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Geographic Variation in Dicamptodon Ensatus (Eschscholtz) with Notes on Life History and Zoogeography

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GEOGRAPHIC VARIATION IN *DICAMPTODON ENSATUS* (ESCHSCHOLTZ) WITH NOTES ON LIFE HISTORY AND ZOOGEOGRAPHY

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INTRODUCTION

The Pacific giant salamander, Dicamptodon ensatus, of western North America is a little-studied species, and almost any aspect of its biology could be fruitfully explored.

This species is generally found in the humid Forested Transition Life-zone, although it can also be found in the Canadian and Hudsonian Life-zones. Its known range, west of the Cascade Mountains, extends from extreme southern British Columbia south along the coast to San Francisco Bay in California. East of the Cascades, it is known from several isolated localities on the eastern slopes of the Cascades and from two areas in the Rocky Mountains of Idaho (see Fig. 1).

To the south, the range of Dicamptodon is probably limited by increasing temperatures and aridity. To the west, the Pacific Ocean is an effective boundary. The inland and coastal populations are separated by the Columbia Basin, an area where arid conditions prevail. At the present time there seems to be no reason why Dicamptodon could not range farther north than its known northern boundary. Conditions to the north in Canada and Alaska seem favorable for the existence of Dicamptodon, and the species may in fact exist in these regions, its presence there, as yet, unreported. Both Savage (1952) and Slater (1962) have suggested this possibility and have further suggested that the inland and coastal population may in reality be connected across some unknown northern corridor, perhaps in the region of the international boundary.

There is evidence that *Dicamptodon* is presently expanding its range northward. For instance, populations of *Dicamptodon* are known from regions in northern Washington and southern British Columbia where they could not possibly have existed 12,000 years ago when continental ice covered the surface of the land. *Dicamptodon* must have dispersed into these areas since the northward retreat of the ice, and there is no reason to assume that its northward dispersal has ceased.

Because the north-south range of *Dicamptodon* is considerable (approximately 825 miles), and because the inland populations are apparently disjunct from the coastal populations, a critical study to determine the degree of variation exhibited by *Dicamptodon* throughout its known range is in order. Knowledge of geographic variation in *Dicamptodon* and a better understanding of its ecological requirements and mode of life history are necessary before an attempt can be made to explain the present distribution and past history of the species.

Concerning geographic variation in *Dicamptodon*, Slater and Slipp (1940) reported the discovery of the salamander in Idaho and mentioned that "... There are only slight differences between these specimens and those of this species which we find in Washington." They went on to mention a few superficial differences, but they were based on only five specimens from Idaho, four larvae and one adult.

The purpose of this paper is to present information concerning geographic variation between three northern populations of *Dicamptodon*, and to add to the meager store of information concerning the life history and biology of the species.

METHODS AND MATERIALS

Description of the Collection Sites

Both larval and adult *Dicamptodon* were collected in and near Mannering Creek, a tributary of the Palouse River located in Benewah County, Idaho (See Fig. 1). Mannering Creek is a rather small stream which flows in a southerly direction from the slopes of Bald Mountain. Following the spring run-off of normal years, Mannering Creek continues to flow strongly throughout the summer, fed by several large springs situated along its course. In exceptionally dry summers, such as the summer of 1967, Mannering Creek is reduced to a trickle, and flow is stopped in places. The dominant conifers along the stream include western red cedar, western white pine, and grand fir. Streamside shrubs include species of *Amelanchier*, *Vaccinium*, *Rubus*, *Rosa*, *Acer*, *Ribes*, and others. Crayfish, eastern brook trout, and *Ascaphus truei* larvae and adults are associated with *Dicamptodon* in the stream. *Bufo boreas*, *Rana pretiosa*, *Ambystoma macrodactylum*, *Charina bottae*, *Thamnophis sirtalis*, and *Gerrhonotus coeruleus* are streamside associates. The elevation at the collection site is approximately 3,500 feet. United States Weather Bureau data indicate that the area receives an average annual precipitation of approximately 30 inches, with about seven inches of this falling in the warm season (April to September,

Fig. 1. Correlation between the known range of *Dicamptodon* (hatched area) and average annual precipitation. The stippling indicates areas that receive 30 inches or more of precipitation per year. Thirty inches may be near the minimum requirement for *Dicamptodon*. Numbers 1, 2, and 3 represent the approximate locations of the Mannering Creek, Mount Pilchuck, and Marratta Creek collection sites respectively. The isolated population of *Dicamptodon* shown in west-central Idaho may be a glacial relict.

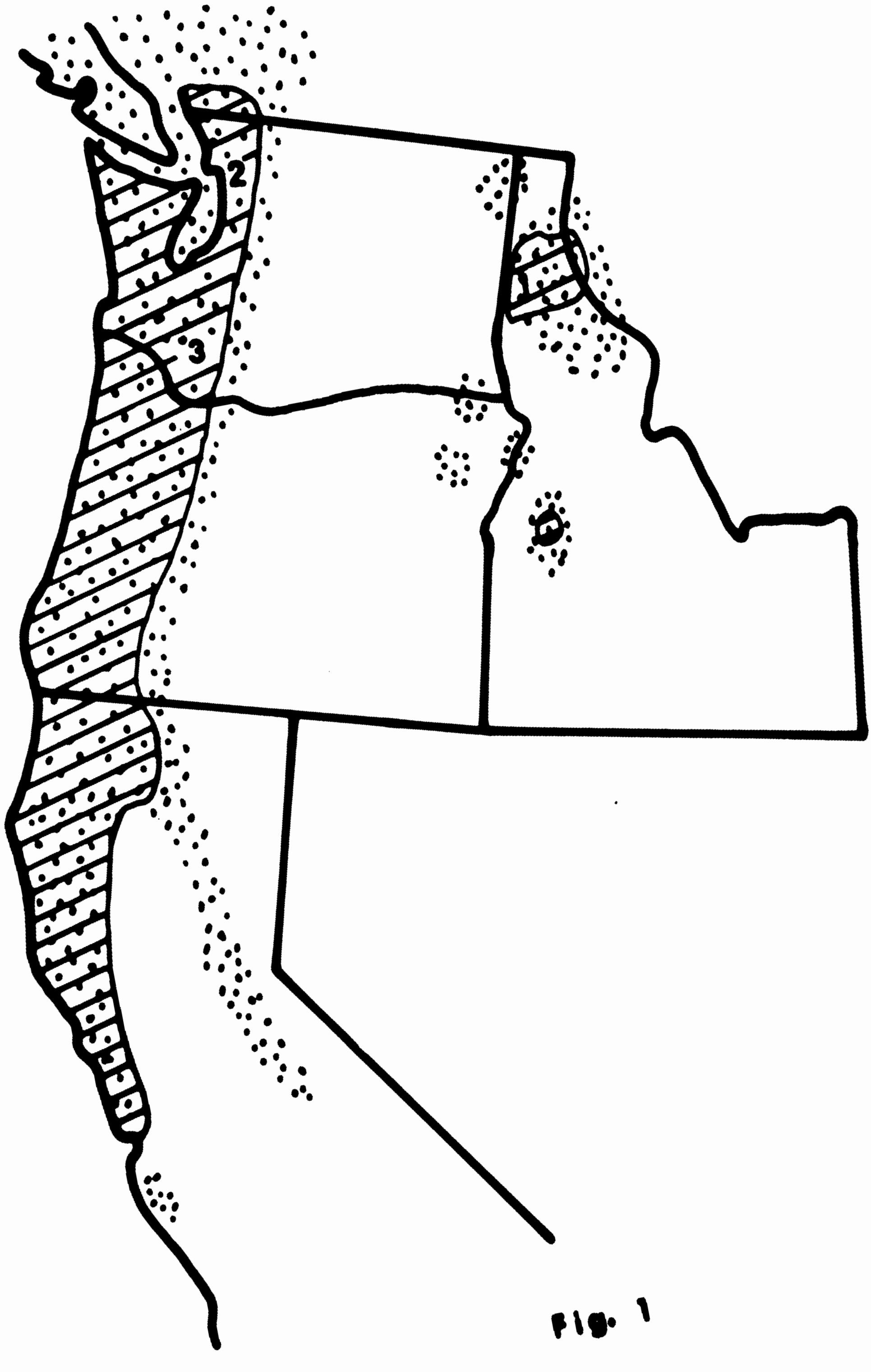

Fig. 1

inclusive). January temperatures average -2° C., while July temperatures average 19° C. Mid-afternoon temperatures average about 10° C. higher than early morning temperatures.

A second collection site is situated on the northern slopes of Mount Pilchuck in Snohomish County, Washington. The area is in the western Cascades approximately 250 air miles west and slightly north of the Mannering Creek site. _Dicamptodon_ larvae and a few adults were taken from a small nameless stream which flows north from Mount Pilchuck to the nearby South Fork of the Stillaguamish River. The elevation at this site is approximately 1,500 feet. The dominant conifer in this area is Douglas fir. Recent logging operations have destroyed much of the surrounding forest. Devil's club and salmonberry are the most common shrubs along the stream. Other than _Dicamptodon_ the only other vertebrates found in the stream were a few _Ascaphus truei_ larvae. _Rana aurora_, _Bufo boreas_, _Plethodon vehiculum_, _Ensatina eschscholtzi_, _Ambystoma gracile_, _Taricha granulosa_, and _Thamnophis sirtalis_ are common streamside residents. Weather Bureau data show that this area receives an average annual precipitation of 60 inches, with about 20 inches of this falling in the warm season. The average temperature for January is 3° C., and July temperatures average 17° C. Diurnal temperature fluctuations are not as extreme here as at the Mannering Creek site. This is primarily due to the ameleorating effect of the nearby Pacific Ocean.

Marratta Creek, the third collection site, is located on the western slopes of the Cascades in Cowlitz County, Washington. It is a tributary of the North Fork of the Toutle River, and is a large stream in comparison to the other two. This site is approximately 230 air miles south of the Mount Pilchuck area. Marratta Creek supports a large population of larval Dicamptodon. Other stream-dwelling vertebrates include at least one species of Cottus, Ascaphus truei, young steelhead (Salmo gairdneri), and Rhyacotriton olympicus. Streamside residents include Rana aurora, Bufo boreas, Plethodon vehiculum, Thamnophis sirtalis and Thamnophis ordinoides. Douglas fir and western red cedar are the dominant conifers at this site. Underbrush is sparse, but salmonberry, devil's club and maple are present. The elevation at the Marratta Creek site is approximately 1,000 feet. Average annual precipitation is 85 inches, with about 20 inches of this in the warm season. January temperatures average -2° C., and July temperatures average 16° C. As with the Pilchuck area, diurnal temperature fluctuations are less extreme due to the proximity of the Pacific Ocean.

An attempt was made to collect Dicamptodon from the population apparently isolated in the west-central part of Idaho (see Fig. 1). Only one animal, a second-year larva, was taken from this population (in Dime Creek along the South

Fork of the Salmon River). To my knowledge, this is only the third specimen known from this area. The other two specimens were reported by Savage (1952) and are in the Natural History Museum of Stanford University. The climate of this region would seem to be unfavorable for Dicamptodon. Average annual precipitation is barely 30 inches, with about six inches of this falling in the warm season. It is interesting to note that with the exception of the Seven Devils region, this area is the only region in the southern half of the state that receives an annual precipitation as high as 30 inches (see Fig. 1 for an interesting correlation between the distribution of Dicamptodon and average annual precipitation). January temperatures at this site average -9° C. and July temperatures average 17° C. The drier aspect of this habitat is reflected by the fact that ponderosa pine is the dominant conifer.

It seems likely to me that Dicamptodon is limited in distribution and abundance in this area due to the relatively dry conditions. The population is probably a southern relict of former glacial times when precipitation over the entire region was higher.

Morphological Comparisons

Because of the rarity of adults, emphasis was placed on morphological characters of larvae for comparison. This procedure is valid when it is realized that natural selection effects all the stages of the ontogeny of a species, and that variation can occur at any one of these stages. Also, in some areas, the breeding population apparently consists almost entirely of sexually mature larvae, these forms then representing the end point of ontogeny.

All measurements were taken with dial calipers and recorded to the nearest tenth of a millimeter. The following thirteen larval measurements were taken from specimens killed in chloral hydrate, positioned and fixed in ten percent formalin, and stored in 50 percent isopropyl alcohol:

1. total length - the distance from the tip of the snout to the tip of the tail.

2. snout-vent length - the distance from the tip of snout to the anterior angle of the vent.

3. snout-gular length - the midline distance from the tip of the snout to the edge of the gular fold.

4. maximum tail height - (self explanatory).

5. axilla-groin length - the distance from the posterior angle of the forelimb with the body wall to the anterior angle of the hindlimb with the body

wall.

6. hindlimb length - the distance from the tip of the longest toe on the hindlimb to the anterior angle of the hindlimb with the body wall.

7. forelimb length - the distance from the tip of the longest toe on the forelimb to the posterior angle of the forelimb with the body wall.

8. maximum head width - the distance across the head at its widest point, usually just behind the orbits.

9. interorbital distance - the distance between the anterior angles of the orbits.

10. internarial distance - the distance between the medial edges of the external nares.

11. orbital-narial distance - the distance between the anterior angle of the right orbit and the posterior edge of the right external nare.

12. orbit length - the distance from the anterior angle of the right orbit to the

posterior angle of the right orbit.

13. Interchoanal distance - the distance between the anterior angles of the openings to the internal nares.

The measurements described above were used in combinations to form ratios for comparative purposes. These ratios, which represent body proportions, were used not only for comparison among the three populations under consideration, but also to determine the amount and kind of allometric growth which occurs within single populations. This was done by dividing each population into three arbitrary size-classes and checking for significant changes in body proportions, with increasing size, within populations.

Because of certain peculiarities in the distribution of ratios, standard statistical formulas cannot be used for determining confidence values for the ratio estimates. In order to overcome this difficulty, the formulas and methods explained by Hansen, Hurwitz, and Madow (1953) were used. The ratio estimate was determined not by averaging the individual ratios, but by dividing the sum of the numerators by the sum of the denominators. Thus:

$$\text{ratio estimate} = r = \frac{\sum Y_i}{\sum X_i} \quad \frac{\bar{y}}{\bar{x}}$$

The variance of the ratio estimate is given by the formula:

$$s^2 = r^2 \ (1-f) \ \left(\frac{V_x^2 + V_y^2 - 2pV_xV_y}{n}\right)$$

where V_x and V_y are the coefficients of variation of the X_i and Y_i, respectively, and p is the coefficient of correlation between the X_i and the Y_i. The sampling fraction, f, is the proportion of the total population sampled and is equal to zero if the population is considered to be infinitely large.

The standard deviation or the standard error of the ratio estimate, s, is the square root of the variance. The Dice-Leraas method for multiple comparison can be used for comparing the ratios by adding and subtracting twice the standard error to each ratio and checking for overlap. If no overlap exists, then the ratios under consideration are usually significantly different at or near the 95 percent confidence level.

The Dice-Leraas method of multiple comparison has been severely criticized in recent years. However, it can be shown (see Eberhardt, 1968) that if the number of means (in this case ratio estimates) being compared is small, then the Dice-Leraas method is reasonably accurate, and the advantages of using the method far outweigh the disadvantages. If the difference between any given pair of ratios is not clearly significant by the Dice-Leraas method, then t-tests can be used to arrive at definite statements.

The last four visceral arches of Dicamptodon larvae have rows of gill rakers. The third visceral arch has one row of gill rakers on the posterior edge. The fourth and fifth visceral arches each have two rows, one on their anterior and one on their posterior edges. The sixth visceral arch has a single row of gill rakers on the anterior edge. Gill raker counts were made for all six rows, and comparisons made between populations.

Gill raker counts are discrete variables, and the simplest way to determine confidence intervals for this type of data is to convert the data to relative frequencies or percentages and to refer to the confidence intervals on a prepared table. A table of 95 percent confidence intervals for binomial distributions in Huntsberger (1961) was used in this instance.

The total number of intercostal grooves, counting one each in the axilla and groin, was recorded for each specimen.

In addition notes concerning color, pattern, sex, and condition of the gonads were taken for each larva examined. No attempt was made to code and quantify qualitative data.

Although no measurements or counts were taken from adults, adult color patterns were compared between the different areas.

Adults were obtained by treating second-year larvae with powdered thyroid. Thirty larvae from Mannering Creek and 30 larvae from Marratta Creek were treated in this manner. In addition, 30 larvae from Eldorado Gulch, a site ten air miles southeast of Mannering Creek were treated with thyroid. This was done to determine if animals from nearby populations showed phenotypic similarities in color pattern. Also, the single larva taken from the west-central Idaho population was treated with thyroid to determine its color-pattern relationship. Only two larvae from the Pilchuck area received the thyroid treatment. However, four adults were collected at the stream, and other natural adults from nearby areas were made available for examination through the collections at the University of Washington and the University of Puget Sound.

It may be argued that these "artificial" adults should not be used for comparison because of possible effects of the thyroid treatment. However, with the exception of the Marratta Creek population, at least a few natural adults from each population were available for comparison, and no significant differences could be detected between the color patterns of the artificial and natural adults within a single population.

Comparison of Blood Serum Proteins

Blood serum proteins were analyzed by electrophoretic separation. Blood was removed from the animals by heart puncture, placed in a capillary tube, and the plasma separated by centrifugation. Serum samples were placed on a biologically inert cellulose acetate membrane, and the serum proteins separated with a Beckman Microzone Cell, Model R-101. The proteins were allowed to migrate for 20 minutes at 250 volts. A barbital buffer solution of pH 8.6 and ionic strength 0.075 was used in the cell.

Migration was stopped by placing the membrane in a fixitive dye solution for eight minutes. The excess dye was rinsed away with a solution of five percent acetic acid. The membrane was then dehydrated for one minute in 95 percent ethanol, transferred to a clearing solution consisting of 25 percent glacial acetic acid and 75 percent ethanol (95 percent) for one minute, and allowed to dry.

The finished membrane was placed in a clear envelope and the pattern scanned with a Beckman Microzone Densitometer, Model R-110. The densitometer is equipped with an automatic integrator, and the relative amounts of the various protein fractions can easily be determined through its use.

A ratio, A/G, was formed by dividing the total amount of blood serum albumin by the total amount of blood serum globulins present in each sample. The averages of the A/G ratios were used for comparative purposes.

Electrophoretic patterns were obtained for six second-year larvae and 12 artificial adults (thyroid produced) from Mannering Creek. In addition, patterns were obtained for three natural adults and one natural, partially metamorphosed individual from Mannering Creek. Four artificial adults from nearby Eldorado Gulch were also subjected to electrophoretic analysis, and the single artificial adult from west-central Idaho was also sacrificed for this purpose. Patterns were obtained for six second-year larvae and two artificial adults from the Mount Pilchuck area. Patterns for Marratta Creek Dicamptodon include four second-year larvae, four neotenic larvae, and three artificial adults.

Life History Studies

An attempt was made to compare certain aspects of life history between the populations. Notes concerning sex, incidence of neoteny, condition of the gonads, and frequency distribution of size-classes were taken for each area.

RESULTS

Morphological Comparison of Larvae

From the 13 measurements taken from each larva, 21 ratios were formed for comparison (see Table 1). By comparing between size-classes within single populations, it was found that 18 of these 21 ratios showed significant differences in at least one of the three populations. Because of this high incidence of allometric growth, it became necessary to compare only larvae of equivalent size-classes between populations.

Considering only the first size-class (larvae up to 69.9 mm in total length) it was found that Mannering Creek larvae differ from Mount Pilchuck larvae in three of the 21 ratios, and from Marratta Creek larvae in seven of the 21. Mount Pilchuck and Marratta Creek larvae differ only in two ratios.

Because allometric growth is not parallel between the three populations, larvae of the second and third size-classes do not differ between populations either by the same ratios or by the same number of ratios as do the larvae of the first size-classes. Within the second size-class (larvae between 70.0 mm and 109.9 mm). Mannering Creek larvae differ from Mount Pilchuck larvae by 8 ratios and from Marratta Creek larvae by 5 ratios. Mount Pilchuck larvae differ from

Table 1. Ratio means and twice their standard errors of measured characters of larval Dicamptodon.

Ratio	size-class	Mannering Creek	sample size	Mount Pilchuck	sample size	Marratta Creek	sample size
2/1	1	.548±.006	30	.543±.015	5	.535±.009	30
	2	.540±.006	30	.536±.011	20	.530±.008	30
	3	.551±.009	30	.536±.008	20	.550±.008	30
	grouped	.547±.006	90	.536±.006	45	.541±.006	90
3/1	1	.163±.011	30	.155±.021	5	.154±.027	30
	2	.152±.014	30	.148±.011	20	.142±.024	30
	3	.147±.009	30	.142±.016	20	.130±.021	30
	grouped	.152±.010	90	.145±.013	45	.138±.019	90
3/2	1	.297±.012	30	.285±.020	5	.288±.028	30
	2	.281±.016	30	.277±.014	20	.269±.030	30
	3	.268±.008	30	.265±.016	20	.236±.024	30
	grouped	.278±.011	90	.270±.012	45	.256±.024	90
5/2	1	.465±.012	30	.487±.024	5	.480±.016	30
	2	.486±.016	30	.497±.010	20	.494±.016	30
	3	.518±.014	30	.506±.014	20	.526±.012	30
	grouped	.498±.012	90	.502±.010	45	.507±.012	90
5/1	1	.255±.014	30	.265±.032	5	.257±.020	30
	2	.263±.020	30	.267±.015	20	.262±.022	30
	3	.285±.017	30	.272±.018	20	.289±.018	30
	grouped	.272±.015	90	.269±.012	45	.275±.017	90
5/6+7	1	.848±.017	30	.928±.045	5	.880±.025	30
	2	.844±.017	30	.897±.015	20	.868±.027	30
	3	.841±.014	30	.845±.025	20	.910±.025	30
	grouped	.843±.010	90	.867±.018	45	.892±.016	90
6+7/1	1	.300±.010	30	.285±.024	5	.292±.015	30
	2	.311±.010	30	.297±.014	20	.302±.012	30
	3	.339±.013	30	.321±.014	20	.318±.013	30
	grouped	.323±.014	90	.311±.016	45	.308±.011	90
6+7/2	1	.547±.011	30	.524±.030	5	.546±.019	30
	2	.576±.009	30	.554±.011	20	.569±.014	30
	3	.616±.011	30	.599±.014	20	.578±.016	30
	grouped	.590±.012	90	.579±.016	45	.569±.011	90

Table 1. (continued)

Ratio	size-class	Mannering Creek	sample size	Mount Pilchuck	sample size	Marratta Creek	sample size
7/1	1	.140±.015	30	.137±.037	5	.142±.013	30
	2	.142±.012	30	.142±.017	20	.142±.014	30
	3	.155±.014	30	.152±.015	20	.148±.015	30
	grouped	.148±.014	90	.147±.015	45	.145±.010	90
7/2	1	.256±.015	30	.253±.034	5	.265±.017	30
	2	.263±.011	30	.265±.014	20	.268±.016	30
	3	.282±.013	30	.283±.014	20	.269±.018	30
	grouped	.271±.012	90	.275±.014	45	.268±.011	90
6/1	1	.159±.015	30	.147±.030	5	.150±.023	30
	2	.169±.012	30	.155±.015	20	.159±.013	30
	3	.184±.014	30	.169±.018	20	.169±.014	30
	grouped	.174±.015	90	.163±.018	45	.162±.013	90
6/2	1	.291±.016	30	.271±.042	5	.280±.026	30
	2	.313±.012	30	.289±.012	20	.300±.015	30
	3	.334±.012	30	.316±.017	20	.309±.016	30
	grouped	.319±.013	90	.304±.018	45	.301±.012	90
7/6	1	.882±.022	30	.935±.046	5	.947±.023	30
	2	.841±.014	30	.916±.015	20	.894±.013	30
	3	.844±.012	30	.896±.015	20	.870±.013	30
	grouped	.850±.009	90	.904±.011	45	.891±.010	90
11/9	1	.571±.022	30	.619±.080	5	.563±.018	30
	2	.579±.020	30	.669±.016	20	.610±.015	30
	3	.559±.021	30	.643±.022	20	.614±.020	30
	grouped	.568±.013	90	.651±.016	45	.602±.013	90
11/10	1	.620±.019	30	.677±.070	5	.660±.016	30
	2	.657±.019	30	.724±.023	20	.687±.018	30
	3	.635±.020	30	.689±.023	20	.649±.018	30
	grouped	.638±.013	90	.701±.023	45	.663±.012	90
4/2	1	.216±.021	30	.213±.029	5	.235±.015	30
	2	.215±.020	30	.226±.050	20	.234±.023	30
	3	.181±.033	30	.213±.020	20	.201±.039	30
	grouped	.198±.027	90	.218±.023	45	.218±.027	90
8/2	1	.242±.018	30	.236±.029	5	.241±.027	30
	2	.235±.018	30	.232±.017	20	.232±.026	30
	3	.226±.010	30	.222±.012	20	.205±.019	30
	grouped	.232±.010	90	.226±.011	45	.220±.021	90

Table 1. (continued)

Ratio	size-class	Mannering Creek	sample size	Mount Pilchuck	sample size	Marratta Creek	sample size
12/11	1	.968±.016	30	.950±.088	5	.885±.038	30
	2	.813±.026	30	.821±.029	20	.794±.028	30
	3	.842±.030	30	.809±.023	20	.754±.031	30
	grouped	.860±.020	90	.822±.020	45	.794±.022	90
12/9	1	.553±.020	30	.588±.036	5	.498±.034	30
	2	.471±.028	30	.530±.026	20	.485±.028	30
	3	.471±.019	30	.520±.023	20	.463±.026	30
	grouped	.489±.018	90	.535±.019	45	.478±.018	90
13/9	1	.701±.013	30	.791±.044	5	.728±.020	30
	2	.686±.016	30	.776±.021	20	.748±.023	30
	3	.689±.014	30	.766±.020	20	.713±.022	30
	grouped	.691±.009	90	.771±.014	45	.728±.014	90
13/8	1	.303±.019	30	.329±.024	5	.322±.031	30
	2	.294±.024	30	.312±.025	20	.312±.022	30
	3	.291±.015	30	.312±.019	20	.291±.025	30
	grouped	.295±.011	90	.313±.014	45	.304±.017	90

Note: The numbers in the ratio column indicate which measured characters were used to form the ratio (see pages 10, 11, and 12). Size-class 1 are larvae up to 69.9 mm in total length, class 2, larvae from 70.0 mm to 109.9 mm, and class 3, larvae greater than 109.9 mm but not including large neotenes. The grouped classification considers all 3 size-classes together.

Marratta Creek larvae by 4 ratios. In the third size-class (larvae 110.0 mm and larger, but not including neotenes), Mannering Creek larvae differ from Mount Pilchuck larvae by 6 ratios and from Marratta Creek larvae by 8 ratios, while Mount Pilchuck larvae differ from Marratta Creek larvae by ten ratios (see Table 2 for a summary).

Gill raker counts indicate that the Mount Pilchuck and Marratta Creek populations are more closely related to each other than either is to the Mannering Creek population, and that Mannering Creek larvae are more similar to Mount Pilchuck larvae than to Marratta Creek larvae (see Table 3).

Every larva examined from all three populations had 13 intercostal grooves, counting one each in the axilla and groin.

Many larval Dicamptodon have dark callosities on the tips of their digits. In some cases the dark, horny skin extends over the palms and soles as well. No correlation could be found between the occurrence of these callosities and locality, season, or sex. It was found that these callosities are more frequent in older larvae. It seems likely that callosities in Dicamptodon develop as a result of friction. Dicamptodon larvae are known burrowers, and this activity would provide the necessary friction. In this regard, it is interesting that several of the mountain

Table 2. Ratios showing significant differences between populations within size-classes.

Mannering Creek vs. Mount Pilchuck			Mannering Creek vs. Marratta Creek			Mount Pilchuck vs. Marratta Creek		
Ratio	Size Class	Confid. Level %	Ratio	Size Class	Confid. Level %	Ratio	Size Class	Confid. Level %
5/6+7	1	99.0	2/1	1	98.0	12/9	1	99.9
7/6	1	95.0	5/6+7	1	95.0	13/9	1	98.0
13/9	1	99.9	7/6	1	99.9	7/6	2	95.0
5/6+7	2	99.9	11/10	1	99.0	11/9	2	99.9
6+7/2	2	99.0	12/11	1	99.9	11/10	2	98.0
6/2	2	99.0	12/9	1	99.0	12/9	2	99.0
7/6	2	99.9	13/9	1	95.0	2/1	3	98.0
11/9	2	99.9	2/1	2	95.0	3/2	3	95.0
11/10	2	99.9	7/6	2	99.9	5/2	3	95.0
12/9	2	99.9	11/9	2	98.0	5/6+7	3	99.9
13/9	2	99.9	11/10	2	95.0	6+7/2	3	95.0
2/1	3	98.0	13/9	2	99.9	7/6	3	98.0
7/6	3	99.9	3/2	3	98.0	11/10	3	99.0
11/9	3	99.9	5/6+7	3	99.9	12/11	3	99.0
11/10	3	99.9	6+7/1	3	95.0	12/9	3	99.0
12/9	3	99.0	6+7/2	3	99.9	13/9	3	99.0
13/9	3	99.9	6/2	3	98.0			
			7/6	3	99.0			
			11/9	3	99.9			
			12/11	3	99.9			

Note: The numbers in the ratio column indicate which measured characters were used to form the ratio (see pages 10, 11, and 12).

Table 3. Summary of gill raker counts.

Visceral Arch	No. Gill Rakers	Mannering Creek rel. freq.	Mannering Creek sample size	Mount Pilchuck rel. freq.	Mount Pilchuck sample size	Marratta Creek rel. freq.	Marratta Creek sample size
3rd (post. edge)	7	— —	100	.16±.11	50	.43±.10	100
	6	.26±.09		.58±.16		.52±.10	
	5	.70±.10		.26±.16		.05±.05	
	4	.04±.05		— —		— —	
4th (ant. edge)	7	— —	100	.16±.11	50	.36±.10	100
	6	.10±.07		.62±.14		.52±.10	
	5	.80±.08		.22±.12		.12±.07	
	4	.10±.07		— —		— —	
4th (post. edge)	7	— —	100	.04±.07	50	.13±.07	100
	6	.09±.06		.74±.13		.77±.08	
	5	.58±.10		.22±.12		.10±.07	
	4	.33±.10		— —		— —	
5th (ant. edge)	7	— —	100	— —	50	.01±.03	100
	6	.03±.08		.84±.11		.79±.08	
	5	.57±.10		.16±.11		.20±.08	
	4	.40±.10		— —		— —	
5th (post. edge)	6	.02±.06	50	.04±.07	50	.22±.09	100
	5	.28±.14		.88±.10		.69±.10	
	4	.70±.13		.08±.09		.09±.06	
6th (ant. edge)	5	.02±.06	50	.02±.06	50	.19±.08	100
	4	.50±.14		.96±.07		.76±.09	
	3	.48±.15		.02±.06		.05±.05	

Note: The numbers following the relative frequency values indicate the 95 percent confidence interval.

stream-dwelling salamanders of the asiatic Hynobiidae have also developed these callosities on their extremities.

Adult Dicamptodon also occasionally have callosities their digits. This condition is probably facultative in adults as well as in larvae, although it may in some way be related to sexual activity. Adult males treated with testosterone developed digital callosities within two weeks.

Larvae from all three populations can be separated by their color patterns. In general, Mannering Creek larvae are darker with less mottling than larvae from Mount Pilchuck and Marratta Creek. This relationship is reflected in the adult color patterns as well (see below). The younger larvae from all three areas are more similar in color and pattern than are the older larvae (see Table 4).

Comparison of Blood Serum Proteins

Separation patterns of blood serum proteins for Mannering Creek (Fig. 2), Eldorado Gulch (Fig. 5, graph 1), and Dime Creek (Fig. 5, graph 2) Dicamptodon indicate that these three inland populations are closely related. Characteristic of these three populations is the relatively high amounts of albumin in the adult forms. Also, the adults from the three populations show well developed alpha-globulin fractions. The Dime Creek adult, from the isolated, west-central Idaho population shows a higher A/G ratio than

Table 4. Color patterns of larvae.

	Mannering Creek	Mount Pilchuck	Marratta Creek
Size Class 1	Dorsum dark brown with lighter mottling. Tail tip with dark spot. Ventral surface white, no melanophores.	Dorsum light (tan) brown very little mottling. Tail tip with dark spot. Ventral surface white, no melanophores.	Dorsum light brown, with lighter mottling. Tail tip with dark spot. Ventral surface white, no melanophores.
Size Class 2	Dorsum trending towards a darker (purplish) brown with obscured mottling. Dark spot on tip of tail recognizable in only 50 percent of larvae. Ventral surface white, no melanophores.	Dorsum light brown, little mottling. Dark spot on tip of tail in all larvae. Ventral surface white, no melanophores.	Dorsum light brown with lighter mottling, but variable. Dark spot on tip of tail recognizable in only 50 percent of larvae. Ventral surface generally white, but some have scattered melanophores.
Size Class 3	Dorsum solid purple-brown, mottling rare. No darker area on tip of tail. Ventral surface dark blue due to invasion of melanophores.	Dorsum light brown, with mottling. Dark spot on tip of tail in 25 percent of larvae. Ventral surface light bluish-brown due to invasion of melanophores.	Dorsum light brown with lighter mottling. No dark areas on tip of tail. Ventral surface dark gray due to invasion of melanophores.

the adults from the other two inland populations.

Patterns for artificial and natural adults from Mannering Creek are very similar, and this is reflected by their A/G values (see Table 5 for a summary of A/G ratios). This indicates that the thyroid treatment does not seriously alter the ontogenetic changes in blood serum proteins.

Patterns for adults from the two coastal populations (Figs. 3 and 4) are similar to each other and different from the patterns for inland Dicamptodon. They differ from the inland populations in their relatively lower albumin fractions and in their poorly defined alpha-globulin fractions. Other differences between the inland and coastal populations can be detected in the region generally referred to as the gamma-globulin fraction.

It should be noted that differences between the larval patterns for the populations are slight, while differences between the adult patterns are marked.

Of interest is the increase in the albumin fraction after metamorphosis for animals from all populations. Also, for the inland populations, the alpha-globulin fraction does not become prominent until the adult stage of ontogeny.

An increase in the amounts of fast-moving blood serum proteins concomitant with metamorphosis was reported by Frieden (1961) for four species of Rana. Frieden suggested that this increase may be adaptive in that an increase in

Table 5. Summary of albumin to globulin ratios.

	A/G	Standard Deviation	Sample Size
Mannering Creek			
larvae	.264	.041	6
partially metamorphosed (natural)	.385	——	1
artificial adults	.454	.045	12
natural adults	.444	.036	3
Eldorado Gulch			
artificial adults	.451	.020	4
Dime Creek			
artificial adult	.545	——	1
Mount Pilchuck			
larvae	.242	.038	6
artificial adults	.349	.064	2
Marratta Creek			
larvae	.260	.036	4
artificial adults	.325	.060	3
neotenic larvae	.287	.046	4

albumin may help to solve osmoregulatory problems faced by the terrestrial animal. He points out that albumin, due to its small molecular weight and highly charged state at physiological pH's, is an ideal protein for this purpose. An increase in albumin increases the total blood serum protein concentration, and this is important from the standpoint of peripheral osmotic exchange, maintenance of blood volume, and transport capacity per unit volume of blood. Whipple (1956, cited by Frieden, 1961) has even suggested that the new osmotic need following metamorphosis may stimulate the synthesis of albumin.

In light of this hypothesis, it is tempting to suggest that the relatively greater increase in the albumin concentration at metamorphosis of inland *Dicamptodon* over coastal *Dicamptodon* is due to the fact that most inland larvae are destined to become adults, while many coastal larvae may never metamorphose. In other words, selection for higher concentrations of albumin following metamorphosis may be greater for inland *Dicamptodon* since almost all inland *Dicamptodon* will become adults and will require high concentrations of blood serum albumin.

Life History Comparisons

Information concerning the life history of *Dicamptodon* is highly speculative and is based on growth studies by Kessel

Fig. 2. Typical electrophoretic patterns of blood serum proteins for Mannering Creek _Dicamptodon_. Graph 1 represents the larval pattern, graph 2 a partially metamorphosed, natural individual, and graph 3 represents the pattern of fully formed artificial (thyroid produced) adults. Note the increase in the albumin fraction with successive ontogenetic stages. Also, note the well developed alpha-globulin fraction in the adult pattern. This seems to be characteristic of the inland populations (for instance, see Fig. 5).

Fig. 3. Typical electrophoretic patterns of blood serum proteins for Mount Pilchuck _Dicamptodon_. Graph 1 represents the larval form, while graph 2 shows the pattern for artificial adults. Although the adult shows an increase in blood serum albumin over the larva, this increase is not as pronounced as in the Mannering Creek series (Fig. 2).

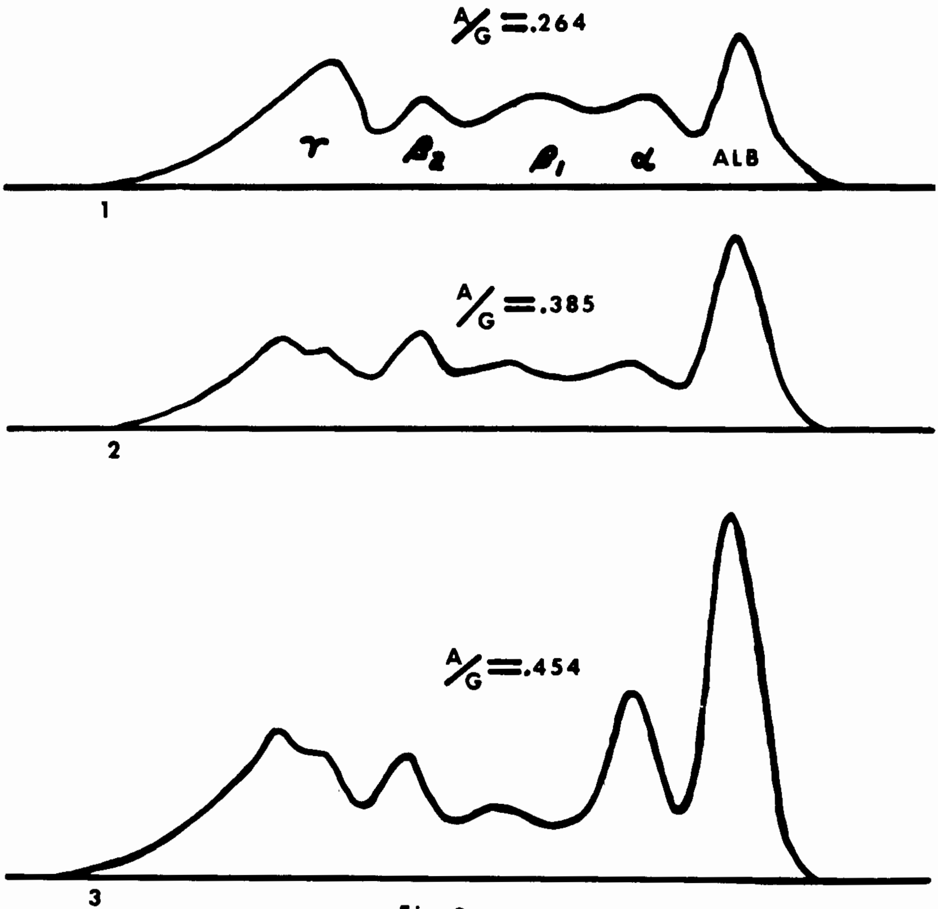

Fig. 2

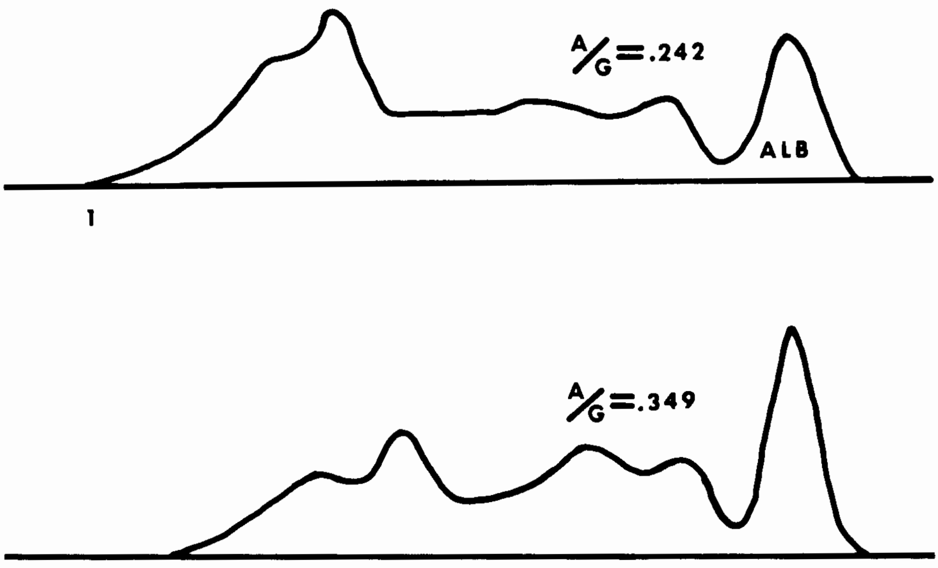

Fig. 3

Fig 4. Electrophoretic patterns of blood serum proteins for Marratta Creek Dicamptodon. Graph 1 is the larval pattern, graph 2 the artificial adult pattern, and graph 3 the pattern for large neotenic larvae. Notice the similarities to the Mount Pilchuck patterns (Fig. 3).

Fig. 5. Electrophoretic patterns of blood serum proteins for aritificial adults from two separate inland populations of Dicamptodon. Graph 1 is the pattern for salamanders from Eldorado Gulch in northern Idaho, and graph 2 is the pattern for the single specimen from Dime Creek in west-central Idaho. Compare these patterns to artificial adults from Mannering Creek (Fig. 2, graph 3).

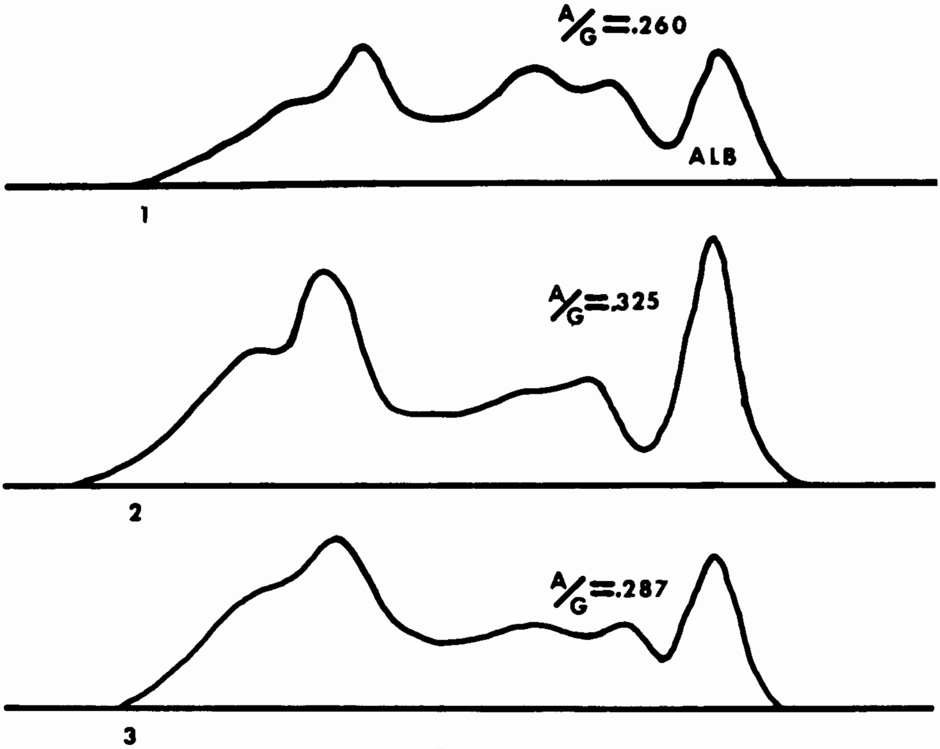

Fig. 4

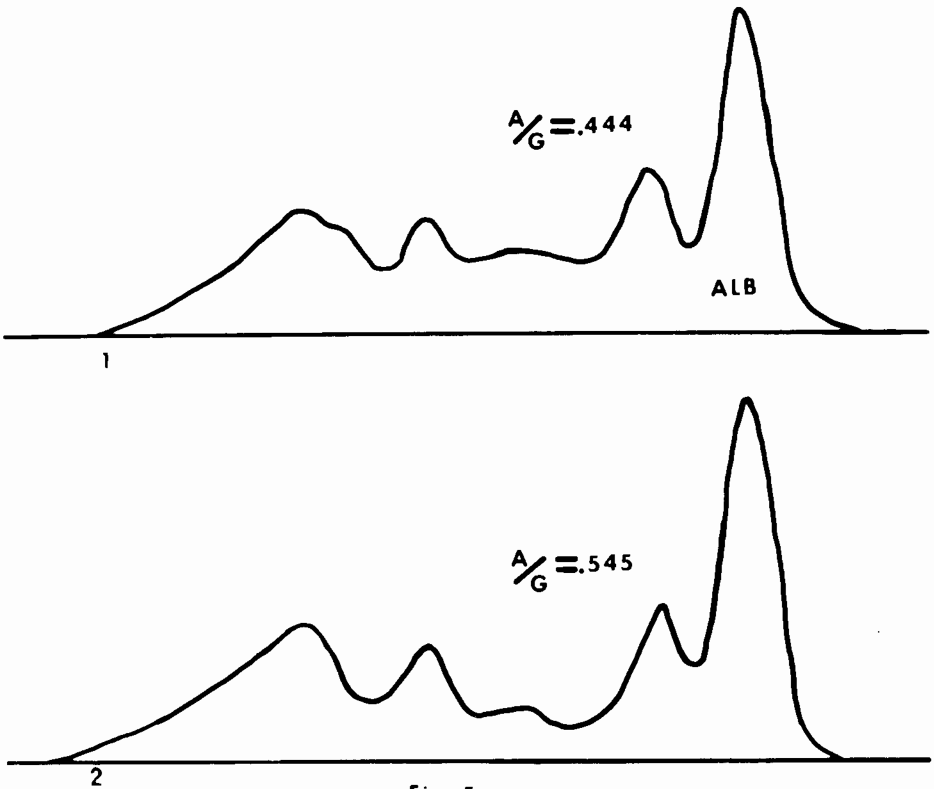

Fig. 5

and Kessel (1943a, 1943b, and 1944), the discovery of three clutches of eggs in California (Dethlefsen, 1948 and Henry and Twitty, 1940), and information from various collecting data.

The eggs of Dicamptodon are deposited in or near the month of March apparently underground in a spring or in the bed of a stream. The eggs are about 5.5 mm in diameter and are completely devoid of pigment. They are deposited singly or in clusters of two and three. Hatching occurs about nine months later in November or December. Storer (1925) reported that the larvae are 17 mm long at hatching. However, Henry and Twitty (1940) argued that Storer's conclusions were based on eggs and larvae of Ambystoma gracile and not Dicamptodon. In support of this arguement, it seems likely to me that a hatching length of only 17 mm is too small for an egg as large as 5.5 mm in diameter. Correlation between the diameter of eggs and larval length at the time of hatching for other urodeles indicates that Dicamptodon larvae should be near 30 mm long at hatching. Also, the smallest larval Dicamptodon I know of is 36 mm long and was collected on August 21, 1967 in Marratta Creek.

By July of their first year, larval Dicamptodon average about 80 mm in total length, and by the time they are one year old in November, they average about 105 mm. Metamorphosis occurs in the following spring and early summer (May through

July) when the larvae are in their second year. They average 135 mm in total length at this time. The young adults leave the stream soon after metamorphosis.

The life history outlined above is based on field studies of California populations of Dicamptodon. If this life history is essentially correct then one would expect to find only one size-class (first-year) of larvae in California streams in the fall months, the second-year larvae having already metamorphosed and deserted the streams. Also, one would not expect to find a high percentage of large neotenic larvae, since metamorphosis normally occurs, and adults should be fairly common.

These requirements seem to be met. In late July of 1967, I collected Dicamptodon along the coast of California from San Francisco Bay to the Oregon boundary. Of the nearly 400 larvae I examined, only four were large enough to be considered as second-year larvae. The remaining larvae averaged 83.40 mm in total length, not far from the figure of 79.09 mm given by Kessel and Kessel (1943a) for the month of July. I observed no large neotenic larvae, but was able to collect five adults in a relatively short time.

Thus it appears that neoteny is not common in Dicamptodon in the southern part of its range. However, that it can occur here is shown by the fact that Storer (1925) reported a 247 mm larva taken in April, 1897 from the Muir Woods, Marin County, California.

That the life history of Dicamptodon varies geographically is suggested by the well-known fact that neotenic larvae of Dicamptodon are fairly common in parts of Oregon and Washington. It is also well known that the adult form is rarely found in some areas of these two states.

Variation in the life histories for the populations under consideration here is very evident, and all three populations seem to have life histories different from the California populations.

Mannering Creek Dicamptodon apparently metamorphose in the fall months (September and October). Many individuals in various stages of metamorphosis were taken during this season at this site. Neoteny rarely if ever occurs in Mannering Creek Dicamptodon. The largest larvae in over 500 individuals examined from this stream was 178.4 mm in total length. This specimen was a female with slightly developed ova. Large adults are fairly common at the Mannering Creek locality. They are most easily found in the late fall and early winter months under large boulders in the stream.

Two distinct groups of first-year larvae exist in the Mannering Creek population (see Fig. 6). This indicates that there are two peaks of egg-laying during the year, one probably in the spring and the other in the fall. That Mannering Creek Dicamptodon can oviposit in the fall is shown

Fig. 6. Histogram for total lengths of larval Dicamptodon from Mannering Creek. All larvae collected on October 1, 1966. The bimodal distribution of the first-year larvae (40 mm to 110 mm) is not evident in the Mount Pilchuck and Marratta Creek populations.

Fig. 7. Histogram for total lengths of Marratta Creek larval Dicamptodon, all captured on August 21, 1967. The first-year larvae (35 mm - 100 mm) do not show the bimodal distribution evident for the Mannering Creek population. The individuals greater than 185 mm in total length are neotenic. There are more of these neotenic individuals present in Marratta Creek than this histogram indicates. Special techniques are necessary for the capture of large, neotenic larvae.

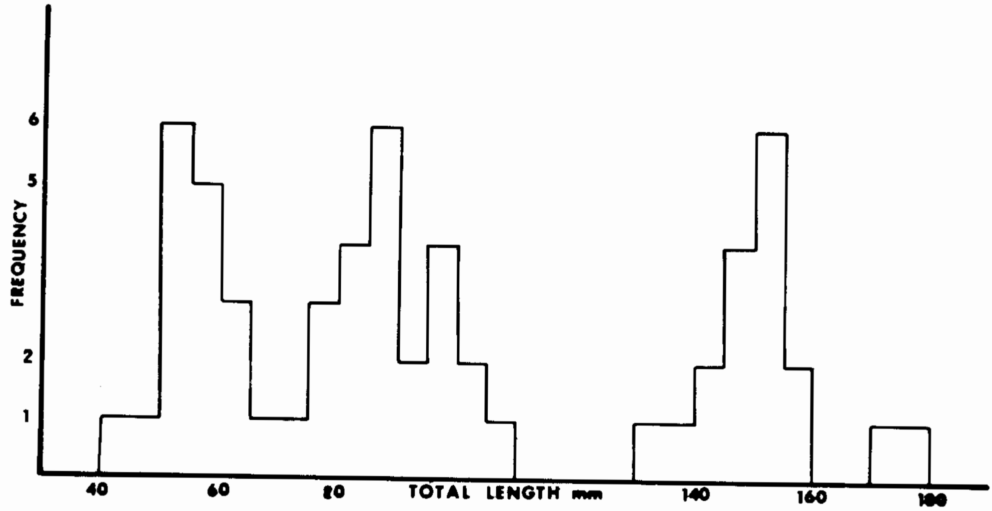
FREQUENCY
6
5
2
1
40
60
80
TOTAL LENGTH mm
140
160
180

Fig 6

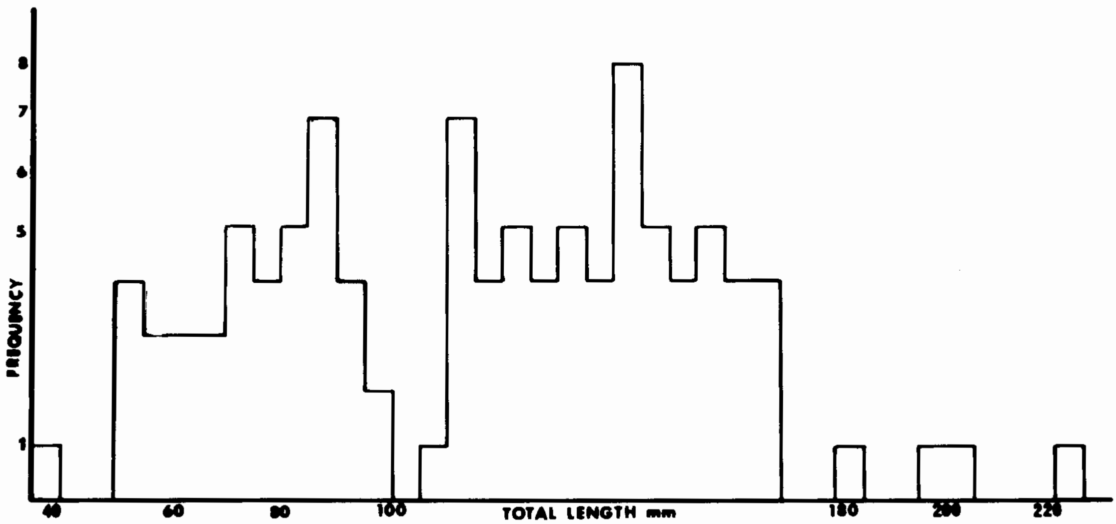
FREQUENCY
8
7
6
5
1
40
60
80
100
TOTAL LENGTH mm
180
200
220

Fig. 7

by the following observations. On September 1, 1967, I discovered an obviously gravid, adult female, 235 mm in total length, buried deep in a rock pile at the base of a waterfall in Mannering Creek. The animal was held in a cooled, darkened aquarium. At the end of one month, on October 1, 1967, the female deposited 185 eggs. These eggs conform in every way to the descriptions of the other three known clutches of Dicamptodon eggs. None of these eggs ever reached the neural fold stage of development. Significant here is the fact that these eggs were deposited six months later (or earlier) than the time proposed for California Dicamptodon.

The gonads of 60 second-year larvae from Mannering Creek were examined to determine sex and breeding condition. Thirty of these larvae were males, which indicates a one to one sex ratio for the population. Only one of the 60 larvae showed any pronounced development of the gonads. This was the 178.4 mm female mentioned earlier. The size of this animal indicates that it is slightly older than the other larvae examined.

No fully adult or partially metamorphosed individuals were found at the Marratta Creek locality, although over 400 animals have been examined. Coupled with this is the fact that many large, neotenic larvae were captured and seen in the stream. These large neotenes are secretive and inhabit the deeper pools. Their abundance in Marratta Creek was revealed by trapping with baited funnel traps. Sometimes two or three animals were caught in one trap, and trapping success was always near 100 percent.

Apparently the period of egg deposition is extended in Marratta Creek Dicamptodon, for young larvae approximately 40 mm in total length can be found at any time during the spring, summer, and fall months. No modality can be detected in the frequency distribution of size-classes (see Fig. 7).

The gonads of 60 second-year larvae from Marratta Creek were examined. Of these 31 were males and 29 females. A one to one sex ratio is indicated.

Surprisingly, the gonads of all but seven of these second-year larvae were in an advanced stage of sexual maturity. Two second-year larval females taken in April had ova 3.6 mm and 4.5 mm in diameter. Second-year larval females taken in June had ova 1.0, 1.5, 4.8, 3.7, 0.9, 1.9, 2.5, and 1.0 mm in diameter. No July larvae were examined, but second-year larvae from August had ova 5.4, 5.7, 3.5, 2.5, 1.0, 1.2, and 1.8 mm in diameter. September larvae had ova 4.7, 4.4, 3.6, 4.5, 4.2, 3.2, 4.5, 3.6, 3.5, 1.0, and 1.8 mm in diameter. The larvae from which these measurements were taken averaged approximately 140 mm in total length. The fact that large ova (4.5 mm in diameter) can be found in larvae collected from April to September is further evidence that the period of egg-laying is extended for the Marratta Creek population.

Second-year larval males collected from April to September also showed varying degrees of sexual maturity. Testes, archinephric ducts, and cloacal glands were greatly swollen in many of these individuals.

The gonads of older neotenic larvae of both sexes were also examined. Apparently the egg-laying season is also extended for these individuals.

The seven second-year larvae examined, which showed no evidence of sexual maturity, are very different in appearance from the other larvae. They have a solid brown coloration with none of the characteristic mottling. Their tail fins are relatively higher and more even, and their heads are relatively larger. The significance of their occurrence is unknown.

Both neotenic larvae and adults were taken from the Mount Pilchuck locality.

No modality is evident in the frequency distribution of size-classes (Fig. 8). This indicates that the period of egg-laying is also extended for this population.

Of 40 second-year larvae examined from the Mount Pilchuck site, 22 were males and 18 were females. This is close to a one to one sex ratio.

Fig. 8. Histogram for total lengths of Mount Pilchuck larvae, all collected on September 7, 1967. No bimodality is evident. Compare to Figs. 6 and 7.

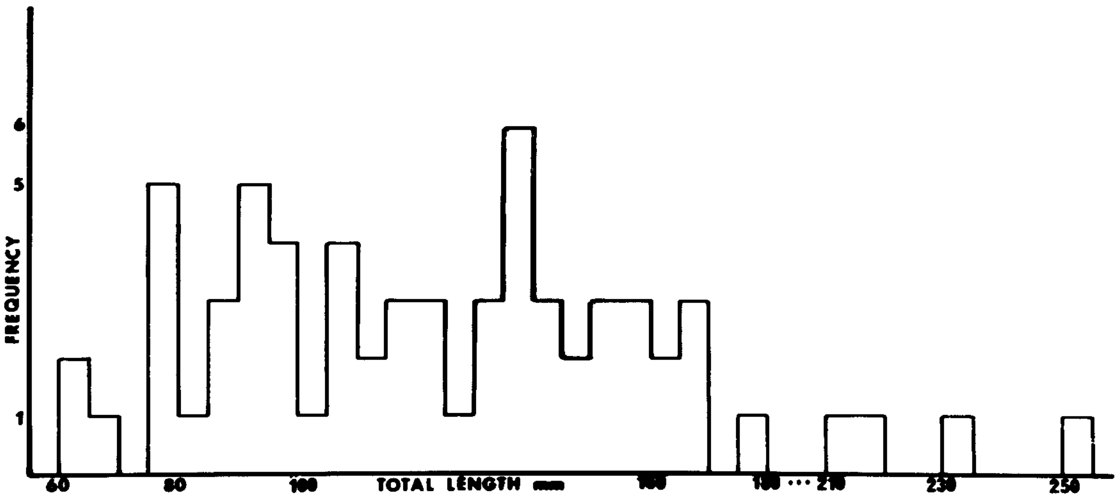

Fig. 8

Although these second-year larvae averaged as large, or slightly larger than the Marratta Creek second-year larvae, none of them showed any signs of sexual maturity. The gonads were so underdeveloped that determination of sex was difficult.

The only sexually mature individual collected at Mount Pilchuck was a neotenic male collected on April 13, 1968. The testes, ducts, and cloacal glands of this animal were well developed.

In summary, the breeding population of Mannering Creek apparently consists almost entirely of adults, and there appears to be a spring and fall peak in egg-laying. The breeding populations of Marratta Creek Dicamptodon consists almost entirely of paedogenetic (precociously sexually mature second-year larvae) and neotenic (older sexually mature larvae) individuals; and the period of egg-laying is extended through the spring, summer, and fall months. Mount Pilchuck breeding animals include both adult and neotenic forms, and egg-laying is extended. The life histories characteristic of these three populations differ from each other, and all three differ from the reported life history for California Dicamptodon. The life history for Mannering Creek Dicamptodon is most similar to California Dicamptodon. However, California Dicamptodon apparently have only one peak (spring) in egg-laying; and second-year larvae in California metamorphose earlier in the season than Mannering Creek *Dicamptodon*.

Comparison of Adult Color Patterns

Two basic differences in color patterns exist between coastal and inland adult *Dicamptodon*. The inland adults are darker, the coppery pigment being less dominant, while the coastal adults are lighter, the coppery pigment being dominant. Also, the grain of the marbling is finer in inland adults than in coastal adults (see Fig. 9).

Comparison of aritificial to natural adults (see Fig. 9. 10, and 11) showed that the thyroid treatment did not effect the normal development of color and pattern.

The 30 artificial adults from Mannering Creek and the 30 artificial adults from Eldorado Gulch were identical in their color patterns, indicating that geographically close populations share common characters.

The single artificial adult from the isolated west-central Idaho population (Fig. 11) is similar to Mannering Creek and Eldorado Gulch adults, although the grain of the marbling is even finer. It was noted earlier that this individual was also similar to the Mannering Creek and Eldorado Gulch animals in its pattern of blood serum protein separation, although it had an even higher concentration of albumin than the coastal adults. This seems to indicate that

Fig. 9. Differences in color and pattern between adult <u>Dicamptodon</u> from the Mannering Creek (upper, darker animal) and Mount Pilchuck (lower, lighter animal) populations. Both animals were fully metamorphosed when collected.

Fig. 10. Two "artificial" adult <u>Dicamptodon</u> captured as larvae and treated with powdered thyroid to force metamorphosis. The upper, darker animal is from Mannering Creek. The lower, lighter animal is from Marratta Creek. It can be seen that the artificial Marratta Creek adult is similar in color and pattern to the Mount Pilchuck natural adult (Fig. 9), while the artificial Mannering Creek adult is almost identical in color and pattern to the natural adult from the same area (Fig. 9). Two artificial adults from Mount Pilchuck were also very similar in color to the Marratta Creek artificial adults, and to the Pilchuck natural adults.

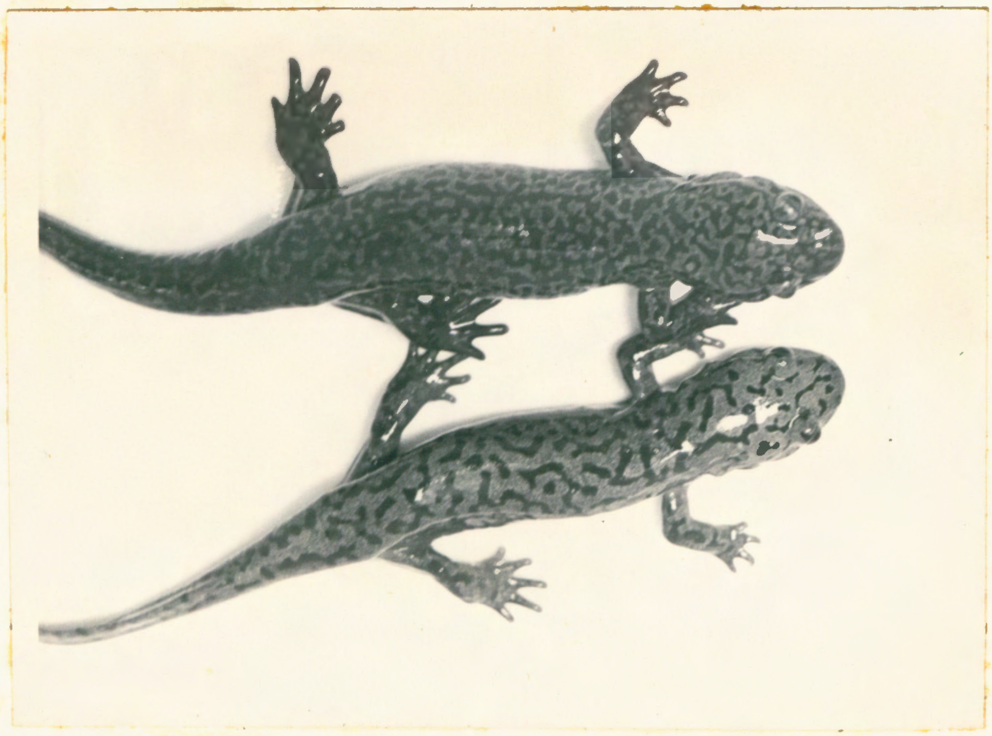

Fig. 9

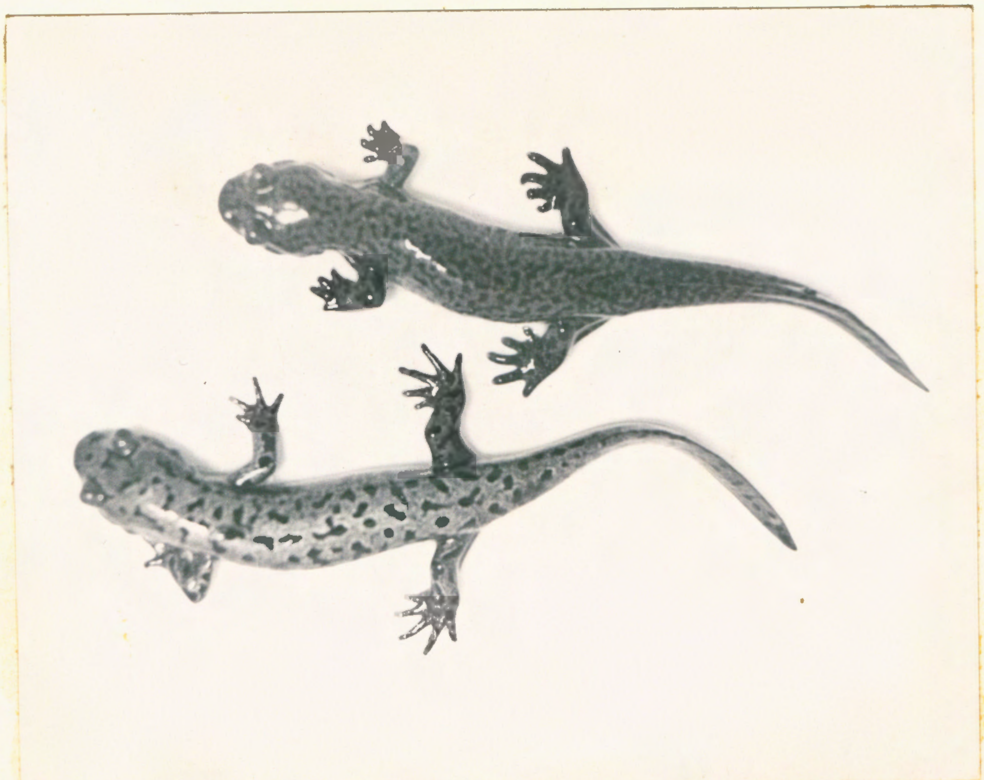

Fig. 10

Fig. 11. Two "artificial" adult *Dicamptodon*. The upper, larger animal is from west-central Idaho (Dime Creek), and the lower, smaller animal is from Mannering Creek. It can be seen that the Dime Creek animal is similar in color and pattern to the Mannering Creek animal, even though the grain of the marbling is finer.

Fig. 11

the west-central Idaho population is even farther removed genetically from the coastal populations than are the two northern Idaho populations.

Of the 30 Marratta Creek adults obtained by thyroid treatment, 22 developed a normal color pattern very similar to Mount Pilchuck natural and artificial adults. One of these larvae is shown in Figure 10. Therefore, these two coastal populations are definitely related as far as color pattern is concerned. The other eight adults from Marratta Creek failed to develop a normal color pattern. Coppery pigment became evident at metamorphosis, but it did not segregated into a distinct marbled pattern. Rather, the coppery pigment was scattered diffusely over the body. Other metamorphic changes appeared normal. The significance of this abnormal development of color pattern in these individuals is presently unknown, but may be related in some way to the fact that larvae from this population apparently do no normally metamorphose into adults.

DISCUSSION AND CONCLUSIONS

Taxonomic Considerations

If examined closely, all breeding populations within a species can be shown to vary genetically; and, as more is known of variation within a species, the harder it becomes to name subspecies. Wilson and Brown (1953) have pointed out several of the difficulties which arise when working with polytypic species. First of all, different taxonomic characters may show independent trends of geographic variation, and some characters may reoccur independently in widely separated populations. These two phenomena seem to be evident in the comparison of larval dimensions of *Dicamptodon*. For instance, Mannering Creek larvae seem to be more closely related to Mount Pilchuck larvae in some characters and to Marratta Creek larvae in other characters, thus making it difficult to assign relationships. To complicate the matter, non-parallel allometric growth between the three populations causes any apparent relationship to change with ontogeny. Because of difficulties such as these, some taxonomists have suggested the abandonment of the subspecific catagory. However, Mayr (1963) and other taxonomists still hold to the concept, pointing out that although the subspecies is not a unit of evolution, it is still a convenient and useful tool for the classifier.

Some of the difficulties which arise in naming subspecies or describing relationships can be overcome if the investigator does not look too closely at the total variation within a polytypic species, and accordingly does not split hairs while assigning relationships.

Taking this view point, one must conclude from the information presented earlier that the inland populations of Dicamptodon, including the Mannering Creek, Eldorado Gulch and Dime Creek populations, are all closely related. Individuals from these populations are similar in larval and adult color patterns and in separation patterns of blood serum proteins.

The two coastal populations are definitely related through similarities in color patterns of both larvae and adults, separation patterns of blood serum proteins, and gill raker counts.

By the criteria outlined by Mayr, Linsley, and Usinger (1953), the coastal and inland populations of Dicamptodon deserve subspecific separation. However, any attempt to describe subspecies at this time would be premature pending analysis of addition populations from other parts of the range.

Biogeographical Considerations

It was stated earlier that some biologists believe the inland and coastal populations of *Dicamptodon* are in reality continuous, the two ranges being connected by a northern corridor. If this is the case, then the Mannering Creek population should be more closely related to the Mount Pilchuck population than to the Marratta Creek population. If the inland and coastal populations are truly disjunct, then the possibility exists that a northern corridor was present in the past and the same relationships should still hold true. On the other hand, there is a possibility that the inland and coastal populations of *Dicamptodon* were connected by a corridor extending from the Cascade Mountains of southern Washington or northern Oregon, east to the Rocky Mountains of central Idaho. The stepping stones could have been the Blue Mountains of northeastern Oregon and southeastern Washington. If this corridor ever existed, then the Mannering Creek population should show closer affinities to the Marratta Creek population than to the Mount Pilchuck population (refer to Fig. 1).

In light of the differences already demonstrated between inland and coastal populations of *Dicamptodon*, it seems unlikely that the range of *Dicamptodon* is presently, continuous between the two areas, or at least that continuous gene flow occurs between the two areas. The problem then, is to determine how the two ranges were continuous in the past.

Comparison of body proportions of larvae sheds little light on this problem. The data in Table 2 seems to suggest that Mannering Creek larvae differ in more characters from the Marratta Creek larvae than from the Pilchuck larvae. This is especially evident if one considers only the first size-classes for comparison. In most taxonomic papers where ratios are dealt with, the authors choose to eliminate the problem of allometric growth by comparing only individuals of the largest size-classes. There seems to be no biological reason for this procedure, and in some cases it seems likely that comparing the smallest size-classes would yield the best results. This is because demonstrable differences (or similarities) in the earliest ontogenetic stages usually reflect the most deeply "ingrained" genetic patterns. In later ontogenetic stages, non-parallel allometric growth, much of which may not be adaptive, could tend to obscure the true relationships. Following this line of reasoning and placing more emphasis on comparison between larvae of the first size-classes, it seems likely that Mannering Creek *Dicamptodon* are most closely related to Mount Pilchuck *Dicamptodon*.

An examination of gill raker counts suggests that the Mannering Creek population is most closely related to the Mount Pilchuck population (refer to Table 3).

Variation in color patterns of larvae sheds no light on this problem.

Electrophoretic patterns of blood serum proteins show scant evidence that the relationship is northern. Most suggestive is the fact that the artificial adult from west-central Idaho has an A/G ratio higher than adults from northern Idaho, suggesting a cline from north to south.

Adult color patterns also show scant evidence for northern relationships. The west-central Idaho adult is even darker and has finer grained marbling than the adults from northern Idaho, again suggesting a cline from north to south.

Differences in modes of life history suggest a northern corridor. For instance, second-year larvae from Mount Pilchuck and Mannering Creek showed no gonadal development, while second-year larvae from Marratta Creek were definitely paedogenetic, some containing mature ova and some with fully developed testes. In addition, adults are present in both the Mannering Creek and Mount Pilchuck populations, while they are rare or absent in the Marratta Creek population.

In summary, there is more evidence for the past existence of a northern corridor between the inland and coastal populations than for any other possibility.

Several aspects of the biology of Dicamptodon must be kept in mind while considering the zoogeography of the salamander. First of all, it must be realized that the adult form possesses far greater powers of dispersal than the larval form. Another fact of importance is that the larval period of Dicamptodon is greater than one year, even in populations where metamorphosis normally occurs. This means that Dicamptodon could not exist in areas where the climate is such that streams commonly dry up in the late summer months. In fringe areas, where streams only occasionally dry up, Dicamptodon may exist; but not if the breeding population consists only of paedogenetic or neotenic individuals, because one drought would destroy the entire breeding population. If adults were present in these hypothetical fringe populations, then occasional droughts would not be too serious as the adults would survive to repopulate the streams the following seasons. In this manner, neoteny and paedogenesis would be selected against in fringe areas.

Dicamptodon is limited in distribution and abundance over the inland portion of its range. This seems to be due to the relatively low average annual precipitation and seasonality characteristic of this region. Many inland populations of Dicamptodon are probably situated in fringe areas as far as their ecological requirements are concerned.

The occurrence of adults and the absence of neotenic and paedogenetic individuals at Mannering Creek is consonant with the hypothesis stated above. That the Mannering Creek population is in a fringe area is indicated by the following observations. The Mannering Creek site receives almost two-thirds less precipitation than the Marratta Creek site, and 77 percent of this falls in the cool season. Also, surface water records (available from the United States Geological Survey) indicate that the Palouse River (the main stream into which Mannering Creek flows) has occasionally stopped flowing, notably in 1910 when the drought that hit the Rocky Mountains caused many disasterous forest fires. Even in normal years the flow of the Palouse River is reduced in the months of August and September to about 0.3 percent of the flow for the month of March. In the dry summer of 1967, it was observed that Mannering Creek had stopped flowing in places.

As stated earlier, Marratta Creek has a high incidence of neotenic and paedogenetic individuals, and few if any adults. Marratta Creek is situated on the western slopes of the Cascade Range. As moisture-laden air masses from the nearby Pacific Ocean rise over these slopes, large amount of precipitation are deposited as the rising air loses its ability to hold moisture. Marratta Creek receives three times as much precipitation as Mannering Creek. Marratta Creek is a tributary of the North Fork of the Toutle River, and surface water data show that this stream has never stopped flowing, at least since the year 1909 when data become available. Flow is reduced to only 15.0 percent of maximum during the warm season for this stream. As far as water supply is concerned, Marratta Creek is definitely not in a fringe area. The high incidence of neoteny and paedogenesis at this site is in agreement with the stated hypothesis.

It was mentioned earlier, that the incidence of neoteny seems to be low in California populations of Dicamptodon. United States Weather Bureau data show that this part of the range of Dicamptodon receives relatively low amounts of precipitation (see Fig. 1). In fact, increasing aridity to the south probably limits the southern range of Dicamptodon in California. Again, the hypothesis seems to be satisfied.

The occurrence of both adults and neotenes at the Mount Pilchuck site, an area with abundant rainfall, need not be a stumbling block. These areas in northern Washington and southern British Columbia were covered with continental ice approximately 12,000 years ago. Dicamptodon has only relatively recently dispersed into these areas, probably from southern, coastal Washington. It seems likely that active dispersal into these areas left barren by the retreating ice was probably accomplished by adults and not by larvae. Active dispersal into new territory is probably another reason for the high incidence of the adult form in fringe areas near the extremes of the range.

If Dicamptodon was absent from the inland regions during the glacial maximum, and this seems likely, individuals trending toward normal metamorphosis probably invaded the area following the retreat of the ice. Once there, increasing aridity as the Altithermal Interval approached, would tend to maintain selection pressure for normal metamorphosis.

Other biologists have demonstrated how ice ages, or periods of maximum precipitation can effect the occurrence of dispersive versus nondispersive forms within a species.

Many beetles of the family Carabidae are dimorphically winged. Both flightless forms with atrophied wings and normal-winged, flying individuals may occur within a single species. Obviously the flightless forms have low powers of dispersal and the flying forms have high powers of dispersal. Lindroth (1949, as cited by Darlington, 1965) has shown that dimorphically-winged carabids are especially numerous in regions that have recently been glaciated. He was able to show that short-winged (flightless) individuals are most abundant in areas (refugia) where they have recently survived a glaciation, and that long-winged individuals are more numerous in regions that have recently been deglaciated. In other words, following the retreat of the ice, new ground was colonized by the morph most capable of rapid dispersal.

Tihen (1955) has demonstrated that fossil populations of Ambystoma tigrinum from deposits associated with major glacial advances are composed of neotenic forms, while those associated with interglacial deposits are composed of individuals that underwent normal metamorphosis (also see Hibbard, Ray, Savage, Taylor, and Guilday, 1965). During glacial advances, precipitation was high and there was probably no selection against the occurrence of neoteny. During interglacial stages when arid conditions returned and threatened the existence of many of the lakes and ponds, and when new territory was opened for colonization, selection probably favored normal metamorphosis, as the adult tiger salamander was better equipped to survive drought and to disperse into new, more favorable territories.

The situations described above for carabid beetles and fossil tiger salamanders support the hypothesis stated earlier for the occurrence of neoteny in Dicamptodon.

If the stated hypothesis is correct, then the post-glacial history for Dicamptodon is probably as follows. During the last glacial maximum, Dicamptodon was confined to the non-glaciated coastal regions from west-central Washington south along the coast of Oregon into California. The southwest corner of Washington probably acted as a refugium for many forest-dwelling species during the glacial maximum. Dumas (1966) has suggested that Rana cascadae may have differentiated from ancestral Rana pretiosa while isolated in this area during Wisconsin Time. During the time Dicamptodon was confined to this area, the incidence of neoteny was probably very high. Following the retreat of the ice, Dicamptodon dispersed northward, the major advances being accomplished by those individual tending to undergo normal metamorphosis. Sometime before the Altithermal Interval, approximately 9,000 years ago, Dicamptodon had reached the Rocky Mountains of Idaho by a northern corridor, perhaps just south of the retreating snow line. During the Altithermal Interval, about 5,000 years ago, increasing aridity caused the disappearance of the connecting corridor. Since the Altithermal Interval, more humid conditions have returned, but the inland and coastal ranges of Dicamptodon remain disjunct.

This hypothetical history of Dicamptodon would explain the present high incidence of neoteny in the Marratta Creek region, as this area is very near the hypothetical glacial refugium. It would also explain the occurrence of adults and neotenes in northern Washington, an area fairly recently invaded by Dicamptodon, but where precipitation is high enough for neoteny to occur.

If selection can favor or disfavor neoteny, then the condition must be at least partially under genetic control, and not entirely under environmental control as is often stated. Neoteny would have to be part of the normal genetic variability of the species, and the frequency of the gene or genes involved would vary between populations both geographically and temporally.

Lindroth (1946) has shown that long and short wings in carabid beetles are inherited in a simple Mendelian manner. Blount (1950) has shown that neoteny in some *Ambystoma* is due to a pituitary derangement, which must in some way be genetically determined. Clothier (1966) has amassed evidence which suggests that a defective pituitary may also be responsible for neoteny in *Dicamptodon*. Thus it appears that natural selection acting on gene pools could favor those individuals with the greatest powers of dispersal depending on the advantages or disadvantages of having these dispersive forms in the population at any given time or place.

It is entirely possible that the pituitary of neotenic *Dicamptodon* is not truly defective, but simply has not received the necessary stimulus from the hypothalamus which would allow metamorphosis to occur. Thus, if a neotenic larva was able to sense a change in the environment which indicated an impending drought, the hypothalamus-pituitary-thyroid pathway could be activated, and the animal could escape death by rapidly metamorphosing. This would seem to be the ideal situation, but there is no evidence that large neotenic larvae ever metamorphose under natural conditions. Rather, those individuals found in the process of metamorphosing in nature are all small, normal-sized larvae (Kessel and Kessel, 1944; Clothier, 1966; Nussbaum, unpublished). In addition, I witnessed a situation where environmentally-induced metamorphosis would have been beneficial but did not occur. In the exceptionally dry summer of 1967, I observed many dead and dying *Dicamptodon* larvae, both neotenic and normal-sized, along a dry section of Marratta Creek. None of these animals showed any signs of metamorphosis, nor did the living animals crowded together in pools along the drying stream bed. That this had been going on for a long time was indicated by the fact that a large aggregation of garter snakes, *Thamnophis sirtalis*, had assembled to feed on the hapless larvae. At least in this case, a changing environment did not produce the desired metamorphosis.

Similar conditions of drought were observed two weeks later at Mannering Creek. The stream was reduced to a trickle, and flow had completely stopped in places along the stream. Here, however, many of the larvae in the crowded pools were in the process of metamorphosing. Many semi-adults were found. The previous summer (1966) was not nearly as dry, and Mannering Creek flowed strongly even in the months of August and September. Even though there was no danger of the stream drying up that summer, most of the second-year larvae collected at that time showed signs of metamorphosing. Many semi-adults were also collected.

The observations presented above seem to indicate that neoteny in Dicamptodon is under direct genetic control, and that environment has only a selective role in the determination of the incidence of neoteny within a given population.

SUMMARY

Comparison of body proportions of larvae between size-classes within populations shows that the incidence of allometric growth is high; and because allometric growth is not parallel between the three populations, determination of relationships is difficult.

Gill raker counts indicate that the two coastal populations are closely related, and that perhaps the Mannering Creek population is most closely related to the Mount Pilchuck population.

An examination of the occurrence of digital callosities and numbers of intercostal grooves revealed no detectable differences between populations.

Color patterns of larvae differ between all three populations under consideration. The two coastal populations are most similar, and the inland population shows no particular affinity to either of the coastal populations.

Separation patterns of blood serum proteins demonstrates a close relationship between the two coastal populations and a close relationship of individuals from three separate inland areas. There is also some indication from blood serum protein analysis that inland *Dicamptodon* are most closely related to Mount Pilchuck *Dicamptodon*.

All three populations differ in their modes of life history, and all three differ from the reported life history for California *Dicamptodon*. The occurrence of adults and the absence of paedogenetic larvae in both the Mannering Creek and Mount Pilchuck populations indicates a northern relationship.

Comparison of adult color patterns demonstrates that the two coastal populations are most closely related, and that the inland populations are closely related. There is some evidence from adult color patterns that the Mount Pilchuck and Mannering Creek populations are more closely related than the Mannering Creek and Marratta Creek populations.

Considering the total evidence, there is little doubt that the inland and coastal populations of *Dicamptodon* deserve subspecific separation, however, no attempt will be made to describe subspecies until additional populations from other parts of the range can be analyzed.

There is more evidence that inland populations of *Dicamptodon* are more closely related to the Mount Pilchuck population than to the Marratta Creek population. This indicates a northern route of dispersal between the two areas rather than a more southerly route.

Certain aspects of the biology of *Dicamptodon ensatus* indicate that neoteny in this species is largely under genetic control, and that the incidence of neoteny in any population at any given time or place is determined by natural selection.

It appears that the incidence of neoteny in Dicamptodon is higher in that portion of its range where precipitation is highest (along the northern coast), and that metamorphosis normally occurs where precipitation is lower (in parts of California, and over the inland portion of the range). Adults have greater powers of dispersal than larvae; and there may be a selective premium on dispersal (therefore on metamorphosis) in areas where precipitation is low and highly seasonal, or in regions where new territory (e.g., recently deglaciated regions) has been opened for colonization.

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