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DATA Nugget

Cheaters in nature – when is a mutualism not a mutualism?
Featured scientist: Iniyan Ganesan from the Kellogg Biological Station

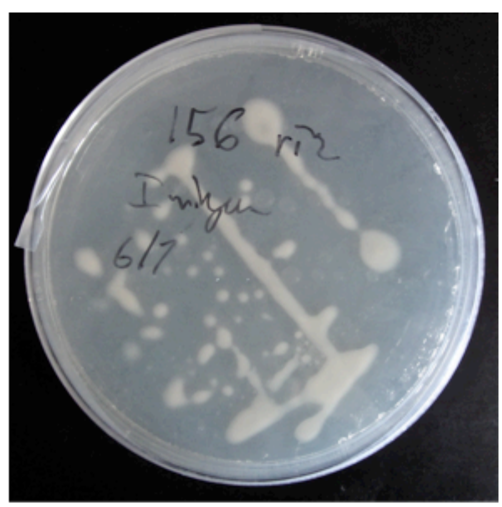
Research Background:

Mutualisms are a special type of relationship in nature where two species work together and both benefit. Each partner trades with the other species, giving a resource and getting one in return. This cooperation leads to partner species doing better when the other is around, and without their partner, each species would have a harder time getting resources.

One important mutualism is between **clover**, a type of plant, and **rhizobia**, a type of bacteria. Rhizobia live in small bumps on the clovers' roots, called nodules, and receive protection and sugar food from the plant. In return, the rhizobia trade nitrogen to the plant, which plants need to photosynthesize and make new DNA. This mutualism works well when soil nitrogen is rare, because it is hard for the plant to collect enough nitrogen on its own, and the plant must rely on rhizobia to get all the nitrogen it needs. But what happens when humans change the game by fertilizing the soil? When nitrogen is no longer rare, will one partner begin to cheat and no longer act as a mutualist?

Worldwide, the nitrogen cycle is off. Not that long ago, before farmers used industrial fertilizers and people burned fossil fuels, nitrogen was rare in the soil. Today, humans are adding large amounts of nitrogen to soils. The nitrogen that we apply to agricultural fields doesn't stay on those fields, and nitrogen added to the atmosphere when we burn fossil fuels doesn't stay by the power plant that generates it. The result is that today,

Clover plant in the field growing with rhizobia mutualist



Rhizobia growing on a Petri Dish in the lab more and more plants have all the nitrogen they need. With high nitrogen, plants may no longer depend on rhizobia to help them get nitrogen. This may cause the plant to trade less with the rhizobia in high nitrogen areas. In response, rhizobia from high nitrogen areas may evolve to try harder to get food from the plant, and may even cheat and become parasitic to plants. If this happens, both species will no longer be acting as mutualists.

When Iniyan was a college student, he wanted to study human impacts on the clover-rhizobia mutualism. To find out more, he contacted Jen Lau's lab at the Kellogg Biological Station one summer, and joined a team of scientists asking these questions. For his own experiment, Iniyan chose two common species of clover: hybrid clover *(Trifolium hybridum)* and white clover *(Trifolium pretense)*. He chose these two species because they are often planted by farmers. Iniyan then went out and collected rhizobia from farms where nitrogen had been added in large amounts for many years, and other farms where no nitrogen had been added.

To make sure that there were no rhizobia already in the soil, Iniyan set up his experiment in a field where no clover had grown before. He then planted 45 individuals of each species in the field. He randomly assigned each plant to one of three treatments. For each species, he grew 15 individuals with rhizobia from high nitrogen farms, and 15 with rhizobia from low nitrogen farms. To serve as a control, he grew the remaining 15 individuals without any rhizobia. To add rhizobia to the plants he made two different mixtures. The first was a mix of rhizobia from high nitrogen farms and water, and the second was a mix of rhizobia from low nitrogen farms and water. He then poured one of these mixtures over each of the plants, depending on which rhizobia treatment they were in. The control plants just got water. No nitrogen was added to the plants.

After the plants grew all summer, Iniyan counted the number of leaves and measured the shoot height (cm) for each individual plant. He did not collect biomass because he wanted to let the plants continue to grow. He then averaged the data from each set of 15 individuals. Plants with fewer leaves and shorter shoots are considered less healthy. He predicted rhizobia that evolved in high nitrogen soils would be worse mutualists to plants, while rhizobia that evolved in low nitrogen soils would be good mutualists.

<u>Scientific Question</u>: How do high nitrogen environments affect the relationship between clovers and rhizobia?

<u>What is the hypothesis?</u> Find the hypothesis in the Research Background and underline it. A hypothesis is a proposed explanation for an observation, which can then be tested with experimentation or other types of studies.

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Scientific Data:

Use the data below to answer the scientific question:

Hybrid Clover Data	**Rhizobia Treatment**		
	Rhizobia from low nitrogen environment	Rhizobia from high nitrogen environment	No rhizobia control
Average Leaf Number	10.19	6.85	9.50
Leaf Number SE*	0.81	1.04	1.50
Average Shoot height (cm)	9.04	8.06	10.65
Shoot height SE	0.83	0.97	1.15

White Clover Data	**Rhizobia Treatment**		
	Rhizobia from low nitrogen environment	Rhizobia from high nitrogen environment	No rhizobia control
Average Leaf Number	4.62	4.65	5.50
Leaf Number SE	0.51	0.37	0.50
Average Shoot height (cm)	9.98	11.83	11.90
Shoot height SE	1.17	0.65	3.90

** Standard error (SE) tells us how confident we are in our estimate of the mean, and depends on the number of replicates in an experiment and the amount of variation around the mean. A large SE means we are not very confident, while a small SE means we are more confident.*

What data will you graph to answer the question?

Independent variable(s): ____

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Dependent variable(s): ____

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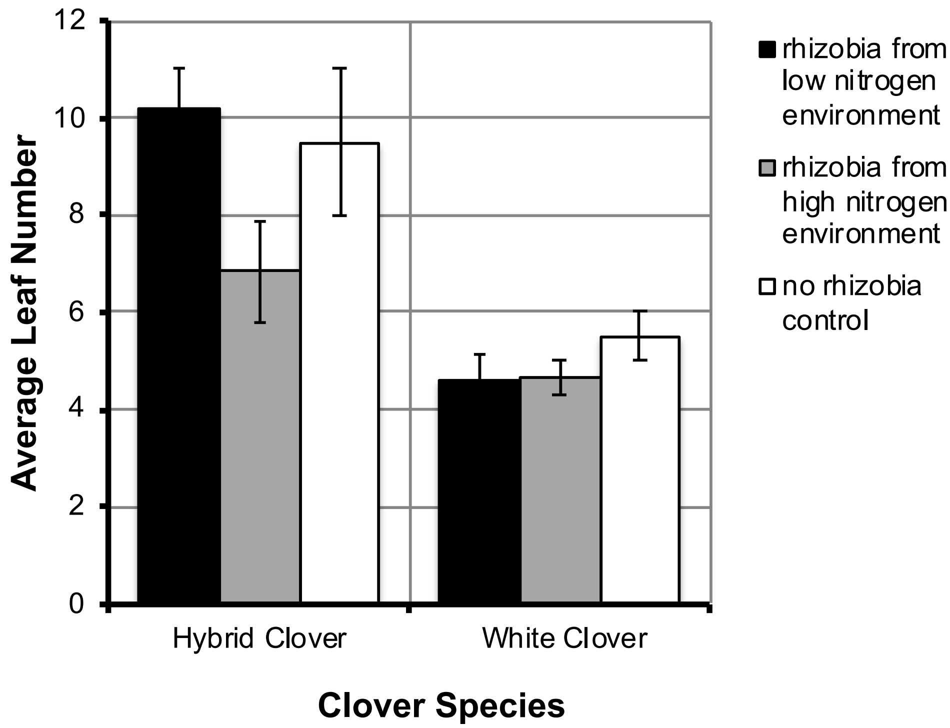
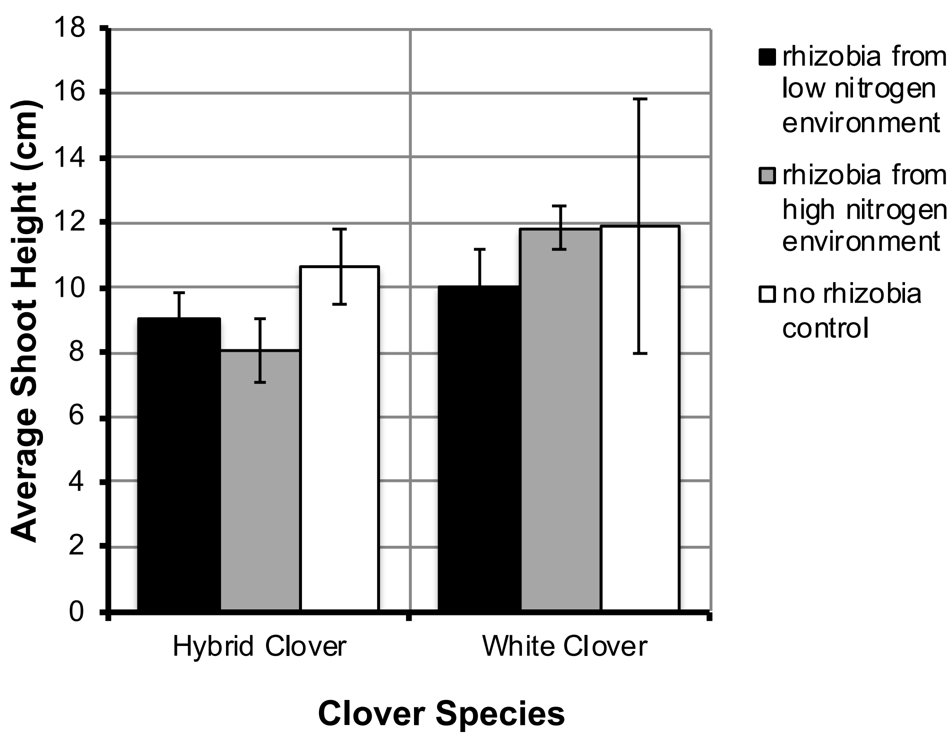
Name____________________

<u>*Below are graphs of the data*</u>: Identify any changes, trends, or differences you see in your graph. Draw arrows pointing out what you see, and write one sentence describing what you see next to each arrow.





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Interpret the data:

Make a claim that answers the scientific question.

What evidence was used to write your claim? Reference specific parts of the tables or graphs.

Explain your reasoning and why the evidence supports your claim. Connect the data back to what you learned about how soil nitrogen affect plants, and why this may in turn affect the plant-rhizobia mutualism.

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Did the data support Iniyan's hypothesis? Use evidence to explain why or why not. If you feel the data were inconclusive, explain why.

<u>Your next steps as a scientist:</u> Science is an ongoing process. What new question(s) should be investigated to build on Iniyan's research? How do your questions build on the research that has already been done?