The Esatina salamanders (*Ensatina eschscholtzii*), shown above, live along the West Coast of North America from Vancouver to Baja California. We will be focusing two populations:

1. individuals that live in Northern California and represent a more ancestral population

2. individuals that live in Southern California.

Researchers have found that these all individuals of this salamander species have two color phenotypes, either red or blotchy.

Genetic analysis has shown that this color difference is linked to the Ensatina melanophore gene (EM Gene). There are 2 alleles present for this gene in the current population. The R allele at this locus results in a red phenotype while the B allele at this locus results in a blotchy phenotype dominated by black, brown and orange coloration. This gene has a co-dominant inheritance pattern, meaning that individuals that are heterozygous for this gene, having the genotype "**RB**", are red and blotchy.

| GENOTYPE | RR | BB | RB |

The research group is interested in whether this population in Southern California is experiencing evolution on the body coloration. The research group will be using the Hardy-Weinberg Equation to test whether evolution is occurring on the EM gene.

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Q1a. What is the null hypothesis for this study?

Evolution for body coloration of the Southern California Esatina salamanders is not occurring and so therefore the salamanders are in Hardy-Weinberg and the allele frequencies for body coloration constant.

Q1b. What is the alternative hypothesis for this study?

Evolution for body coloration of the Southern California Esatina salamanders is occurring and so therefore the salamanders are not in Hardy-Weinberg and the allele frequencies for body coloration are changing.

As part of your summer internship in California, you categorize 100 salamanders collected from several populations across Southern California. You count 50 red and 37 blotchy individuals, the rest are red and blotchy.

Q2a. Using these data you collected as an intern, determine the observed genotype frequencies for the 3 possible genotypes and write them in the appropriate spaces. Show your work below.

RR=   50/100 = .5   BB=   37/100 = .37   RB= 13/100 = .13

Q2b. Calculate the allele frequencies for the two alleles? Show your work and clearly label the values with the appropriate allele letter.

Q2c. Use the Hardy-Weinberg equation to calculate the expected genotypic frequency for this population. And then complete the table below.

Genotypes	# Observed in population	# Expected in population	O- E
RR	50	32	18

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RB	13	49	-36
BB	37	19	18

Q2d. Based on your calculations, do you support your null or alternative hypothesis? Explain your answer.

Based on my calculations, my data calculated using the Hardy-Weinberg equation supports my alternative hypothesis that evolution is occurring because after comparing my observed and expected genotype frequencies I see that they vary greatly and so the population of salamanders are not in Hardy-Weinberg and allele frequencies are changing for body coloration.

You decide to conduct a Chi-Square Test to test for further support for the hypothesis you choose in Q2d.

Q2e. Fill in the right two columns in the table and the box below to determine your Chi-Square value and compare it to the critical values with 1 degree of freedom.

Genotypes	# Observed in population	# Expected in population	O- E	$(O-E)^2$	$\dfrac{(O-E)^2}{E}$
RR	50	32	18	324	10.125
RB	13	49	-36	1296	26.45
BB	37	19	18	324	10.125

Chi-Square Value= 46.7

Chi-Square (χ^2) Distribution
Area to the Right of Critical Value

Degrees of Freedom	0.99	0.975	0.95	0.90	0.10	0.05	0.025	0.01
1	—	0.001	0.004	0.016	2.706	3.841	5.024	6.635
2	0.020	0.051	0.103	0.211	4.605	5.991	7.378	9.210
3	0.115	0.216	0.352	0.584	6.251	7.815	9.348	11.345
4	0.297	0.484	0.711	1.064	7.779	9.488	11.143	13.277
5	0.554	0.831	1.145	1.610	9.236	11.071	12.833	15.086

Q2f. Based on your Chi-Square value do you accept or reject your null hypothesis? Do you conclude that evolution is occurring across these southern populations?   Explain your answer [3 pts].

Based on the very high value of 46.7, I would accept my null hypothesis that evolution is not occurring and that the coloration across southern populations is random. Because the chi-square sum is at such an extreme to say that the changes in coloration are random it is likely that it is correct and so evolution would not be occurring.

Having noted the lack of red and blotchy individuals, you do some research about the environment and the predators and other organisms that live in the areas where these salamanders are found. You notice that on the Western side of the distribution toward the coast, these salamanders co-exist with a species of newt (pictured below) that look awfully similar to the RR salamanders. These newts are also known to be poisonous when eaten, so the predators in the area leave them alone. The ground is mostly rocky or sandy in these areas.

On the Eastern side of the distribution the environment is a forest with rocks, leaves and moss covering the floor of the forest. You notice that the blotchy BB salamanders blend in well with the forest floor in these areas. There are no poisionous newts in this area.

Q3a. The Ensatina salamander species complex dates back to about 10 million years ago and fossil records show that it started in Northern California. The salamanders then migrated south by one of two routes; either by the coast or inland near the forest. The curve on the axes below represents the frequency distribution of the skin coloration patterns of the original population in Northern California.

Draw two new curves on these same axes:

1. A curve representing the distribution of the coloration pattern for a coastal (western) population

2. A curve representing the distribution of the coloration pattern for an inland (eastern) population.

Be sure to label the y axis and provide a figure legend.

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Frequency of Salamander

Key:

Red = Coastal Salamander RR

Blue = Inland Salamander BB

Black = Original population

Red   Red and   Blotchy
             Blotchy
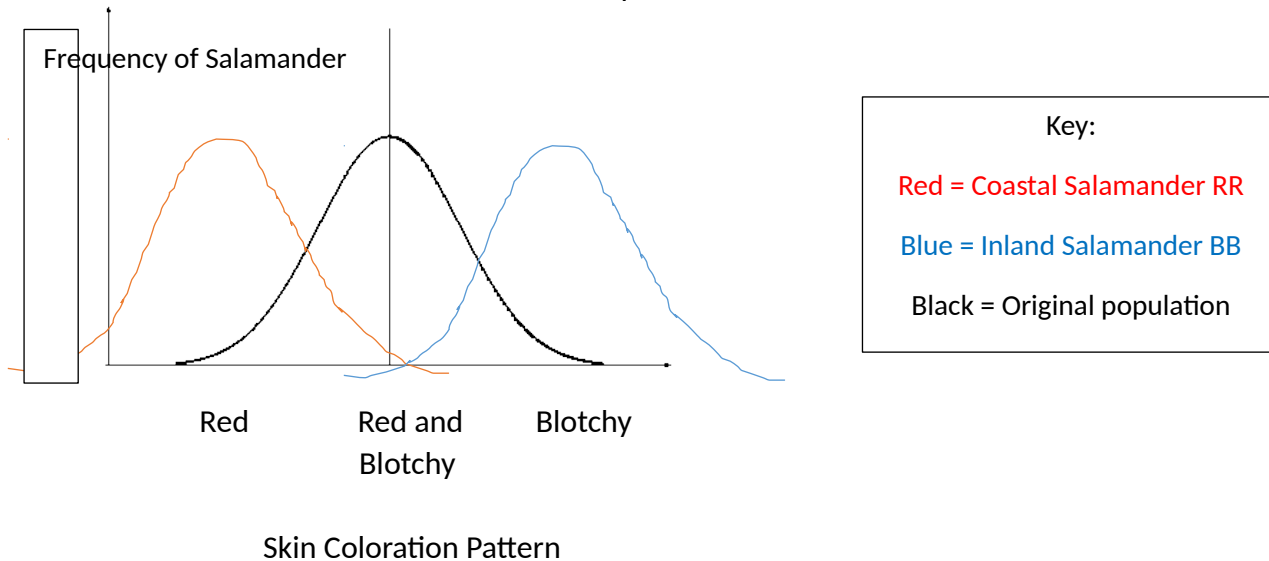
Skin Coloration Pattern

Figure 1.

An original population of coastal (Western) and inland (Eastern) salamanders from Northern California were observed for skin coloration patterns for their ability to survive from predators.

Q3b. What type of selection is modeled by your graph? Explain your choice in 1 sentence. [3 pts]

The selection shown is Disruptive selection because both extremes, red and blotchy skin coloration, are selected for leading to a high probability of speciation.

Q3c. What event could occur if this pattern continued for 20 or more generations in Southern California? Explain your answer in 1 sentence.

The event that could occur if this pattern continued with disruptive selection is speciation because both extremes are being selected for.

Q4 Considering your latest conclusions about the salamanders, explain each of the principles of evolution by selection listed in the left column. In the right column, explain in one sentence how each concept would be represented in this specific case.

Principle	One sentence explanation
Does variation exist for skin coloration pattern in this population? Explain any variation.	**Yes variation exists because there are salamanders with red, red and blotchy and blotchy skin coloration.**
What is the <u>origin</u> of the variation? (How/why is there variation)	**The origin of variation for skin coloration in salamanders is random mutation.**

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Is the variation for skin coloration pattern **heritable**? How would we test for this?	**Yes, the variation for skin coloration is heritable and we could test this by grouping together a population of these salamanders and having them breed together based on skin color to see if the color is passed down to their offspring or we could look at the DNA.**
How was selection a factor in the skin coloration pattern?	**Natural selection occurs by which the red colored salamanders are selected for because they appear to look similar to red poisonous newts which allows them to avoid being eaten and the blotchy salamanders blend in with their environment while red and blotchy salamanders are selected against.**
What is the **evidence** that the population of salamanders is evolving (remember definition of evolution)?	**The evidence that the salamander population is evolving is that there is change in the allele frequencies and the RR and BB genotype is increasing while the RB genotype is decreasing.**

Q5. In the table above, you have shown that this case is evolution by natural selection. Explain in 1-2 sentences how we know it is not genetic drift AND that it is not gene flow.

We know that this case is evolution by natural selection and not genetic drift because genetic drift implies that allele frequencies are drifting up and down overtime and that it is random which we know is not true. We also know that it is not gene flow because it does not involve any other population or any genetic exchange.
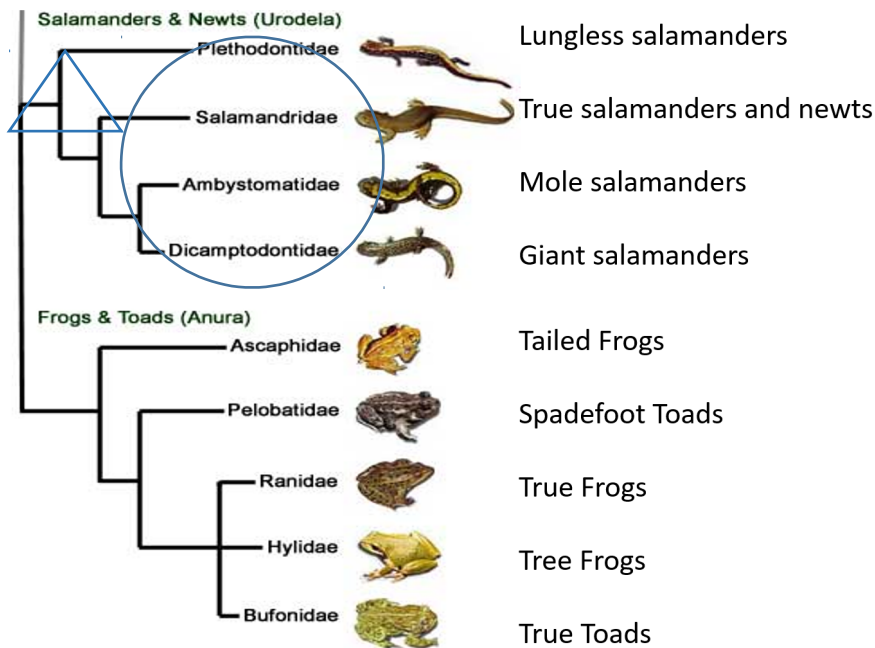
Disease biologist working near you in California have noticed that a large number of salamanders dying.

The disease biologists discover that a virus that is causing "wasting" symptoms in some salamanders that leads to death, but some salamanders survive. The biologists realize that the salamanders that are surviving the disease have a mutation called IM2 which produced proteins that result in an immune system that is better than other salamanders at fighting viral infections.

One of the biologists says, "Wow. It is a good thing that there are some individuals that adapted to this viral infection and developed the IM2 mutation so that the entire species of salamanders doesn't become extinct."

Q6. How do you respond to the comment to educate your co-worker about evolution? A complete answer would include 2 misconceptions and an explanation of each misconception.

One misconception here is that the IM2 mutation was developed which would not be true because mutations occur at random. Another misconception would be that the individual adapted to their environment because organisms can only adjust or acclimate but cannot evolve or adapt to their surroundings.

Lungless salamanders

True salamanders and newts

Mole salamanders

Giant salamanders

Tailed Frogs

Spadefoot Toads

True Frogs

Tree Frogs

True Toads

Above is a phylogenetic tree showing the evolutionary relationships of the several of the major families of amphibians as we understand them today.

Q7a. Circle a monophyletic group that contains exactly four families. There may be more than one correct answer.

Q7b. Draw a triangle around the area of the phylogenetic tree that would represent the common ancestor of giant salamanders and lungless salamanders.

Q7c. If both true frogs and tree frogs have the synapomorphy of having 2 of their wrist bones (carpals) fused, what other group should show this same synapomorphy?.

The True Toads would also have the synapomorphy of having 2 of their wrist bones fused.

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