monophyly.

12. Fusion of otic capsule bones. Lack of fusion in the adult was listed by Tihen (1958) as a primitive character retained in *Rhyacotriton* and *Dicamptodon*, but not seen in *Ambystoma*. This character is variable in *Rhyacotriton* (see Chapter 5).

13. Otic sac. This is a rather complicated character, which was first developed by Lombard (1977) and treated in modified form by Duellman and Trueb (1985) and Larson (1991). *Rhyacotriton* has a bulbar, unvascularized sac. This is a derived state by Larson's interpretation, and is shared by *Dicamptodon*, proteids, hynobiids, and cryptobranchids; alternatively, because this is a simple or simplified state, it might be ancestral.

14. Volume of periotic cistern. A small-volume periotic cistern, interpreted by Larson (1991) as derived, occurs in *Rhyacotriton* and is shared with amphiumids, but also with some salamandrids and plethodontids. Because both of these latter families are clearly monophyletic, this character is a good candidate for homoplastic evolution. In other respects *Rhyacotriton* appears to have ancestral traits for the otic sac.

15. Angular loss. Tihen (1958) listed angular loss as a character of the Ambystomatidae (sensu lato), and Milner (1983) suggested that it is a synapomorphy of *Dicamptodon*, *Ambystoma*, and the Salamandridae. However, Milner was incorrect in his coding of several other salamander taxa. Angulars are present only in cryptobranchids and hynobiids, and are primitive.

16. Loss of the operculum. Milner (1983) suggested that this character is a synapomorphy of *Rhyacotriton*, *Amphiuma*, and the Plethodontidae. However, the operculum is lacking only in larval forms, paedomorphs, and *Rhyacotriton*, and is present in plethodontids. Milner stated that it is replaced by a "neomorph" in plethodontids and *Amphiuma*, but this is by no means clear (Monath, 1965).

17. Cartilaginous hyobranchium. Tihen (1958) suggested that the hyobranchium of *Rhyacotriton* and *Ambystoma* is more cartilaginous than that of *Dicamptodon*. This is a variable character in all salamander families, although in general *Rhyacotriton* resembles plethodontids more than other families in the mainly cartilaginous nature of this complex system.

18. Otoglossal presence. This was proposed by Dunn (1920, 1926) as evidence for a relationship of *Rhyacotriton* with the Ambystomatidae (including *Dicamptodon*) rather than with the Hynobiidae. The otoglossal cartilage is present as a semicircular ring in the hyoid apparatus of adult *Rhyacotriton* and *Ambystoma* and as a plate in *Dicamptodon* (Krogh and Tanner, 1972). It is lacking as such from all other salamanders, but Krogh and Tanner suggested that the interrational cartilage of salamandrids (Özeti and Wake, 1969) and the lingual cartilage or "Sehnenplatte" of some plethodontids (Piatt, 1935; Lombard and Wake, 1977) may be modifications of the same structure. Salamander hyobranchial homologies

are unclear, and this uncertainty casts doubt on the quality of this character as a synapomorphy for *Ambystoma*, *Rhyacotriton*, and *Dicamptodon*; if Krogh and Tanner are correct, no synapomorphy exists for this group. Tihen (1958) pointed out that the annular nature of the otoglossal in *Rhyacotriton* and *Ambystoma* differentiates it from *Dicamptodon*. The *Dicamptodon* condition is an autapomorphy.

19. Loss of the second epibranchial. Dunn (1920) suggested that this character linked *Rhyacotriton* with *Dicamptodon* and the ambystomatids rather than with the hynobiids. Loss of the second epibranchials is seen also in adult salamandrids and plethodontids.

20. Amphicoelous vertebrae. This was listed as a character of the Ambystomatidae (sensu lato) by Dunn (1926) and Tihen (1958), who did not claim that it was a derived character. In fact it is primitive, being seen in all families except the Salamandridae and Plethodontidae, which show separately derived conditions (Wake and Lawson, 1973).

21. Spinal-nerve patterns. Edwards (1976) viewed the pattern of spinal nerves as being of importance to salamander phylogenetic reconstruction. He suggested that the primitive state (seen in cryptobranchids, hynobiids, and proteids) was to have all nerves exiting between vertebrae. *Dicamptodon* and *Rhyacotriton* were considered to be related because they are the only two genera to show spinal nerves exiting mainly between vertebrae anterior to the sacrum, but exiting through vertebral foramina along the tail. In all other families, the spinal nerves exit through foramina in both the presacral and postsacral vertebrae, except in the anterior two or three vertebrae, where the pattern of penetration varies from family to family. Edwards viewed the evolution of spinal nerve patterns as a transformation series from the ancestral state through the state in *Dicamptodon* and *Rhyacotriton* to the various states seen in the other salamander families. However, as pointed out by Milner (1983), the fact that *Dicamptodon* and *Rhyacotriton* show an intermediate condition in this transformation series means that they share no clear synapomorphy; either could be closer to the taxa with a further derived condition.

22. Presence of three larval gill rakers. Nussbaum (1985) demonstrated that *Rhyacotriton* have three gill rakers. This is unique to the genus.

23. Procoracoid foramen. Tihen (1958) listed the lack of a procoracoid foramen as a primitive character present in *Rhyacotriton* and *Dicamptodon* but lacking from *Ambystoma*. The presence or position of this foramen is widely variable in most salamander groups.

24. Number of phalanges in the fourth toe. Tihen (1958) listed the presence of three phalanges in the fourth toe of *Rhyacotriton* and *Dicamptodon* as a primitive character seen in these two genera but lacking from *Ambystoma*, which has four phalanges. Three phalanges are seen generally in salamanders except in some *Ambystoma* (Kraus, 1987, 1988) and taxa with reduced limbs (e.g., Amphiumidae and Proteidae).

25. Abdominal muscles. Dunn (1926) listed "body muscles primitive" as a character of the Ambystomatidae including *Dicamptodon* and *Rhyacotriton*; clearly he did not suggest this "primitive" character as evidence for the monophyly of the family.

26. Enlarged levator scapulae. Enlargement of this muscle so that its posterior insertion occupies the entire anterior edge of the suprascapula was listed by Milner (1983) as a synapomorphy of the Ambystomatidae, Salamandridae, and *Dicamptodon* (with a further derived condition in many salamandrids). It is lacking in *Rhyacotriton*, as it is in other transforming salamanders than those listed above. The condition in larvae and pedomorphs is not comparable.

27. Lack of an anterior projection of the pubis. Dunn (1926) listed this as a character of the Ambystomatidae (including *Dicamptodon* and *Rhyacotriton*). He also listed it as a character of several other salamander families, however, and clearly did not consider it to be evidence for relationship.

28. Lung reduction. Lung reduction was cited by Regal (1966) as a similarity between *Rhyacotriton* and the Plethodontidae. Lung reduction is a derived feature among salamanders, but it is also known in a variety of other, clearly unrelated, taxa (e.g., the hyobiiid *Onychodactylus* and various salamandrid genera) and cannot be taken as strong evidence for relationship. Lung presence was listed by Dunn (1926) as a character of the Ambystomatidae (sensu lato) as well as of all other families except the Plethodontidae.

29. "Cardiovascular modifications." Regal (1966) also cited certain modifications of the cardiovascular system (Noble, 1925) as being similar in *Rhyacotriton* and the Plethodontidae. However, these modifications are directly correlated with lung reduction and cannot be considered as characters separate from it.

30. Loss of the ypsiloid cartilage. This was cited by Milner (1983) as a synapomorphy of *Rhyacotriton*, *Amphiuma*, and the Plethodontidae. However, it too is clearly correlated with lung reduction and therefore should not be considered as a separate character. Dunn (1926) listed its presence as a character of the Ambystomatidae (sensu lato), but did not consider it as evidence for the monophyly of the family.

31. No nasolabial groove. Dunn (1926) listed this as a character of the Ambystomatidae, as well as of all other families except the Plethodontidae. The plethodontid condition is derived.

32. Derived folds from the dorsolateral walls of the cloaca of the male. Sever (1991) found that *Dicamptodon*, *Rhyacotriton*, and *Ambystoma* are unique in having this character state.

33. Extension of the epidermis into the lining of the female cloacal chamber. This character was listed as diagnostic of hynobiids, sirenids, proteiids, plethodontids, and *Rhyacotriton* by Sever (1991).

34. Presence of ciliated epithelium in the cloacal tube and/or cloacal chamber of females. This character was listed by Sever (1991) as being present in the derived state in *Rhyacotriton*, plethodontids, proteiids, and sirenids.

35. Stream-type larvae. Noble (1931), Tihen (1958), and Regal (1966) all pointed out that stream-type larvae occur in both *Rhyacotriton* and *Dicamptodon*. However, this larval type (relatively small gills, reduced tail fins, etc.) is well known to be correlated with habitat and occurs in other salamander families as well.

36. Internal fertilization. This was listed by Tihen (1958) as a character of the Ambystomatidae (sensu lato). It is also characteristic of all other salamanders except the Hynobiidae and Cryptobranchidae (the state in the Sirenidae is unclear).

37. Chromosome number. Duellman and Trueb (1985) suggested a transformation series from a high number of chromosomes with microchromosomes (hynobiids, cryptobranchids, proteiids) through a state with 22-28 chromosomes with microchromosomes (amphiumids, salamandrids, and sirenids) to a state with 26 or 28 chromosomes with no microchromosomes. They suggested that this third state is a synapomorphy of *Rhyacotriton* and *Dicamptodon*, but it is also present in ambystomatids and plethodontids (which, based on their proposed phylogeny, they viewed as convergent).

Table 10. The distribution of character states among the salamander families, and in the genera *Dicamptodon* and *Rhyacotriton* in the 11 phylogenetically informative characters discussed by previous workers in connection with the relationships of *Rhyacotriton* (see text). 0 = ancestral, 1 = derived, U = unknown, NA = not applicable. Unknown states are represented by dashes. Hyn = Hynobiidae, Cry = Cryptobranchidae, Pro = Proteidae, Sir = Sirenidae, Amp = Amphiumidae, Dic = *Dicamptodon*, Rhy = *Rhyacotriton*, Amb = Ambystomatidae, Sal = Salamandridae, Ple = Plethodontidae.

Character	Hyn	Cry	Pro	Sir	Amp	Dic	Rhy	Amb	Sal	Ple
1. Premaxillary nasal process short (0), elongate (1)	0	0	1	1	1	1	1	1	1	1
4. Medial nasal center ossified (0), unossified (1)	0	0	NA	0	1	1	1	1	1	1
5. Lacrimal present in adult (0), absent (1)	0	NA	NA	NA	NA	0	0	1	1	1
15. Angular present (0), absent (1)	0	0	1	1	1	1	1	1	1	1
18. Otoglossal absent in adult (0), present (1) ^a	0	NA	NA	NA	NA	1	1	1	1	1
19. Second epibranchial present in adult (0), absent (1)	0	NA	NA	NA	NA	1	1	1	1	1
21. Spinal nerves all intervertebral (0), presaccrals intervertebral, postsaccrals through foramina (1), pre- and postsaccrals through foramina (2) ^b	0	0	0	2	2	1	1	2	2	2
25. Insertion of levator scapulae narrow (0), broad (1)	0	NA	NA	NA	NA	1	0	1	1	0
31. Folds from the dorsolateral walls of the cloaca of the male	0	0	0	0	0	1	1	1	0	0
32. Extending of the epidermis into the lining of the female cloacal chamber	1	0	1	1	0	0	1	0	0	1
33. Presence of ciliated epithelium in the cloacal tube and/or cloacal chamber of females	0	0	1	1	0	0	1	0	0	1
35. Fertilization external (0), internal (1)	0	0	1	U	1	1	1	1	1	1
36. Chromosomes many, with microchromosomes (0), 22-28 (with microchromosomes) (1), 26-28 (without microchromosomes) (2) ^b	0	0	0	1	1	2	2	2	1	2

^aAssumes homology of the otoglossal with the the salamandrid and plethodontid structures discussed in the text.

^b Transformation series 0-1-2.

38. Tail-wagging display. This behavior was found by Arnold (1977) only in *Rhyacotriton* and is undoubtedly an autapomorphy.

39. Lack of parental care. Nussbaum (1985) found this to be the case in *Rhyacotriton*. Many aquatic-breeding salamanders such as ambystomatids and salamandrids do not exhibit parental care. This may be a primitive or a derived state, but in either case it provides little information concerning the phylogenetic relationships of *Rhyacotriton*.

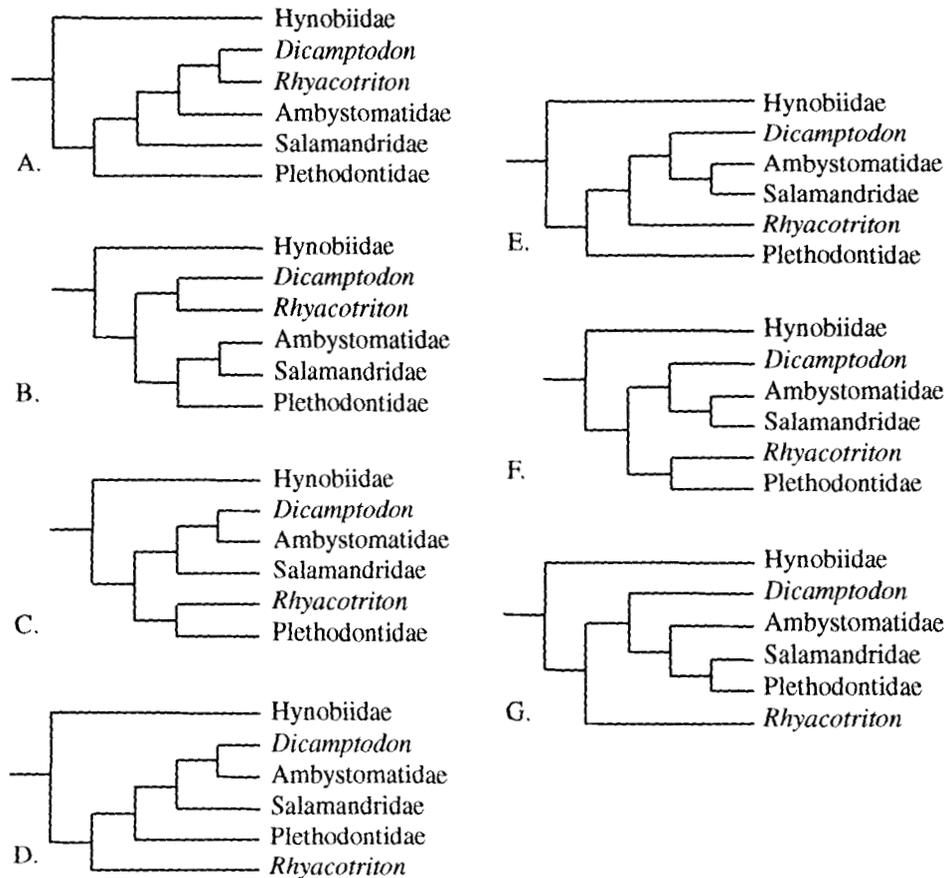


Figure 19. Seven equally parsimonious phylogenetic hypotheses among transforming salamanders, based on 13 characters that have been used in the past to suggest the phylogenetic position of *Rhyacotriton*. Each tree is 20 steps long and has a consistency index of 0.75.

Of the 39 characters discussed above, 26 (characters 2, 3, 6-14, 16, 17, 20, 22-25, 27-31, 35, 38, and 39) provide no useful information for the question of the relationships of *Rhyacotriton*, because they are either too variable to allow for polarization, represent autapomorphies, or are too closely correlated with other characters. The distribution of states in the remaining 13 characters is shown in Table 10.

A major stumbling block in the analysis of relationships of salamander families has always been the scoring of character states in the paedomorphic Cryptobranchidae, Proteidae, Sirenidae, and Amphiumidae, and many of the characters listed in Table 10 are scored as "not applicable" for these taxa because they can be seen only in transformed adults. If the relationships of *Rhyacotriton* are examined with respect only to the transforming salamanders, the results are as follows: Of the 13 characters in Table 10, no

single character suggests a relationship of *Rhyacotriton* with *Dicamptodon*, and only one (character 31) provides a synapomorphy for *Rhyacotriton* and *Dicamptodon* with the Ambystomatidae. On the other hand, 8 characters (1, 4, 15, 18, 19, 21, 35, and 36) suggest that *Rhyacotriton* and *Dicamptodon* are on the "derived" salamander clade, exclusive of the Hynobiidae.

Among these "derived" groups, relationships are ambiguous. Seven equally parsimonious cladograms are obtained (Figure 19) using the data in Table 10. Two place *Dicamptodon* and *Rhyacotriton* as sister taxa, and one of these suggests that *Dicamptodon*, *Rhyacotriton*, and the Ambystomatidae form a monophyletic unit. However, among the five other equally parsimonious cladograms, *Rhyacotriton* is placed variously as the sister taxon of the Plethodontidae, of all other families, and of all families except the Plethodontidae. *Dicamptodon* is placed as the sister taxon to the Ambystomatidae, the Ambystomatidae + Salamandridae, or the Ambystomatidae + Salamandridae + Plethodontidae. Given the data available at present, all phylogenetic hypotheses concerning the Plethodontidae, Ambystomatidae, Salamandridae, Hynobiidae, *Rhyacotriton*, and *Dicamptodon* are essentially equally parsimonious.

Based on the evidence presented above, we are unable to find any basis for an expanded Ambystomatidae to include *Rhyacotriton*, *Dicamptodon*, and *Ambystoma*, or for a Dicamptodontidae to include the first two of these three genera. In fact, there is no evidence that *Rhyacotriton* is close to any other taxon, and there is no candidate sister group. Furthermore, the evidence of Larson (1991) concerning nucleotide sequences for 28s ribosomal RNA argues in favor of an isolated, and relatively basal (in a cladistic sense), position for *Rhyacotriton*. Larson found *Rhyacotriton* to show one of the smallest (together with amphiumids) amounts of molecular change from the ancestral condition as inferred by outgroup comparison. He also found that *Rhyacotriton* is one of four groups of salamanders that show an unusually high number of morphological changes which fall into the categories of losses plus fusions and paedomorphic rearrangements, making phylogenetic analysis difficult. In view of the evidence indicating that *Rhyacotriton* occupies a phylogenetically isolated position, and in the absence of any clear sister group, the most defensible position is to elevate the genus to familial level, as the Rhyacotritonidae. This will insure the monophyly of the Ambystomatidae, Dicamptodontidae, and Rhyacotritonidae. While there is some evidence that the first two families may be close relatives, we are unable to find any character evidence that they form a monophyletic group. It is increasingly clear that *Rhyacotriton* has shared no recent evolutionary history with the other taxa with which it has been associated historically.

OPPORTUNITIES FOR ADDITIONAL RESEARCH ON TORRENT SALAMANDERS

This monograph is a general overview of the present state of our knowledge of the salamander genus *Rhyacotriton*. It should be viewed as a springboard for further work on this fascinating group of salamanders.

Each of the preceding chapters discusses a different aspect of the biology of *Rhyacotriton*, and we recognize that in every instance further work is needed. The allozyme analyses in Chapter 2 and in Good et al. (1987) provide an overview of differentiation in the genus and demonstrate that there are four genetically isolated sets of populations (=species). Although the patterns within these groups at the geographic scale we have used is compatible with an isolation-by-distance model of gene flow, finer-scale analysis is needed to determine in greater detail the pattern and level of gene exchange (or lack of it) among populations. This is particularly true of *R. cascadae*, where within-group comparisons of genetic distance in relation to geography (see Chapter 2) fit isolation-by-distance predictions only poorly. It is possible that the extensive vulcanism in the Cascade Mountains has led to frequent vicariance events and founder effects, and hence to somewhat chaotic patterns. It is even possible that multiple species may occur in the Cascades. This chaotic pattern is also seen in the great diversity in color patterns among populations of this species.

More detailed studies of the history and population biology of *Rhyacotriton* could be provided by analyses of variation in overall mitochondrial DNA (using restriction fragment analysis) and in specific mitochondrial and nuclear genes (using sequencing techniques). Because of differential rates of gene evolution, appropriate genes can be found for in-depth analyses at virtually any level of divergence. In the case of mtDNA, comparison of gene phylogenies with patterns of populational differentiation is also possible.

The contact of two highly differentiated genotypes (*R. kezeri* and *R. variegatus*) in northwestern Oregon without apparent overlap or interbreeding offers several research opportunities. The most compelling question deals with the abrupt parapatry of the two species. What is the mechanism through which this isolation is effected? Behavioral studies are needed to analyze how individuals of these two species interact when brought together under laboratory and field conditions. The basis of intraspecific recognition and

the nature of specific-mate recognition systems could be profitably explored. Arnold (1977) was able to observe courtship under laboratory conditions in *Rhyacotriton*. This raises the hope that courtship and breeding experiments might be devised, so that more can be learned about the nature of factors isolating the taxa. Detailed analyses of potential differences in habitat use are needed. Nussbaum and Tait (1977) analyzed various features of the population biology of two species of *Rhyacotriton*, and such studies of the remaining two species would be useful. Variation within species should also be examined. No one has yet studied the ecology of these salamanders on a more individual level. An analysis of territoriality within and among species, sexes, and age groups would be profitable.

In our present knowledge of the distribution of *Rhyacotriton*, there is only a single contact zone between species, the others being allopatric. However, as can be seen from the discussion in Chapter 5, our understanding of distribution in this genus has by no means been static, and there is no reason to believe that we now understand it perfectly. It is possible that more contact zones exist, particularly in view of the presence of a population of *R. variegatus* in the Cascade Mountains. A detailed search needs to be conducted in the Cascades between the currently known distribution of *R. cascadae* and this population of *R. variegatus*. Similarly, it remains possible that populations of *R. variegatus* will be discovered scattered through the area between the coast and the "disjunct" population in the Cascades.

Chapters 6, 7, 8, and 9 above provide preliminary discussions of patterns seen in osteology, morphometrics, color pattern, and life-history characteristics. The patterns discussed in each of these are complex, and additional study with larger samples from more localities would be useful. The species differ in some important life-history traits, and study of larval life and ontogeny might be very instructive. There is some suggestion that those species with relatively short larval periods develop more fully than those with long larval periods. This phenomenon should be studied among populations within a single species, and more detailed comparative ontogenetic studies are needed in general.

In a number of morphological traits, *Rhyacotriton* displays conditions that suggest that paedomorphosis has been important in its evolutionary history. Some specific examples, in comparison with fully metamorphosed outgroup taxa such as ambystomatids, hynobiids, and dicamptodontids, include: the poorly developed nasal bones (occasionally to always absent in different species); the failure of the coronoid bones to appear in larvae (*Rhyacotriton* may be unique among salamanders in this respect); the incompletely segmented carpus and tarsus; and the undeveloped respiratory system (lungs, larynx, and ypsiloid cartilage). In some other respects, *Rhyacotriton* displays traits that might be interpreted as ancestral: there are three centers of ossification in the otic capsule, as contrasted with one or two in other taxa (Worthington and Wake, 1971); there is a full complement of anterior cranial elements (found otherwise only in dicamptodontids and hynobiids); and some peculiar osteological traits found in occasional individuals might be manifestations of ancestral conditions (e.g., two *R. variegatus* have ossifications of the ascending process of the suspensorium, possibly ectopterygoid bones). A more complete analysis of ontogeny in the species of *Rhyacotriton* and in outgroups would be fruitful. It is possible that the combination of paedomorphosis and very early derivation contribute to the difficulty in assessing the phylogenetic position of the family.

Finally, there are suggestions that *Rhyacotriton* might be useful as a biological indicator of the recovery of degraded habitats (see Chapter 4), and that, in general, it offers opportunities for the development of integrated studies of biodiversity.

Appendix 1

Populations Sampled for Allozyme Analysis of the Contact Zone Between *Rhyacotriton kezeri* and *R. variegatus*

Rhyacotriton kezeri

1. Approximately 0.5 mi northeast of Little Nestucca River Road along Bear Creek (at Siuslaw National Forest boundary), Tillamook County, Oregon [T. 5S, R. 10W, SE 1/4 Section 23] (n=10).
2. Approximately 1.0 mi northeast of Little Nestucca River Road along Bear Creek, Tillamook County, Oregon [T. 5S, R. 10W, NW 1/4 Section 24] (n=9).
3. Approximately 100 yds. west of Little Nestucca River Road along Kellow Creek, Tillamook County, Oregon [T. 5S, R. 10W, SW 1/4 Section 15] (n=5).
4. 2.9 mi east of U. S. Highway 101 on Little Nestucca River Road, Tillamook County, Oregon [T. 5S, R. 10W, SW 1/4 Section 15] (n=45).
5. 3.8 mi north of Little Nestucca River Road on U. S. Forest Service Road 15, Tillamook County, Oregon [T. 5S, R. 9W, NE 1/4 Section 18] (n=7).
6. 4.1 mi south of U. S. Forest Service Road 15 on U. S. Forest Service Road 1588 (0.2 mi from end of road), Tillamook County, Oregon [T. 5S, R. 10W, SW 1/4 Section 14] (n=5).
7. 4.3 mi east of U. S. Highway 101 on Little Nestucca River Road, Tillamook County, Oregon [T. 5S, R. 10W, SW 1/4 Section 23] (n=8).
8. Majors Road, 1.5-2.0 mi north of State Highway 22 at a tributary of Ead Creek, Yamhill County, Oregon [T. 5S, R. 8W, NE 1/4 Section 19] (n=3).
9. U. S. Forest Service Road 2234; 0.2 mi south of U. S. Forest Service Road 2282 (6.3 mi north of State Highway 22), Yamhill County, Oregon [T. 5S, R. 9W, NW 1/4 Section 14] (n=6).
10. U. S. Forest Service Road 2235, 0.3 mi west of U. S. Forest Service Road 2234, Yamhill County, Oregon [T. 5S, R. 9W, N-Central Section 35] (n=10).
11. 0.9 mi east of Little Nestucca River Road on U. S. Highway 22, Yamhill County, Oregon [T. 5S, R. 9W, N-central Section 34] (n=8).

12. 3 mi north of Agency Creek Access Road on Yoncalla Creek Road, Yamhill County, Oregon [T. 5S, R. 8W, NE 1/4 Section 3] (n=15).
13. 4.0 mi north of Grande Ronde Agency on Agency Creek Access Road, Yamhill County, Oregon [T. 5S, R. 8W, SE 1/4 Section 22] (n=10).
14. 5.2 mi north of Grande Ronde Agency on Agency Creek Access Road, Yamhill County, Oregon [T. 5S, R. 8W, SW 1/4 Section 15] (n=8).
15. 5.7 mi north of Grande Ronde Agency on Agency Creek Access Road, Yamhill County, Oregon [T. 5S, R. 8W, SE 1/4 Section 16] (n=12).
16. 7.3 mi north of Grande Ronde Agency on Agency Creek Access Road, Yamhill County, Oregon [T. 5S, R. 8W, SE 1/4 Section 8] (n=7).
17. 0.7 mi south of U. S. Forest Service Road 2280 on U. S. Forest Service Road 2281, Yamhill County, Oregon [T. 5S, R. 9W, N-Central Section 3] (n=1).

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18. Forest Service Highway. 12, 2.9 mi northeast of State Highway. 18 along Deer Creek, Lincoln County, Oregon [T. 6S, R. 10W, NE 1/4 Section 21] (n=2).
19. Tributary on the south side of Schooner Creek, 5.7 mi east of U. S. Highway 101 on Schooner Creek Road, Lincoln County, Oregon [T. 7S, R. 10W, SW 1/4 Section 14] (n=15).
20. U. S. Forest Service Road 1888-112, 2.1 mi southwest of U. S. Forest Service Road 1861, Lincoln County, Oregon [T. 6S, R. 10W, NW 1/4 Section 27] (n=5).
21. 1.5 mi west of Big Elk Forest Service Work Station on Harlan Road, Lincoln County, Oregon (n=10).
22. 1.6 mi north of Hayes Creek Bridge off Hayes Creek Road, Lincoln County, Oregon (n=7).
23. 2.1 mi east of Elk City Road on Harlan Road, Lincoln County, Oregon (n=9).
24. 2.4 mi west of Van Duzer State Park on State Highway 18, Lincoln County, Oregon (n=6).
25. 2.8 mi north of the Ojalla Bridge over the Siletz River on County Route 229, Lincoln County, Oregon (n=4).
26. 4.5 mi east of County Route 229 on U. S. Forest Service Road 19, Lincoln County, Oregon (n=9).
27. 5.6 mi north of Logsdon on County Route 307 along the Siletz River, Lincoln County, Oregon (n=10).
28. 9.9 mi west of Big Elk Forest Service Work Station on Harlan Road, Lincoln County, Oregon (n=10).
29. 0.2 mi north of U. S. Forest Service Road 7-7-5.1 on Gold Creek Road (2.8 mi southwest of point where Gold Creek Road leaves Gold Creek), Polk County, Oregon (n=1).
30. 0.3 mi south of Valsetz Lake Dam, west side of lake, Polk County, Oregon (n=10).
31. 2.3 mi north of Logsdon Road (County Route 410) on Big Rock Road, Polk County, Oregon (n=1).
32. 2.4 mi south of Willamette Valley Lumber Company station ruins, 6.3 mi north and 2.8 mi east Valsetz, Polk County, Oregon (n=11).

33. 3.4 mi northeast of Logsdan Road (County Route 410) on Steere Creek Road, Polk County, Oregon (n=11).
34. Approximately 0.2 mi west of U. S. Forest Service Road 1650 on Little Nestucca River Road, Tillamook County, Oregon [T. 5S, R. 10W, NE 1/4 Section 26] (n=10).
35. Approximately 0.25 mi south of Little Nestucca River Road along Austin Creek, Tillamook County, Oregon [T. 5S, R. 22W, SE 1/4 Section 22] (n=10).
36. Approximately 0.5 mi south of junction of west fork Austin Creek along east fork, Tillamook County, Oregon [T. 5S, R. 10W, NW 1/4 Section 26] (n=3).
37. Approximately 0.5 mi south of Little Nestucca River Road along Austin Creek, Tillamook County, Oregon [T. 5S, R. 10W, SE 1/4 Section 22] (n=10).
38. Approximately 100 yds. up a small stream on the south side of Little Nestucca River Road, 1.6 mi west of State Highway 22, Tillamook County, Oregon [T. 5S, R. 9W, SW 1/4 Section 29] (n=9).
39. Approximately 200 yds. up small creek from Little Nestucca River Road, Tillamook County, Oregon [T. 5S, R. 9W, NW 1/4 Section 29] (n=10).
40. Little Nestucca River Road, 3.9 mi west of State Highway 22, Tillamook County, Oregon [T. 5S, R. 10W, SW 1/4 Section 23] (n=10).
41. Pump station above Hawk Creek Golf Course along Hawk Creek, Tillamook County, Oregon [T. 5S, R. 10W, SE 1/4 Section 25] (n=6).
42. U. S. Forest Service Road 1280, 0.9 m north of U. S. Forest Service Highway 12, Tillamook County, Oregon [T. 6S, R. 10W, SE 1/4 Section 4] (n=4).
43. Upton Creek, approximately 200 yds. east of U. S. Highway 101, Tillamook County, Oregon [T. 5S, R. 10W, SW 1/4 Section 7] (n=10).
44. 0.3 mi south of Little Nestucca River Road on US FSRd 1633 (Squaw Creek Road), then 0.2 mi west on U. S. Forest Service Road, Tillamook County, Oregon [T. 5S, R. 10W, NW 1/4 Section 22] (n=6).
45. 0.3 mi south of Little Nestucca River Road on US FSRd 1633 (Squaw Creek Road), then 0.6 mi west on U. S. Forest Service Road, Tillamook County, Oregon [T. 5S, R. 10W, NE 1/4 Section 21] (n=5).
46. 0.4 mi south of Little Nestucca River Road on Squaw Creek Road/US FSRd 1633, Tillamook County, Oregon [T. 5S, R. 10W, NW 1/4 Section 22] (n=11).
47. 0.5 mi west Forest Service Highway 12 on unmarked service road, just south of Neskowin Creek Campground, Tillamook County, Oregon [T. 6S, R. 10W, NE 1/4 Section 16] (n=12).
48. 0.75 mi south of Little Nestucca River Road on Squaw Creek Road/US FSRd 1633, Tillamook County, Oregon [T. 5S, R. 10W, SW 1/4 Section 22] (n=11).
49. 1.7 mi south of Little Nestucca River Road on Squaw Creek Road/US FSRd 1633, Tillamook County, Oregon [T. 5S, R. 10W, NE 1/4 Section 28] (n=2).
50. 2.9 mi south of Little Nestucca River Road on Squaw Creek Road/US FSRd 1633, Tillamook County, Oregon [T. 5S, R. 10W, SW 1/4 Section 28] (n=1).
51. 3.8 mi east of U. S. Highway 101 on Little Nestucca River Road, Tillamook County, Oregon [T. 5S, R. 10W, SW 1/4 Section 23] (n=58).
52. 4.5 mi southeast of Little Nestucca River Road on US FSRd 1633, Tillamook County, Oregon [T. 6S, R. 10W, NW 1/4 Section 2] (n=11).

53. Waterfall on south bank of Stillwell Creek, approximately 0.5 mi west of US Forest Service Road 12 on U. S. Forest Service Road 1287, Tillamook County, Oregon [T. 5S, R. 9W, SW 1/4 section 33] (n=3).
54. Approximately 1 mi north on road from State Highway 18 toward Fall Creek, Tillamook County, Oregon [T. 5S, R. 9W, NW 1/4 Section 16] (n=3).

Appendix 2

Populations Sampled for Allozyme Analysis of the Zone Between the California and Oregon Subsets Observed by Good et al. (1987) Within *Rhyacotriton variegatus*

1. Elk River Road, 0.5 mi east of road to MacGribble Campground, Curry County, Oregon (n=10).
2. South bank of Rogue River Road, 1.9 mi east of Quosatana Creek Bridge, Curry County, Oregon (n=10).
3. North bank of Pistol River Road, 7.2 mi east of Pistol River, Curry County, Oregon (n=8).
4. North bank of Chetco River Road, 9.9 mi east of U. S. Highway 101 at Brookings, Curry County, Oregon (n=10).
5. Winchuck River Road, 9.9 mi east of U. S. Highway 101, 1.6 mi east of Winchuck River Campground, Curry County, Oregon (n=10).
6. 5.2 mi west of Gasquet on U. S. Highway 199, Del Norte County, California (n=3).
7. South Fork Road, north of Stevens Memorial Bridge, Del Norte County, California (n=10).

Appendix 3

Specimens Sampled for Osteological Analysis in *Rhyacotriton*

Rhyacotriton cascadae

1. Mt. Defiance Trail, Hood River County, Oregon (n=10).
2. Wahkeena Falls, Multnomah County, Oregon (n=50).

Rhyacotriton kezeri

3. Yamhill County, Oregon (n=14).

Rhyacotriton olympicus

4. Mohrweis, Mason County, Washington (n=5).
5. Lake Cushman, Mason County, Washington (n=1).

Rhyacotriton variegatus

6. Polk County, Oregon (n=1).
7. Lincoln County, Oregon (n=17).
8. Tillamook County, Oregon (n=2).
9. Alsea Falls, Benton County, Oregon (n=11).
10. Elk River Road, Curry County, Oregon (n=6).
11. Winchuck River, Curry County, Oregon (n=1).
12. Ti Creek, Siskiyou County, California (n=20).
13. Dark Gulch, Mendocino County, California (n=1).

Appendix 4

Specimens Sampled for Morphometric Analysis of *Rhyacotriton*

Rhyacotriton olympicus

1. Port Angeles, Clallam County, Washington (n=17).
2. Quinault Valley, Grays Harbor and Jefferson Counties, Washington (n=23).

Rhyacotriton kezeri

3. Chehalis River, Grays Harbor County, Washington (n=11).
4. Yoncalla Creek, Yamhill County, Oregon (n=28).

Rhyacotriton variegatus

5. Siletz River, Lincoln County, Oregon (n=27).
6. Reedsport, Douglas County, Oregon (n=14).
7. Chetco River, Curry County, Oregon (n=18).
8. Blue Lake, Humboldt County, California (n=53).
9. Dark Gulch, Mendocino County, California (n=19).

Rhyacotriton cascadae

10. Wahkeena Falls, Multnomah County, Oregon (n=26).
11. Mount Saint Helens, Lewis County, Washington (n=10).

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