Supplemental Material

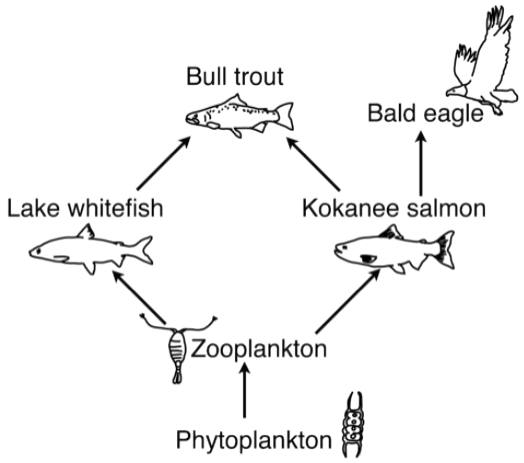
CBE—Life Sciences Education

Summers *et al.*

SUPPLEMENTAL APPENDIX S1. EcoEvo-MAPS question stems with likely/unlikely statements.

Question 1. In 1981, fishery managers introduced mysid shrimp to a Montana lake with the intention of increasing kokanee salmon production.

Below is a food web for this Montana lake *BEFORE* mysid shrimp were introduced. The arrows move from prey to predator. For example, zooplankton are eaten by lake whitefish and kokanee salmon.



A few years after introduction of mysid shrimp to the Montana lake, fisheries managers found that:
- mysid shrimp consumed other zooplankton;
- these smaller zooplankton populations declined;
- and the kokanee salmon population disappeared.

Based on your knowledge about biology and this information, evaluate each statement as **Likely** or **Unlikely** to be true:

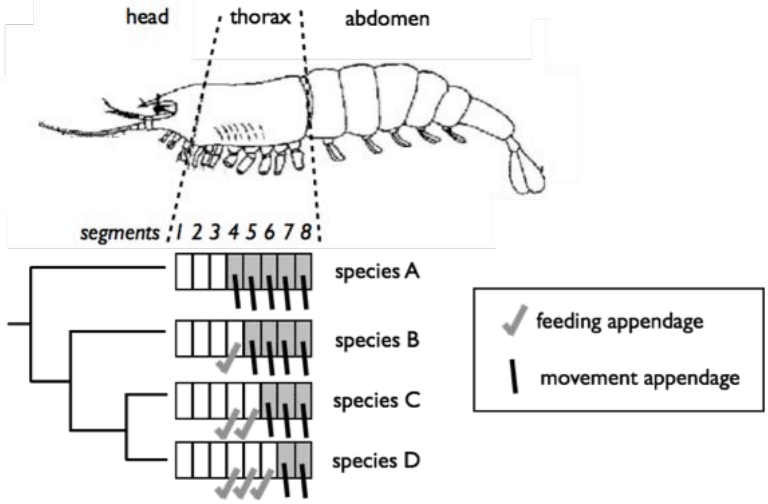
	Likely	Unlikely
1) Bull trout directly prey upon lake whitefish, kokanee salmon, zooplankton, mysid shrimps, and phytoplankton.	❍	❍
2) Kokanee salmon population decline resulted from competition for food resources between mysid shrimp and kokanee salmon.	❍	❍
3) The only factor regulating the number of phytoplankton is predation by zooplankton.	❍	❍
4) If the amount of phytoplankton increases by one ton, a one ton increase in zooplankton is expected.	❍	❍
5) A toxicant present in zooplankton that biomagnifies would be found at a higher concentration in lake whitefish than in bull trout.	❍	❍
6) If phytoplankton in the pond are at carrying capacity, with phosphorous availability limiting growth, nitrogen run-off from a nearby agricultural field will increase phytoplankton growth.	❍	❍
7) If regulations lead to an increase in bull trout, the lake whitefish and kokanee salmon populations will immediately increase in response.	❍	❍

Question 2. The crustacean thorax is divided into segments. Segments may have appendages that are used for either movement or feeding.

Scientists investigated Ubx gene expression in four species of crustaceans with different numbers of movement and feeding appendages, as shown.

Gene expression was measured during early development. In the figure, the thoracic segments are colored:
- grey for Ubx gene expression, or
- white for no Ubx gene expression.

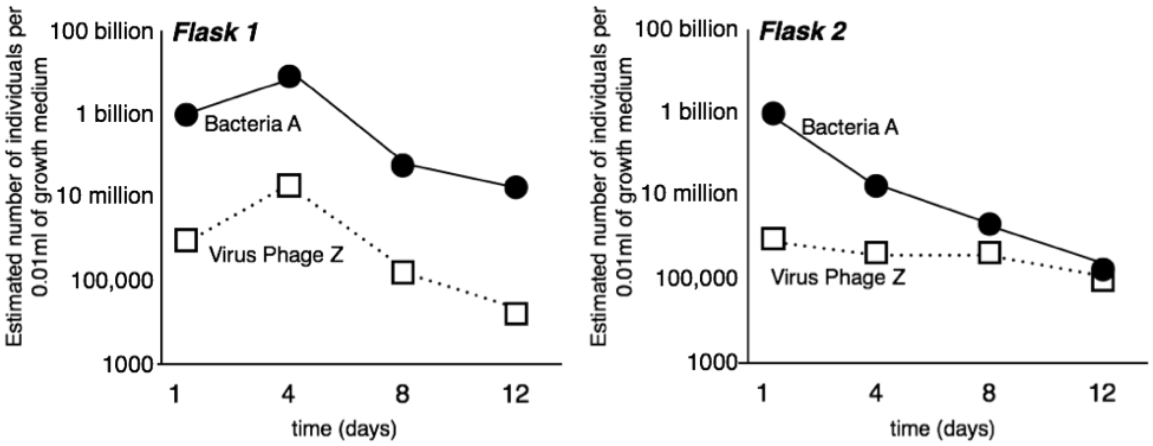
The evolutionary relationships among these four species of crustaceans are shown in the phylogenetic tree below.



Based on this information and your knowledge about biology, evaluate each statement as **Likely** or **Unlikely** to be true:

	Likely	Unlikely
1) For an individual crustacean, segments that have different types of appendages have the same Ubx DNA sequences in their respective cells.	❍	❍
2) Based on the relationships in the phylogenetic tree above, there is only one possible scenario for the evolution of feeding appendages - that first one, then two, then three feeding appendages arose.	❍	❍
3) The phylogentic tree provides support for three feeding appendages resulting in higher fitness than two feeding appendages.	❍	❍
4) All mutations that occur in an individual of species A will be passed down to its offspring.	❍	❍
5) The fact that over 90% of species D individuals have an elongated feeding appendage on segment 4 means that this trait must be controlled by a dominant allele.	❍	❍
6) Any mutation in the DNA sequence of the MSH gene, which only controls feeding appendage length, will impact feeding appendage length.	❍	❍
7) Natural selection results in generating, not eliminating, new phenotypes in these crustaceans.	❍	❍
8) More information is required to determine if the difference in number of feeding appendages between species C and D are adaptations.	❍	❍
9) A federal limit on the total amount of crustaceans harvested will equally protect all of these species.	❍	❍

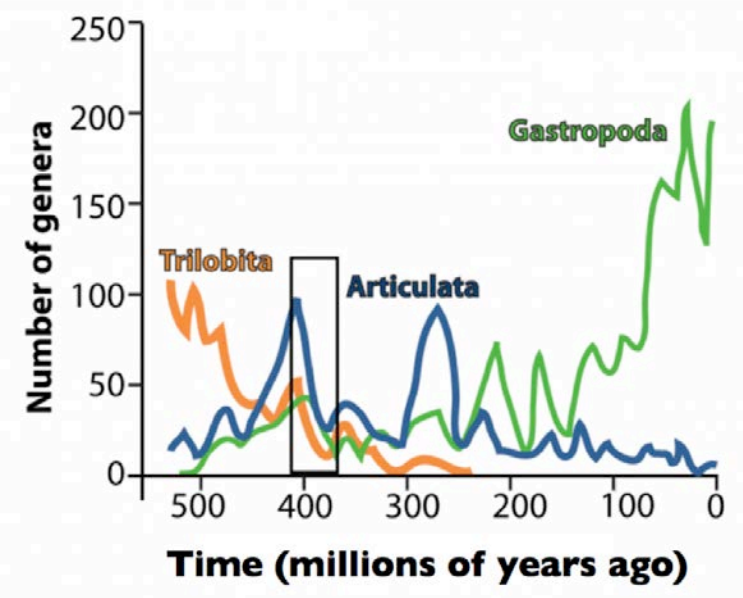
Question 3. Scientists place the same number of bacteria A and virus phage Z, which infects the bacteria, into two different flasks. Each flask contains the same sterile growth medium which is replenished. They then observe the density of bacteria and viruses over 12 days, shown in the graphs below.



Based on your knowledge about biology and this information, evaluate each statement as **Likely** or **Unlikely** to be true:

	Likely	Unlikely
1) On day 4, there is more competition among bacteria A in flask 1 than in flask 2.	❍	❍
2) More information is needed in order to calculate the density of bacteria at eight days in each flask.	❍	❍
3) The rate of bacterial population growth declines in flask 1 because organisms limit their consumption and growth to leave resources for the next generation.	❍	❍
4) The bacterial populations can go locally extinct due to infection by the virus in one of these flasks, even though that would result in the extinction of the virus.	❍	❍
5) Bacteria placed in a separate flask with no virus and unlimited food will grow linearly.	❍	❍

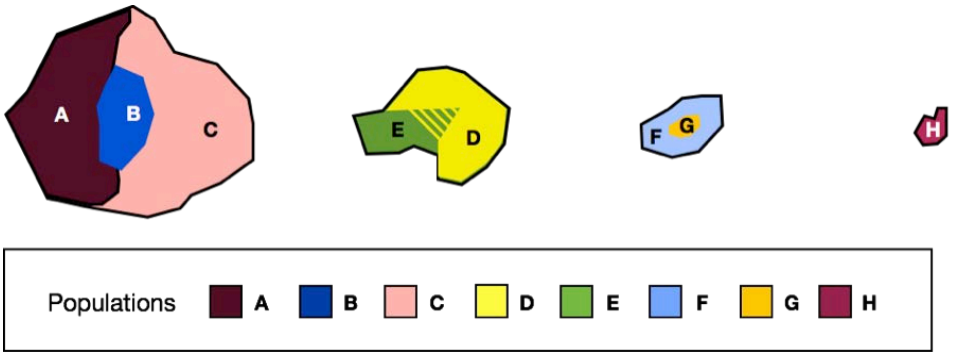
Question 4. The following graph shows the diversity of three important groups of marine animals over the past 500 million years.



Based on your knowledge about biology and this information, evaluate each statement as **Likely** or **Unlikely** to be true:

	Likely	Unlikely
1) If the number of species were graphed instead, the numbers on the y-axis would be smaller.	❍	❍
2) This graph provides evidence for a mass extinction event in the period of time shown in the box.	❍	❍
3) There are more fossils of extinct gastropods with hard shells than extinct soft bodied gastropods without shells.	❍	❍
4) The majority of Trilobita, Articulata, and Gastropoda that ever lived are now extinct.	❍	❍
5) Articulata, Gastropoda, and Trilobita species share a common ancestor.	❍	❍
6) Trilobita species share a common ancestor.	❍	❍
7) Humans have caused the majority of total animal extinctions.	❍	❍

Question 5. Tortoises inhabit a series of volcanic islands off of the coast of South America. Biologists have identified geographically confined populations of tortoises on these islands, shown below. There is strong evidence that tortoises from one population do not naturally reproduce with tortoises from any other population.

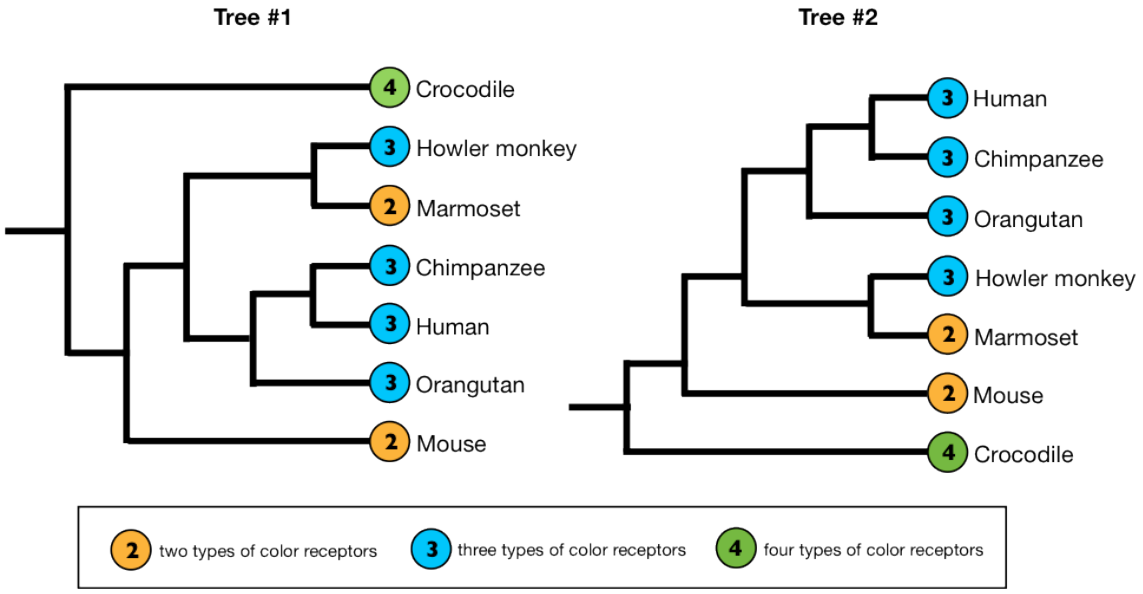


Based on your knowledge about biology and this information, evaluate each statement as **Likely** or **Unlikely** to be true:

	Likely	Unlikely
1) Two tortoises from population H have identical genomes.	❍	❍
2) Past and present genetic drift has increased the number of alleles in population G.	❍	❍
3) Considering only this generation, a tortoise that lived to 100 and had 10 surviving offspring had equal fitness as a tortoise that lived to 30 and had 10 surviving offspring.	❍	❍
4) The temperature at which the eggs incubate determines the sex of these tortoises; therefore, DNA sequence variation from one tortoise to the next is the primary explanation for differences in sex.	❍	❍
5) Conservation biologists can count tortoise hatchlings and estimate hatch rates for population E. This information alone allows prediction of how much the population will increase in size.	❍	❍
6) A tortoise is exposed to a high dose of radiation and develops a mutation in the DNA of a single skin cell. If the tortoise then goes on to have four offspring, at least one of these offspring will inherit the mutation.	❍	❍
7) When conservation biologists inseminated tortoises from population C with sperm from population G, the influence of genetic drift in population C increased.	❍	❍

Question 6. The phylogenetic trees below are based on DNA sequence data. They show the number of color receptors for seven different species.

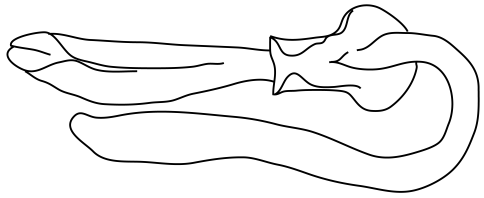
In general, the visual system of most reptiles, for example crocodiles, include four types of color receptors. Mammals in general possess two types of color receptors, however primates, including orangutans, chimpanzees, and humans, have three types of color receptors.



Based on your knowledge about biology and this information, evaluate each statement as **Likely** or **Unlikely** to be true:

	Likely	Unlikely
1) Tree # 2 shows orangutans more closely related to chimpanzees than to humans.	❍	❍
2) Tree #2 shows that marmosets evolved from mice.	❍	❍
3) Tree #2 shows marmosets and howler monkeys sharing a more recent common ancestor than orangutans and howler monkeys.	❍	❍
4) The most recent common ancestor of chimpanzees and humans possessed two, but not three, color receptors.	❍	❍
5) The phylogenetic relationships shown in Tree #1 are the same as the phylogenetic relationships shown in Tree #2.	❍	❍
6) In a population of 100 orangutans, all individuals will reproduce and contribute genetic information to the next generation.	❍	❍
7) In a population of orangutans, 1 in 3000 (0.03%) births have a recessive phenotype affecting vision. Assuming that there are only two alleles for this trait (one dominant and one recessive), the percentage of carriers in this population is less than or equal to 0.03% of the population.	❍	❍

Question 7. Hydrothermal vents are locations in the deep sea where water heated below the seafloor exits. While most of the deep sea is around 2ºC, vent water has been measured at over 400ºC. These vents occur along plate boundaries and can be separated from each other by thousands of kilometers. Chemicals dissolved in this water provide the main energy source to communities of organisms that live near hydrothermal vents.



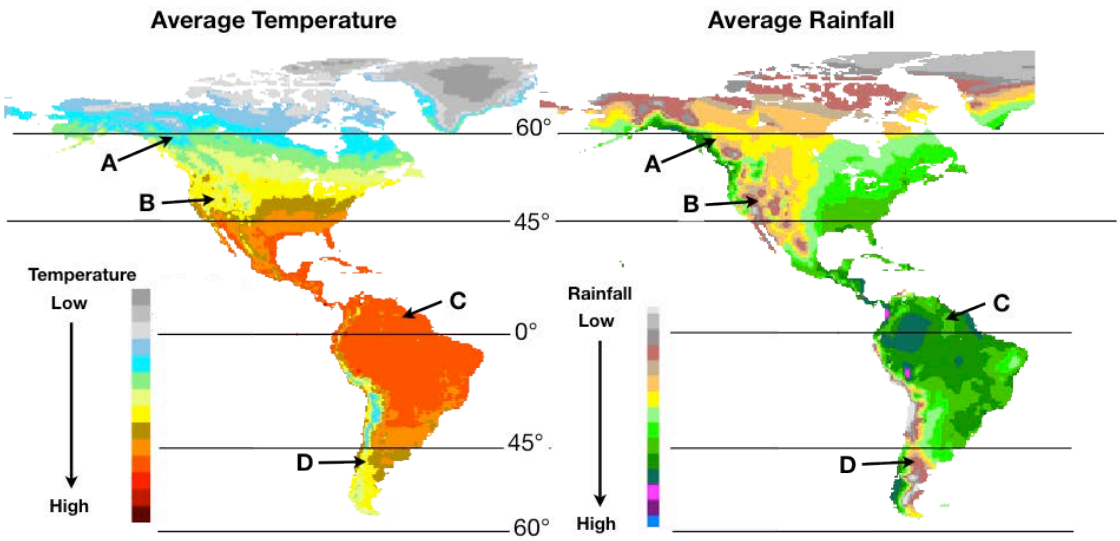
The figure above shows an annelid worm that lives at deep-sea hydrothermal vents. This worm lacks a gut and has an organ that contains intracellular bacteria. These bacteria metabolize hydrogen sulfide for the production of simple sugars in a process called chemosynthesis. An example of this process is written below:

$$CO_2 + 4H_2S + O_2 \rightarrow CH_2O + 4S + 3H_2O.$$

Based on your knowledge about biology and this information, evaluate each statement as **Likely** or **Unlikely** to be true:

	Likely	Unlikely
1) Along the spectrum of symbiotic relationships, the association between this worm and the bacteria is closer to mutualism than parasitism.	❍	❍
2) Carbon dioxide is a direct source of energy in photosynthetic and chemosynthetic systems.	❍	❍
3) Without chemosynthetic organisms, the energy from the hydrothermal vents would be accessible to other organisms.	❍	❍
4) Gene flow resulting from larvae dispersing between hydrothermal vents can act against local adaptation.	❍	❍
5) Gene flow between worm populations found at one vent and another vent is evidence for these worms being the same species.	❍	❍
6) A new allele appearing in an isolated population of these worms is evidence for genetic drift.	❍	❍
7) Some species of deep-sea worms care for their young while others do not. For those that care for young, the survivorship of offspring is greater, but the number of offspring is smaller.	❍	❍

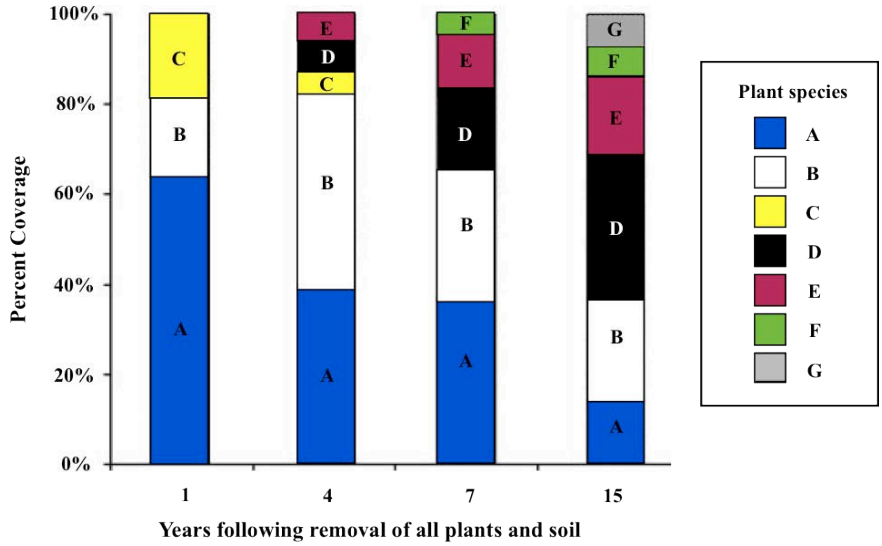
Question 8. The two maps below show the average temperature and yearly rainfall at varying latitudes. Locations of interest are marked A-D.



Based on your knowledge about biology and this information, evaluate each statement as **Likely** or **Unlikely** to be true:

	Likely	Unlikely
1) Climates are more similar at B and D than at A and B.	❍	❍
2) Yearly primary productivity is higher at A than C.	❍	❍
3) Total species diversity is higher at C than at D.	❍	❍
4) Plant species have more similar phenotypes at B and D than at C and D.	❍	❍
5) Primary productivity varies more over the course of a year at A than at C.	❍	❍
6) Mass deforestation at C affects the global climate.	❍	❍
7) If fertilizers are applied to agricultural plants at site D, these fertilizers are a direct source of energy for plant growth.	❍	❍

Question 9. Biologists removed all of the plants and soil in 20 x 20 m2 plots in a Florida wetland. For 15 years, scientists measured the percent coverage of various plant species in these plots. The results of a single plot are shown in the graph below.



Based on your knowledge about biology and this information, evaluate each statement as **Likely** or **Unlikely** to be true:

	Likely	Unlikely
1) The total number of plant species increased over the 15 years.	❍	❍
2) Plants that colonized the plot in year 15 are better competitors for space and resources than plants that colonized the barren plots in year 1.	❍	❍
3) Chloroplasts found in these plants arose from an endosymbiotic relationship.	❍	❍
4) Carbon in the soil is directly incorporated into the tissues of these plants.	❍	❍
5) Soil erosion in these plots removes carbon from the global carbon cycle.	❍	❍
6) Decomposition is important to this ecosystem because it directly provides nutrients, but not energy, to plants.	❍	❍
7) Fungi in these plots destroy atoms of plants during the decay process.	❍	❍

SUPPLEMENTAL APPENDIX S2. Example of student recruitment.

Verbal introduction to students:

"On [day], I will email you a link to take a survey on fundamental concepts in biology. This survey is important because it will help our department improve our undergraduate curriculum. The survey contains nine questions and should take roughly 15-35 minutes to complete. Your answers will not be graded, but you will receive [some participation points] for completing the survey. Once I send the email, you will have until [one week later] to complete the online survey.

Please give your best effort so that the resulting data fully represents the deep knowledge of biology that you have worked so hard to develop. I also ask that you take the survey without consulting outside information."

Email to students:

Dear [CLASS],

Here is the link to the biology survey that I mentioned in class. This survey is intended to help departments nationwide improve their undergraduate curricula. The survey contains nine questions and should take roughly 15-35 minutes to complete. Your answers will not be graded, but you will receive [some participation points] for completing the survey. I ask you to give your best effort so that the resulting data fully represents the deep knowledge of biology that you have worked so hard to develop. I also ask that you take the survey without consulting outside information.

Please follow the link below to access the questionnaire, which will remain posted until [one week later].

[link to Qualtrics survey]

Thank you in advance for your participation.

[YOUR NAME]

SUPPLEMENTAL APPENDIX S3. Student demographic questionnaire.

Note: You may choose to leave any or all of these questions blank. Your answers will be used to better understand characteristics of students taking this survey.

Are you 18 years of age or older?

_____ Yes
_____ No

What is your current class standing?

_____ First-year
_____ Sophomore
_____ Junior
_____ Senior
_____ Postbaccalaureate
_____ Graduate student
_____ Other:_____

Are you a Transfer Student?

_____ Yes
_____ No

If you are a transfer student, what other types of institutions have you attended? (select all that apply)

_____ Two-year college or community college
_____ Four-year college or university
_____ Other:_____
_____ Does not apply

If you are a transfer student, where have you taken introductory biology courses? (select all that apply)

_____ My current institution
_____ A different two year college or community college
_____ A different four-year college or university
_____ Other:_____
_____ Does not apply

Did you take AP biology in high school?

_____ Yes
_____ No

[If yes] What was your score on the AP biology exam?

_____ 1
_____ 2
_____ 3
_____ 4
_____ 5
_____ Not sure
_____ Did not take the AP biology exam

Approximately how many college-level ***biology*** courses have you taken, including any in which you are currently enrolled?

_____ [enter number]

Have you declared or are you planning to declare a major in biology or another life science?

_____ Yes
_____ No

Please check the sub discipline(s) of biology in which you have taken the most courses.

_____ Biochemistry
_____ Molecular/Cell Biology
_____ Physiology
_____ Neuroscience
_____ Ecology
_____ Evolution
_____ No specialization/equal exposure

What is your approximate current overall G.P.A.?

_____ 0.0 – 0.69 (E or F)
_____ 0.7 – 1.69 (D- to D+)
_____ 1.7 – 2.69 (C- to C+)
_____ 2.7 – 3.69 (B- to B+)
_____ 3.7 – 4.00 (A- to A+)

Gender:

_____ Female
_____ Male
_____ Other

Ethnicity (select all that apply):

_____ African American/Black
_____ Asian/Asian American
_____ Caucasian/White
_____ Filipino
_____ Hispanic/Latino
_____ Native American/Alaska Native
_____ Native Hawaiian
_____ Pacific Islander
_____ Other: _____

Did you speak English at home when you were growing up?

_____ Yes
_____ No

[If no] What language did you speak at home? _____

Was English the primary language of instruction in your high school?

_____ Yes
_____ No

Highest level of education completed by at least one of your parent(s):

_____ Did not complete high school
_____ High school/GED
_____ Some college (but did not complete college)
_____ Associate's degree (2-year degree)
_____ Bachelor's degree
_____ Master's degree
_____ Advanced graduate degree (for example, DVM, MD, PhD)
_____ Not sure

SUPPLEMENTAL APPENDIX S4. Item response theory (IRT) model comparison and fit statistics.

Model comparisons

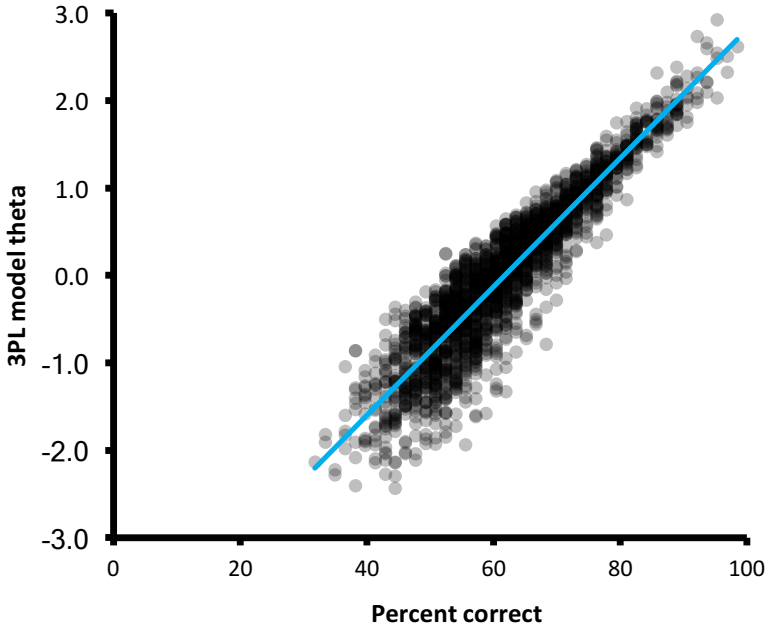
Model	AIC	BIC	Log Likelihood	X^2 compared to 3PL	Degrees of freedom	p-value
1PL	162930	163293	-81401	2725.7	125	0
2PL	161061	161776	-80405	733.2	63	0
3PL	160454	161526	-80038	-	-	-
3PL – multi (VC core concepts)	163269	164340	-81446	2815.1	0	0
3PL – multi (Eco/Evo themes)	163965	165036	-81793	3510.7	0	0

M2 fit statistics of 3PL model

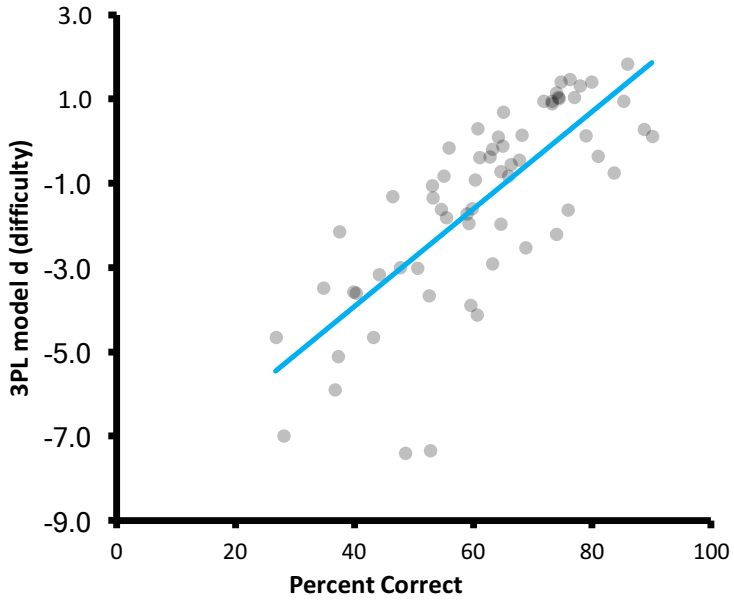
M2	df	p	RMSEA (5-95)	SRMSR	TLI	CFI
4381.05	1827	0	0.0256 (0.0246-0.0265)	0.033	0.859	0.868

SUPPLEMENTAL APPENDIX S5.

Correlation between overall student scores reported as percent correct (classical test theory) and 3PL IRT thetas. r=0.92.



Correlation between statement difficulty estimated based on percent correct (classical test theory) and 3PL model. r=0.76.



SUPPLEMENTAL APPENDIX S6. Three-parameter logistic model item response theory (3PL IRT) single item analysis.

Single item fit statistics of 3PL model

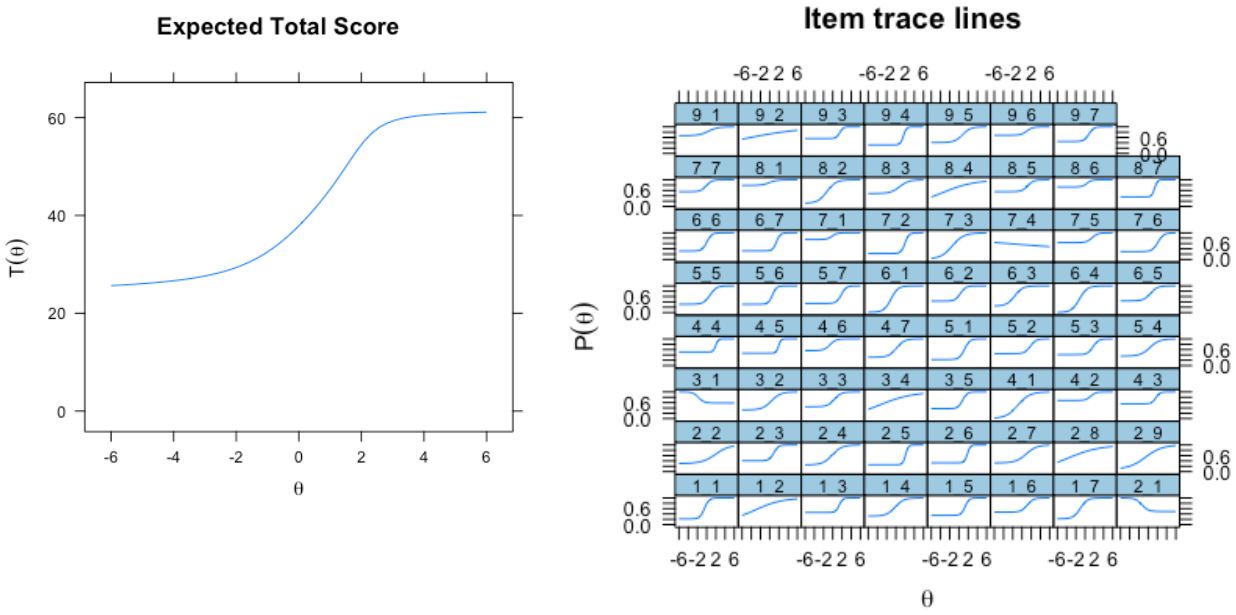
Pearson's Chi Square ($S{-}\chi^2$) fit statistic (Orlando & Thissen, 2000), degrees of freedom (df), and significance (p). The null hypothesis is that the statement fits well; a significant result ($p<0.05$) indicates poor statement fit. The six items with poor fit are indicated with bold italics – 1_5, 2_9, 4_4, 4_5, 7_5, and 8_7.

Statement*	Pearson's Chi Square ($S{-}\chi^2$)	$S{-}\chi^2$ df	$S{-}\chi^2$ p		Statement*	Pearson's Chi Square ($S{-}\chi^2$)	$S{-}\chi^2$ df	$S{-}\chi^2$ p
1_1	26.646	25	0.374		5_5	27.583	32	0.690
1_2	29.678	33	0.633		5_6	31.635	28	0.290
1_3	24.695	32	0.818		5_7	35.829	34	0.383
1_4	35.631	31	0.259		6_1	31.777	27	0.240
1_5	***47.070***	***32***	***0.042***		6_2	38.641	30	0.134
1_6	21.531	33	0.938		6_3	21.983	27	0.738
1_7	37.858	27	0.080		6_4	33.550	29	0.256
2_1	43.598	36	0.180		6_5	34.992	31	0.284
2_2	33.075	35	0.561		6_6	20.494	28	0.846
2_3	29.910	31	0.522		6_7	28.588	32	0.640
2_4	34.526	33	0.395		7_1	20.383	25	0.726
2_5	44.346	34	0.110		7_2	28.589	34	0.730
2_6	29.295	34	0.698		7_3	39.628	31	0.138
2_7	25.151	33	0.834		7_4	22.475	36	0.962
2_8	27.654	33	0.730		***7_5***	***47.578***	***33***	***0.048***
2_9	***58.026***	***33***	***0.005***		7_6	25.050	34	0.868
3_1	27.182	36	0.855		7_7	13.092	28	0.992
3_2	20.043	33	0.963		8_1	23.207	27	0.674
3_3	31.221	31	0.455		8_2	38.060	30	0.148
3_4	30.999	34	0.616		8_3	39.437	33	0.204
3_5	22.371	31	0.871		8_4	46.963	33	0.055
4_1	42.290	33	0.129		8_5	38.538	33	0.233
4_2	32.811	31	0.378		8_6	28.193	28	0.454
4_3	33.127	33	0.461		***8_7***	***54.973***	***32***	***0.007***
4_4	***48.906***	***33***	***0.037***		9_1	27.829	30	0.579
4_5	***57.289***	***33***	***0.005***		9_2	36.803	34	0.340
4_6	24.322	26	0.558		9_3	28.182	32	0.660
4_7	34.095	31	0.321		9_4	43.020	32	0.092
5_1	42.757	32	0.097		9_5	41.733	33	0.142
5_2	33.166	34	0.508		9_6	25.711	31	0.735
5_3	43.459	34	0.128		9_7	36.124	31	0.241
5_4	35.027	33	0.372					

*The first number refers to the question and the second to the statement (e.g., "1_1" is question 1, statement 1).

Orlando, M., & Thissen, D. (2000). Likelihood-based item-fit indices for dichotomous item response theory models. *Applied Psychological Measurement, 24*(1), 50-64. doi: 10.1177/01466216000241003

Test characteristic curve and item trace lines for 3PL model



Rationale for including statements with negative IRT slopes

Three statements with negative slopes (2_1, 3_1, and 7_4) are included in the assessment because:

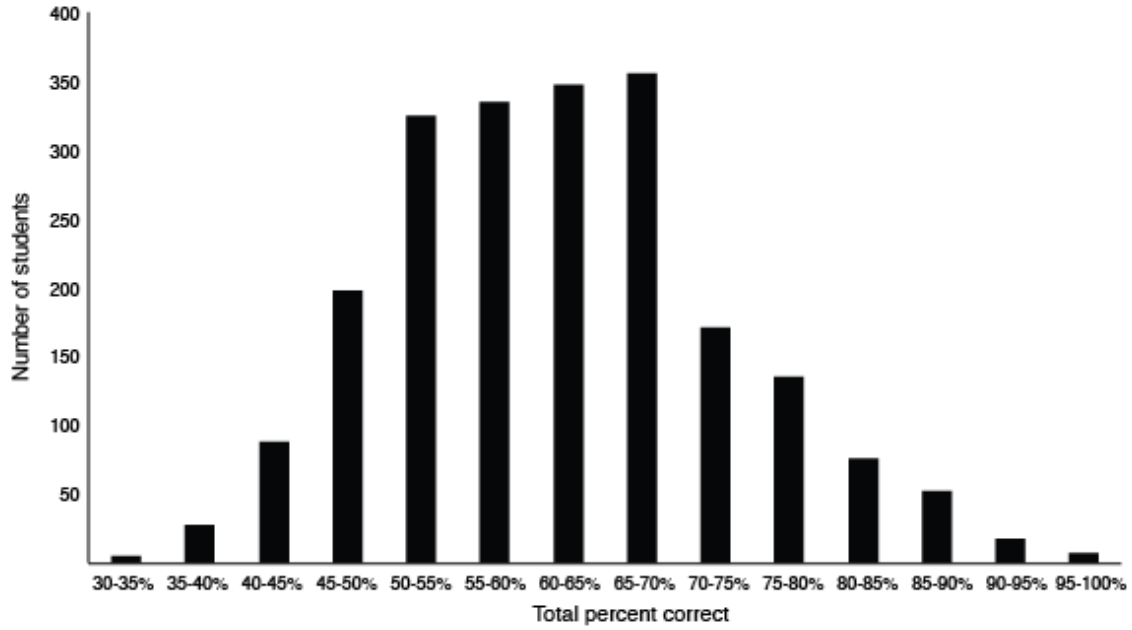
1) faculty reviewers stated they were testing important concepts where students often have conceptual difficulties (e.g., gene expression, intraspecific competition, and gene flow);

2) results from student interviews also indicate that students who answered incorrectly also did not have sound reasoning (see table below); and

3) the overall score estimates for students with or without these three statements were strongly correlated (Pearson's r > 0.99), regardless of whether student ability was calculated using IRT thetas or classical test statistics.

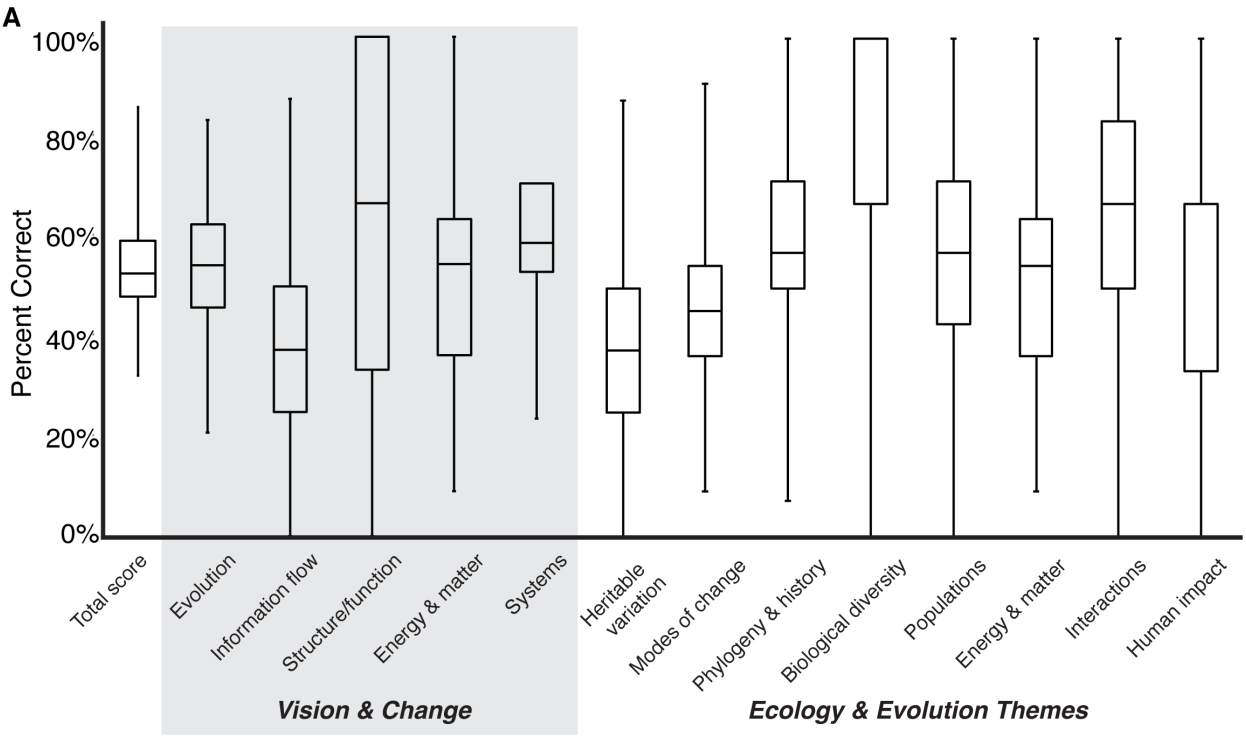
	No. student interviews where students answered:			
Statement	**Correctly with sound reasoning**	**Correctly with not sound reasoning**	**Incorrectly with sound reasoning**	**Incorrectly with not sound reasoning**
2_1	9	1	0	19
3_1	5	0	0	10
7_4	10	5	0	13

SUPPLEMENTAL FIGURE S1. Frequency distribution of overall student scores for the final version of EcoEvo-MAPS. N=2142 students.



SUPPLEMENTAL FIGURE S2. Example of EcoEvo-MAPS automatically-generated score report for a single administration. (A) Distribution of percent correct for the overall assessment, *Vision & Change* core concepts, and ecology and evolution themes. Central bars represent course median scores, boxes represent inner quartiles, and whiskers represent minimum/maximum scores. (B) Percent correct and student thinking for two likely/unlikely statements within the ecology and evolution theme "heritable variation." The score report provides percent correct for all 63 likely/unlikely statements along with associated student thinking.

A



B

Topic	Percent correct	Example of correct student thinking	Example of incorrect student thinking
Inheritance	39%	*Not all mutations can be passed down, only those in germ cells.*	*All mutations would be passed down; at least 1/4 offspring would have the mutation.*
Genotype/ phenotype	63%	*All appendages have the same DNA, it is expression, the gene is there, it just is turned on or off.*	*Different segments are caused by different DNA sequences. If DNA is the same, then same appendage.*

SUPPLEMENTAL TABLE S1. The number of questions in each category in the final version of EcoEvo-MAPS.

Concepts	**No. statements**
Ecology	30
Evolution	33
Vision and Change	
1. Evolution	24
2. Information flow	8
3. Structure function	3
4. Transformations of energy and matter	11
5. Systems	17
Ecology and Evolution	
1. Heritable variation	8
2. Modes of change	11
3. Phylogeny and evolutionary history	14
4. Biological diversity	3
5. Populations	7
6. Energy and matter	11
7. Interactions within ecosytems	6
8. Human impact	3

SUPPLEMENTAL TABLE S2. Demographics of students who participated in "think-aloud" interviews. N=86.

Gender	Number	Percentage of Total
Female	61	71%
Male	24	28%
	75	
English as a second language	8	9%
Transfer students	9	10%
First generation college status	18	21%

Class standing	Number	Percentage
First-year	30	35%
Sophomore	15	17%
Junior	24	28%
Senior	17	20%

Estimated GPA	Number	Percentage
A	38	44%
B	35	41%
C	10	12%
D	3	3%

Ethnicity	Number	Percentage
Asian/Asian American	7	8%
Caucasian/White	62	72%
Filipino	1	1%
Hispanic/Latino	6	7%
Native American/Alaska Native	0	0%
African American/Black	1	1%
Hawaiian Native	0	0%
Pacific Islander	0	0%
Other	2	2%
More than one	4	5%

SUPPLEMENTAL TABLE S3. Demographics of EcoEvo-MAPS student participants in final administration. N=2142.

Gender	Number	Percentage of Total
Female	1367	64%
Male	745	35%
Other	12	1%
English as a second language	274	7%
Transfer students	317	15%
First-generation college status	754	35%

Class standing	Number	Percentage
First-year	1023	48%
Sophomore	467	22%
Junior	339	16%
Senior	313	15%

Estimated GPA	Number	Percentage
A	732	34%
B	1156	54%
C	172	8%
D	6	0%
F	10	0%

Ethnicity	Number	Percentage
Asian/Asian American	291	14%
Caucasian/White	1382	65%
Filipino	20	1%
Hispanic/Latino	144	7%
Native American/Alaska Native	12	1%
African American/Black	63	3%
Hawaiian Native	0	0%
Pacific Islander	0	0%
Other	68	3%
More than one	142	7%

SUPPLEMENTAL TABLE S4. Faculty responses to survey on question accuracy and clarity for final version of the assessment. N=11.

Question	**Percent Agreement:** *Is this question:*	
	scientifically accurate?	*clear?*
1	100%	100%
2	91%	82%
3	100%	100%
4	100%	90%
5	100%	100%
6	100%	90%
7	100%	82%
8	100%	100%
9	100%	91%

SUPPLEMENTAL TABLE S5. Examples of the most common thinking encountered during think-aloud student interviews for the final version of the assessment.

Learning Target	Learning Outcome	Example of Correct Student Thinking	Example of Incorrect Student Thinking	Percent Correct	Question-Statement Number(s)
Heritable variation					
Inheritance	Offspring only inherit DNA from the germ cells.	Not all mutations are passed down, only the ones in the germ cells.	All mutations would be passed down. At least one out of four would have the mutation.	64% 53%	2-4 5-6
Genotype versus phenotype (i.e., gene expression)	Cells within an individual contain the same DNA.	All appendages have the same DNA, the gene is there, it is just turned on or off (i.e., expression).	Different segments caused by different DNA sequences. If DNA is the same, then same appendage.//Temperature must change DNA sequences to have different sexes.	59% 63%	2-1 5-4
Allele frequencies	The frequency of alleles in a population can be summarized and calculated using the Hardy Weinberg equation ($1=p^2+2pq+q^2$).	There are likely more carriers than the percent with phenotype.	Students calculate 3:1, using a Punnett square.	40%	6-7
Variation	Individuals within a population are genetically variable.	Genome is all of the genes, so even though very isolated, the individuals are not clones of each other, so there will be some variation.	Two individuals from the same population will have the same genes, especially if they are isolated.	37%	5-1
Mutation	Not all mutations result in phenotypic change.	Could be a silent mutations, where there is a change in the sequence of mRNA, but not the proteins that arise from translation.	If mutation occurring, most likely phenotypic impact. There is no way that the mutation wouldn't since that is all that that gene controls.	37%	2-6

Dominance	Dominant and recessive refer to relative expression, not the frequency of an allele in a population.	The most common traits can be recessive. Unless looking at gene itself and how it is expressing, cannot determine if dominate or recessive.	If most have a certain trait, most likely because it is dominant.	28%	2-5
Modes of change					
Fitness	Fitness is determined by reproductive success.	Species having 2 and 3 both co-existing, not filtered out by evolution. Phylogenetic tree doesn't show fitness, just relationships among species and their evolutionary history. Even though one lived longer, equal fitness because both had 10 offspring.	Three feeding appendages better than two, natural selection would prefer 3, evolved from 1 and 2; The more feeding appendages, the more feeding, which is better.//Not equal fitness, live to 100 had a lot more fitness.	55% 48%	2-3 5-3
Genetic drift	Genetic drift can only change the frequency of present alleles or decrease the number of alleles, it does not increase the possible number of alleles or genetic diversity.	In an isolated population it is not likely to have new allelic diversity occur, so should be stable or decrease. Creating genetic diversity by artificial means, so less genetic drift. Genetic drift is the loss of alleles, a new allele could result from mutation.	Drift is random, so it could either increase or decrease in diversity. Drift is not likely in an isolated population. Genetic drift from outside sources, if from different islands would definitely create diversity. (i.e., genetic drift = gene flow). If new allele appears, probably genetic drift.	51% 40% 35%	5-2 5-7 7-6
Adaptation/ convergent evolution	Convergent evolution can produce similar phenotypes in distantly related organisms.	With similar temperature and rainfall, similar adaptations.	More likely to have the same phenotypes on the same continents.	74%	8-4
Adaptation	To be considered an adaptation, a trait must meet certain criteria, such as having a function and being selected for.	The only information given is that these are two different phenotypes.	Any difference in number of appendages can be assumed to be an adaptation.	74%	2-8

Gene flow/Species	Evidence of reproduction between individuals is one way to delimit a species.	If interbreeding, they are likely the same species.	Presence at different locations means that they are different species.	69%	7-5
Differential reproduction	Not all individuals in a population reproduce/pass on their genetic information.	Some individuals might not survive to reproduce or their offspring could die, but the ones that do reproduce will contribute genetic information to the next generation.	All individuals have the opportunity and means to reproduce.	65%	6-6
Gene flow	Gene flow can result in new allelic variation entering a population.	If other larvae come, slow local adaptation; bring in alleles special location starting to get rid of.	Unsure how gene flow and local adaptation relate to one another.	56%	7-4
Mutation/Natural selection	Mutations generate new phenotypes and natural selection increases and/or decreases the frequencies of phenotypes.	Mutations generate new phenotypes. Natural selection eliminates phenotypes.	A phenotype cannot be eliminated by natural selection.	55%	2-7
Phylogeny and evolutionary history					
Common ancestry	All life shares common ancestry.	All life goes back to common ancestor.	Very different, so most likely different ancestors.	49% 85%	4-5 4-6
Tree-reading	Relatedness is shown by common ancestors, represented as nodes, on a phylogenetic tree. Reading from top to bottom or left to right does not indicate evolutionary timing, relatedness, or trait evolution.	Species with one and two feeding appendages from same common ancestor, so there is more than one possible evolutionary scenario. Humans and chimps have more recent common ancestor. Howler monkey and marmosets share a more recent common ancestor. Most common ancestor likely had three color receptors.	The tree shows that first one, then two, then three feeding appendages evolved. (i.e., reading from top to bottom). Humans branched off further away. They are equally related.	46% 75% 86% 76%	2-2 6-1 6-3 6-4

Tree-reading	Extant species evolved from common ancestors, not from each other.	Marmoset and mice share a common ancestor, marmosets do not stem from mice directly.	One branch from mice, also means humans evolved from mice.	66%	6-2
Tree-reading	Relatedness is shown by common ancestors, represented as nodes, on a phylogenetic tree. The shape of the tree does not determine the relationships.	The relationships are the same.	The trees have different shapes, so they show different relationships.	65%	6-5
Extinction	Evidence of a mass extinction includes a decrease in biodiversity (i.e., increase in extinction rates) exceeding typical background levels.	Dramatic drop in the amount of genera suggests an extinction that wiped out a lot of diversity.	A mass extinction means that no species would be present, something happened here but did not cause them to go extinct. Because decrease happens multiple times, could not be a mass extinction.	74%	4-2
Taxonomic rankings	Each species belongs to a genus.	Most likely larger amount of species than genera, species more specific.	Species more specific, so number of species would be smaller than genera.	65%	4-1
Deep time	Humans have only been present for a very short time in the history of life.	Humans were not around for most of earth history and previous mass extinctions, but humans have increased the rate of extinction by a few hundred percent.	Humans have caused most extinctions.	63%	4-7

Symbiosis	Endosymbiosis has resulted in major events in evolutionary history and important structures like chloroplasts and mitochondria.	The endosymbiont theory of mitochondria and chloroplasts, have own DNA and ribosomes.	Chloroplasts are not their own life form, they are part of the plant for photosynthesis.	61%	9-3
Fossilization	The chance of fossilization is higher for organisms with hard compared to only soft body parts.	Easier for harder materials to become fossilized.	Hard shell is a beneficial adaptation, so more likely to survive.	60%	4-3
Extinction	Most of what has ever lived on earth is now extinct.	Species go extinct all the time, so rapidly and often, now cannot compare to the entirety of earth being here. 99% of all species are extinct.	More species are alive now than were in the past.	53%	4-4
Biological diversity					
Biomes	In general, climate varies by latitude.	Sites with similar latitude likely have similar climates.	Sites that are closer together (within the same hemisphere) have more similar climates.	90%	8-1
Species diversity	A graph can be interpreted to determine the number of species. One measure of diversity is the number of species.	Interpret graph to see change in number of species from 3 to 6.	Incorrectly interpret graph to mean that there are the same number of species and only their relative abundance has changed.	81%	9-1
Biodiversity trends	In general, species diversity is highest nearest the equator and decreases toward the poles (latitudinal diversity gradient).	In general, species diversity increases toward the equator.	There is more variability at higher latitudes, so there is more diversity there.	66%	8-3
Populations					

Reproduction/ life history	There is a life-history trade-off between number of offspring and survivorship.	Organisms with higher number of offspring typically have lower survivorship.	If young are cared for more, there is greater reproduction.	79%	7-7
Intraspecific competition	Intraspecific competition increases as the population size increases.	When there are more bacteria in the flask, they compete more with each other for resources and space.	There is more competition only where there are more of another species.	63%	3-1
Density	Density (one measurement of population size) is the number of individuals per unit volume or area.	Density is measured and shown in the figure.	More information is required in order to calculate density.	61%	3-2
Population growth/decline	Carrying capacity is the maximum population size a particular habitat can sustain and is influenced by regulating mechanisms.	If at carrying capacity because of limited phosphrous - need addition of phosphorous for population to increase.	If more nitrogen, more growth because nitrogen and phosphorous cancel out.	59%	1-6
Population growth/decline	Populations that grow without limitation follow an exponential growth pattern.	Would more likely grow exponentially while unlimited food and resources.	There is nothing to make the population decrease, so probably linear growth.	55%	3-5
Population growth/decline	Population size is dependent on many factors.	The population size is dependent on many factors in addition to predation - water, temperature, resources, competition, amount of sunlight, disease, changes in pH, habitat space, birth/death rates, environmental factors, etc.	Only one organism is shown in the food web to eat phytoplankton, which is the only regulating mechanism.	53%	1-3
Energy and matter					
Primary production	In general, yearly primary productivity is higher and more regular near the equator	The most amount of sunlight is near the equator, so this area would have the highest	Higher temperatures and greater rainfall results in greater variability in productivity.	78% 65%	8-2 8-5

	and lower and more varied near the poles.	photosynthesis/productivity. Where winter occurs there is less photosynthesis.			
Energy & matter	Decomposition recycles matter, and in doing so provides nutrients to plants, but not energy (plants obtain their energy from the sun).	Decomposition provides nutrients, but the main driver of photosynthesis is the sun.	Energy is created through decomposition, dead plants had energy and it has to go somewhere.	76%	9-6
Primary production	Primary producers provide accessible energy to other organisms.	The energy is converted by chemosynthetic organisms.	All organisms would be able to use some form of energy in the environment.	74%	7-3
Energy & matter	Energy transfer is inefficient.	Energy is lost as it transfers between levels in a food web.	If prey increase, predators increase.	68%	1-4
Energy & matter	The total amount of matter is fixed in global cycles - it moves from one reservoir to another and cannot be created or destroyed.	Carbon is never removed; it can be moved to another location or recycled, but stays in the cycle.	Soil erosion and decomposition removes carbon.	60%	9-5
Energy & matter	Matter cannot be created or destroyed (by biological organisms).	When fungi eat they do not destroy atoms, they break down compounds.	As decomposers, fungi destroy atoms.	60%	9-7

Energy & matter	When a toxicant biomagnifies, it is found at a higher concentration at higher levels of the food web.	In biomagnification, a toxicant increases and concentrates higher up in the food chain because those organisms are consuming more of the toxicant. Bull trout are at a higher trophic level than whitefish and therefore more would accumulate more.	A toxicant is diluted as it is consumed, so higher trophic levels will have a lower concentration. Whitefish are directly eating toxicant. The toxicant is digested twice before the bull trout, so although the bull trout still receives the toxicant, it is diluted because less direct.	44%	1-5
Energy & matter	Fertilizers provide nutrients, but not energy, for photosynthesis.	Fertilizers are a source of nutrients and the sun is the source for energy in photosynthesis.	Any of the main elements, (P, K, N) are direct sources of energy.	43%	8-7
Energy & matter	The source of carbon in plants is the atmosphere, not soil.	Carbon is taken in from the atmosphere, while nutrients and vitamins are from the soil.	Plants take up carbon from the soil and use to respire oxygen.	37%	9-4
Energy & matter	Carbon dioxide does not provide energy for biological processes.	Energy for photosynthesis comes from light (the sun). Energy for chemosynthesis comes from chemicals.	Carbon dioxide is required for both photosynthesis and chemosynthesis and is used as the source of energy because otherwise neither process would occur.	27%	7-2
Interactions within ecosystems					
Population growth/decline / Predation	Organisms do not limit their consumption to leave resources for future generations.	The population can go extinct if it runs out of resources.	Organism is looking out for itself as well, wouldn't try to deplete source of food population because then nothing to live off of.	68% 73%	3-3 3-4

Symbiosis	Symbioses can be classified depending on the effect of the interaction on both partners - from parasitism to commensalism to mutualism.	Both partners are benefitting, so closer to mutualism - the worm benefits from chemosynthesis and the bacteria have a place to live.	N/A	89%	7-1
Predation	An increase in the population size of a predator likely leads to a decrease in the population size of prey.	An increase in predator population leads to a decrease in prey population.	N/A	80%	1-7
Interspecific competition	Organisms that consume the same resource will be affected by interspecific competition.	Since both consume zooplankton, competition is likely to have had an effect.	Salmon declined because their food resource declined, not because of competition.	77%	1-2
Succession	Organisms that colonize later experience more competition for space and resources than those that colonize first.	In year 1, not as many to compete with, in year 15, more species, more competition for space.	Plants that colonize first grow faster and are likely better competitors for space and resources.	73%	9-2
Predation	Arrows in a food web show the organisms that are consumed.	Bull trout directly feed on whitefish and salmon (where the arrows point).	Bull trout consume all of the other organisms.	72%	1-1

Human impact					
Global change	Mass deforestation has global implications.	Mass deforestation could change global climate because if less forest, more CO2 in air.	Mass deforestation will only have an effect on that area, not globally.	84%	8-6
Conservation	Conservation efforts will affect species differently depending on their ecology and life history.	No gaurantee that all are equally procted because: some species easier to harvest, some types favored over others and harvested more, different population sizes, or there could be more of one species in area where harvested.	These crustaceans are close enough to being the same species that a federal limit will protect all of them.	61%	2-9
Conservation	Population growth models require information about birth and death rates.	Need to look at many factors - predation, food, habitat, environmental disaster, death rates, disease - to make accurate population predictions.	If know hatch rates about to see changes in population size if growing or decreasing in size.	53%	5-5

SUPPLEMENTAL TABLE S6. Summary of differential item functioning (DIF) analyses for the 63 likely/unlikely statements (i.e., items) on the final version of EcoEvo-MAPS.

	No. of statistically significant DIF items	Range of effect sizes	No. of non-negligible DIF items
Gender	16	0.0001 – 0.0086	0
English as a second language	11	0.0000 – 0.0086	0
Transfer student status	7	0.0000 – 0.0048	0
First generation college status	9	0.0000 – 0.0082	0
Ethnicity	11	0.0000 – 0.0073	0