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A Cross-Course Investigation of Integrative Cases for Evolution Education†

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Evolution is a cornerstone theory in biology, yet many undergraduate students have difficulty understanding it. One reason for this is that evolution is often taught in a macro-scale context without explicit links to micro-scale processes. To address this, we developed a series of integrative evolution cases that present the evolution of various traits from their origin in genetic mutation, to the synthesis of modified proteins, to how these proteins produce novel phenotypes, to the related macro-scale impacts that the novel phenotypes have on populations in ecological communities. We postulated that students would develop a fuller understanding of evolution when learning biology in a context where these integrative evolution cases are used. We used a previously developed assessment tool, the ATEEK (Assessment Tool for Evaluating Evolution Knowledge), within a pre-course/post-course assessment framework. Students who learned biology in courses using the integrative cases performed significantly better on the evolution assessment than did students in courses that did not use the cases. We also found that student understanding of evolution increased with increased exposure to the integrative evolution cases. These findings support the general hypothesis that students acquire a more complete understanding of evolution when they learn about its genetic and molecular mechanisms along with macro-scale explanations.

INTRODUCTION

Evolutionary principles provide the overarching theoretical framework for biological sub-disciplines ranging from molecular and cell biology to genetics, physiology, ecology, and medicine. However, evolutionary theory presents undergraduate science students with a particularly difficult set of concepts and principles to master, in part due to their intrinsic conceptual difficulty, but also due to societal resistance (18). Student misconceptions about evolutionary principles have been well explored (13, 14, 8, 2, 16). Gregory (10) reported that less than 50% of the students in typical post-secondary science classrooms have an accurate understanding of natural selection or the origin of phenotypic variation, while common misconceptions include students confusing biological fitness with physical fitness (10) and thinking that phenotypic variation arises in response to environmental stimuli (5, 1). Mis- and preconceptions ultimately prevent some students from accepting evolution as a legitimate and empirically-supported theory (see, for example, 5, 2, 3, 20, 12, 16).

Another potential barrier to student learning and understanding of evolutionary concepts is the inability of novice learners to integrate concepts across scales (e.g., molecular, cellular, organismal, etc. (7)). Experts have the ability to operate across scales, while novices tend to focus on one scale at a time (4). An ability to integrate concepts across scales (systems-thinking) requires cultivation. Unfortunately, evolutionary biology is often taught with a primary, and sometimes sole, emphasis on ecological selection principles, presented in isolation from the micro-scale genetic, biochemical, and cell biological processes that underlie them (15).

Our overarching hypothesis is that biology students develop a more complete and deeper understanding of evolutionary principles when they learn to integrate knowledge across biological scales and disciplines (21). Rather than treating natural selection and fitness as the sole standard-bearing concepts for evolution, an integrative approach provides a way for students to interact with concrete examples of evolution that include, yet extend far beyond, ecological principles. Along these lines, we developed a set of online evolution cases[1] (Evo-Ed Cases, www.evo-ed.org) that address the biological basis of evolution within single study systems from genes to protein function, cell biology, and selectable phenotypes (21). Each case focuses on the

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[1] We distinguish "Cases" from "Case Studies" and discuss this difference, as well as the advantages of case-based approaches, in White et al. (21).

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complete evolution of a trait from its origin in DNA mutation (the "micro" scale) to its culmination in a selectable phenotype affecting ecological communities (the "macro" scale). To date, we have developed six cases of trait evolution that explicitly tie biochemical evolution to natural selection (see Box 1 and White et al. (21) for descriptions of cases). The primary learning objective of each case is for students to use principles of molecular and cell biology when they describe evolutionary processes. They can accomplish this by explaining the connection between a genotype and a phenotype, using descriptions that incorporate protein function and genetic differences between alleles.

An initial study (22) describing implementation of Evo-Ed cases in an introductory molecular and cell biology course showed that knowledge of evolution was related to a student's ability to describe an integrative case of trait evolution, namely white fur color evolution in beach mice (*Peromyscus polionotus*). Here, we extend this study by addressing three main questions arising from our overarching hypothesis. First, do students using an integrative case approach in an introductory biology course have an improved understanding of evolution compared with students not using this approach? Second, can students in an introductory *organismal* course demonstrate learning gains pertaining to the *molecular* underpinnings of evolution when integrative evolution cases are used? Finally, does increased exposure to integrative cases in both semesters of the introductory biology sequence lead to a better understanding of evolution among students?

METHODS

Experimental design

We investigated four courses in which cases were implemented and four courses in which cases were not implemented: C_1^{CMB}, C_2^{CMB}, C_3^{OB}, C_4^{CMB}, NC_1^{OB}, NC_2^{OB}, NC_3^{EVO}, and NC_4^{CMB} (Table 1). The "C" indicates the use of cases; the "NC" indicates that cases were not used. The subscript differentiates courses from one another; the superscript identifies each course as a cell and molecular biology (CMB), an organismal biology (OB), or an evolution course (EVO). Table 2 summarizes teaching methods used by each participating instructor in his/her course. An evolution assessment tool was administered in the first week of class and again in the last week of class in each course. Case implementation details and course descriptions can be found in Supplemental Materials, Appendix 1. The project was reviewed and approved by the MSU Institutional Review Board (IRB# X10-1086) prior to data collection.

The Case of Light Fur Evolution in Beach Mice: The first case describes the evolution of light fur in *Peromyscus polionotus* beach mice. A single nucleotide substitution mutation in the melanocortin-1-receptor gene (mc1r) results in a non-functional mc1r protein that is critical in the synthesis of the dark pigment eumelanin. The novel light fur phenotype has higher fitness in light sand environments resulting in geographically and phenotypically distinct *P. polionotus* populations.

The Case of Sweet Taste Evolution in Peas: The second case describes the evolution of sweet tasting seeds in *Pisum sativum* pea plants. An 800-nucleotide insertion mutation into the starch-branching enzyme 1 gene (sbe1) results in a non-functional starch branching enzyme. Pea plants with this mutation therefore make less starch and more sugar within the cells of their seeds. This resulted in sweeter peas that were artificially selected by ancient farmers.

The Case of Color Vision Evolution in Primates: The third case describes the evolution of color vision in old world primates. A few single nucleotide substitution mutations on a duplicated medium-wave sensitive opsin gene results in an opsin protein with different spectral absorption properties (i.e., the long-wave sensitive opsin protein). Primate species with this extra type of opsin protein have trichromatic vision and have an advantage in color discrimination and, by extension, food foraging efficiency.

The Case of Toxin Resistance in Soft-Shell Clams: The fourth case describes the evolution of toxin resistance in *Mya arenaria* soft-shell clams. Single nucleotide substitution mutations in the voltage gated sodium channel gene results in the formation of a transmembrane protein that is immune to the paralyzing effects of the neurotoxin saxitoxin. These clams have a higher fitness in areas with recurring red tides (algal blooms).

The Case of Citrate Use in *E. coli*: The fifth case describes the evolution of citrate use in *E. coli* in Dr. Rich Lenski's Long-Term Evolution Experiment. *E. coli* cannot use citrate as an energy source in oxic conditions. However, a mutation in the *cit* operon allowed bacteria to metabolize citrate in the presence of oxygen, giving them a more efficient way to produce cellular energy. This increased cell growth in the mutated population.

The Case of Lactase Persistence in Humans: The sixth case describes how humans evolved the ability to digest lactose as adults. A mutation in a regulatory region of the LCT gene allowed the transcription of the lactase enzyme to continue throughout human adulthood. This mutation gave Neolithic farmers an alternate and reliable calorie source in the form of livestock milk.

BOX 1. A brief description of six integrative cases for evolution education in undergraduate biology courses.

TABLE 1.
Details of the courses in which student understanding of evolution was measured.

Course Abbreviation[a]	Course Title[b]	Instructor(s)	Semester	# of Students Enrolled (*n*) (*n* that Wrote the ATEEK)	Course Taught Using Integrative Cases?	Duration of In-Class Case Teaching (hrs)
C_1^{CMB}	LB145, Introductory Cell and Molecular Bio	A	Spring 2012	66 (63)	Yes	~ 6 hours
C_2^{CMB}	LB145, Introductory Cell and Molecular Bio	A + B	Fall 2012	40 (33)	Yes	~ 6 hours
C_3^{OB}	LB144, Introductory Organismal Bio	C	Fall 2012	124 (108)	Yes	~ 6 hours
C_4^{CMB}	LB145, Introductory Cell and Molecular Bio	B	Spring 2013	79 (72)	Yes	~ 7 hours
NC_1^{OB}	BS162, Introductory Organismal Bio	D	Spring 2012	120 (94)	No	~ 0 hours
NC_2^{OB}	LB144, Introductory Organismal Bio	E	Spring 2012	116 (81)	No	~ 0 hours
NC_3^{EVO}	ZOL445, Evolution	F	Fall 2012	106 (91)	No	~ 1.5 hours[c]
NC_4^{CMB}	LB145, Introductory Cell and Molecular Bio	G	Spring 2013	80 (51)	No	~ 0 hours

[a] C designates courses where cases were used; NC designates courses where cases were not used. Both the C and NC sets are numbered sequentially. The superscript denotes the course subject: CMB – Cell and Molecular Biology, OB – Organismal Biology, EVO – Evolution.

[b] Course descriptions and case implementation details can be found in Supplemental Materials, Appendix 1.

[c] This course instructor implemented approximately 1/3 of the curriculum for a single case, but did not implement any cases in their entirety.

Evolution Assessment Tool (ATEEK)

We used the Assessment Tool for Evaluating Evolution Knowledge (ATEEK) as described in White et al. (22) (Supplemental Materials, Appendix 2), designed to measure student ability to solve problems that require knowledge of evolutionary processes across different scales. This tool was developed using an iterative design process (e.g., Bishop and Anderson (5)) with inter-rater validation of student responses. Question 1 asks students to describe the molecular basis (protein function) of two different phenotypes associated with three genotypes. The second question tests whether students can identify genetic mutation as being responsible for the appearance of new phenotypes, while the third question tests whether students can define the concept of natural selection and use it to explain changes in allele frequency across populations. The fourth question (parts i and ii) determines whether students can explain what a genetic mutation is and how it leads to a new phenotype through a DNA-to-RNA-to-protein pathway.

Student responses for each question were graded on a three-point scale (0, 1, or 2). A score of 2 indicates that a response was correct or mostly correct. A score of 1 was awarded when a response was partially correct; certain information may have been missing, or incorrect information may have been included with correct information. A score of 0 indicates that a question was answered incorrectly or mostly incorrectly. PJTW and MKH modified the scoring rubric described in White et al. (31). The original criteria for questions 1, 2, and 4(i) were kept; the criteria for questions 3 and 4(ii) were refined. Credit was not given for question 3 for talking about *fitness* per se (as it was in White et al. (22)). Rather, the meaning of fitness had to be clearly articulated. Additionally, students were not given full credit in question 4(ii) unless they mentioned mRNA in describing how a new phenotype arises as a result of genetic mutation. Scoring of student responses on the ATEEK was done by PJTW. Scoring was blind with respect to both course identification and pre-course versus post-course assessments. Prior to scoring, PJTW and MKH scored 52 randomly chosen ATEEKs (from C_4^{CMB}) to independently verify the analysis of student responses. PJTW and MKH gave identical scores in 220 of 260 questions (84.6% agreement, unweighted Cohen's kappa = 0.768 (6)); there was no statistical difference in the mean score of any of the ATEEK questions. A description of the scoring rubric along with sample student responses can be found in White et al. (22).

Evaluating case knowledge

We used an open-ended survey to assess student case-specific knowledge for three cases: Mouse Fur Color evolution (4 questions), Pea Seed Taste evolution (4 questions), and

TABLE 2.
The frequency of instructional approaches used by instructors A through G who participated in our study.

Instructor	Use of Lecturing[a]	Use of Student Discussions[a]	Use of Student Writing[a]	Use of Clicker Questions[a]	Use of Group Work[a]	Use of Demonstrations[a]	Use of Assigned Readings[b]	Other Pedagogies Used
A	Always	Very often	Very often	Very often	Sometimes	Sometimes	Every week	Mastering Biology (online homework), Classroom role-plays, building molecular models.
B	Very often	Always	Very often	Very often	Sometimes	Rarely	Every week	Classroom role-plays. Building molecular systems with modeling clay.
C	Always	Sometimes	Sometimes	Very often	Very often	Sometimes	Most weeks	Case Studies – some scripted from National Center for Case Studies in Science Teaching website and others informal.
D	Very often	Very often	Very often	Very often	Very often	Sometimes	Most weeks	No additional information provided.
E	Always	Always	Always	Always	Always	Rarely	Every week	Case Studies – one from the National Center for Case Studies; one interrupted case study created by the instructor.
F	Very often	Sometimes	Sometimes	Very often	Very often	Sometimes	Every week	Case Studies from various sources.
G	Always	Always	Very often	Very often	Very often	Never	Every week	Classroom role-plays. "Shotgun journal clubs" (where the entire period is going from Figure to Figure in a paper and calling on students to explain it).

[a] Instructors were asked to indicate how frequently they use each pedagogical tool in a typical class session. A Likert scale was used as follows: Always–Very often–Sometimes–Rarely–Never.

[b] Instructors were asked to indicate how frequently they assign readings. A Likert scale was used as follows: Every week–Most weeks–Some weeks–Rarely–Never.

Primate Opsin evolution (4 questions) (Supplemental Materials, Appendix 3). The questions associated with each case were isomorphic. The first two questions probed for information at a genetics and protein level, the third probed for information at a cell biology level, and the fourth probed for information at a natural/artificial selection level. Case knowledge was evaluated in two separate courses: C_4^{CMB} and NC_4^{CMB}.

Data analyses

We performed three sets of data analyses to (i) examine student absolute gain and normalized gain among courses, (ii) examine the relationship between case knowledge and post-course ATEEK score, and (iii) track ATEEK scores of students across the introductory biology sequence under varying levels of case use.

ATEEK gains among courses

Absolute gain on a given ATEEK question, *q,* is calculated as:

$$\text{Absolute gain}_q = \text{Post-course ATEEK score}_q - \text{Pre-course ATEEK score}_q$$

In the case of individual questions, calculating absolute gain is preferred to calculating normalized gain because normalized gain calculations based on the three-point grading system used for individual questions do not provide meaningful data (i.e., because gains from 0 to 2 and from 1 to 2 both result in a normalized gain score of 100). Instead, normalized gain (11) was calculated from total pre-ATEEK to total post-ATEEK student scores in each course. The normalized gain was calculated as:

$$\text{Normalized gain} = [(\text{Post-course ATEEK\%} - \text{Pre-course ATEEK\%}) / (100\% - \text{Pre-course ATEEK\%})] \times 100$$

We computed a Kruskal-Wallis test with post-hoc Dunn comparisons to determine instances where the absolute gain on each question differed among courses; this test is preferred for non-parametric data. We computed an ANOVA with post-hoc Tukey-Kramer comparisons to determine instances where the total ATEEK normalized gain differed among courses. The Dunn and Tukey-Kramer procedures identify pairs of courses within the Kruskal-Wallis and ANOVA tests (respectively), where scores differ significantly on a given question; it also identifies pairs of courses where the total ATEEK scores differ significantly.

Case knowledge and ATEEK score

Secondly, we related post-course ATEEK scores to student Case Knowledge, a direct measure of how well each student understood particular case content. We performed multiple regression analyses (R: Development Core Team;

www.r-project.org; 19), for two LB145 courses, C_4^{CMB} and NC_4^{CMB}, using Case Knowledge score and three control variables to predict post-course ATEEK score. Pre-course ATEEK score served as a control for the ATEEK-related knowledge that students had prior to instruction; course grade served as a control for student learning in the course, independent of case learning; cumulative grade point average (GPA) served as a control for overall student academic ability. We chose post-course ATEEK score as our dependent variable rather than ATEEK normalized gain because it allowed us to examine the statistical effect of pre-course ATEEK as an independent variable.

Tracking student ATEEK scores

Lastly, we tracked students' ATEEK scores across three cohorts in the LB144–LB145 introductory biology course sequence (Table 3). The first cohort had case exposure in both LB144 and LB145. The second cohort had case exposure in LB144 but did not have case exposure in LB145. The third cohort did not have case exposure in LB144 but had case curriculum exposure in LB145. Unfortunately, due to low sample size (*n* = 3), we were not able to track a fourth cohort of students, those who were not exposed to case curriculum in either LB144 or LB145. ATEEK scores for the three cohorts of students were examined with a Kruskal-Wallis test (with post-hoc Dunn's comparison) at three time-points: 1) prior to receiving instruction at the beginning of the first introductory biology course, LB144, 2) at the end of the first introductory biology course, LB144, after having received one semester of instruction, and 3) at the end of the second introductory course, LB145, after having received two semesters of instruction.

Data collection. Over our two-year study period, 308 students were taught biology in courses where evolution cases were integrated into the curriculum, and 395 students were taught biology in courses where evolution cases were not integrated into the curriculum. We collected data from 276 and 317 students in these two groups, respectively (the remaining students did not complete the pre- and post-course ATEEK).

RESULTS

ATEEK gains among courses

Generally, students who learned biology in courses using cases had higher ATEEK gains than students who learned biology in courses that did not use cases (Fig. 1). This result was consistent for the absolute gains on questions 1, 2, 4(i), 4(ii), and for the normalized gain across the entire ATEEK instrument. While the average absolute gain of students on question 3 was higher in courses that used cases, the Dunn's post-hoc pairwise comparisons between courses using cases and those not using cases were not often significant. When the data were pooled across courses, students who learned evolution using an integrative approach had higher post-course ATEEK scores, higher total ATEEK normalized gains, and higher question-by-question absolute gains on all ATEEK questions (Table 4).

Case knowledge and ATEEK score

Case knowledge score and pre-course ATEEK score were statistically significant variables used to describe post-course ATEEK score (Table 5); there was a positive linear relationship between case knowledge score and post-course ATEEK score (standardized coefficient = +0.44); there was also a positive linear relationship between pre-course ATEEK score and post-course ATEEK score (standardized coefficient = +0.18). Student course grade and overall GPA were not statistically related to post-course ATEEK score in this model. A comparison of the relative magnitude of the standardized coefficients shows that case knowledge score was the most important variable in the model, accounting for the highest proportion of the model's descriptive power. The model explained 43% of the variance in post-course ATEEK score (i.e., adjusted R^2 = 0.43).

Tracking student ATEEK pre/post scores

We tracked 45 students in the first cohort (case exposure in LB144 and in LB145), 23 students in the second

TABLE 3.
Course progression of three different student cohorts.

Cohort	Number of Students	LB144 w/ Cases[a]	LB144 w/o Cases[a]	LB145 w/ Cases[a]	LB145 w/o Cases[a]
1	45	C_3^{OB}, Fall 2012	—	C_4^{CMB} Spring '13	—
2	23	C_3^{OB}, Fall 2012	—	—	NC_4^{CMB}, Spring 2013
3	25	—	NC_3^{OB}, Spring 2012	C_2^{CMB}, Fall 2012 / C_4^{CMB}, Spring 2013	—

[a] C designates courses where cases were used; NC designates courses where cases were not used. The superscript denotes the course subject: CMB – Cell and Molecular Biology, OB – Organismal Biology. Course descriptions and case implementation details can be found in Supplemental Materials, Appendix 1.

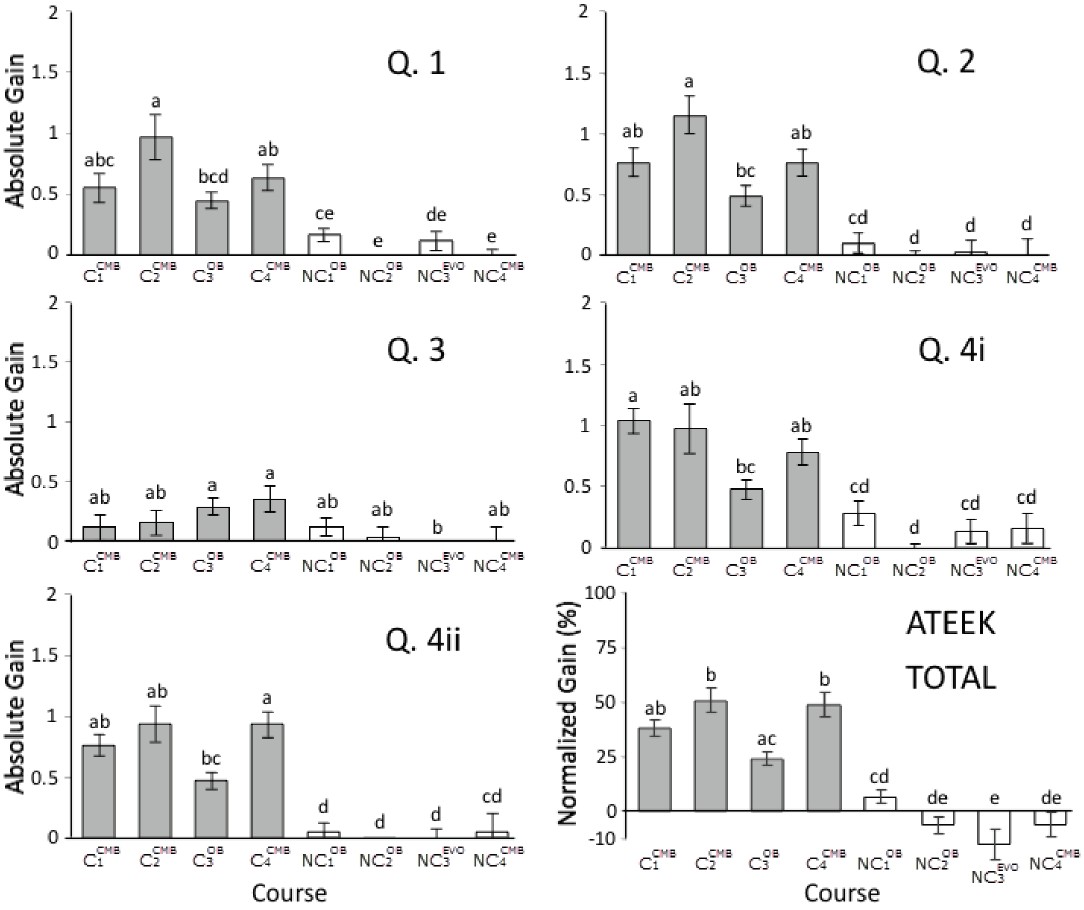

FIGURE 1. Average student gain on ATEEK questions in four courses using cases and in four courses where cases were not used. Gains (mean ± SE) on ATEEK questions were significantly higher among students in courses that featured integrative evolution cases (dark bars) than among students in courses that did not feature integrative evolution cases (light bars). Bars on individual questions are not shown where the average gain is negative, though error bars are still visible in some cases. Gains with the same letter mark within a graph do not differ significantly (Kruskal-Wallis with a post-hoc Dunn's test, or, in the case of Normalized Gain, ANOVA with a post-hoc Tukey-Kramer test). ATEEK = Assessment Tool for Evaluating Evolution Knowledge; C = course in which integrated cases were used; NC = course in which integrative cases were not used; CMB = cell and molecular biology course; OB = organismal biology course; EVO = evolution course; SE = standard error.

cohort (case exposure in LB144; no case exposure in LB145), and 25 students in the third cohort (no case exposure in LB144; case exposure in LB145).

Prior to instruction, students in cohort 3 did significantly better on the pre-course ATEEK (time-point 1, Fig. 2) than did the students in the other two cohorts (Dunn's p values < 0.05, Kruskal-Wallis H(2) = 7.98). Students in cohort 1 (with cases) did significantly better on the second ATEEK (time-point 2, Fig. 2) than did students in cohort 3 (without cases) (Dunn's $p < 0.05$; Kruskal-Wallis H(2) = 6.70). At this second time-point, ATEEK scores from students in cohort 2 (with cases) were not significantly different from the scores of the students in either of the other two cohorts (Dunn's $p > 0.05$). At time-point 3 (Fig. 2), students in cohort 1 (with cases – with cases) had significantly higher ATEEK scores than students in cohort 2 (with cases – without cases) and students in cohort 3 (without cases – with cases) (Dunn's $p < 0.05$ for each comparison; Kruskal-Wallis H(2) = 14.96).

DISCUSSION

Students who used integrative cases showed increased learning of evolutionary concepts

We sought to address three questions in this study. Our first question asked whether students using an integrative case approach to learn evolutionary concepts in an introductory biology course would demonstrate an improved understanding of evolution compared with students not using this approach. Taken as a whole, our results indicate that implementation of the integrative cases indeed leads to increased learning of evolution concepts.

Our second question asked whether students in introductory organismal courses would demonstrate learning gains pertaining to the molecular underpinnings of evolution when integrative evolution cases were used in the curriculum. Here, the answer is a qualified "yes";

TABLE 4.
Question-by-question and total student ATEEK gains across courses that used cases and across courses that did not use cases.[a]

	Absolute Scores					
	Q. 1 (2 points) Score (SE)	**Q. 2 (2 points) Score (SE)**	**Q. 3 (2 points) Score (SE)**	**Q. 4(i) (2 points) Score (SE)**	**Q. 4(ii) (2 points) Score (SE)**	**Total (10 points) Score (SE)**
Students in courses using cases						
Pre	0.35 (0.04)	0.41 (0.04)	0.45 (0.04)	0.53 (0.04)	0.25 (0.03)	1.99 (0.14)
Post	0.93 (0.05)	1.11 (0.05)	0.70 (0.04)	1.27 (0.05)	0.96 (0.05)	4.97 (0.18)
Gain	0.58 (0.05)	0.70 (0.06)	0.25 (0.05)	0.73 (0.06)	0.72 (0.05)	2.98 (0.17)
Students in courses not using cases						
Pre	0.34 (0.04)	0.58 (0.05)	0.43 (0.03)	0.81 (0.05)	0.49 (0.04)	2.65 (0.13)
Post	0.40 (0.04)	0.62 (0.05)	0.44 (0.03)	0.93 (0.05)	0.49 (0.04)	2.89 (0.14)
Gain	0.06 (0.04)	0.04 (0.05)	0.01 (0.04)	0.13 (0.05)	0.00 (0.04)	0.24 (0.12)

	Percent Scores					
	Q. 1 (2 points) Percent (SE)	**Q. 2 (2 points) Percent (SE)**	**Q. 3 (2 points) Percent (SE)**	**Q. 4(i) (2 points) Percent (SE)**	**Q. 4(ii) (2 points) Percent (SE)**	**Total (10 points) Percent (SE)**
Students in courses using cases						
Pre	17.3 (1.9)	20.5 (2.2)	22.4 (1.8)	26.7 (2.2)	12.4 (1.6)	19.9 (1.4)
Post	46.4 (2.6)	55.6 (2.7)	34.7 (2.0)	63.5 (2.5)	48.2 (2.5)	49.7 (1.8)
Gain	29.1 (2.7)	35.1 (2.8)	12.4 (2.5)	36.7 (2.8)	35.8 (2.5)	29.8 (1.7)
Normalized Gain[b]						35.6 (2.3)
Students in courses not using cases						
Pre	17.2 (1.9)	28.9 (2.3)	21.5 (1.5)	40.4 (2.3)	24.6 (1.9)	26.5 (1.3)
Post	20.2 (2.0)	30.8 (2.3)	22.1 (1.7)	46.7 (2.3)	24.6 (1.8)	28.9 (1.4)
Gain	3.0 (1.9)	1.9 (2.3)	0.6 (2.1)	6.3 (2.7)	0.0 (2.0)	2.4 (1.2)
Normalized Gain[b]						-4.5 (2.8)

[a] Mean pre- and post-course ATEEK scores and learning gains of students in courses where cases were used (275 students across 4 courses) and in courses where cases were not used (317 students across 4 courses).

[b] Calculated as (post-course ATEEK – pre-course ATEEK) / (100% – post course ATEEK) × 100%.

ATEEK = Assessment Tool for Evaluating Evolution Knowledge; SE = standard error.

TABLE 5.
A multiple linear regression using pre-course ATEEK score, overall course grade, cumulative grade point average, and case knowledge score to describe post-course ATEEK score ($F_{4,115} = 23.01$, adjusted $R^2 = 0.43$).[a]

Variable	Coefficient	Standardized Coefficient	Standard Error	t-value	*p* value
Pre-course ATEEK	0.16	0.18	0.077	2.1	0.039
Cumulative GPA	1.14	0.21	0.66	1.7	0.085
Overall course grade	-0.001	-0.003	0.032	-0.03	0.98
Case knowledge score	0.18	0.44	0.037	4.8	5.3×10^{-6}

[a] Standardized coefficients show the relative effect that each independent variable has on the calculation of the post-course ATEEK value. The R^2 value indicates that this model successfully explains 43% of the variance in post-course ATEEK scores.

ATEEK = Assessment Tool for Evaluating Evolution Knowledge; GPA = grade point average.

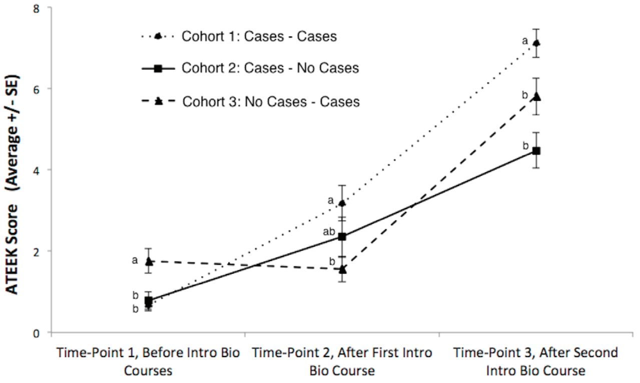

FIGURE 2. ATEEK score for three cohorts of students, tracked across two introductory biology courses. Students in three cohorts were surveyed using the ATEEK. Students in cohort 1 (*n* = 45) had case exposure in both intro bio courses. Students in cohort 2 (*n* = 23) had case exposure in the first intro bio course but not in the second. Students in cohort 3 (*n* = 25) had case exposure in the second bio course but not in the first. Data points with the same letter mark within a time-point do not differ significantly (Kruskal-Wallis with post-hoc Dunn's test). ATEEK = Assessment Tool for Evaluating Evolution Knowledge; SE = standard error.

inclusion of integrative cases in organismal biology courses has the potential to help biology students expand their understanding of evolution to incorporate molecular and cellular mechanisms (Fig. 1). Students in C_3^{OB} had significantly higher gains than students in NC_2^{OB} for total ATEEK score and most individual ATEEK questions. These were two iterations of the same introductory organismal course, LB144, taught in different semesters with different instructors (although see the Limitations section below). In contrast, differences in ATEEK gains between C_3^{OB} and NC_1^{OB} were not statistically different. Although C_3^{OB} and NC_1^{OB} are both organismal biology courses, they are taught within different biology sequences. NC_1^{OB} is a version of introductory organismal biology within the university that is *preceded* by introductory cell and molecular biology whereas C_3^{OB} is a version that is *followed* by introductory cell and molecular biology. Thus, one possible explanation for the lack of differences in the comparisons of courses C_3^{OB} and NC_1^{OB} is that the latter group of students had prior cell and molecular biology instruction, though without cases, that may have allowed them to perform moderately better than the former group on their post-course ATEEK.

Our third question asked whether increased exposure to integrative cases led to increased understanding of evolutionary theory. We established cohorts of students who took C_3^{OB} or NC_2^{OB} and then subsequently C_2^{CMB}, C_4^{CMB} or NC_4^{CMB}. Thus, some students had two "doses" of the cases, while others had only one (Table 3). When tracking students across the introductory biology sequence, students who experienced integrative cases in both introductory courses showed the highest gains on the ATEEK.

ATEEK Q3 and student difficulty explaining natural selection

ATEEK question 3 probed student understanding of natural selection by asking students to define natural selection and then to use that principle to explain a given evolutionary scenario. In this case the scenario was the loss of toxicity and the corresponding change in allele frequency in a population of mushrooms. Only two Dunn's post-hoc comparisons (C_3^{OB} and C_4^{CMB} vs. NC_3^{EVO}), showed significant differences (Fig. 1).

Part of the reason for the low scores on ATEEK Q3 may have been the fact that we set a high bar for answer correctness. "Natural selection" is the primary concept related to evolution that students learn in K to 12 education, generally taught with macro-scale topics. Thus, we required that a student's definition of natural selection link increased reproductive output to environmental conditions. This proved difficult, and many students gave no definition of natural selection whatsoever (see Box 2). Other students provided what we called "Fitness-esque Answers," in which students evoked the term "fitness" but failed to articulate what fitness means or how it relates to natural selection. Other students left out any mention of environmental conditions when alluding to higher reproductive rates amongst the non-toxic mushroom phenotype. Still others appeared to personify nature, whereby some kind of selecting agent or force, akin to Mother Nature, chooses traits and/or species that benefit a particular ecosystem, community, or population. In the second part of ATEEK Q3, many students had a hard time conceptualizing how the loss of a protective trait (i.e., toxicity) could result in higher fitness. Part of the difficulty with ATEEK Q3 may have stemmed from our choice of a specific scenario involving trait loss. Students tend to struggle when it comes to applying natural selection principles to scenarios describing the loss of a trait (5, 10), particularly in non-animal organisms (17). Future Evo-Ed case implementation studies may need to incorporate an animal trait-gain question into the assessment question set.

Limitations of the present study

While our data clearly show that students who used the integrative cases made strong gains on the ATEEK, it can be noted that this evolution assessment instrument measures a subset of core evolutionary concepts. It is unclear how stronger knowledge of the molecular and genetic underpinnings of evolution translates to stronger knowledge of specific higher-order evolutionary concepts such as dynamic population genetics, speciation, and tree-thinking. However, the positive relationship between ATEEK score and case knowledge score seems to indicate that the cases help students apply these principles to different evolutionary scenarios. Perhaps more importantly, the cases have succeeded in bringing cell and molecular concepts to

Fitness-esque answers where it is not clear that students understand what fitness is. Typical answers include:

- *Natural Selection is the survival of the fittest.*
- *Natural Selection: when more fit specimen produces more offspring.*
- *Natural selection is a factor that says the more fit species will reproduce.*

Many students did not include any environmental aspect in their definition of Natural Selection. Typical answers include:

- *Natural selection is the process by which populations undergo change due to the individuals' heritable characteristics that increase fitness.*
- *Natural selection is where a species with certain traits have [sic] better fitness & reproduce more than species w/o those traits.*
- *Natural selection is the occurrence where particular traits of organisms that can increase fitness and general survivability are passed down generation to generation.*

A smaller subset of students personified nature in some shape or form. Typical answers include:

- *Natural Selection: when personal preference of an environment favors a species and it results in a higher frequency of that species.*
- *Natural Selection: Nature's way of weeding out the weakest.*
- *Natural selection chooses the fittest individuals for survival and reproduction.*

Additionally, many students had difficulty describing a scenario where non-poisonous mushrooms had a fitness advantage over poisonous mushrooms.

- *Animals or other organisms began to prefer the nonpoisonous Toxican mushrooms causing the poisonous Toxican mushrooms to become rare.*
- *Non-toxic mushrooms are probably eaten more → need more offspring to survive.*
- *Because animals learned to avoid the toxic mushroom due to its toxic nature, the toxian [sic] was no longer needed.*
- *After an extended period of time, the toxins stop being produced because the mushrooms stop being eaten.*

BOX 2. Answers to Assessment Tool for Evaluating Evolution Knowledge (ATEEK) Question 3. Students had difficulty providing succinct and correct answers. A variety of common erroneous answers are shown. All answers provided below are from post-course ATEEK surveys.

bear on topics normally discussed only at organismic and population scales.

There were also limitations with the experimental design of our study. Ideally, our study would have featured a comprehensive design where treatments (use of cases) were randomized with respect to instructor and where instructors were tracked over many years in iterations of their courses with and without case use. As with many studies of this nature, there were a number of factors, many beyond our control, that prevented us from implementing an optimal design. For example, it is common at our institution for core biology courses to have different instructors from semester to semester and from year to year. This prevented the use of an experimental design where the same instructor used "cases" one year and "no cases" the next (or vice versa), notwithstanding changes that might naturally occur in a course from one year to the next. Thus, in our study, the courses where cases were used were not perfectly "matched" to courses where cases were not used. In fact, the final set of eight courses included all of the courses that were available to us in which to implement the ATEEK and/or case material during the two-year window of our study. Although these eight courses involved seven different instructors, each instructor was well-respected as an educator, and they all included active learning pedagogies in their classrooms.

Summary and implications

Our initial report on student learning in courses where cases were used (22) suggested that the use of these cases could facilitate students' learning of evolution. Thus, we undertook this more rigorous and extensive study to extend the initial findings by making comparisons between courses where cases were used and those where cases were not used. While it is apparent that many students still struggle with the concepts of natural selection and biological fitness, learning case material appears to have helped many students integrate micro-scale biological knowledge into an evolutionary context. Students in courses in which entire cases were used learned more than did students in courses not using cases. Moreover, students in an introductory organismal course could use and apply molecular and cellular ideas in their learning of evolution, and students exposed to cases in both introductory sequence courses learned more by our measures than did those using cases in only one course.

One of our primary objectives when developing the cases was to provide a way for students to integrate content and concepts across the biology curriculum. In particular, we expected that students would be able to integrate principles of molecular and cell biology when they described evolutionary processes. We incorporated multiple biological sub-disciplines into each case and it appears that the integrative evolution cases can help students move beyond a solely ecological understanding of evolution. We envisioned these materials to be used as a "thread" woven across the curriculum that would positively impact student learning. Our results speak to the way that biology is traditionally taught, with molecular and cellular processes presented in isolation from the organisms in which they occur. Evolution has tremendous explanatory power across the discipline (9) and we, as educators, would do well to teach with elements and examples that span the curriculum.

SUPPLEMENTAL MATERIALS

Appendix 1: Course descriptions and case implementation details
Appendix 2: ATEEK questionnaire
Appendix 3: Evolution knowledge assessment

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REFERENCES

1. **Abraham, J., E. Meir, J. Perry, J. Herron, S. Maruca, and D. Stal.** 2009. Addressing undergraduate student misconceptions about natural selection with an interactive simulated laboratory. Evol. Educ. Outreach **2**:393–404.
2. **Alters, B. J., and C. E. Nelson.** 2002. Perspective: teaching evolution in higher education. Evolution **56**:1891–1901.
3. **Anderson, D. L., K. M. Fisher, and G. J. Norman.** 2002. Development and evaluation of the conceptual inventory of natural selection. J. Res. Sci. Teach. **39**:952–978.
4. **Bahar, M., A. H. Johnstone, and M. H. Hansell.** 1999. Revisiting learning difficulties in biology. J. Biol. Educ. **33:**84–86.
5. **Bishop, B., and C. W. Anderson.** 1990. Student conceptions of natural selection and its role in evolution. J. Res. Sci. Teach. **27:**41–57.
6. **Cohen, J.** 1960. A coefficient of agreement for nominal scales. Educ. Psychol. Measure. **20**:37–46.
7. **Dauer, J. T., J. L. Momsen, E. B. Speth, S. C. Makohon-Moore, and T. M. Long.** 2013. Analyzing change in students' gene-to-evolution models in college-level introductory biology. J. Res. Sci. Teach. **50**:639–659.
8. **Demastes, S. S., J. Settlage, and R. Good.** 1995. Students' conceptions of natural selection and its role in evolution: cases of replication and comparison. J. Res. Sci. Teach. **32**:535–550.
9. **Dobzhansky, T.** 1973 Nothing in biology makes sense except in the light of evolution. Amer. Biol. Teach. **35**:125–129.
10. **Gregory, T. R.** 2009. Understanding natural selection: Essential concepts and common misconceptions. Evol. Educ. Outreach **2**:156–175.
11. **Hake, R. R.** 1998. Interactive-engagement versus traditional methods: a six-thousand-student survey of mechanics test data for introductory physics courses. Am. J. Physics **66**:64–74.
12. **Ingram, E., and C. Nelson.** 2006. Relationship between achievement and students' acceptance of evolution or creation in an upper-level evolution course. J. Res. Sci. Teach. **43**:7–24.
13. **Lawson, A. E., and W. A. Worsnop**. 1992. Learning about evolution and rejecting a belief in special creation: effects of reflective reasoning skill, prior knowledge, prior belief and religious commitment. J. Res. Sci. Teach. **29**:143–166.
14. **Lord, T., and A. Marino.** 1993. How university students view the theory of evolution. J. Coll. Sci. Teach. **22**:353–357.
15. **Nehm, R., et al.** 2009. Does the segregation of evolution in biology textbooks and introductory courses reinforce students' faulty mental models of biology and evolution? Evol. Educ. Outreach **2**:527–532.
16. **Nehm, R., and I. Schonfeld.** 2007. Does increasing biology teacher knowledge of evolution and the nature of science lead to greater preference for the teaching of evolution in schools? J. Sci. Teacher Educ. **18**:699–723.
17. **Nehm, R. H., and M. Ha.** 2014. Darwin's difficulties and students' struggles with trait loss: cognitive-historical parallelisms in evolutionary explanation. Sci. Educ. **23**:1051–1074.
18. **Nelson, C.** 2012. Why don't undergraduates really "get" evolution? What can faculty do? *In* Karl, S. K. B., S. Rosengren, E. Margaret Evans, and Gale M. Sinatra (eds.), Evolution challenges: Integrating research and practice in teaching and learning about evolution, Oxford University Press, Oxford.
19. **Theobald, R., and S. Freeman.** 2014. Is it the intervention or the students? Using linear regression to control for student characteristics in undergraduate STEM education research. CBE Life Sci. Educ. **13**:41–48.
20. **Trani, R.** 2004. I won't teach evolution; it's against my religion. And now for the rest of the storytelling. Am. Biol. Teach. **66**:419–427.
21. **White, P. J. T., M. K. Heidemann, M. Loh, and J. J. Smith.** 2013. Integrative cases for teaching evolution. Evol. Educ. Outreach **6**:1–7
22. **White, P. J. T., M. K. Heidemann, and J. J. Smith.** 2013b. A new integrative approach to evolution education. BioScience **63**:586–594.