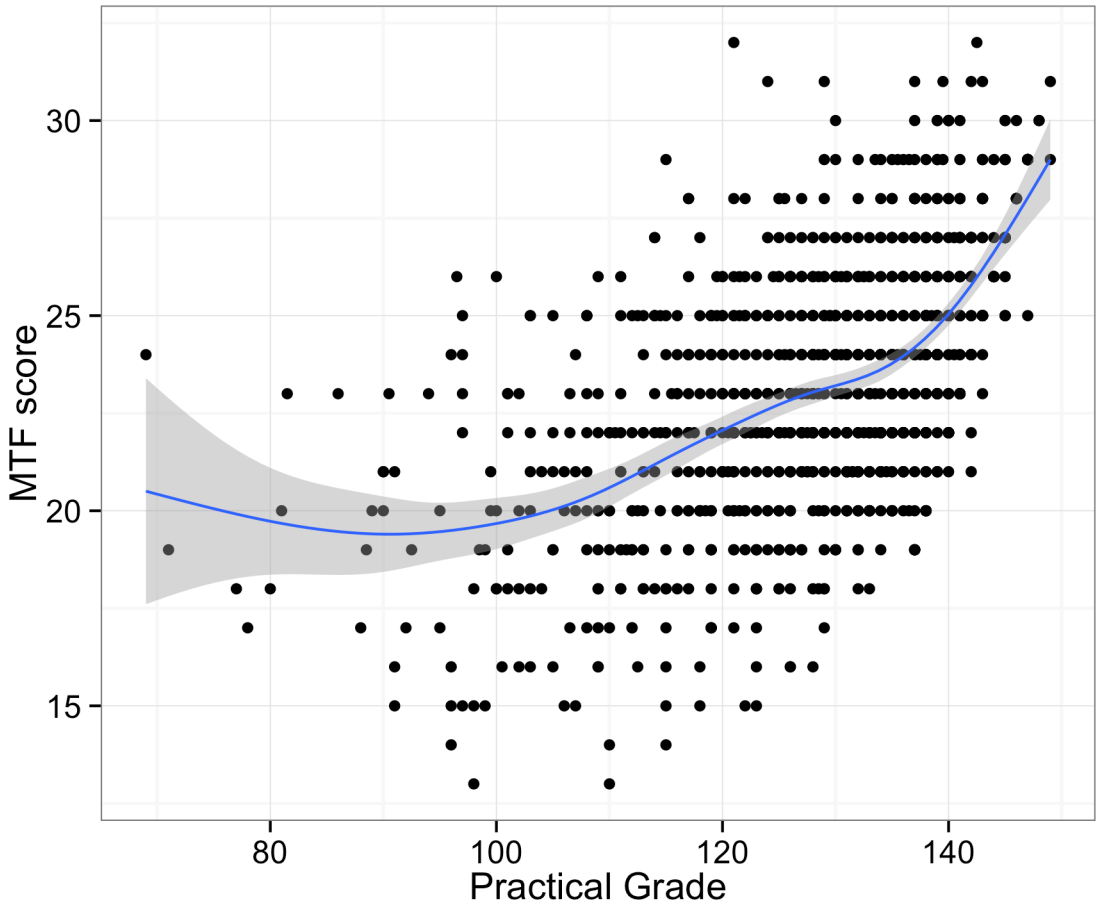
Supplemental Material

CBE—Life Sciences Education

Deane-Coe *et al.*

Figure S1: Student performance on multiple true false questions (MTF score, out of 32 points total) as a function of total score from the second lab practical (Practical Grade) in which the questions were included. The number of total points in the practical varied across semesters thus MTF score is plotted in relation to total score. The trend line represents the best-fit nonlinear spline given the data, and the gray bands indicate the 95% confidence interval.

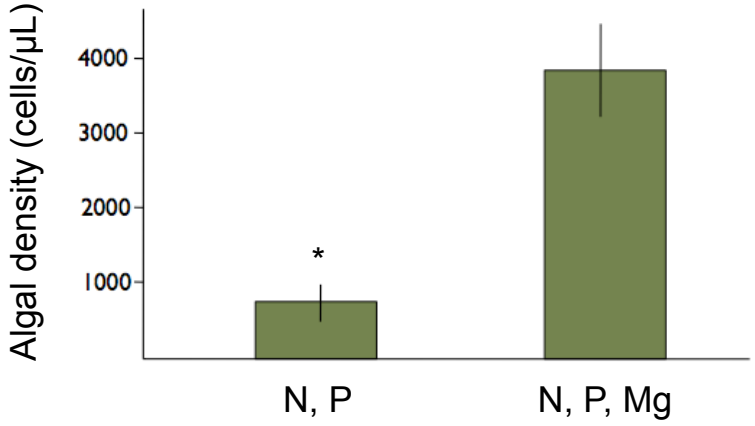


Pages to follow: Multiple true-false questions used in study. In each set of questions, statements 1-8 were Category A (familiar content and low complexity), statements 9-16 were Category B (novel content and low complexity), statements 17-24 were Category C (familiar content and increased complexity), and statements 25-32 were Category D (novel content and increased complexity).

QUESTION 1

Please take this question to your seat to answer the questions below, and then return it to the station when finished.

Use the Scantron bubble sheet, and mark your answers clearly. Use "A" for True and "B" for False in each of the eight questions below. Be sure to start with the correct number!




```
> shapiro.test(N_P_Mg)

 Shapiro-Wilk normality test

data:  N_P_Mg
W = 0.9178, p-value = 0.178

> shapiro.test(N_P)

 Shapiro-Wilk normality test

data:  N_P
W = 0.9681, p-value = 0.8297
```

In your nutrient enrichment experiment, you compared algal density after adding N and P (n=10), *or* N, P, and Mg (n=10) to test tubes containing filtered water from Beebe Lake. After one week, you collected your data using optical density measurements, produced the figure above to summarize your findings, and generated the *R* output shown.

Based on your *R* output, you can conclude that:

1) The results of the Shapiro-Wilk tests indicate that solutions with Mg did not have significantly higher algal density than those without Mg (P>0.05)
2) We cannot reject the null hypothesis of the Shapiro-Wilk tests
3) A t-test is appropriate for comparing the N, P group to the N, P, Mg group
4) There is no correlation between Mg amount and algal density

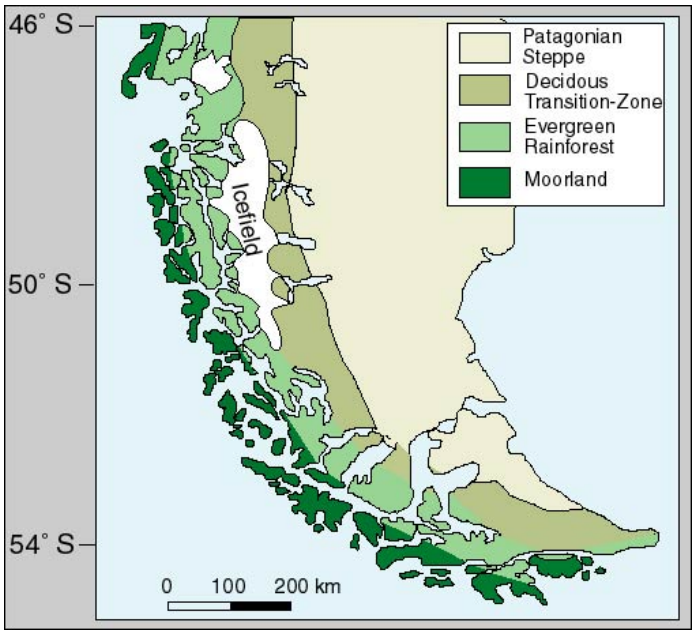
If the asterisk in your figure indicates a significant difference in algal density between the two treatment categories, which of the following statements is/are correct based on your results?

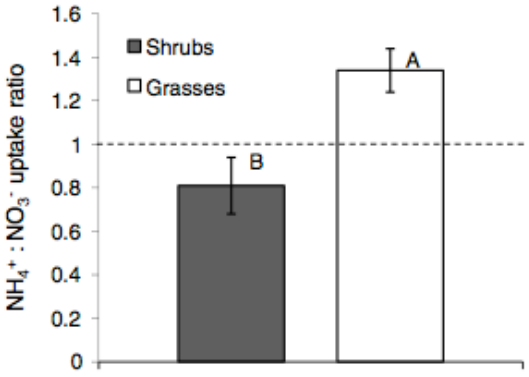
5) Mg is the tertiary limiting nutrient in this system
6) Experimentally removing Mg from the N, P, Mg group would result in reduced chlorophyll a concentration
7) In an analogous natural system, eutrophication is more likely in when Mg is added compared to when it is not
8) Algal reproduction rates are higher when Mg is added

QUESTION 2

Please take this question to your seat to answer the questions below, and then return it to the station when finished.

Use the Scantron bubble sheet, and mark your answers clearly. Use "A" for True and "B" for False in each of the eight questions below. Be sure to start with the correct number!





In the South America, the arid Patagonian steppe is dominated by two plant functional types: shrubs and grasses. Nitrogen limits productivity in this ecosystem, and exists in the soil as both ammonium (NH_4^+) and nitrate (NO_3^-). Grasses and shrubs can take up both nitrogen forms for growth and maintenance, but do these plant types differ in their preferred form for uptake?

Researchers set out to answer this question using a hydroponic experiment in which Patagonian grasses and shrubs were grown in solutions containing NH_4^+ and NO_3^-. Uptake rates were extrapolated from differences in nutrient concentrations before and after the experiment in solutions in which plants were grown. Results for $NH_4^+ : NO_3^-$ uptake ratio in both plant types are shown in the figure above. Different uppercase letters represent significant differences in uptake ratios between the two plant types (P=0.03) following statistical analysis using a t-test.

TURN OVER for questions

Which of the following is a valid conclusion drawn from the data presented?

9) Grasses have a significantly higher % nitrogen in their tissues compared to shrubs
10) Following the experiment, hydroponic solutions in which grasses were grown will have a lower NH_4^+ : NO_3^- ratio than at the start of the experiment
11) For every 1 mg NO_3^- absorbed, shrubs absorb < 1 mg NH_4^+
12) Because nitrogen exists as both NO_3^- and NH_4^+, shrubs and grasses are unlikely to compete for nitrogen in this system

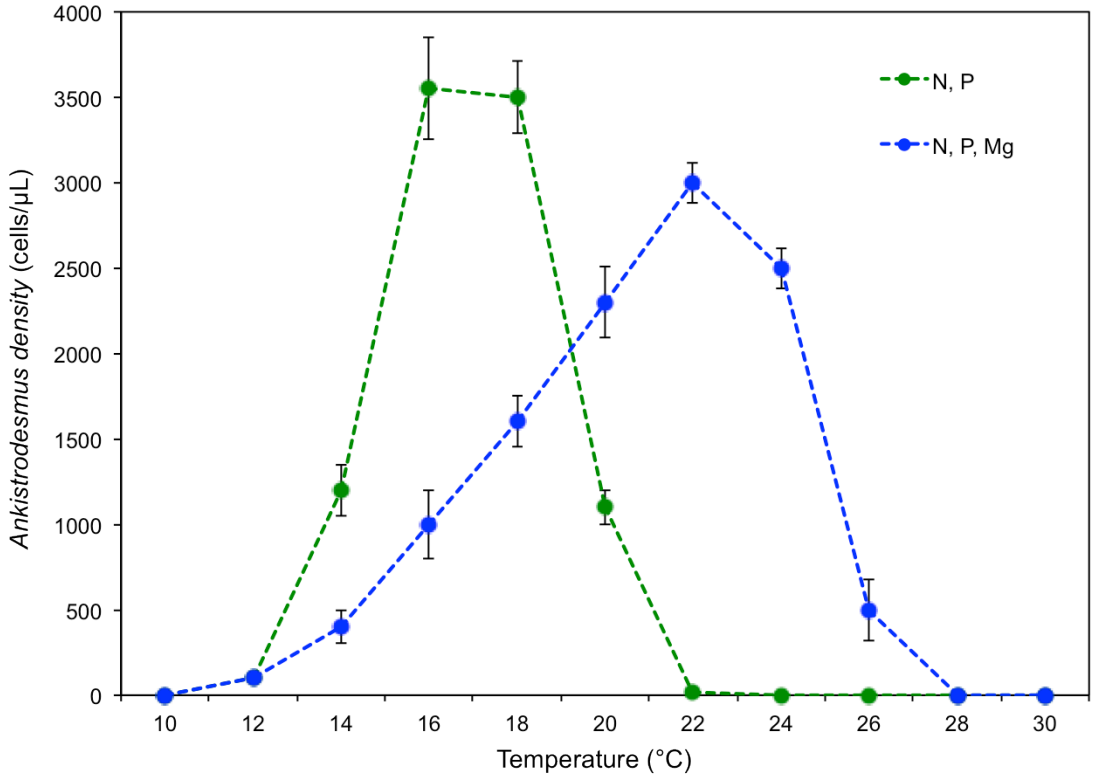
Based on the information given, which of the following are true statements regarding the researchers' experimental design?

13) If raw uptake ratio data were not normally distributed, they were transformed prior to statistical analysis
14) A valid control group would have been: solutions with no plants grown in them left for the duration of the experiment
15) Design should include grasses and shrubs grown alone as well as together in solutions
16) Regardless of the **form** of nitrogen supplied, identical starting **concentrations** of nitrogen must have been present in each hydroponic solution

QUESTION 3

Please take this question to your seat to answer the questions below, and then return it to the station when finished.

Use the Scantron bubble sheet, and mark your answers clearly. Use "A" for True and "B" for False in each of the eight questions below. Be sure to start with the correct number!



You are interested in determining if nutrient enrichment influences the temperature tolerance of algae. In the lab, you perform an experiment in which *Ankistrodesmus* is grown in test tubes containing sterile pond water containing N and P, *or* N, P, and Mg. Groups of 10 test tubes from each of the two groups were then incubated at 11 different temperatures at 2 °C intervals between 10 and 30 °C for two weeks. You measured optical density of the samples and converted measurements to cell density. Results are shown in the figure above (dotted lines represent extrapolated values between points).

Which of the following are true statements regarding your experimental design and analysis?

17) Identical ratios of N:P:Mg must be applied to control and experimental replicates
18) A rank sum test can be used to analyze data from the two groups grown at 20°C
19) The group containing N and P only can serve as the control
20) H_0 = there is no influence of temperature on solution nutrient levels

TURN OVER for questions 21-24

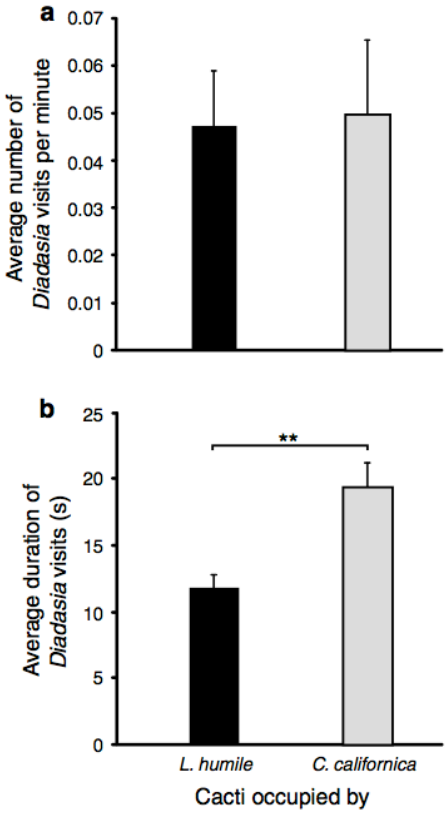
What can you conclude from your results presented in the figure above?

21) *Ankistrodesmus* will not display growth at 26°C unless Mg is added
22) *Ankistrodesmus* has a single temperature optimum at approximately 17 °C
23) Mg does not appear to affect minimum viable temperature in *Ankistrodesmus*
24) Nutrient enrichment results for *Ankistrodesmus* in this experiment can be attributed to directional selection

QUESTION 4

Please take this question to your seat to answer the questions below, and then return it to the station when finished.

Use the Scantron bubble sheet, and mark your answers clearly. Use "A" for True and "B" for False in each of the eight questions below. Be sure to start with the correct number!




```
> cor.test(visit_duration,pollination_success, method="spearman")

 Spearman's rank correlation rho

data:  visit_duration and pollination_success
S = 9.6243, p-value = 2.939e-12
alternative hypothesis: true rho is not equal to 0
sample estimates:
      rho
0.9858466
```

The coast barrel cactus *Ferocactus viridescens* is an example of a plant that depends on insects for survival and reproduction. Flowers of the cactus produce nectar that is consumed by ants; the ants, in turn, reside on the cactus and offer protection from herbivores. This cactus species also depends on different insects, bees of the genus *Diadasia,* for pollination of their flowers.

The figure above shows data collected by researchers on (a) frequency of bee visits to flowers and (b) duration of bee visits to flowers on cacti occupied by one of two different ant species (*Linepithema humile* or *Crematogaster californica*). The researchers were later interested in determining of any relationship existed between pollinator visit duration and pollination success. The results of a correlation test performed on these two variables are also shown above.

TURN OVER FOR QUESTIONS

Based on the results shown in the figure and in the correlation test, it can be concluded that:

25) Fewer flowers will be pollinated if *L. humile* occupy the cacti than if *C. californica* occupy the cacti
26) Bees visit cacti occupied by both ant species equally as frequently, therefore pollination success is independent of ant species
27) Association with *C. californica* confers a selective advantage to cacti
28) If pollination success has declined, pollinators are spending less time on flowers

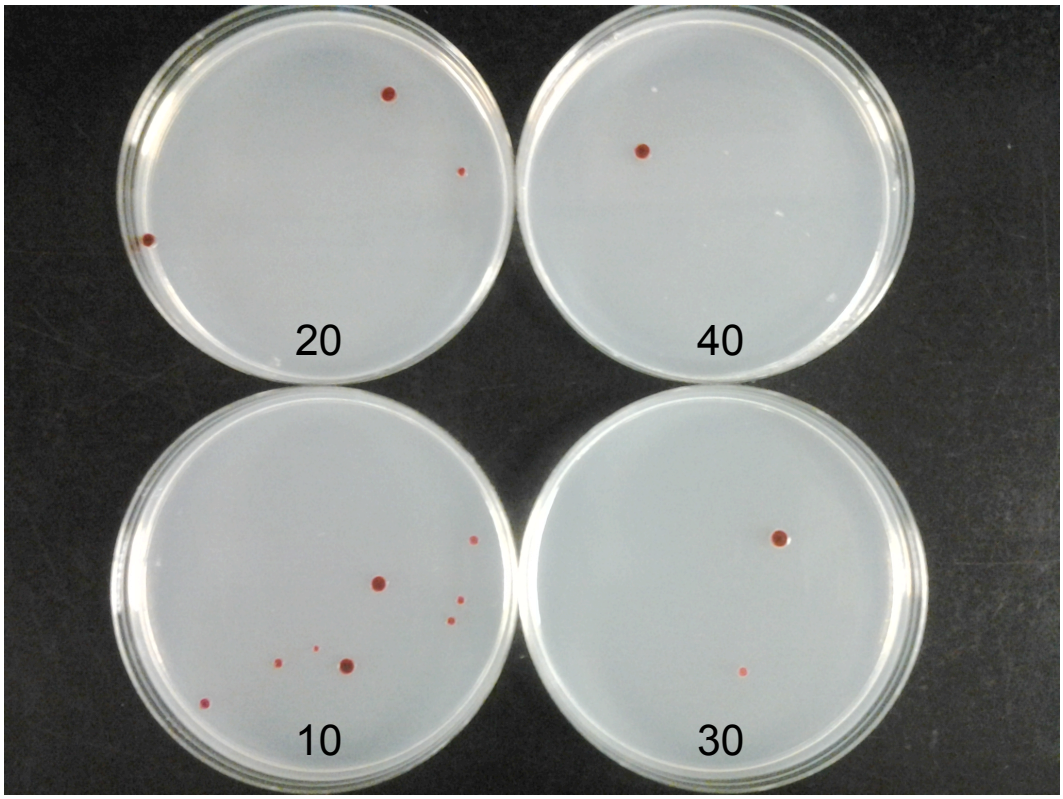
Which of the following is a <u>research hypothesis</u> that could be used to explain the results shown in panel (b) in the figure:

29) *C. californica* facilitates pollination by not defending cacti from bees
30) *L. humile* displaces pollinators from flowers
31) Nectar is a limiting resource, and *C. californica* and *L. humile* are displaying resource partitioning
32) H_0 = There is no effect of ant species on *Diadasia* visit duration

QUESTION 1

Please take this question to your seat to answer the questions below, and then return it to the station when finished.

Use the Scantron bubble sheet, and mark your answers clearly. Use "A" for True and "B" for False in each of the eight questions below. Be sure to start with the correct number!



You came across an interesting primary research article that showed how hundreds of different mutations can occur in *Serratia* that confer resistance to aminoglycoside antibiotics. After sharing this information with your lab group, you worked together to design your mutation and selection experiment: To test for antibiotic resistance, you grew *Serratia*, a gram-negative bacterium, in the presence of Kanamycin, an aminoglycoside antibiotic that functions to inhibit translation. You plated equal numbers of *Serratia* cells from a parent culture on agar with four different Kanamycin concentrations (10 µg/ml; 20 µg/ml; 30 µg/ml and 40 µg/ml) as well as on a control plate with no antibiotics in the agar. Following the one-week incubation, the control plate produced a lawn of growth, and four representative experimental plates are shown in the photo above (numbers on the plates represent different Kanamycin concentrations).

TURN OVER for questions

Which of the following is a valid research hypothesis you may have posed *before* setting up this experiment?

1) Kanamycin induces a greater number of mutations at lower concentrations
2) Mutations are not equally effective in maintaining protein synthesis in the presence of Kanamycin
3) Natural Selection occurs spontaneously in *Serratia*
4) Of the mutations present in the parent culture, only very few will confer resistance to the highest concentration of antibiotic

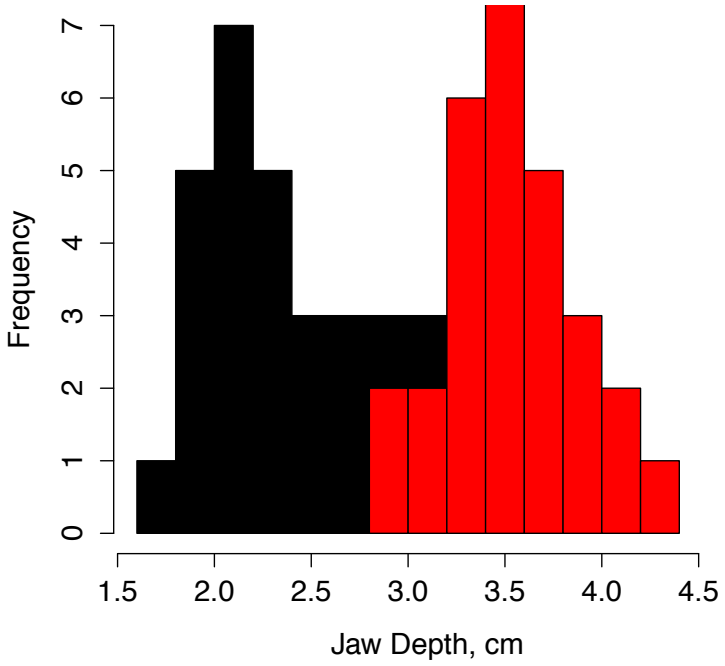
Based on the raw data shown in the photo of your plates, which of the following statements about this experiment is/are true?

5) If a harmful outbreak of this *Serratia* strain occurred, application of Kanamycin at the 40 μg/ml level would eradicate the outbreak by preventing the bacteria from evolving
6) A Spearman Rank Correlation test can be used to analyze the relationship between Kanamycin concentration and colony counts
7) The frequency of mutants is highest on the 10 μg/ml plate
8) Phenotypic diversity in resistant *Serratia* is likely to be highest on the10 μg/ml plate

QUESTION 2

Please take this question to your seat to answer the questions below, and then return it to the station when finished.

Use the Scantron bubble sheet, and mark your answers clearly. Use "A" for True and "B" for False in each of the eight questions below. Be sure to start with the correct number!



In Cameroon, two different species of *Tilapia* inhabit Lake Ejagham: one with dorsal black coloration (*T. fusiforme*), and one with dorsal red coloration and (*T. deckerti*). Total population size for each species is currently thought to be in the thousands. As these two species share an extinct common ancestor, researchers were interested in determining to what extent they shared another morphological trait, jaw depth. In a pilot study, the researchers caught and measured jaw depth in 30 individuals of each species of *Tilapia* from Lake Ejagham. The number of individuals in each species (*T. fusiforme* = black bars, *T. deckerti* = red bars) with particular jaw depth values are shown in the figure above.

Which of the following is correct based on the pilot data shown?

9) A rank-sum test can be applied to determine if the two species differ significantly in their jaw depth values
10) At the jaw depth value of 3 cm, *T. fusiforme* and *T. deckerti* are indistinguishable
11) Jaw depth is a strong predictor of dorsal coloration (black or red) in *Tilapia*
12) No *Tilapia* in Lake Ejagham have a jaw depth of 1 cm

TURN OVER for questions 13-16

If the extinct ancestral *Tilapia* species had a mean jaw depth of 3 cm, what is a hypothesis that could be derived from the pilot data?

13) Individuals with 3 cm deep jaws are not contributing genes to future generations
14) The genus *Tilapia* in Lake Ejagham has undergone disruptive selection
15) *T. fusiforme* and *T. deckerti* have adapted to feed on different prey
16) Dorsal coloration traits and jaw depth traits are inherited together

QUESTION 3

Please take this question to your seat to answer the questions below, and then return it to the station when finished.
Use the Scantron bubble sheet, and mark your answers clearly. Use "A" for True and "B" for False in each of the eight questions below. Be sure to start with the correct number!

Peptidoglycan concentration, ng/cell	**1 µg/ml Streptomycin**		**5 µg/ml Streptomycin**	
	# cells, wk 0	**# cells, wk 6**	**# cells, wk 0**	**# cells, wk 6**
0-100	21	61	23	0
100-200	39	72	38	0
200-300	52	41	51	0
300-400	70	20	73	28
400-500	63	17	70	41
500-600	49	38	53	68
600-700	40	69	38	99
700-800	18	73	17	119
Total cells sampled:	*352*	*391*	*363*	*355*

In this course, you learned in the mutation and selection module that the antibiotic Streptomycin inhibits translation in *Serratia.* But what happens when *Serratia* is exposed to low doses of Streptomycin that kill some cells but permit others to survive? Could other cellular changes occur in *Serratia*? You decide to examine if exposure to non-fatal concentrations of Streptomycin has any effect on the cellular concentration of the cell wall polymer peptidoglycan.

You prepared two liquid cultures of *Serratia*, and before adding any antibiotics, measured the initial peptidoglycan concentration in cells (week 0), taking a tally of the number of cells that had peptidoglycan concentrations in particular ranges. Next, you added 1 µg/ml Streptomycin to one of the cultures, and 5 µg/ml Streptomycin to the other, waited six weeks, and measured peptidoglycan concentration again in the same manner. Your results for number of cells, grouped into peptidoglycan concentration ranges, are shown for each antibiotic treatment in the table above.

TURN OVER for questions

Proud of you results from this small-scale study, you eagerly prepare and submit a manuscript to the journal *Cell*, yet within a week your paper is rejected on the grounds of insufficient sample size! If you wanted to repeat this experiment with a larger sample size, which of the following are testable hypotheses for your study?

17) Exposure to Streptomycin selects for different cell wall phenotypes depending on antibiotic concentration
18) Exposure to Streptomycin selects for similar cell wall phenotypes regardless of antibiotic concentration
19) Streptomycin resistance increases with increasing Peptidoglycan concentration
20) There is a correlation between Streptomycin concentration and Peptidoglycan concentration

Which of the following are true with respect to selection in this experiment?

21) Exposure to Streptomycin, regardless of concentration, selected **against** 300-400 ng/cell Peptidoglycan concentrations in cell walls
22) Selection began after six weeks of growth
23) Exposure to 1 μg/ml Streptomycin resulted in disruptive selection in *Serratia*
24) Exposure to 5 μg/ml Streptomycin resulted in directional selection in *Serratia*

QUESTION 4

Please take this question to your seat to answer the questions below, and then return it to the station when finished.
Use the Scantron bubble sheet, and mark your answers clearly. Use "A" for True and "B" for False in each of the eight questions below. Be sure to start with the correct number!

Ethnicity	Dengue cases per month (mean ± SD)	HLA-1 Locus Genotype			HLA-2 Locus Genotype		
		AA	BB	AB	CC	DD	CD
Chinese	89.5 ± 6.1 *	24%	17%	59%	0%	33%	67%
Malaysian	60.2 ± 7.8	79%	17%	4%	100%	0%	0%

* T-test (α=0.01) for difference in number of Dengue cases per month between ethnicities: $P = 0.0034$

Dengue virus is a mosquito-borne pathogen that infects an estimated 50-100 million people annually worldwide. It was recently suggested that ethnicity could play a role in susceptibility to the virus. Researchers interested in this question set up a pilot study comparing cases of Dengue over one year in humans in China compared to humans in Malaysia. They examined the frequency of Dengue occurrence by counting the number of cases in individuals of each ethnicity monthly, and also gained permission to obtain genetic information about each patient with Dengue. Specifically, they targeted two loci occurring on chromosome six in humans that are known to function in immune response (HLA-1 and HLA-2). HLA-1 can have either allele A or B, and HLA-2 can have either allele C or D. The table above shows the researchers' results from this preliminary study: average Dengue cases per month in Chinese and Malaysian individuals, and the percent of individuals with Dengue sampled for each ethnicity that had the different possible genotypes at each locus.

Which of the following must be true about the researchers' experimental design / analysis for the results of this pilot study to be valid?

25) Alleles must occur in equal frequency at each locus (F(A)=F(B) for HLA-1, F(C)=F(D) for HLA-2) in each sampled population (ethnicity)
26) Genotypes at each locus must show that each population (ethnicity) is in Hardy Weinberg Equilibrium
27) A valid control group is genotype frequencies at each locus for members of each ethnicity that are *not* infected with Dengue
28) HLA-1 and HLA-2 must *both* be present in all individuals sampled

TURN OVER for Questions 29-32

Based on the initial data presented in this pilot study, an appropriate hypothesis for a full-scale study would be:

29) Heterozygosity at either locus increases susceptibility to Dengue in Chinese populations
30) Homozygosity for allele A reduces Dengue susceptibility in Malaysians compared to Chinese
31) The presence of the Dengue virus is selecting for homozygosity at both loci
32) Based on the alleles examined, susceptibility to Dengue can be explained exclusively based on the presence or absence of allele D in a population