Insights from introducing natural selection to novices using animations of antibiotic resistance

Gustav Bohlin, Andreas C. Göransson, Gunnar Höst and Lena Tibell

This is an electronic version of an article published in:
Bohlin, G., Göransson, A. C., Höst, G., Tibell, L., (2017), Insights from introducing natural selection to novices using animations of antibiotic resistance, *Journal of Biological Education*, , 1-17. https://doi.org/10.1080/00219266.2017.1368687

Original publication available at:
https://doi.org/10.1080/00219266.2017.1368687

Insights from Introducing Natural Selection to Novices using Animations of Antibiotic Resistance

Gustav Bohlin[1*], Andreas Göransson[1], Gunnar E. Höst[1], & Lena A. E. Tibell[1]

[1]*Department of Science and Technology, Linköping University, Norrköping, Sweden*

*Corresponding author: gustav.bohlin@liu.se +46 (0)11 363079. Linköping University, Department of Science and Technology, Campus Norrköping, 601 74 Norrköping, Sweden

Insights from Introducing Natural Selection to Novices using Animations of Antibiotic Resistance

Antibiotic resistance is typically used to justify education about evolution, as evolutionary reasoning improves our understanding of causes of resistance and possible countermeasures. It has also been promoted as a useful context for teaching natural selection, because its potency as a selection factor, in combination with the very short generation times of bacteria, allows observation of rapid selection. It is also amenable to animations, which have potential for promoting conceptual inferences. Thus, we have explored the potential benefits of introducing antibiotic resistance as a first example of natural selection, in animations, to novice pupils (aged 13-14 years). We created a series of animations that pupils interacted with in groups of 3-5 (total n=32). Data were collected at individual (pre-/post- test) and group (collaborative group questions) levels. In addition, the exercise was video-recorded and the full transcripts were analysed inductively. The results show that most of the pupils successfully applied basic evolutionary reasoning to predict antibiotic resistance development in tasks during and after the exercise, suggesting that this may be an effective approach. Pedagogical contributions include the identification of certain characteristics of the bacterial context for evolution teaching, including common misunderstandings, and factors to consider when designing animations.

Keywords: natural selection; antibiotic resistance; animation; mutations; lower secondary education.

Introduction

Evolution is one of the foundations of biology. Through its generality and power to both explain biological history and allow predictions it transcends biological sub-disciplines such as biochemistry, ecology and botany. Evolution is also an important aspect of many of today's great societal challenges, including climate change, antibiotic resistance and biodiversity management (Meagher, 2007). Thus, it is important for citizens to have at least some knowledge of evolutionary concepts, and advocates of evolution education often highlight its potential for improving our ability to address

societal problems (e.g. Bull & Wichman, 2001). Antibiotic resistance is a commonly mentioned example, since increasing resistance in bacteria is causing major global health threats, which can only be understood and addressed with knowledge of evolutionary processes (Antonovics, 2016; Genereux & Bergstrom, 2004; Gluckman et al., 2011).

A major problem is that biology education researchers have consistently shown that evolution is a difficult subject to teach and learn (e.g. Gregory, 2009; Smith, 2010). There are many reasons to believe that antibiotic resistance is a useful context in which to situate teaching of evolution and natural selection (Delpech, 2009; Smith et al., 2015). However, these benefits are usually grounded in assumptions that are poorly supported by empirical data or explanatory rationale. This paper addresses the assumptions' validity by empirically exploring potential benefits of connecting teaching of evolution and antibiotic resistance. In addition, there are clear indications that visual animations promote conceptual inferences. Thus, it also addresses the educational value of animations illustrating key aspects of antibiotic resistance, and factors that may influence their effectiveness.

Literature review

Bacterial resistance to antibiotics is a major threat to human health rendering previously curable diseases untreatable. Given that functional antibiotics are a prerequisite for several other medical treatments such as chemotherapy, transplantations and invasive surgery the implications are even more fearsome (e.g. Cars et al., 2008). Unless radical actions are taken, antibiotic resistance has been estimated to cause 10 million lives each year and cost the global economy 100 trillion US Dollars up to 2050 (O'Neill, 2014). Studies measuring the public's knowledge and beliefs about antibiotic resistance

consistently reveal that the public both have an incomplete understanding of antibiotic resistance (e.g. Gualano et al., 2015; Carter, Sun & Jump, 2016) and believe that they do not contribute to its development (McCullough et al., 2016). The last issue might be associated with findings that newspapers often frame antibiotic resistance as a responsibility for society rather than for the general public (Bohlin & Höst, 2014). Against this background, numerous campaigns and interventions to raise public awareness have been conducted (Cross, Tolfree, & Kipping, 2017) and a global public awareness campaign, focusing specifically on educating children and teenagers was the first intervention suggested by the Review on Antimicrobial Resistance (O'Neill, 2016). Clearly, increasing public knowledge about the processes leading to antibiotic resistance is of crucial importance.

In Swedish compulsory biology education, both evolution and antibiotic resistance are parts of the central curricula for pupils aged 13-15 years (Skolverket, 2011). This is the last mandatory biology course that all pupils must take, and thus should provide the basic biological knowledge needed by citizens. Although microbial resistance to antibiotics evolves through natural selection, in the commonly used textbooks in Sweden antibiotic resistance is generally covered in the context of microbiology whereas evolution is found in a separate chapter (Bohlin & Höst, 2015). A tendency to separate evolution from other biological contents in textbooks has also been observed, and criticised, in other countries, e.g. the USA (Nehm et al., 2009). This separation may at least partially explain why most pupils, including high-performers, had problems correctly answering items on antibiotic resistance that were included in nation-wide Swedish tests for 15-year-olds in 2014 and 2015. Commonly identified misconceptions (clearly related to evolutionary misunderstandings) were that bacteria

purposefully develop resistance and that humans, rather than bacteria, become resistant (Lind Pantzare et al., 2014; 2015).

Reciprocally, research suggests that learning evolution can be facilitated through examples based on antibiotic resistance (e.g. Delpech, 2009). Suggested reasons for this include the short generation times and small sizes of bacteria, which allow large populations to live in small physical spaces and hence enable observations of rare events, such as survival of bacteria exposed to antibiotics through resistance-conferring mutations. Thus, exploitation of these traits can avoid the conceptual problems of grasping the enormous time frames needed to study, for example, mammalian evolution (Cheek, 2010). Moreover, very large numbers of bacteria can be cultivated in a test tube or studied under a microscope, providing convenient opportunities to study evolution in real-time in a school laboratory (Elena & Lenski, 2003; Smith et al., 2015).

Further acknowledged problems with teaching evolution are so-called item feature effects, or surface features (Nehm & Ha, 2011; Nehm & Ridgway, 2011). This means that students have troubles seeing that general mechanisms act on all taxa, and tend to apply different explanatory patterns to different taxa, or groups of taxa, that share certain features. However, several studies indicate that interventions based on microbial antibiotic resistance can increase students' likelihood to include randomness and submicroscopic mechanisms in explanations of natural selection (Cloud-Hansen et al., 2008; Robson & Burns, 2011; Göransson, Fiedler, Orraryd & Tibell, unpublished data). As randomness is often an obstacle for understanding evolution (Garvin-Doxas & Klymkowsky, 2008; Tibell & Harms, 2017), this implies that antibiotic resistance may be a valuable context for evolution education. Moreover, since DNA is crucial for variation (as the material in which variation arises and is inherited) and one of the

common denominators for mammals, bacteria and plants, drawing pupils' attention to the molecular genetic events involved in the evolution of resistance may help them to perceive the generality of evolutionary mechanisms. Another reason why microbial antibiotic resistance may be suitable for teaching evolution is that it has high affective potential, due to its enormous societal relevance (Krist & Showsh, 2007; Wolf & Akkaraju, 2014), and thus meets a need noted by Hillis (2007) to make evolution teaching relevant and engaging for students. Lastly, in places where the theory of evolution is controversial, teaching antibiotic resistance may provide a way to convey the mechanisms of natural selection without explicitly referring to evolution (DeSantis, 2009; Scharmann, 1994).

Natural selection consists of a number of linked processes that occur at different organisational levels. For example, variation originates through random mutations and recombination within the hereditary material (DNA). These are subcellular molecular events, but consequences of slight variations in DNA sequences may include cellular-level changes in proteins and meter-scale changes in physical characteristics. Furthermore, establishing patterns of changes in frequencies of traits over time in populations of many taxa generally requires observations over large areas and multiple generations. For example, the time frame required for the divergence of separated populations into different species is often millions of years. Thus, we cannot perceive many key evolutionary processes and require abstract thinking or visual aids to comprehend them. Hence, use of visual tools in the teaching of interrelations between evolutionary concepts and processes beyond our perceptual boundaries shows great promise (e.g. Lee & Tsai, 2013). Dynamic visualisations, such as animations, are particularly effective for promoting conceptual inferences, especially when scaffolds such as reflective prompts and interactive elements are present (McElhaney, 2015).

Thus, they may be highly valuable for teaching evolution and natural selection, but more research is needed on their educational effects in this context.

Objectives

As outlined above, introducing the evolution of bacterial antibiotic resistance to novice pupils through a series of interactive animations could be a powerful approach to promote the learning of evolution and natural selection. To assess this possibility, the presented study explores educational aspects of the approach, specifically addressing the following research questions:

(1) What characterises novice pupils' understanding of the origin of resistance, and how is their understanding affected by an interactive animation and accompanying exercise (described below)?'
(2) What obstacles and/or opportunities can be discerned for teaching the evolution of antibiotic resistance to novice pupils through a series of interactive animations in terms of (a) the origin of resistance, and (b) the ability to make predictions?
(3) What evolutionary aspects do the pupils choose to include when asked to transfer reasoning from a bacterial to a mammalian context?

Methods

We employed mixed-methods in a study design combining individual written pre-tests, an intervention in which groups of pupils interacted with animations and solved problems, and individual written post-tests.

Sample

The participants were 32 Swedish 8th grade pupils (16 male/16 female) aged 13-14 years. The study was performed during two consecutive days (half of the pupils each day) as a part of their biology class. The participants had taken a segment of a course on microbiology a year before the exercise, but none had received any formal teaching on evolution. To resemble a normal situation for group work in the class, we let the teacher assign the pupils into groups consisting of 3-5 pupils. Informed consent was gathered from all participants and their parents. Five of the pupils agreed to take part in the study but did not want to be filmed. These formed a separate group from which only written responses were collected. The other pupils took part in all levels of data collection. All pupils were given an identification number to enable tracking of individuals through the exercise while ensuring anonymity. The pupils wore the numbers on stickers that were visible during the video-recordings, and wrote them on hand-in responses. A brief oral introduction was given to the whole class before the exercise. This included a presentation of the study, a short description of natural selection and an explanation of the relationships between DNA, genes and inheritance.

Description of the interactive animations

The animations describe how antibiotic resistance evolves in bacteria through mutations and natural selection.

Overall design considerations

A linear overall structure was chosen for the presentation of the animations (Figure 1). The junction at C indicates that the parts D and E can be accessed in any order. The main reason for adopting this largely linear structure was to introduce concepts sequentially, in the order required to understand them.

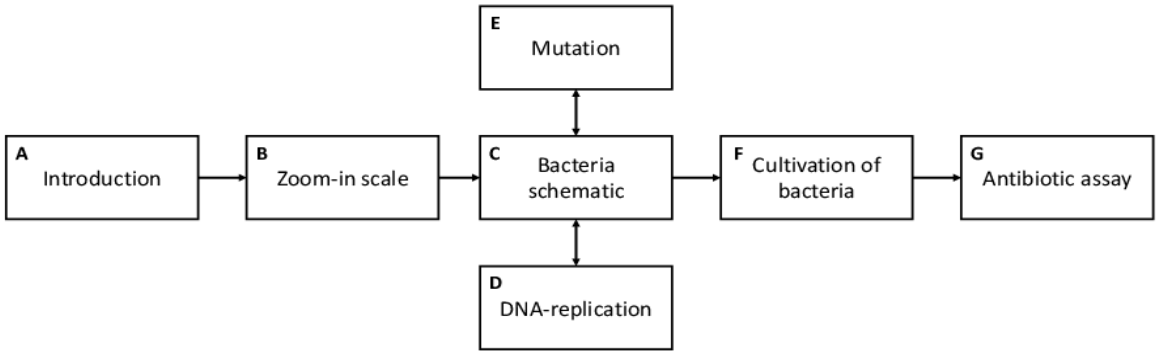

Figure 1. Overall structure of the interactive animation. Letters correspond to descriptions in the following text section.

First, a short introductory text explains the general function of antibiotics and how to navigate the interactive animation (A in Figure 1). The overall context, a laboratory with test tubes, is also presented (Figure 2a). A scale transition from test tubes to bacteria is shown as an animation (B in Figure 1) with scale bars added. It aims to convey the large number of bacteria and their small size in comparison to a known frame of reference, the test tube (Figure 2a).

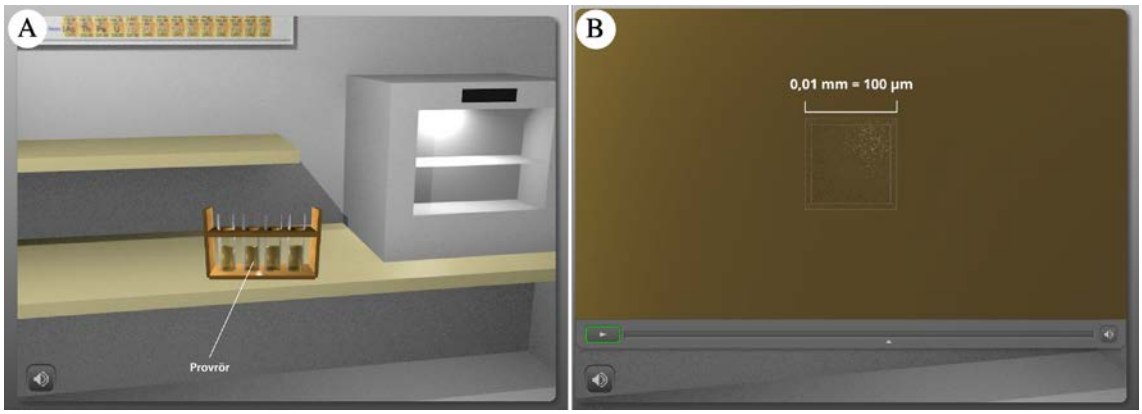

Figure 2. A) The laboratory context. B) Zoom-in scale animation.

DNA-replication and mutations

The next scenes (D and E) are centred around a schematic representation of a bacterium with two linked animations, one about DNA-replication and the other about mutations (Figure 3a). The first animation (D) shows the molecular basis of DNA-replication with randomly moving nucleotides arriving at DNA polymerase (Figure 3b). It also shows strand separation and base pairing. The second animation (E) shows base pairing with one base mismatch (i.e. a point mutation).

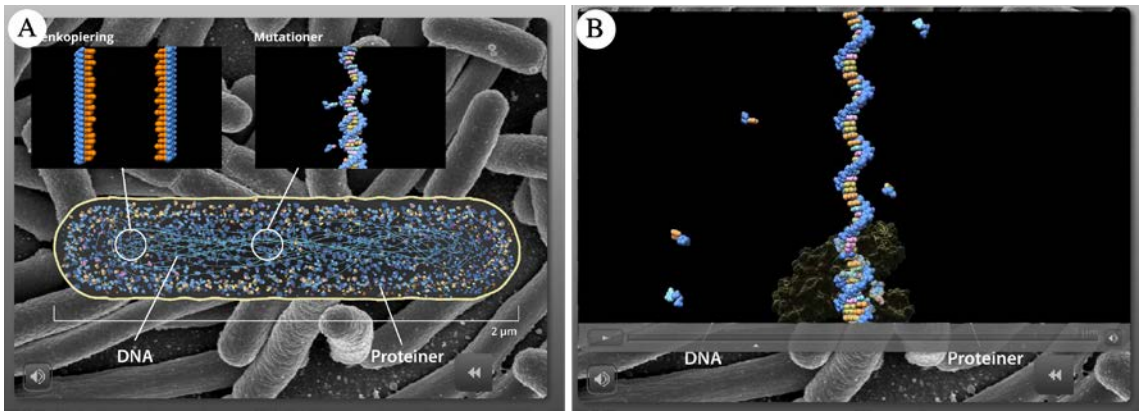

Figure 3. A) Schematic representation of a bacterium with linked animations. B) Animation of nucleotides arriving at DNA polymerase by random walk.

In both animations, base letters are also used to provide both symbolic and iconic representations of the correct pairing (Figure 4a). Since the visual difference between correct and incorrect base pairing is quite subtle in space-filling molecular models, a rotation of the chain is animated to show the base-pairing (Figure 4b).

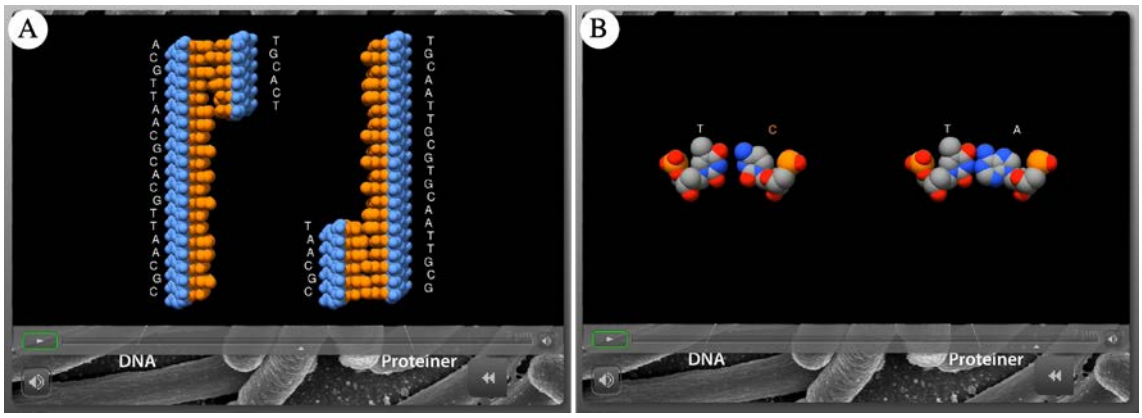

Figure 4. A) Two concurrently replicating DNA-strands represented using space-filling atom models and letters corresponding to the types of bases. B) Mutation animation - base pairing showing one correctly matched base pair (right) and one incorrectly matched base pair (left).

Subsequently, a schematic sequence of replicating bacteria is shown to place the mutation in a population context and highlight inheritance of the mutation (Figure 5a). Cell divisions of several generations are depicted by animations, and inheritance is indicated by using different color cues for wild type and mutated genotypes. The next sequence presents a graph showing exponential growth of a bacterial population in a test tube (Figure 5b). The number of bacteria in the population and the number of mutations are also shown. The animation continues with streaking of the bacteria (Figure 6a) on three agar plates: a control plate containing a medium with no antibiotic (AB0), a plate containing an antibiotic (AB1) and a plate containing another antibiotic (AB2). Lastly, the animation shows how colonies appear during incubation of the plates at 37 C (Figure 6b).

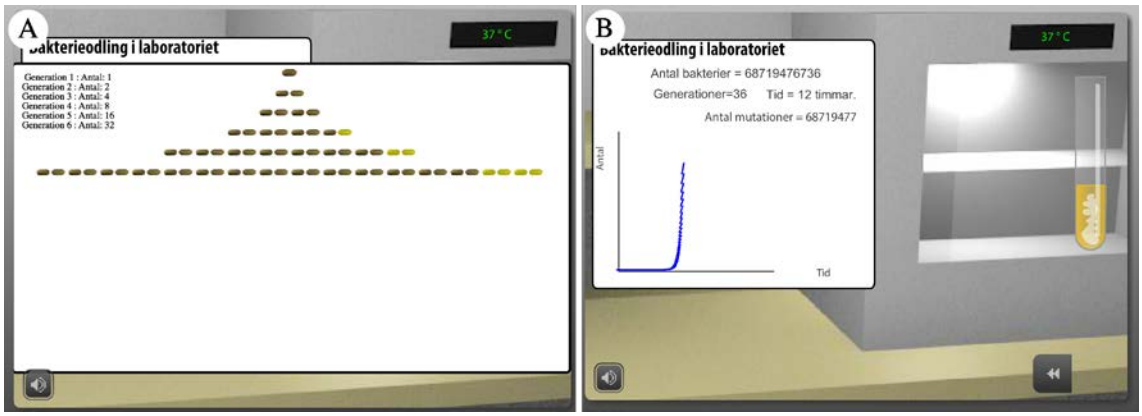

Figure 5. A) Schematic representation of exponential bacteria growth by mitosis, mutated genotypes depicted with yellow colour cue. B) Graph and numbers showing exponential growth of bacteria in the test tube to the right.

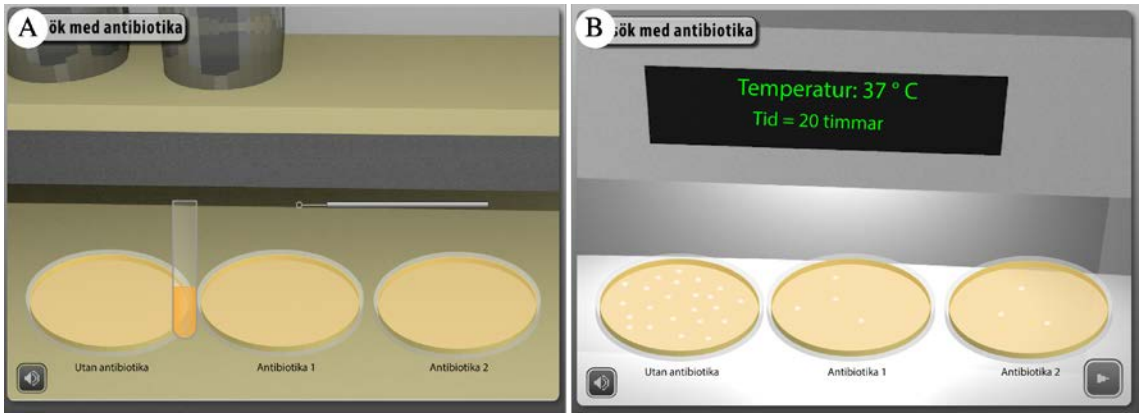

Figure 6. A) Animation of streak plating. B) Animation state after incubation simulation. First plate – control (AB0), second and third plate – antibiotic-containing plates (AB1 and AB2).

Data collection

Following the common introduction, all pupils were asked to individually respond to a closed-response item with four alternatives (similar to the one used in the 2014 national test). The first two response-options in the item correspond to two common misconceptions about resistance development: that resistance develops as a response to

need and that humans, rather than bacteria, become resistant (e.g. Lind Pantzare et al., 2015). The third false alternative describes the common advice to finish a whole course of antibiotic treatment once started as the main reason resistance appears.

In groups they were then allowed to interact with the series of animations described above. During and after this interaction, they were asked to collectively answer six questions, designed to: probe their ability to make evolutionary predictions (three items), explain the origin of resistance (two items) and evaluate the animations (one item).

Their discussions around the questions during interactions with the animations were video-taped and transcribed (verbatim). The questions were written on separate sheets that were handed to the pupils one after another. Thus, they could not see the next question before handing in their answer to the previous one. Each pupil group worked independently with the animations and the questions, while the role of the researchers was only to administer the question-sheets and clarify any ambiguities in the questions. The groups completed the interaction and group questions in times ranging from 47 to 72 minutes, and the recordings provided 7 hours and 9 minutes of transcribed video footage in total.

Lastly, the students were individually asked to reconsider the initial closed item, either revise or retain their previous response, and justify their decision to change or retain it. At this point, the pupils were also asked to answer an open-response item targeting possible transfer of knowledge from a bacterial to a mammalian context. An overview of the collected data is provided in Table 1 and Figure 7. The questions that were posed to the pupils are displayed in Table 2.

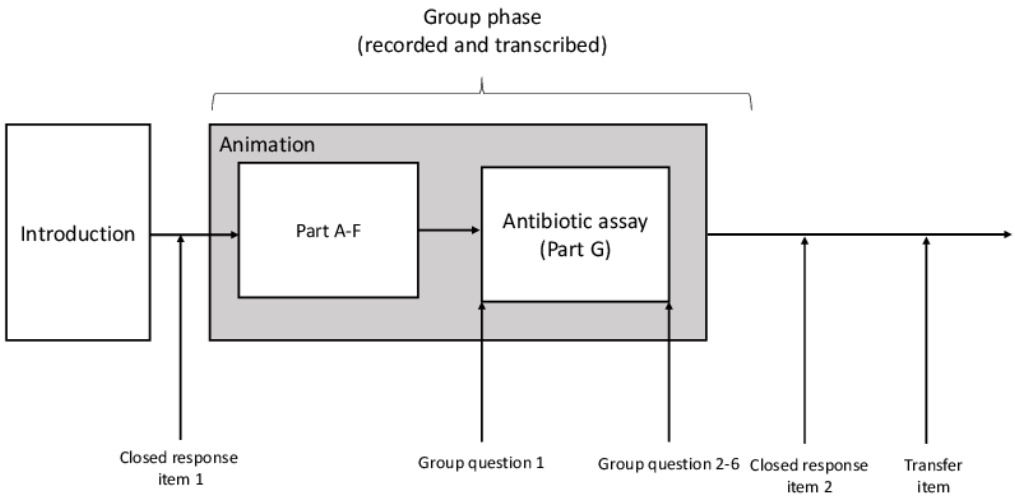

Figure 7. Overview of the study design.

Table 1. Overview of the data collected in the study.

	Pre/during/after interaction	Qualitative/quantitative	Individual level/group level
Closed-response item #1	Pre	Quantitative	Individual
Group responses	During	Qualitative	Group
Transcripts from discussions	During	Qualitative	Group/individual*
Closed response item #2	After	Quantitative	Individual
Justification to closed response item #2	After	Qualitative	Individual
Transfer item	After	Qualitative	Individual

*One group (five pupils) chose to be excluded from this part of the data collection.

Table 2. The questions included in the study.

Pre- (and post-*) exercise (individual,	Which of the following best describes how bacteria develop resistance to antibiotics?

closed-response)	A. Bacteria always try to develop resistance when they are exposed to antibiotics. So, those that succeed will be protected the next time. B. Bacteria can become resistant if they infect a person who is already resistant because he or she has used too much antibiotics previously. **C. Bacteria that have become resistant through random mutations can survive and spread when antibiotics kill non-resistant bacteria (*correct option*).** D. Bacteria that have made a person ill will develop resistance if the person does not finish his or her course of treatment.
Group questions (open-response)	*After streaking bacteria on three plates (one neutral and two containing different types of antibiotics):* 1. What do you think the plates will look like after incubation? Provide as much detail as possible. *After retrieval of the plates from the incubator:* 2. Were the results consistent with your expectations? Try to explain what has happened and why the plates look as they do. 3. Now imagine that you isolate and grow bacteria from the AB2 plate, then streak them out on three new plates like the first set. How would these plates look after incubation? Explain why. 4. When and how does the resistance arise in the bacteria shown in the animations? 5. Mutations occur randomly and very rarely. Explain how the establishment and growth of resistant strains can still happen so quickly in the presence of antibiotics on the plates.
Post-exercise (individual, open-response)	Earlier generations of giraffes did not have as long necks as those found today. Try to describe similarities and differences between the neck-development of giraffes and bacterial development of antibiotic resistance.

*This item was presented to the pupils again after the exercise, and then they were asked to justify their decision to change/not change their initial response.

Analysis

To detect any learning progress by the pupils, every individual's responses to the closed items and the justifications for their responses were compared. A McNemar test was conducted to discern whether a statistically significant change ($p < 0.05$) in the proportion of correct responses to the closed item before and after the exercise had occurred. Group responses were thematically coded and three to five response categories were created for each question. The transcripts were analysed in several steps using MaxQDA®. First, all authors read the complete transcripts, to get a sense of the material and identify key components and patterns (so-called pawing) (Ryan & Bernard, 2003). Then, three of the authors independently conducted an inductive coding procedure (Graneheim & Lundman, 2004). The results were merged and processed in several rounds, based on the posed research questions, until an acceptable categorisation scheme had been established. Responses to the transfer item were imported into MaxQDA and coded with respect to pupils' use of the three general principles of natural selection: variation, inheritance of traits and selection of individuals with beneficial traits (Tibell & Harms, 2017).

Results

The number of correct responses to the closed item increased from six (of 32) before the exercise to 17 after it (Figure 8). A McNemar test indicated that the change in proportion of correct responses was statistically significant ($p=0.007$). Analysis of the group discussion transcripts identified themes corresponding to the two aspects encapsulated in research question 2: the ability to make predictions and understanding

of the origin of resistance. The results from the group assignments are presented in the following sections under headings derived from the research questions: predictions about resistance development, origin of resistance, influence of the animations on pupil reasoning and transfer to a mammalian context.

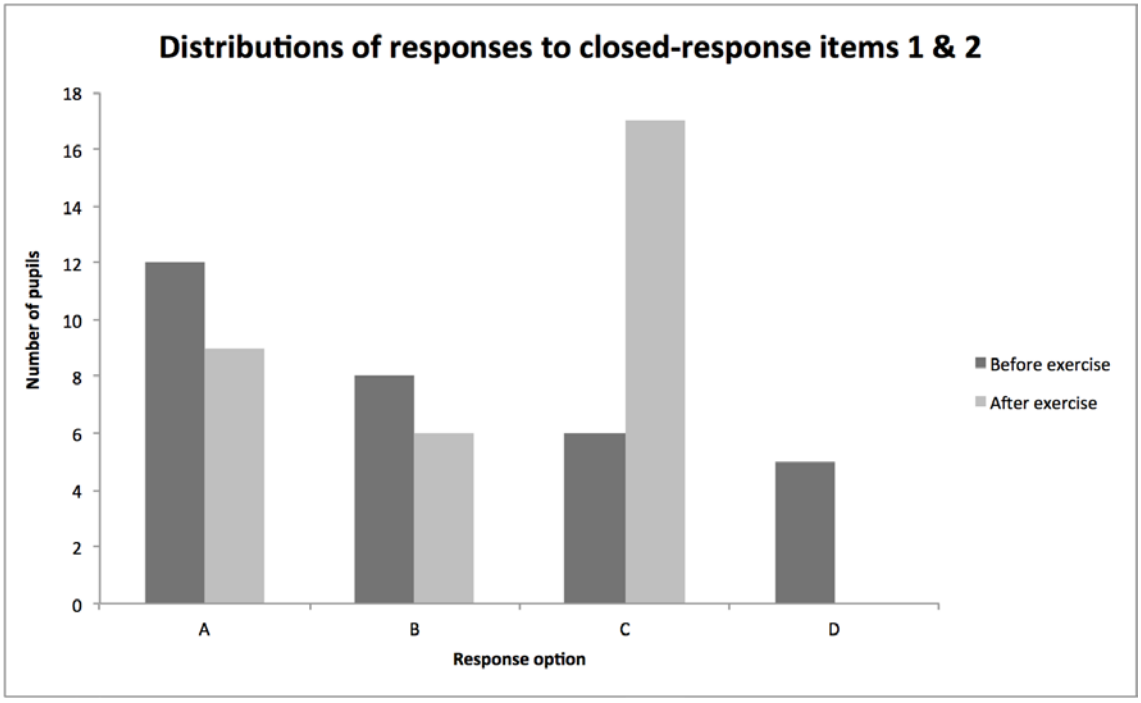

Figure 8. Distributions of responses to the closed-response items 1 and 2, before and after the exercise (see Table 2). The increase in correct responses (option C) was statistically significant (p=0.007).

Predictions about resistance development

Seven out of the eight groups provided reasonable predictions in response to group question 1 regarding the agar plates' appearance after the initial incubation (growth of many bacterial colonies on the AB0 plate and occasional colonies on the AB1 and AB2 plates). The eighth group replied that colonies would grow on all plates without specifying to which relative degree. However, the transcript indicates that the required knowledge to predict relative numbers of colonies seems to have existed in the group:

Pupil #41: In the one without antibiotics the bacteria will grow visibly… …How will the other two… how will they… …But some of the bacteria will procreate. Those that are resistant. And the rest will die.
Pupil #42: Incredible.

In response to group question 2, asking them to explain the plates' appearance, most (five of the eight groups) replied that antibiotics kill bacteria, without giving explanations for the few surviving colonies on the plates containing antibiotics. Of the remaining groups, two correctly explained that surviving colonies are due to bacteria that had acquired resistance and the explanation of the other group was that there was too little time for the antibiotics to eliminate all the bacteria.

In response to question 3 (regarding patterns after streaking surviving bacteria from the AB2 plate containing one of the antibiotics on a fresh set of plates), half of the groups gave satisfactory answers. These included statements that the largest numbers of colonies will be found on AB0 and AB2 plates, due to the selection of AB2-resistant bacteria in the first round of cultivation, and again there will be occasional colonies on the AB1 plate. The other groups responded that there would be: more bacteria on the AB2 plate, with no explanations (one group); fewer bacteria on the AB0 plate due to the misunderstanding that not only bacteria, but also the antibiotic, will be transferred from the initial AB2 plate (one group); a general decrease in numbers of colonies on all plates (one group); and a non-coherent answer (one group).

The inductive transcript analysis focusing on predictions about resistance development identified three general themes connecting the bacterial context to evolutionary reasoning. One was that the variation (resistance) is heritable and that the biotic potential of bacterial cell division might lead to a rapid increase of resistant

individuals. Another was that resistance is a trait that you either have or do not have, which will inevitably lead to survival or death (fitness). The third was that even rare changes could happen in a reasonable time due to short generation times. In addition, a fourth theme emerged from instances where the pupils integrated these aspects. These themes are all exemplified with quotes below.

Inheritance:

Pupil #3: It is somewhat like this, that bacteria divide into two copies. And if it is a resistant bacterium that divides then there will be more of these. So, in the end when… if it is one resistant then it becomes two, and then it becomes four and then it becomes eight and then it continues like that.

Fitness:

Pupil #41: But like, some of the bacteria will procreate. Those that are resistant, and the rest will die.

Rare changes accumulate:

Pupil #31: Sometimes an error could happen… and then, multiple generations pass without any errors. Because this happens fast and… then maybe the errors appear very rarely.

Integration of aspects:

Pupil #40: Now I am thinking about these… I don't know but… the number of bacteria that already from the beginning… if any of these were mutants of whatever it was called.
Pupil #35: Mm, they are mutants.
Pupil #40: Yes, then these won't go away.
Pupil #35: No, and then there will…
Pupil #40: Is it… could it be those that are still there in this…
Pupil #35: Yes, it is those that will continue to…
Pupil #40: They will increase again later.

Pupil #35: While those that are not resistant will die out. So, they adapt.
Pupil #40: But I mean… then you could say that… they talk about this small, small chance that they… they will become mutificated (sic).
Pupil #35: Yes, but given that these are the only ones left it will be…
Pupil #40: They will become abundant and they will like copy themselves.
Pupil #35: Yes… it is only those who will live.
Pupil #40: Because they copy their DNA too.
Pupil #35: Yes.
Pupil #40: So, they will be like copies of themselves.
Pupil #35: Mm.
Pupil #40: And then they will reach high numbers.
Pupil #35: Yes, in the end.
Pupil #40: In the end… Aha!

Origin of resistance

When asked when and how resistance arises in bacteria (group question 4), the role of mutations was recognised in answers of six of the eight groups. Four of these also specified that mutations happened in the DNA in connection with replication and cell division. Two groups mistakenly attributed resistance to an active choice of the bacteria, which were implicitly assumed to have brains or willpower.

When asked why resistant strains establish and grow relatively quickly, although mutations occur both rarely and randomly (group question 5), six groups replied that the variation is inherited through cell division and that this happens relatively quickly in bacteria. One group provided a teleological answer based on the bacteria 'learning' that resistance is good for them and one group did not give any answer.

The transcript analysis around this topic identified two main themes for explaining the origin of resistance, one involving mutations and the other teleological explanations, which were divided into sub-themes as shown in Table 3.

Table 3. Themes emerging from analysis of transcripts of pupils' explanations of the origins of resistance.

Theme
Mutations
Mutations are errors
Mutations are due to nucleotide mismatches
Teleological explanations
On organism level
Bacteria learn to be resistant
Bacteria want to live and reproduce
On nucleotide level
Nucleotides are agents
Nucleotides are programmed

Examples from subthemes in Table 3 are provided in the following excerpts.

Mutations are errors:

Pupil #41: Ok, why? Because some error happens in the copying of DNA when the cell is about to divide.

Mutations are due to nucleotide mismatches:

Pupil #16: T and A (inaudible). Most often it happened like that and… G and C. But sometimes it could happen that they ended up with the wrong partner. And that…
Pupil #10: And that's a mutation.

Bacteria learn to be resistant:

Pupil #31: When and why does the resistance arise? Well, it is because… when you use a lot of antibiotics… then it doesn't help against… because the bacteria have gotten used to what it is.
Pupil #43: Yes.
Pupil #31: So, they can protect themselves against it.

Pupil #43: Yes, they have like... cracked the code.

Bacteria want to live and reproduce:

Pupil #43: It occurs because they want to live. Because everything wants to live and reproduce. It's like the meaning of life.
Pupil #31: *laugh* Well. Maybe not our lives but maybe the lives of the bacteria.
Pupil #39: The meaning of life is to live.
Pupil #43: No, the meaning of life is to procreate.

Nucleotides as agents:

Pupil #35: Yes, but what. They flew together there and then like wee… and then I discovered… oh we belong together here and… and then they discovered… no we don't belong together… ah we can't stand moving again.

Nucleotides are programmed:

Pupil #41: They are like programmed, I mean… in some way. Because they copy each other. And the others know where to be placed. I mean the other DNA-chain.

Influence of the animations on pupils' reasoning

In several instances, the pupils made direct references to the events displayed in the animations when discussing their responses. This was especially apparent in connection to DNA-level molecular events:

Researcher: When did the bacteria become resistant, if you think about what happened?
Pupil #3: Something went wrong in the DNA-chains. And then they became like resistant.
Pupil #7: Mm, because it was.. was it A and T that belonged together?
Pupil #3: Mm, and then it was C and G or something like that.
Pupil #7: Mm, should I write that when the different letters come in contact with the wrong letters… Wait, what were the letters actually?
Pupil #3: It's like the DNA-chains in the bacteria.

Another example from a different group:

Pupil #13: So, it's like when… when a bacterium like… or when antibiotics don't help much because they sort of resist it.
Pupil #10: Yeah, I understand now.
Pupil #13: Mm.
Pupil #16: Couldn't it be during those events that it happens? When they are dividing?
Pupil #13: It's mutation or something.
Pupil #14: Mm.
Pupil #13: Here I think.
Pupil #16: But couldn't it be that there is a mutation that makes them become…
Pupil #13: Yes.
Pupil #14: I think so. Do you want to write 16 (inaudible)?
Pupil #16: What should I write?
Pupil #14: Hello…
Pupil #13: It was like… for example… if C attaches to an A, then it becomes a mutation.

When asked specifically, one pupil replied that random processes were easier to understand with the help of the animation:

Researcher: What do you think it helped you understand?
Pupil #13: That an animation so carefully demonstrated… how eh.. it happens randomly.

Four of the participating pupils explicitly mentioned the animations as the cause for changing their responses to the closed-response item about how antibiotic resistance develops:

Pupil #40: Previously, I didn't know that resistant bacteria had to do with mutations, as it was explained in the animation.

Pupil #41: The animation explained how the mutated bacteria were formed.

Pupil #44: Because now I know how it happens after the animation.

Pupil #7: I haven't chosen the same since I learned more from the animation.

However, one mentioned that he or she had not changed response since he or she *'didn't really get it'*.

Transfer to a mammalian context

None of the pupils included variation, inheritance and selection in their comparison of bacterial development of resistance and giraffes' development of longer necks. However, 16 of 32 pupils mentioned changes in genes or DNA in their answers. Four of these made explicit links between changes in the hereditary material (DNA or genes) and phenotypic changes, thus linking sub-micro and macro-level phenomena. For example:

Pupil #3: Both maybe got a change in their DNA-strands which caused a change in their properties.
Pupil #41: But then a DNA got mutated which led to a longer neck**.**

One of the 32 pupils included multiple evolutionary concepts in his/her answer (randomness, genetic change leads to phenotypic change, inherited variation and change in population). Eight of 32 pupils explicitly mentioned similarities between the bacteria and mammal, often including genetic or DNA/changes, but other examples were also found, such as:

Pupil #40: Both adapt to the environment […] both have to do with evolution.

Two of the answers also identified dissimilarities between the organisms*:*

Pupil #16: The bacteria do not change shape while the giraffes do.
Pupil #4: Differences could be in the size.

In addition, four pupils based their answers on obviously teleological thinking, for example claiming that both the giraffes and the bacteria 'needed' to evolve in order to survive.

Discussion

This is the first study to provide empirical data regarding the potential efficacy of using dynamic visual material to teach natural selection and microbial antibiotic resistance simultaneously. The results indicate that most pupils who participated in the study could successfully apply basic evolutionary reasoning to bacterial resistance development after merely one hour of interacting with and discussing a series of animations. This has proved very difficult for pupils of the same age during national tests at the end of their standard biology courses (Lind Pantzare et al., 2014; 2015). Thus, antibiotic resistance, which is often cited as one of the reasons we need to learn about evolution, also seems to be a promising context in which to initiate teaching about evolution.

The answers to the closed-response item reveal that pupils' views on resistance development before the exercise were often based on a teleological conception, and/or the conception that resistance develops in humans rather than bacteria. There was a clear shift towards a correct explanation after the exercise, calling for deeper consideration of the nature of the peer discussions as well as the design of the animations.

In accordance with the famous remark by Dobzhansky (1973) that nothing in biology makes sense except in the light of evolution, there are good reasons for advocating the teaching of evolution as an integrated theme throughout biology curricula (Nehm et al., 2009). Moreover, our results indicate that it is not essential to

teach general evolutionary mechanisms before bacterial contexts, instead they can be taught simultaneously. Our results also corroborate previous findings (e.g. Robson & Burns, 2011) that microbial contexts facilitate acquisition of an understanding of the origin of variation. Applying the reasoning in other contexts, especially regarding the selection processes, is harder. This may be due to surface features in the bacterial context (discussed below), and uneven understanding arising from design choices in the learning material (the animations).

The transcript analyses suggest that some biological aspects are perceived differently in the bacterial context than in typical mammalian contexts. For example, in terms of reproduction, the pattern of cell division where a population is duplicated each generation facilitates pupils' acceptance that a mutation will be present in increasing numbers of individuals over time. The short bacterial generation times and the possibility to cultivate large populations in small volumes are also helpful. The participating pupils appeared to readily accept that such circumstances allow very rare point-mutations to spread widely over the course of a few generations. In the animation, we assumed a generation time of 20 minutes and grew the bacteria in the test tube for 12 hours, allowing 36 generations to pass. A comparable number of generations in a human context would require us to follow a total human population over more than 1 000 years (assuming a generation time of ca. 30 years). Although we do not know how the same pupils would reason about this context, it is well-established that inferring processes over long time periods is a problematic issue in evolutionary reasoning (e.g. Cheek, 2010). Thus, starting with a bacterial context and directly perceivable time scales before translating acquired conceptions to corresponding processes in populations of other organisms, which could span enormous absolute time scales, may enable avoidance of conceptual problems associated with deep time in classroom situations.

With regard to fitness, the pupils perceived resistance as a discontinuous trait in the sense that an organism either does or does not have it. Specifically, in this context, a bacterium is either resistant and will live and reproduce, or it is not resistant and will consequently die, because it is born either with or without the relevant variation (due to its genetic make-up). This may be more advantageous for novice learners than learning about more subtle differences in levels of fitness associated with quantitative traits, typically used as textbook examples (for example, lengths of giraffes' necks). These often tend to be mistakenly interpreted through soft inheritance mechanisms such as Lamarckian explanations that acquired traits are passed on to offspring (Gregory, 2009). This might be because more subtle changes have stronger resemblance to non-hereditary changes that we do have individual control over, for example stimulation of muscle growth by physical training. The difference in the way that resistance is perceived might explain why pupils find it easier to explain with congenital changes.

In their responses to the transfer item, the pupils included variation much more frequently than selection. This is intriguing, given previous indications that random factors such as genetic mutations (giving rise to variation) are among the most difficult aspects in learning about evolution (e.g. Garvin-Doxas & Klymkowsky, 2008). It should also be noted that the pupils had not been exposed previously to the general principles underlying natural selection (variation, inheritance and selection, see e.g. Tibell & Harms, 2017) and that the animations did not address all these principles explicitly, mainly focusing on origin of variation at the molecular level. This could well be one reason for the pupils' recognition of DNA-level similarities between mammals and bacteria.

It is a well-documented problem that learners tend to use different types of explanations for different biological phenomena (Nehm & Ha, 2011). For example, explanations have been found to vary with the context of questionnaire items in terms of both evolutionary phenomena (e.g. trait loss or gain) and biological taxa (Nehm & Ha, 2011). Until the causes of the different types of reasoning are elucidated and addressed it is clearly important to treat associated issues seriously and cautiously. In test situations, for example, including similar items with references to different organisms may enable distinction between understanding linked to surface features and broader understanding of general principles. Moreover, in teaching, insights into which contexts are associated with particular affordances permit the design of effective teaching tools. In this case, the results indicate that the bacterial context is promising for grasping random mutations and their role in generating variation. This might be at least partly due to the short physical distance between the location of genes and sites of their functions in unicellular organisms, and perhaps the absolute difference in fitness that antibiotic resistance is perceived to grant. Since DNA is the basis for variation and one of the unifying essences in evolution, given its ubiquity in all living organisms, effective teaching in this domain may be a key to successful transfer across organisms.

For a full understanding of biological phenomena, submicroscopic processes need to be considered and represented (Tsui & Treagust, 2013). The findings that 50 % of the pupils mentioned genetic changes or DNA as a common denominator between mammals and bacteria is therefore interesting and encouraging in the light of the animation's focus on the genetic level. This indicates that visualisations could provide an effective way of conveying the principles of natural selection and enable their transfer between taxa of organisms. However, only four of 32 pupils made links between organisational levels. Future versions of the animations may benefit from

including clearer transitions between, for example, gene-alterations, modified proteins and cellular functions to individual characteristics. Other potential developments of the teaching intervention could include adding examples of evolution from diverse taxa and enabling the learner to abstract the mechanisms involved from these examples by linking multiple representations.

It is difficult to separate the relative learning effect of the bacterial context and the medium (the interactive animations). Undoubtedly, the design choices and narrative in the animations influenced the pupils' understanding of the subject. While this might be seen as a limitation of the study, our aim was to qualitatively explore beneficial and troublesome aspects of learning the evolution of antibiotic resistance through animations. Hence, we were also interested in visual tools' potential to clarify the evolutionary mechanisms involved in the development of bacterial resistance. Future studies could control for possible confounding of medium and context by using an experimental design that, for example, compares one group using animations with another group using only text and/or two groups using animations focusing on the same mechanisms in different contexts.

Instances of teleological reasoning could be found across the data, from explanations of nucleotide mismatches to how organisms or even species choose to adapt in different environments. These are not surprising results, given that the participating pupils had no previous training in evolutionary biology and that learners are generally limited to a repertoire of simple causal models (Perkins and Grotzer 2005). In fact, teleological thinking could be viewed as a cognitive instinct and deserves to be addressed explicitly with, for example, meta-cognitive activities (Gonzales Galli & Meinardi, 2011). Our data provide no indications of the best way to counter

teleological reasoning in the classroom. However, we note that teleological statements do not necessarily reflect a person's actual understanding (e.g. Zohar & Ginossar, 1998), and that discerning whether pupils use such statements in a concrete sense or as metaphors is a challenging task for science teachers (Höst & Anward, 2017).

In conclusion, what are the implications for biology teachers? We have found a number of characteristics related to the bacterial context for teaching natural selection. Among these are that acceptance of occurrence and spread of rare point-mutations are facilitated by the bacterial biotic potential. Further, the perception of resistance as a discontinuous trait provides a way to counter Lamarckian explanations commonly found in more subtle fitness traits. Our results also confirm earlier research that the students are more prone to include molecular explanations in bacterial evolution compared to the evolution of animals. However, teleological explanations are still frequently occurring and these need to be considered in the classroom. Evolution is undoubtedly a hard subject to learn and requires a longer period of teaching to be successful (e.g. Andrews et al., 2011). Thus, a single introductory exercise is unlikely to affect pupils' wider understanding profoundly. However, given that grasping the origin of variation is one of the hardest aspects for students to accomplish (e.g. Speth et al., 2014), and the relative improvement of the pupils in our sample, we conclude that using antibiotic resistance is a promising context in which to initiate teaching about evolution and natural selection.

Funding

This work was supported by the Swedish Research Council (Vetenskapsrådet) [grant number 2012-5344].

References

Andrews, T. M., Leonard, M. J., Colgrove, C. A., Kalinowski, S. T. (2011). Active learning not associated with student learning in a random sample of college biology courses. CBE – Life Sciences Education, 10, 394-405.

Antonovics, J. (2016). The value of concept: Lessons from the evolution of antibiotic resistance. *Global Policy, 7*(S1), 97-106.

Bohlin, G., & Höst, G. E. (2014). Is it my responsibility or theirs? Risk communication about antibiotic resistance in the Swedish daily press. *Journal of Science Communication, 13*(3), A02.

Bohlin, G., & Höst, G. E. (2015). Evolutionary explanations for antibiotic resistance in daily press, online websites and biology textbooks in Sweden. International Journal of Science Education, Part B: Communication and Public Engagement, 5(4), 319-338.

Bull, J. J., & Wichman, H. A. (2001). Applied evolution. *Annual Review of Ecology and Systematics, 32*, 183-217.

Cars, O., Högberg, L. D., Murray, M., Nordberg, O., Sivaraman, S., Lundborg, C. S., … & Tomson, G. (2008). Meeting the challenge of antibiotic resistance. *BMJ: British Medical Journal, 337*, a1438.

Carter, R. R., Sun, J., & Jump, R. L. (2016). A survey and analysis of the American public's perceptions and knowledge about antibiotic resistance. *Open Forum Infectious Diseases, 3*(3), ofw112.

Cheek, K. A. (2010). Why is geologic time troublesome knowledge? In: J. H. F. Meyer, R. Land & C. Baillie (Eds.), *Threshold Concepts and Transformational Learning* (pp. 117-129). Rotterdam: Sense Publishers.

Cloud-Hansen, K. A., Kuehner, J. N., Tong, L., Miller, S., & Handelsman, J. (2008). Money, sex, and drugs: A case study to teach the genetics of antibiotic resistance. *CBE life sciences education, 7*(3), 302-309.

Cross, E. L. A., Tolfree, R., & Kipping, R. (2017). Systematic review of public-targeted communication interventions to improve antibiotic use. *Journal of Antimicrobial Chemotherapy, 72*, 975-987.

Delpech, R. (2009). Evolution in an afternoon: Rapid natural selection and adaptation of bacterial populations. *Journal of Biological Education. 43*(3), 129-133.

DeSantis, L. R. G. (2009). Teaching evolution through inquiry-based lessons of uncontroversial science. *American Biology Teacher, 71*(2), 106-111.

Dobzhansky, T. (1973). Nothing in biology makes sense except in the light of evolution. *The American Biology Teacher, 35*(3), 125-129.

Elena, S. F., & Lenski, R. E. (2003). Evolution experiments with microorganisms: the dynamics and genetic bases of adaptation. *Nature Reviews Genetics, 4,* 457-469.

Garvin-Doxas, K., & Klymkowsky, M. W. (2008). Understanding randomness and its impact on student learning: Lessons learned from building the biology concept inventory (BCI). *CBE - Life Sciences Education, 7,* 227-233.

Genereux, D. P., & Bergstrom, C. T. (2004). *Evolution in Action: Understanding Antibiotic Resistance.* Paper presented at the Evolutionary Science and Society: Educating a New Generation, Chicago, IL.

González Galli, L. M. & Meinardi, E. N. (2011). The role of teleological thinking in learning the Darwinian model of evolution. *Evolution: Education & Outreach, 4*(1), 145-152.

Gluckman, P. D., Low, F. M., Buklijas, T., Hanson, M. A., & Beedle, A. S. (2011). How evolutionary principles improve the understanding of human health and disease. *Evolutionary Applications, 4*(2), 249-263

Graneheim, U. H., & Lundman, B. (2004). Qualitative content analysis in nursing research: concepts, procedures and measures to achieve trustworthiness. *Nurse Education Today, 24*(2), 105-112.

Gregory, T. R. (2009). Understanding natural selection: Essential concepts and common misconceptions. *Evolution: Education and Outreach, 2*(2), 156-175.

Gualano, M. R., Gili, R., Scaioli, G., Bert, F., & Siliquini, R. (2015). General population's knowledge and attitudes about antibiotics: A systematic review and meta‐analysis. *Pharmacoepidemiology and drug safety, 24*(1), 2-10.

Hillis, D. M. (2007). Making evolution relevant and exciting to biology students. *Evolution, 61*(6), 1261-1264.

Höst, G. E., & Anward, J. (2017). Intentions and actions in molecular self-assembly: perspectives on students' language use. *International Journal of Science Education,* 1-18.

Krist, A. C., & Showsh, S. A. (2007). Experimental evolution of antibiotic resistance in bacteria. *The American Biology Teacher, 69*(2), 94-97.

Lee, S. W. Y., & Tsai, C. C. (2013). Technology-supported learning in secondary and undergraduate biological education: Observations from literature review. *Journal of Science Education and Technology, 22*(2), 226-233.

Lind Pantzare, A., Abrahamsson, M., Almarlind, P., & Lundgren, C. (2014). *Ämnesproven i grundskolans årskurs 9 och specialskolans årskurs 10: Vårterminen 2014*. Umeå Universitet.

Lind Pantzare, A., Abrahamsson, M., Almarlind, P., & Lundgren, C. (2015). *Ämnesproven i grundskolans årskurs 9 och specialskolans årskurs 10: Vårterminen 2015*. Umeå Universitet.

McCullough, A. R., Parekh, S., Rathbone, J., Del Mar, C. B., & Hoffmann, T. C. (2016). A systematic review of the public's knowledge and beliefs about antibiotic resistance. *Journal of Antimicrobial Chemotherapy, 71*, 27-33.

McElhaney, K. W., Chang, H.-Y., Chiu, J. L., & Linn, M. C. (2015). Evidence for effective uses of dynamic visualisations in science curriculum materials. *Studies in Science Education, 51*(1), 49.

Meagher, T. R. (2007). Is evolutionary biology strategic science? *Evolution, 61*(1), 239-244.

Nehm, R. H., & Ha, M. (2011). Item feature effects in evolution assessment. *Journal of Research in Science Teaching, 48*(3), 237–256.

Nehm, R. H., Poole, T. M., Lyford, M. E., Hoskins, S. G., Carruth, L., Ewers, B. E., & Colberg, P. J. S. (2009). Does the segregation of evolution in biology textbooks and introductory courses reinforce students' faulty mental models of biology and evolution? *Evolution: Education and Outreach, 2*(3), 527-532.

Nehm, R. H., & Ridgway, J. (2011). What do experts and novices "see" in evolutionary problems? *Evolution: Education and Outreach, 4*(4), 666-679.

O'Neill, J. (2014). Antimicrobial resistance: Tackling a crisis for the health and wealth of nations. *The Review on Antimicrobial Resistance*. Retrieved from: http://www.jpiamr.eu/wp-content/uploads/2014/12/AMR-Review-Paper-Tackling-a-crisis-for-the-health-and-wealth-of-nations_1-2.pdf

O'Neill, J. (2016). Tackling drug-resistant infections globally: Final report and recommendations. *The Review on Antimicrobial Resistance*. Retrieved from: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf

Perkins, D. N., & Grotzer, T. A. (2005). Dimensions of causal understanding: The role of complex causal models in students' understanding of science. *Studies in Science Education, 41*(1), 117–165.

Robson, R. L., & Burns, S. (2011). Gain in student understanding of the role of random variation in evolution following teaching intervention based on Luria-Delbruck experiment. *Journal of Microbiology & Biology Education, 12*(1), 3-7.

Ryan, G. W., & Bernard, H. R. (2003). Techniques to identify themes. *Field Methods, 15*(1), 85-109.

Scharmann, L. C. (1994). Teaching evolution: Designing successful instruction. *Journal of Science Teacher Education, 5*(4), 122-129.

Skolverket. (2011). *Läroplan för grundskolan, förskoleklassen och fritidshemmet 2011*. Stockholm: Edita.

Smith, M. U. (2010). Current status of research in teaching and learning evolution: II. Pedagogical issues. *Science & Education, 19*, 539-571.

Smith, G., Golomb, M., Billstein, S., & Smith, S. (2015). The Luria-Delbrück fluctuation test as a classroom investigation in Darwinian evolution. *The American Biology Teacher, 77*(8), 614-619.

Speth, E. B., Shaw, N., Momsen, J., Reinagel, A., Le, P., Taquieddin, R., & Long, T. (2014). Introductory biology students' conceptual models and explanations of the origin of variation. *CBE – Life Sciences Education, 13*, 529-539

Tibell, L., & Harms, U. (2017). Biological principles and threshold concepts for understanding natural selection – Implications for developing visualizations as a pedagogic tool. *Unpublished manuscript.*

Tsui, C-Y., & Treagust, D. F. (2013). Introduction to multiple representations: Their importance in biology and biological education. In: D. F. Treagust & C-Y. Tsui (Eds.), *Multiple Representations in Biological Education* (pp. 3-18). Dordrecht: Springer.

Wolf, A., & Akkaraju, S. (2014). Teaching evolution: From SMART objectives to threshold experience. *Journal of Effective Teaching, 14*(2), 35-48.

Zohar, A. & Ginossar, S. (1998). Lifting the taboo regarding teleology and anthropomorphism in biology education – Heretical suggestions. *Science Education, 82*(6), 679-697.