
Research Paper

Development and Evaluation of an Activity to Teach Molecular Phylogeny, Deep Time and Classification Systems for Japanese High School Students

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In this study, we attempted to develop a teaching material about molecular phylogeny, deep time and classification systems to Japanese high school students. On the basis of the pre- and post-test results and the positive impressions of the activity by students and teachers, our protocol was considered useful for teaching these macroevolutionary concepts. Combined use of this activity and other materials (e.g. Westerling 2008) to teach the accumulation mechanism of neutral variations in DNA molecules will promote understanding of the link between DNA and biodiversity.

Keywords: *deep time, mammalian evolution, molecular phylogeny, molecular clock, neutral theory*

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Introduction

Many studies on evolution education have concentrated on microevolutionary topics (Catley 2006). However, research on teaching macroevolution has been actively performed, primarily in the USA. The major scope of these studies was to understand how to effectively teach the topology of phylogeny, that is, 'tree thinking' (Baum *et al.* 2005, Meisel 2010, Morabito *et al.* 2010).

There are three issues to promote teaching macroevolutionary topics in evolution education. First, research on how to teach phylogeny, particularly molecular phylogeny, is lacking particularly in non-English-speaking countries. Most teaching materials about molecu-

lar phylogeny have been developed in English-speaking countries (e.g. Maier 2001, Kuzoff *et al.* 2009, Franklin 2010, Lents *et al.* 2010, Kvist *et al.* 2011). Molecular phylogeny is an essential tool in current biological studies, and consequently, topics related to molecular phylogeny are included in many biology textbooks for both high school and university students (Cain *et al.* 2002, Campbell and Reece 2005, Raven *et al.* 2005, Sadava *et al.* 2009). In addition, the development of effective teaching materials for molecular phylogeny is strongly required because related topics, such as the neutral theory, molecular clock and effects of natural selection on molecules, appear difficult for students to compre-

hend (Ware 2008, Yamanoi *et al.* 2012a).

Second, materials for teaching deep time are required (Hills 2007). Catley and Novick (2009) indicated that American university students did not understand deep time irrespective of their biological background. Teaching the construction of a molecular phylogenetic tree and the calculation of the divergence time using a molecular clock in parallel can be an effective method of teaching deep time.

Third, materials for teaching biodiversity are necessary to promote its conservation. Classification is one of the important basic concepts for understanding biodiversity. Activities to teach these three contents (molecular phylogeny, deep time, and classification) to students simultaneously have not been developed, although there are many activities to teach molecular phylogeny (Maier 2001, Campo and Gracia-Vazquez 2008, Kuzoff *et al.* 2009, Franklin 2010, Lents *et al.* 2010, Kvist *et al.* 2011).

Biology education based on the new Japanese national curriculum framework, the Course of Study (CS), and its guidelines for secondary schools is being implemented in high schools in 2012 (Ministry of Education, Culture, Sports, Science and Technology 2009). This new CS places a greater emphasis on links between micro- and macro-biology and evolution (Shimada 2010, Yamanoi *et al.* 2012b). For instance, contents on molecular evolution (e.g., the neutral theory, molecular phylogeny, the molecular clock) and taxonomy (e.g., domain) are included in the new CS. Understanding molecular phylogeny, deep time and classification systems will promote biology education based on the new CS. However, effective teaching materials linking micro- and macro-biology have hardly been developed in Japan.

Our goal in this study was to develop an effective activity which can teach molecular phylogeny, deep time and classification systems simultaneously. Kuzoff *et al.* (2009) developed an excellent protocol of molecular phylogeny for inquiry learning using a free software MEGA (Tamura *et al.* 2007). In this study, we translated this protocol into Japanese and then modified it by incorporating two additional activities: the calculation of divergence time using a molecular clock and the examination of the classification of mammalian species thorough the Internet. We verified the effectiveness of this new activity for Japanese high school students by comparing their test scores before and after its implementation. Moreover, we conducted a free training course of this teaching protocol for Japanese high school biology teachers and administered questionnaires to reveal the effectiveness from the teachers' perspectives.

Methods

Question for Inquiry Learning

The following question was used for students: 'Which species are more closely related to whales in mammals?' This question was proposed by Kuzoff *et al.* (2009) for students' question when they depict molecular phylogeny, and it is also seen as a column in one biology textbook for Japanese high school students. We assumed that this question was suitable for many Japanese students because they were familiar with mammals.

Molecules, Species and Topology

Kuzoff *et al.* (2009) recommended using the amino acid sequence of cytochrome c oxidase subunit 1 when answering the above-mentioned question. However, in this study, the amino acid sequence of alpha-globin chain was used for constructing phylogenetic

trees because this molecule can be utilized as a molecular clock (Barton *et al.* 2007, Futuyma 2009) and most Japanese students are aware of this molecule because the structure and function of hemoglobin are described in all Japanese biology textbooks for high school students (8/8 textbooks). We chose protein sequence data instead of DNA sequence data for constructing molecular phylogenetic trees because the amount of protein sequence data registered was small compared with that of DNA sequence data and it appeared to be easier for Japanese students to obtain relevant data in an English database; we used those of the National Center for Biotechnology Information (NCBI).

We selected seven mammalian species (minke whale, sperm whale, hippo, cat, kangaroo, quoll and platypus) for constructing molecular phylogenetic trees. From this topology, we expected students to learn two important topics, namely rough systematics of mammals (monotreme, marsupial and placental mammals) and convergent evolution (cat and quoll), in addition to answering the above-mentioned question. However, this topology would be invalid if another species (e.g. human, camel) were added to these seven species because the peptide length of alpha-globin is short and does not contain sufficient genetic information to create a valid topology. In summary, these species were arbitrarily selected to teach the above-mentioned concepts (rough systematics of mammals, convergent evolution).

Japanese Protocol for Students

We translated the protocol of Kuzoff *et al.* (2009) into Japanese with some modifications.

First, in our protocol, we did not use Basic Local Alignment Search Tool but repeatedly inputted the scientific and protein names in the

search bar of the NCBI search engine (web-based resource) because we considered it might be difficult for Japanese students to find valid protein sequences in English databases.

Second, students additionally examined the protein sequence differences between species using MEGA as a source for estimating the divergence time (Fig. 1).

After constructing phylogenetic trees, students calculated the divergence time using the differences in protein sequences between species and the alpha-globin molecular clock. The rate of amino acid substitution in alpha-globin (the number of amino acids: 141) is estimated to be 1.2×10^{-9} /amino acid positions/year (Kimura 1983, Barton *et al.* 2007). Thus, the average time required for one amino acid substitution is calculated to be approximately 'six million years.' Students used this numeric value to calculate the divergence time between minke whale and sperm whale and that between the common ancestor of these whales and the species (hippo) most closely related to whales among the rest (five mammalian species).

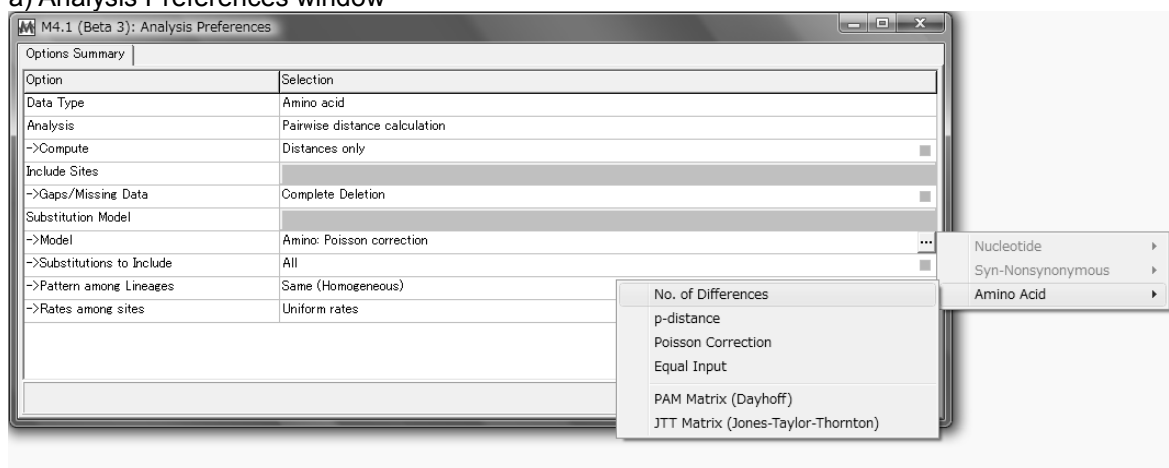
Third, we introduced a 'taxonomic sheet' (Fig. 2). Students examined the classification (kingdom, phylum, class, order, genus and species) of seven mammals through the Internet and depicted the topology on this sheet.

Fourth, pictures of these seven species provided on the students' protocol were pasted on the tip of the phylogeny depicted by students on the taxonomic sheet (Fig. 2). We believe that these pictures would increase students' motivation for this activity.

Student Activity

We performed the activity for Japanese students (N = 60, male: 16, female: 44) at three Japanese high schools from October to December 2010 (High School A: N = 20, High

a) Analysis Preferences window



b) Pairwise Distance window

	1	2	3	4	5	6	7
1. <i>O. anatinus</i> HBA							
2. <i>B. acutorostrata</i> HBA	45.000						
3. <i>H. amphibius</i> HBA	41.000	26.000					
4. <i>M. giganteus</i> HBA	46.000	39.000	28.000				
5. <i>D. viverrinus</i> HBA	46.000	42.000	32.000	22.000			
6. <i>F. catus</i> HBA	43.000	31.000	24.000	30.000	35.000		
7. <i>P. catodon</i> HBA	46.000	18.000	21.000	36.000	35.000	35.000	

Figure 1 How to examine amino acid differences between each species using MEGA

From the ‘Distances’ menu at the top of the MEGA window, select ‘Compute Pairwise.’ When the ‘Analysis Preferences’ window (a) opens, click the box on the right side of the ‘Model’ bar and select ‘Amino Acid’ and ‘No. of Differences.’ A table presenting the amino acid differences between each species will then appear in the ‘Pairwise Distances’ window (b).

School B: N = 23, High School C: N = 17). Two class hours (100 min) were used for this activity. These students were science majors and had previously studied evolution and molecular biology, including molecular phylogeny, the neutral theory, the molecular clock, the molecular structure of hemoglobin and classification systems, through normal lectures and textbooks.

Before the student activity, a teacher explained the following two points because this information is not adequately provided through normal biology class lectures in Japanese high schools. As biology textbooks with outdated scientific theories about evolutionary mecha-

nisms were used in Japanese high schools (Yamanoi 2008, Yamanoi *et al.* 2012b), it was considered that students did not have enough opportunity learning about neutral theory (including multiple sequence alignment).

(1) Some protein sequence regions have varied during evolutionary history by genetic drift because they are neutral to protein function, whereas the other regions have been conserved by stabilized selection because they are vital to the protein function. If these essential regions are changed by mutation, the organism will die. Thus, you can depict molecular phylogeny by utilizing neutral variations of the protein sequence.

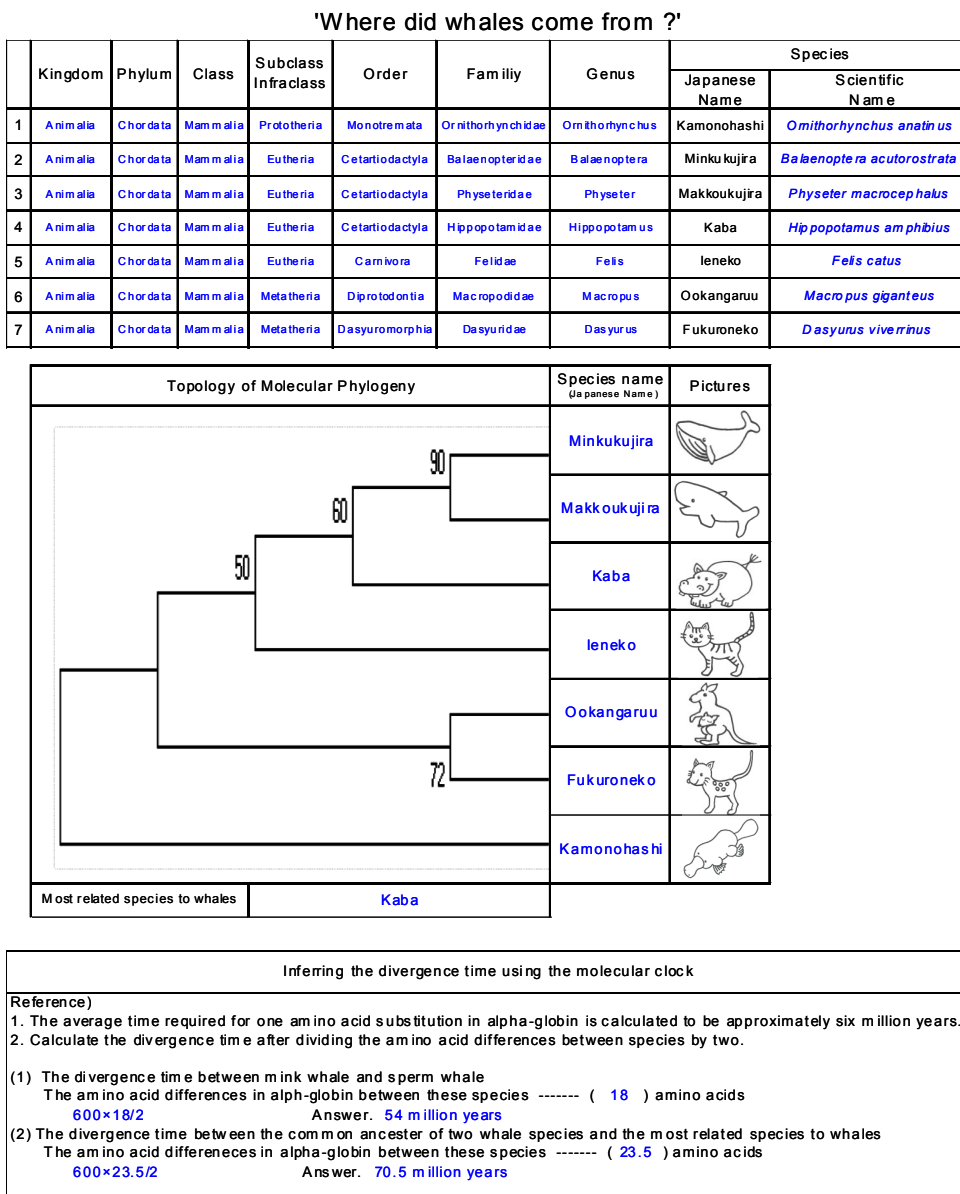


Figure 2 Taxonomic sheet

Students provided the classification of seven mammalian species, depicted the molecular phylogeny of these species, and pasted digital pictures cut from the student's protocol (mammal illustration drawn in this figure are shown as samples). In addition, they calculated the divergence time between two whale species and that between a common ancestor of these whales and hippo using the alpha-globin molecular clock. The blue characters and the topology with bootstrap values illustrate examples.

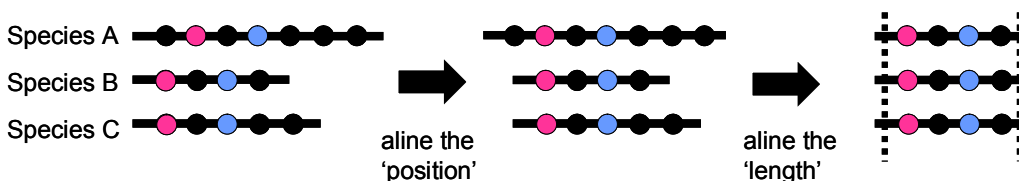


Figure 3 An illustration used for explaining multiple sequence alignment

Circles represent amino acids. Different colours represent different amino acids.

(2) You perform ‘multiple sequence alignment’ after downloading the amino acid sequences of alpha-globin in seven species that are used for constructing molecular phylogenetic trees. Multiple sequence alignment is a method that aligns the peptide lengths and amino acid sequences of alpha-globins among species (Teachers can use Figure 3 to explain this method). Some mutations, such as deletions and insertions, alter the peptide length of the protein. Thus, the peptide length of alpha-globin differs among species.

To start the student activity, students were divided into pair groups (two students per group). We asked the students groups to answer the question “Which species were more closely related to whales in mammals?” presented previously and to determine which species are closely related to each other by comparing their pictures. We then prompted students in each group to determine their roles: one student downloaded the protein sequences and the other examined the classification of each mammal through the Internet. The students exchanged roles after downloading the amino acid sequences of three or four species. The students managed to complete this activity by reading the protocol and confirming the procedure with their partner without any teacher’s help.

An example of a molecular phylogeny depicted by students is shown in Figure 2. After constructing the phylogenetic tree, the students calculated the divergence time using the following formula: **the number of amino acids being different between species/2 × 6 (million years)**. The divergence time between minke whale and sperm whale was calculated to be 54 million years and that between the common ancestor of these whales and hippo was calculated to be 70.5 million years.

Because minke whale and sperm whale, and the common ancestor of whales and hippo are considered to have diverged approximately 33 and 60 million years ago, respectively (Gatesy 2009), the values estimated in this activity are not valid. However, we considered these values useful for students to understand the deep time necessary for macroevolution. To prevent students from keeping these values as their knowledge, a teacher explained after the activity that these values were rough estimate values inferred from only one molecular clock, and that we needed to use more information to get more precise values through estimations by other molecular clocks and fossil records.

Evaluation of the Activity Using Pre- and Post-Test

We made a test that included 13 questions to evaluate this activity (Box 1). The main topics of the test were molecular evolution, the molecular clock, the timescale of macroevolution and classification systems. The same test was conducted before (pre-test) and after carrying out the student activity (post-test). We did not tell students to do post-test until finishing the student activity. One point score was given to each correct answer (maximum score: 13). The pre- and post-test scores were compared by paired *t*-test and Wilcoxon’s signed-rank test using the statistical software Systat 8.0. We also evaluated their impressions after this activity with some questions and descriptions (Box 1).

Evaluation of the Activity by High School Biology Teachers

For popularization of this teaching protocol, it must be considered effective by both students and teachers. We held a workshop of this activity for Japanese high school biology teachers without charge. We invited teachers and communicated with them via a mailing list

network of Japanese biology teachers prior to the workshop.

In the training course, we requested teachers to perform the activity in the same

Box 1 Questionnaire for students

A. Fill in the blank () or circle the appropriate in [].

Q1. Proteins are molecules that consist of () by bounded together by peptide bounds.

Q2. Most organisms commonly bear certain proteins. [true/false/beyond comprehension]

Q3. Organisms are classified into the following, in sequence: kingdom, (), (), (), (), (), species.

Q4. Scientific name comprises a () name and a () epithet

Q5. Mammals are classified into three subgroups: (), () and ()

Q6. When amino acid sequences are compared between species A and B, amino acid sequences are perfectly identical. [true/false/beyond comprehension]

Q7. In particular genes, irrespective of species, DNA sequences and amino acid sequences of protein originated from the gene have changed at a constant rate during evolutionary history. [true/false/beyond comprehension]

Q8. Organisms with analogous morphology are not necessarily closely related species. [true/false/beyond comprehension]

Q9. Whales are more closely related to () in mammals.

Q10. When same proteins are compared, their amino acid sequences show more resemblance among closely related species. [true/false/beyond comprehension]

Q11. When amino acid sequences are compared among some species, their sequences show both evolutionarily labile and hard-wired regions. [true/false/beyond comprehension]

Q12. We can calculate the divergence time between species using a molecular clock.

Q13. The average time spent in speciation is about

[one/one hundred/one thousand/ten thousand/hundred thousand/over million] year(s).

B. Evaluate this activity on a five-level rating system. Circle the appropriate in [].
interesting [5 / 4 / 3 / 2 / 1] boring

increase understanding [5 / 4 / 3 / 2 / 1] not increase understanding

digital work difficult [5 / 4 / 3 / 2 / 1] easy

English expression of MEGA difficult [5 / 4 / 3 / 2 / 1] easy

C. Please write any of your impressions of this activity.

Box 2 Questionnaire for teachers

A. Please fill in the blank () or circle the appropriate in [].

1. Sex [male/female]

2. Teaching experience () year(s)

3. Do you think this activity is easy to practice in high school biology class?

agree [5 / 4 / 3 / 2 / 1] disagree

4. Do you think you will practice this activity in your next year's biology class?

agree [5 / 4 / 3 / 2 / 1] disagree

B. Do you think this activity will help students understand the following contents?

Please circle the appropriate number.

'Molecular phylogeny' increase understanding [5 / 4 / 3 / 2 / 1] not increase understanding

'Molecular clock' increase understanding [5 / 4 / 3 / 2 / 1] not increase understanding

'Convergent evolution' increase understanding [5 / 4 / 3 / 2 / 1] not increase understanding

'Classification system' increase understanding [5 / 4 / 3 / 2 / 1] not increase understanding

'Mammalian evolution' increase understanding [5 / 4 / 3 / 2 / 1] not increase understanding

C. Please write any of strengths and weakness to this activity.

D. Please write any of your impressions to this activity.

manner as students and to complete a questionnaire (Box 2) for evaluating this teaching protocol. We also asked them to describe the strong points and weak points of the activity and to provide their impressions by descriptions (Box 2).

Results

Evaluation by the Students Pre- and Post-test

The average score of post-test was significantly higher than that of the pre-test (Figure 4; paired *t*-test, $df = 59$, $t = -16.19$, $p < 0.0001$). The average pre-test and post-test scores were 7.2, 10.8, respectively. In addition, the scores of nine questions out of 13 showed a significant rise after the activity, whereas there was no questions whose scores showed a significant fall (Figure 5, Wilcoxon's signed-rank test).

The students' impressions after the activity are presented in Figure 6. Most students be-

came more interested in molecular phylogeny through this activity, and their understanding of molecular phylogeny was deepened (Fig. 6 a–b).

We checked all of their descriptions at several times and extracted common impression. Their impressions ($n > 2$) were categorized as follows (some students' descriptions were distributed to several categories): understood that it is not always possible to judge evolutionary relationships between species only by phenotypes ($n=13$), surprised that whales and hippo were most closely related among the shown species despite being found in different habitats ($n = 11$), found depicting molecular phylogeny to be easier than expected ($n = 7$), understood molecular phylogeny better ($n = 5$), wanted to depict another molecular phylogeny ($n = 4$), and thought that the use of computer included any difficulties ($n = 4$).

Conversely, Figure 6 also shows that ap-

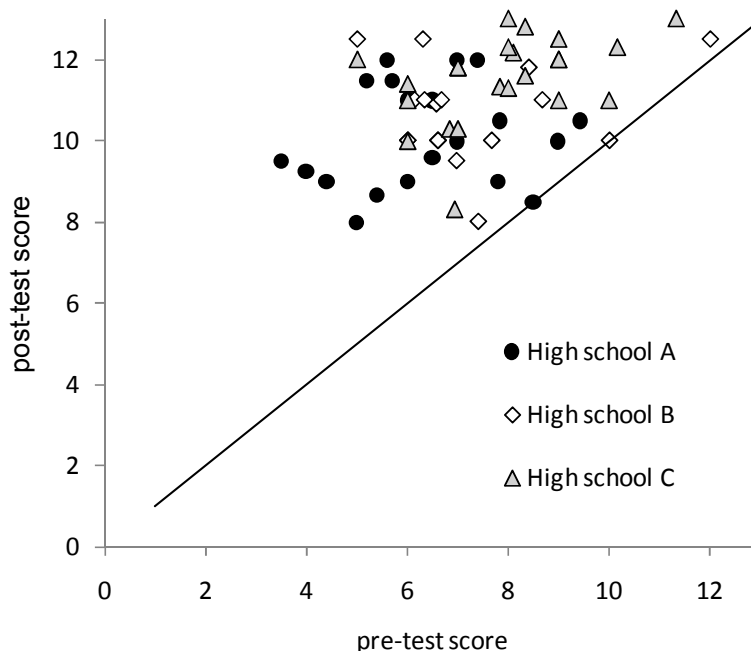


Figure 4 Comparison between the pre- and post-test values

The straight line in the graph indicates $y = x$. Thus, plots above this line represent rises in scores after the activity.

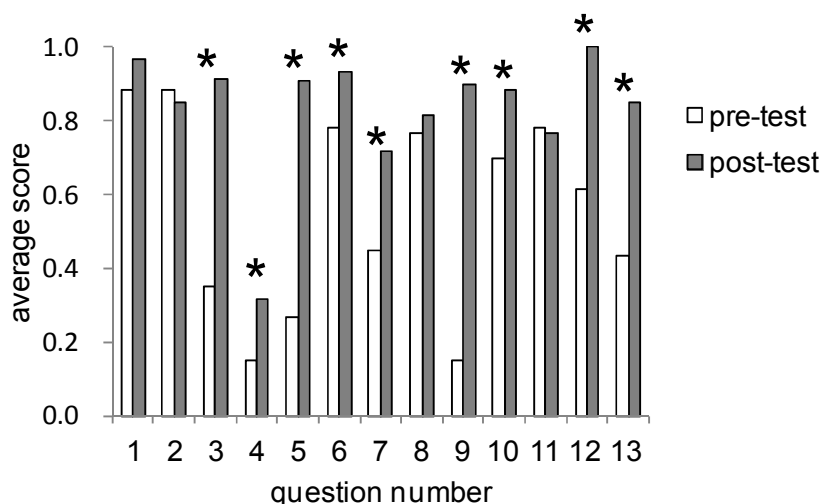


Figure 5 Changes in the average correct answer ratio for individual questions

Asterisks indicate significant differences between pre-test and post-test at the 5% level.

proximately a half of the students felt the digital practice and the English expressions of MEGA were difficult to comprehend (Fig. 6 c–d).

Evaluation by High School Biology Teachers

Twenty-six teachers with an average teaching experience of 16.2 years participated in this course (male: 16, female: 8, no description about sex: 2). They positively evaluated the designed activity (Fig. 7). Most of them answered that they wanted to implement this activity in their next year's classes and that this activity was useful for teaching molecular phylogeny.

More than two teachers pointed out four strong points of the activity: students will be able to understand contents related to molecular phylogeny through hands-on activity, which is difficult to understand through only lectures ($n = 8$); students will be able to have a researcher-like experience ($n = 4$); the visualization of the differences in protein sequences among species will be easier because students will actually use colourful depiction ($n = 3$); and this activity could be useful to understand evolution especially in Japan where teaching

materials for evolutionary biology are scarce so far ($n = 3$). In addition, the teachers noted two drawbacks of this activity ($n > 2$): the English expressions of MEGA were difficult to understand ($n = 6$) and this activity was useful only for students wishing to proceed to higher education ($n = 3$). Other descriptions ($n > 2$) were interesting ($n = 5$) and inspiring ($n = 3$).

Discussion

Increase in the students' understanding of molecular evolution (Box 1: Q 6, 7, 10 and 12), deep time (Q 13) and taxonomy (Q 3, 4, 5) resulted in a higher average test score after the activity. On the basis of these test results and the positive impressions of the activity by students and teachers, our protocol is considered to be useful for at least to Japanese high school students to study these concepts. The students' descriptions in the questionnaire suggested that the activity was effective for understanding convergent evolution and homoplasy. While the activities on molecular phylogeny developed so far were mostly designed for university students in English-speaking countries (Kuzoff *et al.* 2009, Franklin 2010, Lents

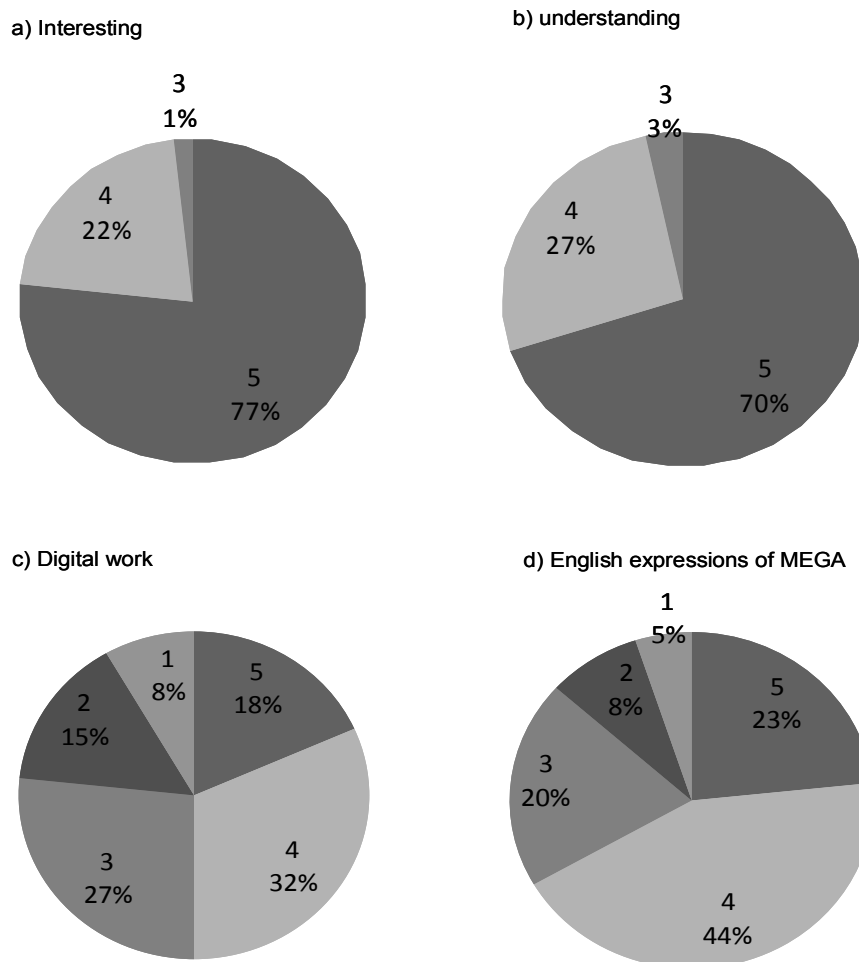


Figure 6 Students' impressions after the activity

Students graded their impression on a scale of one (negative) to five (positive) (see Box 1 for the scale).

et al. 2010, Kvist *et al.* 2011), our activity was shown to be effective for high school students in one non English-speaking country. Because misconceptions about deep time and homoplasy among university students in the USA have been reported (Catley and Novick 2009, Morabito *et al.* 2010), our activity may be effective for high school students and university students in countries other than Japan.

However, some students and teachers felt the English expressions of MEGA were difficult to comprehend. Although this activity offers a valuable opportunity for Japanese students to notice the importance of English in

scientific research field, it would be more valuable to many students and teachers if a Japanese version of MEGA be developed.

The development of this activity is one of the important steps in teaching the links between micro- and macro-biology and evolution. This activity does not include learning how amino acid variations in a particular protein molecule among species arose. Therefore, combined usage of this activity and other teaching materials about the accumulation mechanism of neutral variations in molecules (e.g. Westerling 2008) will lead to further understanding of the links between DNA and

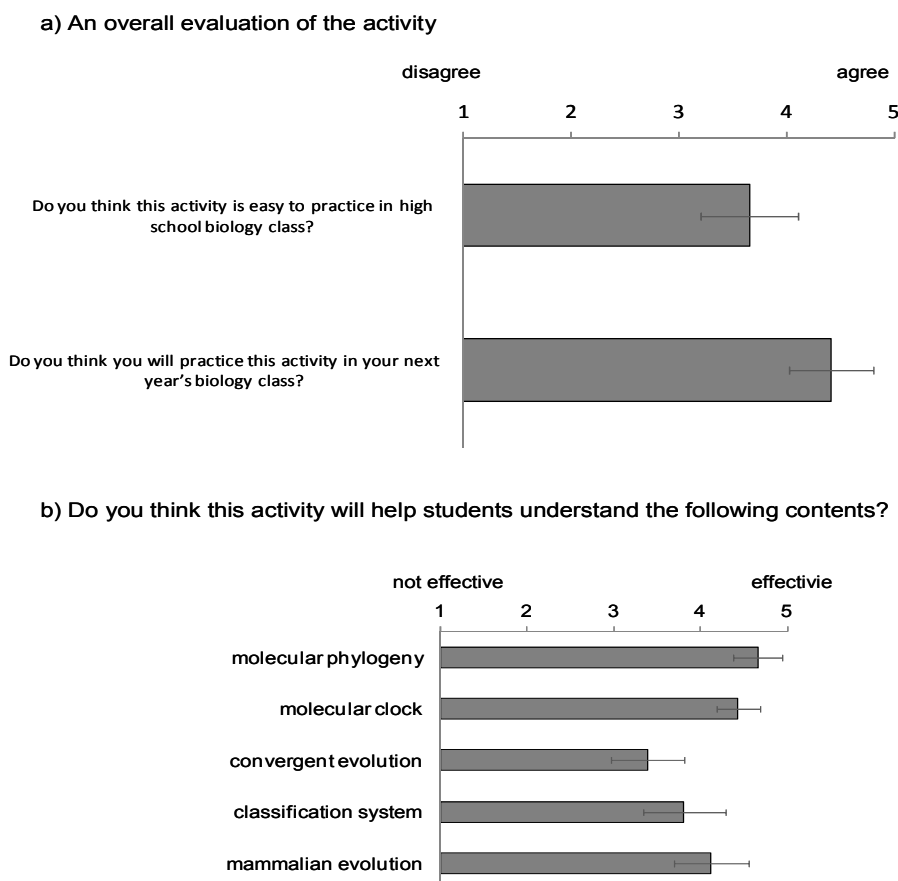


Figure 7 Biology teachers' impressions after carrying out the practice
 Teachers graded their own impression on a scale of 1 (negative) to five (positive).

biodiversity.

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