Genetic diversity and gene flow of coastal tailed frogs (*Ascaphus truei*) and Cope's giant salamander (*Dicamptodon copei*) across three regions of western Washington

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Introduction

Goal: To describe baseline genetic structure in coastal tailed frogs and Cope's giant salamander across the Type N experimental study region.

Objectives:

1. Distinguish between the two species of giant salamander from samples collected in the field
2. Estimate metrics of genetic diversity to identify differences between regions for both tailed frogs and Cope's giant salamander
3. Evaluate the extent of gene flow across each study region

Approach: Sample individuals of both species at focal Type N study basins as well as surrounding neighborhood sites

Significance: Response of coastal tailed frogs and Cope's giant salamander is an important aspect of the Type N experimental study. Baseline genetic evidence for both species is necessary both to provide a comparison for future treatment effects and to determine if population structure of amphibians is similar among the three main study regions.

Methods: Study Area and Genetic Analysis

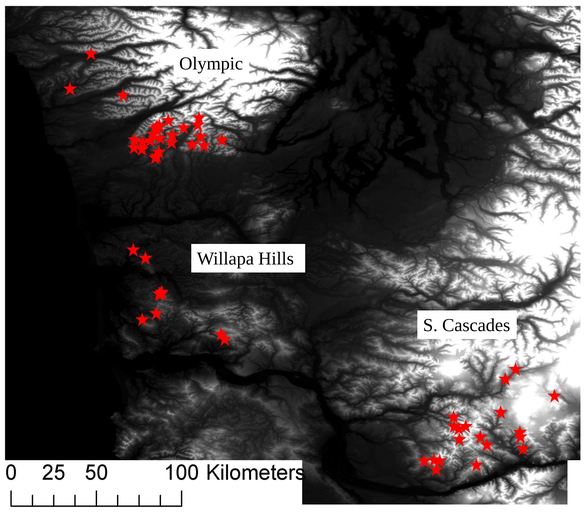


Figure 1. Map of genetic sampling sites for coastal tailed frogs and Cope's giant salamander across the 3 Type N regions. Background is a digital elevation model (light areas indicate high elevation)

Field and Laboratory Methods

- Sampled all Type N focal basins and adjacent neighborhood sites for coastal tailed frogs and Cope's giant salamander
- Collected tissue from 20-50 individuals per site
- DNA extracted from tissue and amplified at 13 microsatellite loci using Polymerase Chain reaction (PCR)

Genetic Analysis

- Used NewHybrids (Anderson & Thompson 2002) to distinguish Cope's giant salamander from the morphologically similar coastal giant salamander
- For both coastal tailed frogs and Cope's giant salamander at each experimental basin, calculated indices of genetic diversity such as average alleles per locus and expected heterozygosity.
- Bayesian clustering program BAPS (Corander & Marttinen 2006) used to cluster both experimental basins and neighborhood sites

Results: Species Identification and Genetic Diversity

Table 1. Numbers of Cope's giant salamander and coastal giant salamanders at each site. In addition, F_2 and backcross hybrids were identified at several sites, primarily in the Willapa Hills

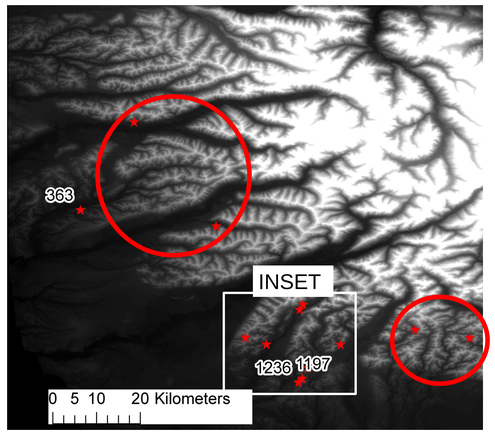
Region	Site	Cope's	Coastal	F_2	Bc_{cope}	Total
Olympics	363	31	0	0	0	31
	1099	62	0	0	0	62
	1197	28	0	0	0	28
	1236	43	0	0	0	43
	H1	21	0	0	0	21
	H3	3	0	0	0	3
	H11	15	0	0	0	15
	H12	9	0	0	0	9
	W1	7	0	0	0	7
	S1	12	0	0	0	12
	JB1	23	0	0	0	23
	JB2	20	0	0	0	20
	SAM	18	0	0	0	18
	Total	**292**	**0**	**0**	**0**	**292**
Willapa Hills	2260	95	0	0	0	95
	2468	58	0	0	1	59
	3074	21	4	0	0	25
	3098	12	41	0	0	53
	3110	21	79	1	2	103
	3111	23	140	2	0	165
	3437	67	5	0	3	75
	3576	37	16	4	1	58
	3914	5	70	7	0	82
	5785	26	40	5	1	72
	Total	**365**	**395**	**19**	**8**	**787**
South Cascades	5595N	19	66	0	0	85
	5595S	69	101	1	0	171
	5378	52	31	0	0	83
	6000	100	107	0	0	207
	Y1	13	9	0	0	22
	PC	12	3	0	0	15
	KC	8	1	0	1	10
	Total	**273**	**318**	**1**	**1**	**593**
	Grand Total	**637**	**713**	**4**	**9**	**1145**

Tables 2 & 3. Genetic diversity estimates for Cope's giant salamander (top) and coastal tailed frog (bottom). N is sample size, A represents average alleles/locus, and H is heterozygosity. Note that heterozygosity is high across both species and study regions. This indicates that populations of both species typically have high genetic diversity There is, however, lower genetic diversity in Cope's giant salamander across the Olympics.

	Basin	A	H
Olympics	363	4	0.524
	1099	8	0.657
	1197	6	0.654
	1236	6	0.639
	Average	**6**	**0.618**
Willapa Hills	2260	9	0.735
	2468	9	0.750
	3074	11	0.842
	3098	9	0.829
	3110	10	0.816
	3111	11	0.839
	3437	15	0.864
	3576	13	0.846
	3914	6	0.836
	5785	12	0.846
	Average	**11**	**0.820**
South Cascades	5378	9	0.689
	6000	13	0.783
	5595N	9	0.834
	5595S	12	0.833
	Average	**11**	**0.785**

	Basin	N	A	H
Olympics	1099	42	17	0.913
	1197	19	11	0.878
	1236	13	11	0.914
	Average	**25**	**13**	**0.902**
Willapa Hills	2260	50	17	0.881
	2468	33	15	0.884
	3074	25	17	0.907
	3098	9	9	0.877
	3110	51	22	0.919
	3111	52	20	0.918
	3437	35	18	0.906
	3576	48	20	0.912
	3914	25	18	0.917
	5785	54	23	0.923
	Average	**38**	**18**	**0.904**
South Cascades	5378	26	17	0.879
	6000	48	19	0.871
	Average	**37**	**18**	**0.875**

Results: Cope's Giant Salamander Genetic Neighborhood



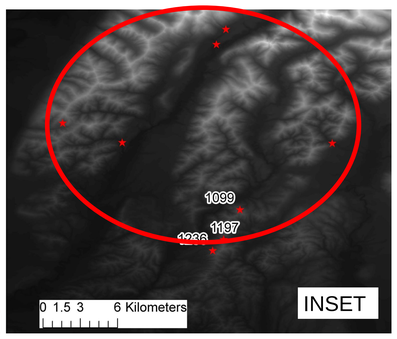


Figure 2. Genetic clustering across Olympic region for Cope's giant salamander. Circled sites are clustered together. In the Olympics, typical neighborhood size is large (10-20 km), but note isolation of 1236 (inset) despite close proximity to other sites.

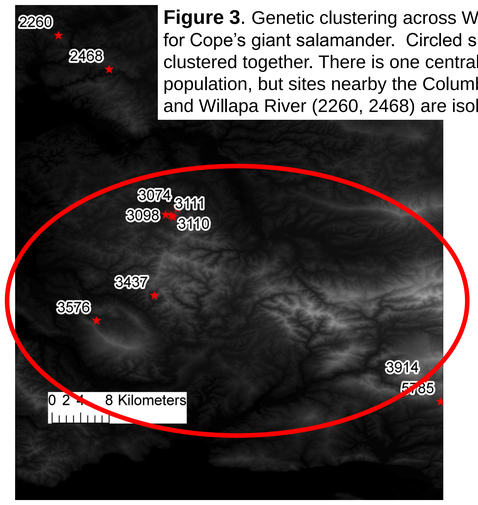


Figure 3. Genetic clustering across Willapa Hills for Cope's giant salamander. Circled sites are clustered together. There is one central large population, but sites nearby the Columbia (5785) and Willapa River (2260, 2468) are isolated

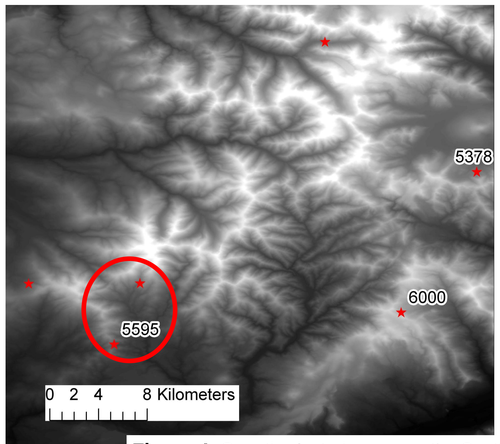


Figure 4. Genetic clustering across the South Cascades for Cope's giant salamander. Circled sites are clustered together. This region displays the most restricted gene flow for this species

Results: Coastal tailed frog genetic neighborhood

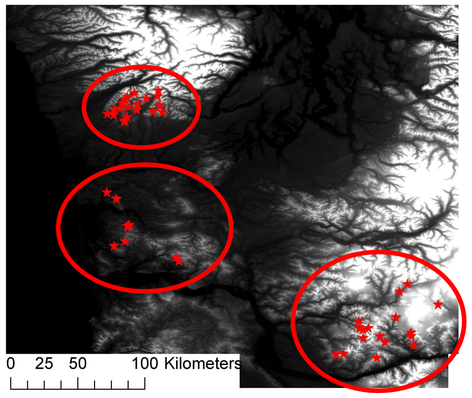


Figure 5. Genetic neighborhood of coastal tailed frogs. Circles represent population clusters, which correspond exactly to the Olympic , Willapa Hills, and South Cascades. Therefore, gene flow is typically very high in this species, with a genetic neighborhood of 50+ km.

Summary & Conclusions

1. More than half of collected giant salamander samples are non-target coastal giant salamanders, but North of Willapa River only Cope's giant salamander are found
2. There is some evidence of hybridization, particularly in the Willapa Hills region.
3. Both species are characterized by a high degree of genetic diversity. Lower diversity of Cope's giant salamander on Olympics may be a historic founder effect, but this pattern is not seen in tailed frogs
4. The size of the genetic neighborhood for Cope's giant salamander is quite variable based on study region, with patches of high connectivity in the Olympics and Willapa Hills. However, all three regions have isolated sites despite close proximity to other basins. Therefore, distance alone cannot satisfactorily explain salamander gene flow.
5. Coastal tailed frogs have consistently high gene flow across all study regions, although there is variation in gene flow within population clusters (data not shown).

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