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Cover page

Does integrating Natural Selection throughout Upper Secondary Biology Education result in a better understanding? A Cross-national Comparison between Flanders, Belgium and the Netherlands.

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Abstract

Secondary school teaching of evolution through natural selection is very important because for most people, it is the only formal introduction to the scientific understanding of this theory. However, there are major concerns over its unsatisfactory teaching. In several European countries, including the Flanders region in Belgium, natural selection is treated as a side-topic that is referred to only after all other biological content has been covered. It has been suggested that improved understanding can be achieved by teaching it in a more integrated manner throughout the biology curriculum, as is largely the case in the Netherlands. We tested this hypothesis by a standardized comparison of the understanding of natural selection between university freshmen who had completed high level biology secondary education in Flanders or the Netherlands. We used the Conceptual Inventory of Natural Selection (CINS), designed to measure the understanding of 10 underlying key concepts (KC), including four core concepts (CC), and the magnitude of alternative conceptions. Regression analysis was used to control for potentially confounding student parameters. Dutch graduates indeed obtained a significantly higher CINS-score than Flemish graduates. They also scored significantly higher on eight key concepts. The 10 KC were employed to varying degrees, with the relative rank being highly comparable between both student populations, and the CC *origin of variation* and *variation inheritable,* both linked to genetics, being more challenging than the CC *existence of variation* and *differential survival*. The relative frequency of alternative conceptions elicited by the CINS was almost identical in both student populations.

Keywords: Conceptual Understanding, Evolution, Natural Selection, Alternative Conceptions, Secondary Education, Flanders, the Netherlands, CINS

Introduction

Biological evolution is a major concept that needs to be understood by all citizens because it influences their lives and decisions. For example, biodiversity and the need to make careful choices in medical treatment of illnesses are both direct results of evolutionary processes. Natural selection is an important mechanism in the biological theory of evolution. It is considered a threshold concept that integrates the learning of many sub-concepts and is necessary to make sense of the field of biology (Dobzhansky, 1973). The importance of evolution through natural selection as a unifying theory able to account for the unity and variability of all present and past living forms is nowadays unanimously accepted among scientists. It is considered central to scientific literacy, providing the basics to understand a wide range of topics from biology to medicine (Hazelkorn et al., 2015).

However, the general public's acceptance and knowledge of evolution lag behind , as shown in a large number of international studies that highlight how difficult the general public find evolution to grasp, although there are significant differences between countries (e.g. Coyne, 2012; Dennis & Borgerding, 2018; Miller et al., 2006). In the past decades, scientists and educators have explored acceptance and understanding of evolution across a variety of publics, in order to identify possible causal explanations and barriers that make evolution so difficult to accept or understand (Crivellaro & Sperduti, 2014). Considering cross-national comparisons, these mainly focused on the factors that influence the acceptance of evolution, revealing a negative relationship with fundamentalist religious beliefs and religiosity, and a positive relationship with genetic and scientific literacy, and school-life expectancy (see e.g. Heddy & Nadelson, 2012; Miller et al., 2006). As research findings regarding the relationship between acceptance and understanding of evolution are inconsistent, with some studies finding positive correlations (Nehm & Reilly, 2007; Rutledge & Warden, 2000) and others reporting no statistical correlations (Bishop & Anderson, 1990; Sinatra et al., 2003), there is a need for more cross-national comparisons focusing specifically on the understanding of evolution.

To date, many studies have investigated the factors that contribute to the misunderstanding in individuals of different ages and cultural backgrounds in the United States (e.g. Alters & Nelson, 2002; Thagard & Findlay, 2010), where there is a particular public resistance to evolution (Gallup, 2009), contrary to Europe (Miller et al., 2006). The widespread poor understanding in the United States has been attributed to a wide variety of cognitive, epistemological, religious, and emotional factors (for reviews, see Alters & Nelson, 2002; Glaze & Goldston, 2015; Rosengren et al., 2012). Although more data have also been recently gathered for European countries (e.g. Athanasiou & Mavrikaki, 2014; Carvalho et al., 2004; Grosschedl et al., 2014; Konneman et al., 2016; Mead et al., 2017; Southcoth & Downie, 2012), at present, less information is available with respect to the factors affecting understanding of evolution in Europe.

To improve the general public's scientific literacy in evolution, the teaching of evolution in secondary school in particular is very important as, for most people, it is the only formal introduction to the scientific understanding of the theory. However, there are major concerns over its unsatisfactory teaching (Alters & Nelson, 2002; Glaze & Goldston, 2015; Leopoldina, 2017; Mead et al., 2017; Nehm et al., 2009). The failure of secondary school teaching to promote full comprehension of evolution and natural selection has been attributed to diverse factors such as an inappropriate curriculum, student alternative conceptions and insufficient (pedagogical) content knowledge in evolution instructors.

Considering the specific role of the evolution curriculum, it has been suggested that understanding of evolution can largely be improved by restructuring the specific place and treatment of evolution in the biology curriculum and supporting textbooks (Leopoldina, 2017; Nehm et al., 2009). The syllabi of many secondary educational programs in Europe indeed appear to treat evolutionary theory as a side-topic that is, at best, usually referred to after all other biological content has been covered, and is, therefore, often neglected or even omitted altogether (Carvalho et al., 2004; Dennis & Borgerding, 2018). Until recently, this was also the case in Flanders, the Dutch speaking region of Belgium, where the teaching of evolution was largely restricted to the last weeks of the final year of general secondary education, as a separate and last chapter in the textbooks (De Schutter et al., 2005; D'Haeninck, et al., 2009b). Consequently, it was taught in isolation from the ongoing development of understanding of other aspects of biology throughout the secondary curriculum. However, there are exceptions (Dennis & Borgerding, 2018). For example, in the Netherlands, evolution and natural selection are already explicitly addressed in the fourth year of general secondary education and in a more integrated manner throughout the biology curriculum of upper secondary education (Geraedt & Boersma, 2006; Smith et al., 2004). Surprisingly, the hypothesis that a better integration of evolution throughout the secondary school biology curriculum promotes understanding has not yet been formally tested.

The main goal of this study is, therefore, to compare the effectiveness of upper general secondary education (or pre-university level) in promoting understanding of evolution through natural selection between Flanders (Belgium) and the Netherlands. In particular, we will describe and compare the conceptual understanding of natural selection between Flemish and Dutch university freshmen enrolling in the Biomedical Sciences or Veterinary Sciences bachelor programs. The fact that traditionally a high number of Dutch students enroll in these programs at the University of Antwerp, in Belgium provided this research opportunity. By comparing Flemish and Dutch students enrolling in these two related university programs, we control for the potentially confounding effects of different study interests, which may influence the way evolutionary concepts are dealt with, and different programs attended during secondary education, which may affect the amount of biology and evolution instruction. Other potentially confounding variables, such as age, gender and cognitive ability, will be statistically controlled for. Since most participating students appeared to have completed the secondary education program with the highest possible level of biology education in their country (see below), this allowed us to also specifically compare, on a cross-national level, its effectiveness in fostering understanding of natural selection.

Theoretical Background

Comprehension of Evolution through Natural Selection

Natural selection is the process responsible for the evolution of adaptive features. Without a working knowledge of natural selection, it is impossible to understand how or why living things, including humans, have come to exhibit their diversity and complexity. Full comprehension of evolution through natural selection is only made possible through the understanding of the underlying fundamental concepts that comprise the theory (Anderson et al., 2002). There is some discussion in the literature regarding the number of essential key concepts (KC), but at a minimum, the following KC are generally considered necessary and sufficient to explain evolutionary patterns using the natural selection model: *the origin and presence of variation*, *the heritability of this variation*, and *the differential survival and/or reproduction of individuals that differ in heritable traits* (Anderson et al., 2010; Nehm & Ha, 2011; Nehm & Schonfeld, 2010, but see e.g. Fiedler et al., 2017, 2019; Tibell & Harms, 2017 with respect to the deceptively simplicity of this framework), and these KC are, therefore, defined as core concepts (CC) (Göransson et al., 2020; Nehm & Ha, 2011). Some authors acknowledge the additional KC: *hyper-fecundity* or *overproduction of offspring*, *natural resources*, *limited survival*, and *a change in the distribution of produced phenotypic/genotypic variation in the next generation* (Endler, 1986; Nehm & Schonfeld, 2010), while others in addition include *population stability* and *origin of species* as KC (Anderson et al., 2002; Mayr 1982).

Despite its centrality in the life sciences, and although the CC that underpin natural selection have been shown to be fairly straightforward and simple, evolutionary change by natural selection appears to be one of the most difficult topics in biology to teach and understand. A fundamental problem is that many students hold remarkably high levels of alternative conceptions about basic evolutionary principles (see Gregory, 2009; Nehm & Schonfeld, 2008). Alternative conceptions are ideas that differ from the corresponding normative scientific understanding. Most alternative conceptions relating to natural selection are variations of the belief that individuals, instead of populations, evolve. Students often believe that individuals change because the environment changes them directly, because they need to, because they want to or because they use or do not use specific body parts, and that these changes are passed on to offspring (Gregory, 2009). Alternative conceptions can be seen as 'misconceptions' that are obstacles to be overcome. At the same time, they can also serve as anchoring conceptions from which to move to a scientific conception using suitable instructional strategies focusing on conceptual change (Anderson et al., 2002; Sinatra et al., 2008). In both perspectives, teachers need to identify their students' ideas in order to design instructional activities that confront these ideas or draw attention to the contexts in which these ideas are useful or inappropriate. Concept inventories are "research-based instruments designed to measure student conceptual understanding in areas where rigorous research has shown that students hold common alternative conceptions" (Garvin-Doxas et al., 2007). The Conceptual Inventory of Natural Selection (CINS, Anderson et al., 2002) is such an inventory that has been widely used by evolution education researchers (see Mead et al., 2019), and that was also used in the present study (see below). Although the actual alternative conceptions students may have about natural selection are well-documented, studies comparing their prevalence at the cross-national level are scarce (Nehm & Schonfeld, 2008).

The Role of the Specific Evolution Curriculum

Nehm et al. (2009) suggested that the well-established finding that substantial confusion and alternative conceptions about evolution and natural selection persist after evolution instruction, may be due to the segregation of evolution as separate ‘units’ or chapters in biology textbooks, which reinforces students’ faulty mental models of biology and evolution. By integrating evolution throughout the biology curriculum, educators would model for students how biologists employ evolution as a conceptual organizer for their own cognitive understanding of fields and ideas as diverse as genetics and ecology (see also Leopoldina, 2017). The latter implies that evolution has to be introduced much earlier in the secondary school curriculum than as a final chapter after all other biological content has been covered. Moreover, if natural selection is taught only during the last weeks of secondary education, there will only be limited or no opportunities to realize knowledge transfer (i.e. the ability to apply knowledge learned in one context to other novel contexts or to use it generatively), and hence deep conceptual understanding, of this major concept in biology. Most students appear to have numerous difficulties regarding knowledge transfer (Anderson & Schönborn, 2008). For example, Pugh et al. (2014) described the patterns of transfer displayed by secondary school biology students learning about natural selection over time and concluded that transfer and duration of transfer were fairly limited.

In addition, the effectiveness of evolution instruction in secondary education may also depend on a variety of other factors, such as the specific attention and time devoted to instruction of the CC and KC of natural selection and underlying fundamental concepts such as genetics and ecology that support the theory (Anderson et al., 2002; Mayr, 1982; Miller et al. 2006), which may largely depend on the specific program students are enrolled in. For example, students enrolled in a Science program will experience more science and biology instruction, and hence likely also more instruction of these fundamental concepts, than students following a Languages program. It has been shown that the more students are exposed to biology and evolution instruction, the larger the learning gain (Athanasiou & Mavrikaki, 2014; Deniz, Donnelly, & Yilmaz, 2008). In addition, Nadelson and Southerland (2010) found a positive correlation between science literacy and individual understanding of evolution. Through exposure to science ideas and deeper exploration and explanation of evolution and natural selection, students can develop a greater knowledge of scientific thinking and evidence, which in turn leads to greater understanding of evolution (Heddy & Nadelson, 2012). Hence, when comparing understanding of natural selection between secondary education systems on a cross-national level, graduates from a comparable program should be compared to obtain a valid comparison.

The evolution curriculum in upper general secondary education in Flanders and the Netherlands

In the present study, we compare the effectiveness between the Flemish and Dutch upper general secondary education (i.e. granting admission to university education), in promoting understanding of natural selection. In particular, we also focus on a comparison of the effectiveness between the program with the highest possible timetabled hours of biology education in Flanders (i.e. the 'Science' program) and the Netherlands (i.e. the 'Nature and Health' program). Below, we compare the structure and organization, and the specific evolution curriculum at the time of the present study between both programs.

In Flanders, general secondary education has a uniform structure. Students enter the first of six years around the age of 12. The six years are grouped into three cycles of two years each. Students enrolling in the second cycle can choose between more than 10 different fields of study, which can be categorized in three major programs: a program with a Science component, a Mathematics component or a program without one of these two components. Students enrolled in the Science program experience the highest level of biology instruction (two hours per week) a student can possibly receive in general secondary education in Flanders. At the time of the present study (2012-2014), the idea of evolution of living species was very briefly introduced for the first time in the textbooks of the fourth year (grade 10, focusing largely on ecology), using the idea of unity and diversity of living things and the phylogenetic classification (see De Schutter et al., 2001; D'Haeninck, et al., 2009a), but the mechanisms of the process of natural selection were not explicitly addressed at that time. It was only mentioned that organisms are adapted to their environment. Evolution and natural selection were only explicitly addressed towards the end of the last year of secondary education, as the last chapter in the textbooks, preceded by the topics reproduction, genetics and heritability (De Schutter et al., 2005). Also, the process of natural selection was not mentioned explicitly in the three attainment targets about evolution (out of a total of 24) in the Flemish curriculum for the third cycle (ATB-3nd grade ASO, 2011), although it was covered in the supporting textbooks.

In the Netherlands, during the last three years (called second phase) of general secondary education, students focus on one of four subject clusters, each of which emphasises a certain field of study in addition to satisfying the general education requirements. Only students attending the 'Nature and Health' subject cluster have compulsory biology classes, while for students attending the 'Nature and Technology' subject cluster, biology is an optional course. Evolution and natural selection are already explicitly addressed in grade 10, and are, as in Flanders, also preceded by the topics reproduction and genetics, whereas ecology is addressed afterwards (Smith et al., 2004). In contrast to the situation in Flanders, students also have to participate and succeed in a National Central Examination (CE) at the end of the last year of secondary education. This CE assesses their knowledge of the majority of learning contents of the second phase (grades 10-12). Evolution and natural selection are explicitly mentioned in the syllabus describing the attainment targets for this CE, which also emphasizes that pupils have to be able to apply all concepts in different contexts, given the strong focus on a concept-context approach in the curriculum (Boersma et al., 2007; College voor examens, 2010). Hence, this different assessment system implies that, to prepare their pupils for this CE, Dutch biology teachers should apply natural selection in different contexts throughout biology instruction during the second phase, providing more opportunities for knowledge transfer and deep conceptual learning.

The Conceptual Inventory of Natural Selection (CINS)

The CINS is a 20-question multiple-choice test, designed to measure knowledge of 10 KC of natural selection and to identify alternative conceptions (Anderson et al., 2002). Whereas other tests assess the process of natural selection itself, the CINS also addresses the students' understanding of the underlying concepts of genetics and ecology that provide a foundation for using natural selection as an explanatory theory. It consists of three reading passages and 20 items which are based on actual scientific studies of natural selection. The 3 distractors, or wrong answers, in each item represent common alternative conceptions regarding natural selection and related KC. Each reading passage on the CINS describes a brief background of a particular population of organisms (e.g. the Galapagos finches) and establishes the evolutionary setting for the series of questions that follow it (see Table A1). The 10 KC related to natural selection, with two questions per concept, are the four CC '*origin of variation', 'existence of variation (in a population)', 'variation is inherited' and 'differential survival'* and the six KC *'limited survival',' biotic potential', 'limited (natural) resources', 'change in a population', 'population stability' and 'origin of species'*. The CINS is considered 'a valid and generally reliable measure of knowledge and alternative conceptions about natural selection' (Athanasiou & Mavrikaki, 2014; Nehm & Schonfeld, 2008, 2010). However, it also received some criticisms. One of these concerns the claim that it assesses understanding of the 10 different KC related to natural selection. In Anderson et al.'s (2002) original sample of non-majors, principal component analyses (PCA) results indicated that nine of the 10 pairs of items representing the 10 KC indeed emerged together on the same component, indicating strong support for the internal validity. However, Nehm and Schonfeld (2008) and Athanasiou and Mavrikaki, (2014) did not find support for different PCA components representing 10 distinct evolutionary concepts in their student samples. Hence, more empirical work examining the efficacy and generalizability of the CINS among students from different racial and ethnic groups, geographic regions, socio-economic and language backgrounds is still needed to clarify these contradictory results with respect to the validity of the different KC (Nehm & Schonfeld, 2008, 2010). The present study contributes in this respect as we also examined the internal validity for the Flemish and Dutch student populations. Flanders (Belgium) and the Netherlands can be characterized as highly 'evolutionary' educated societies in the modern world, possessing the 8th and 12th position out of 34 countries on the evolutionary acceptance scale proposed by Miller et al. (2006).

Aim and Research Questions

To summarize, the major goals of this study were: 1) to test the hypothesis that integrating natural selection throughout upper general secondary education (as is the case in the Netherlands) results in a better understanding than when it is treated as a final chapter after all other biological content has been covered (as in Flanders, Belgium), 2) to compare the observed levels of understanding in Flemish and Dutch secondary graduates with those reported for comparable student populations in other countries. The following main research questions guided our study:

- Do Dutch secondary graduates obtain a higher overall CINS score than Flemish graduates?

- Does the understanding of specific CC and KC differ between Dutch and Flemish secondary graduates? What is the value of the CINS in measuring knowledge of specific CC and KC?
- Does the relative frequency of alternative conceptions elicited by the CINS differ between Dutch and Flemish graduates?
- How do overall CINS scores, knowledge of specific CC and KC, and alternative conceptions in Dutch and Flemish secondary graduates compare to results availalbe for student populations in other countries?

Methods

Sample Characteristics

This study was conducted using two cohorts of students enrolling in university education at the University of Antwerp in Flanders, Belgium in 2012 (N=196) and 2014 (N=193). Based on both cohorts, two samples were defined. The first sample consisted of 238 students enrolling in the Biomedical Sciences bachelor program (N=112 in 2012 and N=126 in 2014), of which 198 had completed secondary education in Flanders and 40 in the Netherlands. The second sample consisted of 151 students enrolling in the Veterinary Sciences bachelor program (N=84 in 2012 and N=67 in 2014). In this second sample, 93 students had completed secondary education in Flanders, and 58 in the Netherlands. Hence, in total we sampled 291 'Flemish' students and 98 'Dutch' students. The results of 17 (16 Flemish and 1 Dutch) of these 389 students who did not answer all 20 CINS questions or selected more than one answer for an item, were not included for further analyses.

Considering the remaining 372 students, males comprised 29.5% of the Flemish (N=275) and 22.7% of the Dutch sample (N=97). Most (62.9%) Flemish students had just completed secondary education and the mean age of this sample was 18.4 years (SD=1.2), while

Dutch students were on average slightly older ($t=-5.65$, $df=134$, $p<0.0001$), with a mean age of 19.5 years (SD=1.7). At least 79.2% (218/275) of the Flemish students had attended the Science program. Of the 260 Flemish students that reported the weekly number of biology classes during the last year of secondary education, 192 (73.8%) reported 2 hours a week, which is the standard number for the Science program, while 10 and 5 students reported 3 and 4 hours a week, respectively and 42 and 11 students reported 1 hour or no biology education. Considering Dutch students, at least 67.0% (65/97) had attended the Nature and Health program, while 3 students had followed another profile and 29 provided no information. Of the 42 Dutch students that reported the weekly number of biology classes during the final year, the majority had 3 (40.5%) or 4 (28,6%) hours per week, with in total 83,3% having at least 3 hours or more.

Research Instrument and Procedure

We used a questionnaire that consisted of two parts. In the first part, we asked students to report about their secondary school education (e.g. the specific program followed, weekly number of biology classes in grade 12, global final grade score) and students' details (age, gender). Not all students provided all of the requested information, resulting in different sample sizes (see above). In the second part, students were asked to complete the CINS. The students were asked to 'choose the one answer that best reflects how an evolutionary biologist would answer' (see Table S1). This wording emphasizes that students' understanding of biological concepts was assessed, rather than their personal opinions. In order to avoid bias of translation, the questionnaire was translated from English to Flemish/Dutch (both languages are similar when written) by the first author. Another lecturer at the Department of Biology, teaching a semester-long evolution course and fluently speaking English checked the translation for any subtle differences from the original questionnaire and agreed to the final text.

The questionnaire was distributed in person at the beginning of a mandatory practical class (animal dissection) of the semester-long zoology course, approximately three weeks before the end of the first semester in 2012 and 2014 (i.e. early December, after 10 weeks of lectures). After a short introduction to clarify the purpose of the research, the students were informed that the questionnaire was not obligatory and completely anonymous. Students were asked to complete the questionnaire within 30 minutes. Student understanding of natural selection as revealed by the CINS was considered to be the result of their secondary education, as there was no evolution course included the program of the first semester. The final score students obtained on the Zoology exam (i.e. a pooled score based on the practical classes and the written exam about the lectures), was provided by the lecturer and considered as a proxy of student cognitive ability (see Anderson & Lebière, 1998), in addition to the global final grade score for secondary education.

Data analyses

Data were analysed using the statistical packages IBM SPSS 19.00 and R Studio 1.0.136/R 3.1.3. For all statistical tests, a significance level of 0.05 was adopted. To measure the internal consistency of the test, we calculated Cronbach's alpha for the total student sample (N=372).

Average CINS-scores of Flemish and Dutch students were compared using the non-parametric Mann-Whitney U test for two independent samples when including all students (as the hypothesis of normal distribution was not satisfied for one of the two samples). A t-test was used when only students who attended the Science program or the Nature & Health program were included. We applied a Levene's test to check whether the variances of both samples significantly differed and if this was the case, we used the Welch two-sample t-test. In addition, we performed a regression analysis in R to examine in detail the specific effects of additional parameters, other than country of secondary education, on the obtained CINS-scores. For the latter analysis, only students for which all parameters were available were included, resulting in a reduced sample size of 340 students (N= 255 Flemish, N= 85 Dutch). The full model was constructed with as fixed effects country of secondary education (Flanders *vs.* the Netherlands), field of study (BS *vs.*VS), year when tested (2012 *vs.* 2014), age (range 18-28), gender, global final grade score (50-59%, 60-69%, 70-79%, or 80-89%) and the score (out of 20) on the Zoology exam (range 2-18). The latter was also included since the final grade score of secondary education may not be fully comparable between schools and countries (however, both scores were significantly correlated: r=0.32, p<0.001). The score on the Zoology exam was comparable between Dutch and Flemish students: 11.1/20 (SD=2.9) versus 11.8/20 (SD=3.2, t=1.85, p=0.066). In the full model we also included the three-way interaction country of secondary education*field of study*year when tested, to check, for example, whether the two-way interaction effect country of secondary education*field of study on the CINS-score varied significantly over the years. As we wanted to avoid overfitting of our model, we did not include any interactions with gender or age. In an additional analysis, we used the same full model but included only Flemish Science program graduates (N= 199) and Dutch Nature & Health program graduates (N= 58).

When comparing the proportion of times that 1) Flemish and Dutch students answered correctly to the two items representing a specific concept, and (2) specific alternative conceptions were selected as the correct answer in both student populations, we applied Fisher's exact tests, which were computed using the QuickCalcs software from the GraphPad website (http://www.GraphPad.com). *P*-values were adjusted to counteract the problem of multiple pairwise comparisons, using the sequential Bonferroni correction (Holm, 1979).

Results

Internal Consistency

The internal consistency reliability of the CINS-scores was satisfactory: we found an acceptable Cronbach's alpha value of 0.74 for the whole sample of bachelor freshmen (which is comparable to previously published work).

CINS Scores in Flemish and Dutch students

The overall score obtained on the CINS was 12.8 $\pm$ 3.6 (range 3-20, N=372). Students who had completed their secondary education in the Netherlands obtained a significantly higher score (14.3 $\pm$ 2.8, range 7-20, N=97) than Flemish students (12.2 $\pm$ 3.8, range 3-20, N=275, U-test, $p<0.001$), earning approximately two points more. The same result was found when only considering students who had attended the high level biology program ($t=-4.67$, $p<0.0001$), with corresponding values for Dutch and Flemish students being equal to 14.4 $\pm$ 2.6 (range 7-19, N=65) and 12.5 $\pm$ 3.8 (range 4-20, N=218) respectively, and an overall score of 12.9 $\pm$ 3.7. Anderson et al. (2010) suggested that students scoring 16/20 or higher understand natural selection quite well. The proportion of Dutch students scoring 16/20 or higher (33/97 = 34.0%) was significantly higher than the proportion of Flemish students (52/275= 18.9%, $p<0.01$). A comparable result was found when considering only students who attended the high level biology program: 36.9% (24/65) of Dutch students versus 22.0% (48/218) of Flemish students ($p<0.01$).

Factors affecting CINS scores

We used a regression analysis to examine the effect of country of secondary education on the CINS score, while controlling for several additional parameters. The final model ($F = 16.24$; $p<0.001$; all model assumptions fulfilled) revealed that both the country of secondary education and the score on Zoology very highly significantly affected the CINS-score, while the effect of age and final grade score for secondary education was also significant, but less strong (Table

1). Field of study, year when tested and their (two-way/three-way) interaction with country did not significantly affect the CINS-score. The effect of gender also turned out to be non-significant. Flemish students scored on average 2.65 points less than Dutch students. Students earning 1 point more on the Zoology exam, obtained on average 0.28 points more on the CINS. Likewise, students obtaining a score of 80% or more when graduating from secondary school, obtained on average 1.40 points more than students that obtained a grade point in the range of 50-59%. CINS scores also decreased with student age: an increase of one year in age will on average result in a decrease of 0.38 points.

[Table 1 near here]

Including only students who attended the high level biology program in the analysis provided a highly comparable result, with the exception that the effect of age was no longer significant (Table 2). Flemish students now obtained on average 2.1 points less than Dutch students. Students earning 1 point more on the Zoology exam obtained on average 0.29 points more on the CINS. Likewise, students obtaining a score of 80-89% when graduating, obtained on average 1.77 points more than students who got a grade point in the range of 50-59%.

[Table 2 near here]

The Value of the CINS in Measuring Knowledge of Specific Key Concepts

A varimax rotated PCA was used to explore correlation patterns among the CINS items. The PCA was conducted on the 20x20 matrix of item phi correlation coefficients (as in the original study by Anderson et al., 2002). Theoretically, the CINS's final PCA should have 10 components that explain the variation among the 20 test items, with each component representing a separate concept of natural selection. In addition, each set of two items designed to measure a single concept should both load on the same component. We maintained the same criteria for determining the final PCA solution as in Anderson et al. (2002). These criteria include: ‘a) having a large proportion of the total matrix variation explained, b) having a high number of items with strong (>0.40) loading on at least one component, c) having a minimum number of complex items (i.e. with strong loadings on more than one component), and d) having a component pattern that was theoretically interpretable’ (p. 966). We found six components with eigenvalues >1, which collectively accounted for 50% of the variance in the data set. The rotated component matrix values are shown in Table 3.

[Table 3 near here]

Apart from item 17, all items had loadings >0.4 on at least one component. Only one item (5) had a loading >0.40 on multiple components (PC2: 0.47 and PC3: 0.46). It had its highest loading in component 2, together with its pair item 15 (representing *limited survival*). In total seven (3 CC and 4 KC) of the 10 pairs of items did emerge together on the same component. Given that the paired items 7 and 17, designed to measure the core concept *variation inheritable,* also loaded together on PC4, although the loading of item 17 was only 0.38 (and not >0.40), this CC may also be considered as valid, implying that all four CC were ‘correctly addressed’ by the CINS. It should be noted that item 17 also had a loading of 0.38 on PC1. The paired items designed to measure the KC *natural resources* and *population stability* loaded on different components and may thus be considered as more problematic. Three components contained two pairs of items: component 1 contained *origin of variation* (CC) and *origin of species* (KC), component 2 contained *differential survival* (CC) and *limited survival* (KC), and component 4 contained *variation inheritable* (CC) and *change in a population* (KC).

Most common key concepts in both student samples

Tables A2 and 4 present and summarize the percentage of correct answers and the corresponding rank of the 10 concepts of natural selection as revealed by the CINS. The goal of this analysis was to identify and compare the most common concepts used by Flemish and Dutch graduates. It was done in an identical way as in the Nehm and Schonfeld (2008) paper for biology majors in their second semester, after instructional units on evolution and natural selection were completed (results shown in Table 4 for comparison), apart from the fact that in their analyses the two CC *origin of variation* (CC1a) and *existence of variation* (CC1b) were originally grouped into one single core concept. In the present analysis, these were considered separately. We calculated the percentage of correct answers (at the population level) to the two items designed to represent each specific concept.

[Table 4 near here]

In both samples, students employed the 10 concepts of natural selection to varying degrees (Table 4, Fig. 1). The percentage of times Flemish students responded correctly to items representing a specific concept varied between 28% and 81%, while for Dutch students the corresponding values were 38% and 93%. The five most common employed concepts were similar in Flemish and Dutch students, although their specific rank differed, while the rank of the five less common employed concepts was identical. In Flemish students *population stability* (81%) was the most commonly elicited concept , while the second and third most abundant were *natural resources* (71%.) and *biotic potential* (69%.). In Dutch students *limited survival* was the most common elicited (93%.) and *population stability* (89%) and *origin of variation* (77%.) were the second and third most abundant concept. Recall that for *population stability* and *natural resources*, PCA did not provide strong support for the two items representing a distinct evolutionary concept. In both samples *change in a population* was the less elicited concept of the CINS. For eight of the 10 concepts (except *biotic potential* and *natural resources)*, the percentage of times that students responded correctly to the items bearing on this concept was significantly higher ($p<0.05$) in the Dutch sample than in the Flemish sample (Fig. 1).

[Fig. 1 near here]

Considering the percentage of times that the items representing the four CC *origin of variation*, *existence of variation*, *variation inheritable* and *differential survival* were correctly answered, *these* varied between 49% and 65% for Flemish students and 66% and 77% for Dutch students (Table 4). When considering the percentage of *individual* students who answered correctly to *both* items representing a CC, the corresponding figures for these four CC were substantially lower: 33.5%, 47.3%, 23.3% and 43.3% for Flemish students and 44.3%, 60.8%, 42.3% and 55.7% for Dutch students. The latter again performed significantly better for each CC. In both student groups, the items representing the CC *origin of variation* and *variation inheritable*, which are both linked to genetics, were significantly less frequently correctly answered than the other two CC. Overall, 14.4% (14/97) of Dutch students answered correctly to *all eight items* representing the four CC, compared to only 5.5% (15/275) of Flemish students, which again was a significantly better performance.

In the sample of USA biology majors (Table 4), the most commonly elicited concept was the CC *differential survival,* with 82% of correct answers, which was significantly higher than in Dutch and Flemish students, and which was followed in rank by *limited survival* (74.5%, significantly less elicited than in Dutch students) and *limited resources* (70.5%). As in both our students samples, *change in a population* was the less elicited concept of the CINS, although the percentage of correct responses (40%) was significantly higher than in Flemish students.

Relative frequency of alternative conceptions in both student groups

We noted the relative percentage and rank of 23 alternative conceptions that were employed as CINS distractors (see Table A3). It was done in an identical way as in the Nehm and Schonfeld (2008) paper for USA biology majors. The specific number of each alternative conception corresponds to the specific rank in the latter study, thus with alternative conception #1 (*Intention/need relating to speciation)* being the most common (13%) .

Of the total of 2212 alternative conceptions elicited in the Flemish students (Table A3), the five most common were alternative conception #1 (12.2%), #5 (*Mutations occur to meet the demands of the population*, 9.8%), #9 (*Variations only affect outward appearance, not survival*, 9.1%), #15 (*Traits that are positively influenced by the environment will be inherited by offspring,* 9%), and #11 (*Fitness is equated with strength, speed, intelligence, longevity*, 7.7%). The proportion of times that #1 was selected was significantly higher than #5, #9 and #15 (which all three did not differ significantly from each other), and #11. The proportion of times that #5 was selected was significantly higher than the fifth ranked, #11. All other differences were not significant.

Considering the 577 alternative conceptions that were documented among the Dutch students, the five most common were identical to those among the Flemish students, except that #3 (*Organisms can always obtain what they need to survive*), instead of # 11, was included in the top 5: #1 (12.6%), #5 (10.4%), #15 (9.2%), #3 (8.5%), and #9 (8,5%). Alternative conception #3 was ranked sixth in Flemish students while #11 was ranked seventh in Dutch students (Table A3). The proportion of times that the first three ranked (#1, 5, 15) were selected did not differ significantly, while #1 was significantly more frequently selected than the fourth ranked # 9 and # 3. All other differences were not significant.

The relative frequency of the six alternative conceptions ranked in the top five in both populations did not differ significantly. This was also the case considering the first 10 ranked in the Flemish population, apart from #7 (*Learned behaviours are inherited*), which was significantly more present in Dutch students (8.0% versus 6.9%).

Considering the 730 alternative conceptions documented in USA biology majors (see Table 2 in Nehm & Schonfeld, 2008) for comparison, the most common, #1 (13%), was the same as in the Flemish and Dutch students. The proportion of times that #1 was selected did not differ significantly among all three student populations. However, in this USA population, #1 was followed in rank by #2 (*Intention/need related to genetic change*', 8.6%), #3 (8.1%), #4 (*Population level off*, 7.8%) and #5 (7.4%). Alternative conception #2 was selected significantly more often than in both the Dutch and Flemish sample, while for #3, #4 and #5 there was no significant difference between the three student populations. Comparing the proportion of times that each of the five most frequently used alternative conceptions in the Flemish (# 1,5,9,15,11) and Dutch populations (# 1,5,15,9,3) were selected with the proportion of times they were selected by USA biology majors, #9 (*Variations only affect outward appearance, not survival)* was selected significantly more often by both Flemish and Dutch students, while #11 (*Fitness is equated with strength, speed, intelligence, longevity*, 7.7%) was selected significantly more often in Flemish students only.

Discussion

In the present study, we compared in a standardized way the level of understanding of natural selection between graduates from the Flemish and Dutch type of secondary education which grants admission to higher education. We hypothesized that, due to the different place and treatment of natural selection in the biology curriculum (see Introduction), Dutch secondary graduates would have a better understanding. As a research instrument we used the CINS (Anderson et al., 2002). Our results showed that Dutch secondary graduates indeed obtained a significantly higher overall CINS score. They also scored significantly higher on most KC of natural selection, including all four CC. By contrast, the relative frequency of alternative conceptions, as revealed by the CINS, was highly comparable in both student groups. In both student samples, the 10 concepts were used to varying degrees, with the relative rank being highly comparable, and the CC *origin of variation* and *variation inheritable,* being more challenging than the CC *existence of variation* and *differential survival*. Below, we discuss these main findings and also compare the CINS-scores of Flemish and Dutch secondary graduates with those reported for various student samples in other countries. In addition, we discuss the implications of our findings for educational practice.

Factors affecting CINS scores

Our result that Dutch university freshmen obtained a significantly higher CINS-score strongly suggests that secondary education preparing for higher education in the Netherlands, and in particular the high level biology program, is indeed more effective in promoting understanding of natural selection than the comparable program in Flanders, Belgium. This better understanding in Dutch secondary graduates is most likely largely due to the more appropriate place and treatment of natural selection in the upper secondary biology curriculum. However, it should be taken into account that most Dutch secondary graduates received at least 3 hours of biology education per week, compared to only two hours for most Flemish graduates. This may also have contributed, to some extent, to their better understanding, since knowledge about evolution has been shown to increase with an increase in the amount of biology education (Glaze & Goldston, 2015). It may be argued that, as CINS scores were not obtained immediately after students graduated from secondary school, but on average only about five months afterwards, these may not be fully representative of the knowledge gain through formal secondary education. It cannot completely be excluded that some students may have gained additional understanding of evolution following graduation through, for example, informal science education activities or the media. However, it is very unlikely that this would apply to more than a few students, given the specific topic of natural selection. In addition, the observation that Dutch university freshmen were found to perform better irrespective of the year of sampling, strongly suggests that this was not a major factor contributing to the higher CINS scores in Dutch graduates.

Our final regression model indicated that student intellectual ability also significantly positively affected the CINS score, which is not surprising. By contrast, student age appeared to negatively affect the CINS score. Hence, although Dutch students were on average approximately one year older than Flemish students, resulting in a decrease in their CINS-score of about 0.38 points, they still performed significantly better.

Level of Understanding in Flemish and Dutch Secondary Graduates

Taking into account that students should obtain a CINS-score of at least 16/20 for having a good understanding of natural selection (Andersson et al., 2010), the average performance of the two student samples, and in particular the Flemish population, appears to be rather weak at first sight. Only 22% of the Science program graduates in Flanders had a good understanding, compared to 37% of the Nature & Health graduates in the Netherlands. However, to correctly interpret the level of understanding based on these CINS-scores, it should be noted that the CINS was originally designed for measuring knowledge of natural selection in undergraduate biology non-majors, as an in-class pretest before instruction on any topic related to natural selection during a general biology course. Although Anderson et al. (2002) concluded that the CINS was well-suited for non-majors, the average scores in the two samples that they studied were in fact very low (8.2/20 and 10.4/20, see Table 5). This result reinforces the suggestion of Nehm and Schonfeld (2008), based on the marginal scores (12.6/20*)* their own sample of biology majors obtained after instructional units on evolution and natural selection, that the CINS is difficult for undergraduate non-majors and better-suited for first year biology-majors. When comparing the performance of the Flemish (12.5/20) and Dutch (14.4/20) high level biology secondary graduates with the latter results and other CINS-scores obtained in various undergraduate and graduate student populations (Table 5), they seem to perform better than Greek non-majors with a biology course attended, Greek biology majors of first, second and third year, and Canadian biology majors with no evolution course attended. Moreover, Dutch Nature & Health graduates even appear to have a comparable understanding with Canadian biology majors with an evolution course attended, German pre-service biology teachers and Greek biology majors of the fourth year, while Flemish Science graduates have a comparable understanding as USA biology majors after having attended an evolution course. These results suggests that Flemish and, in particular, Dutch high level biology secondary education programs have a comparable effectiveness in promoting understanding of natural selection than various biology university programs in other countries, and hence perform rather well.

[Table 5 near here]

Understanding of specific Concepts of Natural selection

The theory of natural selection is challenging for students because it entails the understanding and integration of several CC and KC. In the present study we used the CINS, which was designed to measure the magnitude of knowledge of 10 KC. In Anderson et al.'s (2002) original sample of USA non-majors, PCA results indeed demonstrated strong support for the internal validity of the CINS's underlying measurement structure. In contrast to both Nehm and Schonfeld (2008) and Athanasiou and Mavrikaki (2014), our PCA results provided some additional support for the CINS's internal validity. Overall, the reported PCA findings from these different student populations indicate that the value of the CINS in measuring students' knowledge of specific CC and KC is not generalizable across different student populations, except for the key concepts *biotic potential*, *limited survival* and *change in a population*, whose two items load on the same component in all studies so far. Trying to explain the different PCA results between our student samples and others falls outside the scope of this study.

Comparing understanding of specific KC, Dutch secondary graduates were found to perform significantly better for seven of the eight concepts we considered to be correctly addressed by the CINS, including all four CC, which can probably also be explained by increased opportunities for knowledge transfer and deep conceptual learning of these specific concepts throughout upper secondary education (see above). The effectiveness of evolution instruction may also depend on the specific attention and time devoted to instruction of underlying fundamental concepts such as genetics and ecology. However, a detailed comparison of biology textbooks between Flanders and the Netherlands was not the focus of the current study.

Our results also showed that concepts of natural selection were employed to varying degrees by both Flemish and Dutch secondary graduates, with the relative ranking of the different concepts being highly comparable. These findings confirm previous findings regarding students' use of key concepts in explanations of natural selection, showing that some concepts are more challenging to students than others (Nehm & Ha, 2011, Athanasiou & Mavrikaki, 2014). For example, *change in a population* was the less elicited concept of the CINS, not only in both Flemish and Dutch students, but also in North-American biology majors (Nehm & Schonfeld, 2008).

Focusing on the level of understanding of the four CC of natural selection, our results suggest knowledge was rather weak in both student groups, and in particular in Flemish graduates, taking into account they are supposed to be the best possible performing secondary school students in Flanders and the Netherlands. In particular the proportion of students answering *both* items designed to measure understanding of the CC *origin of variation (33.5% and 44.3%, respectively)* and *variation inheritable (23.3% and 43.3%)* correctly, was unsatisfactory low. These findings also confirm previous findings in other student groups, showing that most commonly, learners seem to apply the CC of *differential survival* and *existence of variation*, while the *origin of variation* and *inheritance* tend to be less frequently used (Nehm & Ha, 2011; Nehm & Schonfeld, 2008; Opfer et al., 2012). This indicates that concepts linked to genetics are more challenging for learners, which may be due to the invisible nature of genes and/or the multitude of organizational levels needed to link genes to phenotypes (Göransson et al., 2020). In addition, as genetics and evolution were treated as separate topics in biology teaching in Flanders and the Netherlands (see introduction), this may also have contributed.

Alternative Conceptions in Flemish and Dutch secondary graduates

One of the major barriers to learning natural selection comes in the form of students' alternative or prior conceptions (Sinatra et al., 2008). The origin and cognitive structures that give rise to student alternative conceptions about natural selection are the subject of ongoing research (Kampourakis & Nehm, 2014; Nehm, 2018), but the actual alternative conceptions students may have about natural selection are well-documented. In the CINS, common alternative conceptions were used as distractors. Our results revealed that the *relative* frequency of alternative conceptions that were selected by Flemish and Dutch secondary graduates as the correct answer was almost identical. In contrast, a comparison of the relative frequency of the five most common alternative conceptions in Flemish/Dutch secondary graduates versus those in North-American biology majors, revealed a significantly different prevalence for some alternative conceptions. These findings support the conclusion of Nehm & Schonfeld (2008) that the prevalence of alternative conceptions of natural selection may differ among students from different cultural, ethnic and/or class background. Based on the results of the present study, we are not able to explain which of the latter factors specifically contributed to the different prevalence between Dutch/Flemish secondary graduates and Biology majors. It should be noted that the idea that organisms can intentionally become new species over time (#1) appeared to be the most common alternative conception in both Flemish and Dutch students, as well as in biology majors in North-America (see Nehm & Schonfeld, 2008).

Limitations of the study

Despite the relevance of the results obtained, the following limitations should be acknowledged. At the start of the present study in 2012, there were only few validated instruments available to measure understanding of natural selection: two open-response instruments (Bishop & Anderson, 1990; Nehm & Schonfeld, 2008), and the CINS (Anderson et al., 2002). We choose the latter as, being a multiple-choice test, it was very useful for investigating conceptual parameters in large groups of students. However, the CINS has some limitations (Nehm & Schonfeld, 2008, 2010). As a multiple-choice test, the CINS attempts to assess understanding of different KC of natural selection, but it cannot provide any measure of the ability of students with respect to the degree to which they can assemble the different pieces into a coherent and functional explanatory structure. In addition, the CINS cannot elicit all possible alternative conceptions, since students can only select alternative conceptions that were used as distractors. To more thoroughly evaluate explanatory models of natural selection and the prevalence of alternative conceptions, open-response tests are better suited. In addition, the CINS was not designed to assess evolutionary reasoning in multiple contexts. However, Nehm & Ha (2011) showed that the specific scenarios/contexts in which students are asked to reason, evoke different types, magnitudes, and arrangements of KC and alternative conceptions. Although Kalinoswki et al. (2016) developed and validated a new instrument, the CANS (Conceptual Assessment of Natural Selection) that does satisfy the criteria needed for thoroughly assessing how students think about the core concepts of natural selection, Fiedler et al. (2019) recently showed the CANS and the CINS, when both being used on the same students, nevertheless provide highly comparable results. In addition, we could not use a longitudinal approach to compare the effective learning gain throughout secondary education in both education systems.

Hence, longitudinal research in other secondary educational programs, using open-response tests (such as for example the ACORNS; Nehm et al., 2012) to more thoroughly assess and compare exploratory models of natural selection, is necessary to examine further the role of the specific place and treatment of natural selection in the biology curriculum in secondary education.

Educational Implications

Our findings suggest a number of implications for educational practice. First, in those secondary education systems where evolution and natural selection are still only taught as a final chapter after all other biological contents has been covered, understanding can be improved by a curricular revision in which the process of natural selection, and underlying concepts, will be introduced much earlier in the biology curriculum, and subsequently taught alongside other aspects of biology. Moreover, researchers as well as educational organisations have been highlighting the importance of exploring evolution and evolutionary processes already from a stage as early as kindergarten (Nadelson et al., 2009; NRC, 2012; Campos & Sá-Pinto, 2013). It should be emphasized that the specific place of natural selection in the biology curriculum in

Flanders has also been revised since the time of the current study, with natural selection already being introduced a first time in the fourth year of secondary education.

Second, our finding that, even in the best possible performing secondary graduates in Flanders and the Netherlands, comprehension of the four CC, was unsatisfactory low, confirms that there should be a much stronger focus on fostering a better understanding of these concepts in types of secondary education. This should preferentially be achieved by using specific 'teaching toward conceptual change' instructional strategies (see Sinatra et al. 2008; Tanner & Allen, 2005). Recently, Nehm (2018), exploring the conceptual difficulties inherent to teaching and learning about evolutionary change, suggested that understanding of the CC of natural selection can be improved by encouraging students during instruction to compare and contrast different examples of natural selection across taxa and trait polarity. Reorganizing common curricular examples into such contrasting cases (e.g. Darwin's finches vs. antibiotic resistance; loss of thorns in plants vs. loss of eyes in fish) provides an opportunity for students to build abstract cognitive models that transcend exemplar cases, and address the well-documented fragmentation and context specificity of students' evolutionary reasoning (Nehm & Ha, 2011). Evolutionary examples that highlight surface feature dissimilarity while emphasizing causal unity are one approach to this challenge.

In particular, the finding that the CC *origin of variation* and *variation inheritable*, appear to be the most challenging for students, confirms earlier findings that biology teachers should also pay more attention to fostering understanding of the underlying genetic processes (population variation, mutation and the genetic basis for diversity) and hence genetic literacy (Anderson et al., 2002; Miller et al. 2006). Students appear to be more likely to state that new traits arise as a result of an organism's needs or in response to environmental changes rather than occurring through multiple, random genetic processes, such as recombination of genes through sexual reproduction, gene shuffling and random mutations in genetic sequences, (Bishop & Anderson 1990; Geraedts & Boersma 2006). In addition, recent findings emphasize that to fully comprehend natural selection, it is essential to also understand the general abstract threshold concepts that underlie the biological processes such as, for example, randomness or probability (Fieldler et al., 2017, 2019; Göransson et al., 2020). Hence, statistical literacy should also be integrated into evolution education efforts.

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Appendices: Tables A1, A2, A3

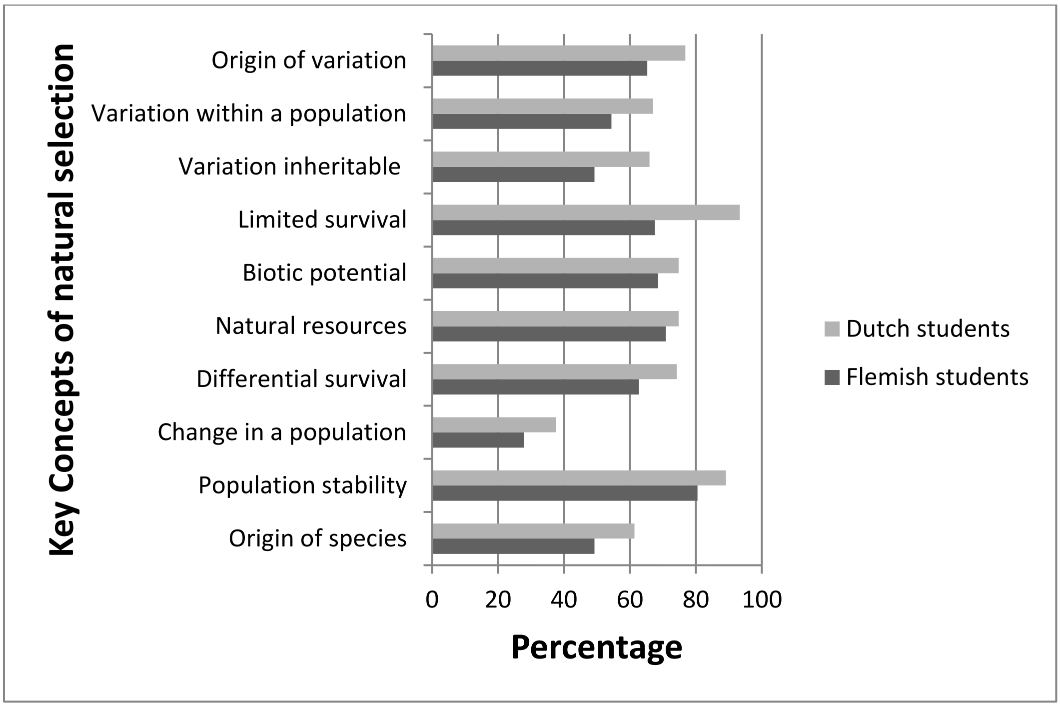

Figure 1. Percentage of correct answers (at the population level) to the two items designed to represent each of the 10 key concept in the CINS for Dutch and Flemish secondary graduates.

Table 1. Coefficient estimates in the final regression model examining the effect of country of secondary education (Flanders vs the Netherlands) on CINS scores in university freshmen (Biomedical and Veterinary sciences), while controlling for the parameters: field of study, year when tested, age, gender, global grade score secondary education, and the score on the Zoology exam in the bachelor program.

Coefficients	**Estimate**	**Std. Error**	**t value**	**p-value**
Intercept	18.57	2.95	6.29	< 0.001(***)
Country	-2.65	0.45	-5.93	< 0.001(***)
Zoology exam	0.28	0.06	4.73	< 0.001(***)
Age	-0.38	0.14	-2.63	< 0.01 (**)
Grade score 80-89%	1.40	0.68	2.08	< 0.05 (*)

Table 2. Coefficient estimates in the final regression model (see legend Table 1 for tested parameters) when including only students graduating from high level biology secondary education in Flanders and the Netherlands.

Coefficients	**Estimate**	**Std. Error**	**t value**	**p-value**
Intercept	11.11	0.87	12.72	< 0.001(***)
Country	-2.10	0.51	-4.08	< 0.001(***)
Zoology exam	0.29	0.07	4.19	< 0.001(***)
Grade point 80-89%	1.77	0.82	2.15	< 0.05 (*)

Table 3. A varimax rotated PCA of the 20 CINS items produced six components with eigenvalues >1. High loadings on each component are highlighted and indicated in bold (as per Andersson et al., 2002). (cc) = core concept of natural selection.

Item	**Component**					
	1	2	3	4	5	6
Origin of variation (cc)						
Item 6	**0.52**	0.28	0.07	0.06	0.32	0.17
Item 19	**0.74**	0.04	0.08	0.13	-0.09	0.12
Existence of variation (cc)						
Item 9	0.14	0.13	0.11	0.06	0.10	**0.65**
Item 16	0.09	0.04	-0.14	0.02	0.04	**0.75**
Variation inheritable (cc)						
Item 7	0.12	0.12	0.26	**0.52**	0.24	-0.08
Item 17	**0.38**	0.01	0.08	**0.38**	0.31	-0.02
Limited survival						
Item 5	-0.07	**0.47**	**0.46**	0.20	-0.16	0.06
Item 15	0.13	**0.61**	-0.03	0.19	-0.10	0.05
Biotic potential						
Item 1	0.14	-0.20	**0.72**	-0.04	0.12	0.16
Item 11	0.10	0.02	**0.69**	0.03	0.05	-0.16
Natural resources						
Item 2	-0.09	**0.41**	0.36	0.19	-0.01	0.39
Item 14	-0.08	-0.01	-0.01	0.14	**0.76**	0.01
Differential survival (cc)						
Item 10	0.35	**0.41**	-0.12	-0.25	0.25	-0.15
Item 18	0.16	**0.64**	0.06	0.01	0.13	0.01
Change in a population						
Item 4	0.17	0.05	0.03	**0.80**	0.01	0.12
Item 13	0.22	0.05	-0.08	**0.74**	0.02	0.07
Population stability						
Item 3	-0.01	**0.56**	-0.14	-0.04	0.14	0.20
Item 12	0.09	0.10	0.09	0.01	**0.55**	0.11
Origin of species						
Item 8	**0.51**	0.05	-0.03	0.30	0.08	-0.02
Item 20	**0.73**	0.08	0.14	0.17	-0.05	0.12

Table 4. Overview of the most common concepts used in the sample of Flemish and Dutch university freshmen and in USA biology majors*. The percentages of correct responses to the two items representing each specific concept are indicated. N= 550 responses (by 275 Flemish students), 194 responses (by 97 Dutch students) and 200 responses (by 100 USA students). Concepts that are considered 'correctly addressed (= both items loading on the same PCA component)' by the CINS (see Table 3) are in bold. Core concepts of natural selection are underlined.

Flemish students	Dutch students	USA biology majors*
1. Population stability (80.5)	**1. Limited survival (93.3)**	**1. <u>Differential survival (82.0)</u>**
2. Natural resources (70.9)	2. Population stability (89.2)	**2. Limited survival (74.5)**
3. Biotic potential (68.5)	**3. <u>Origin of variation (76.8)</u>**	3. Natural resources (70.5)
4. Limited survival (67.6)	4. Natural resources (74.4)	4. Population stability (67.0)
5. <u>Origin of variation (65.3)</u>	**4. Biotic potential (74.4)**	<u>5. Existence of variation (67.0)</u>
6. <u>Differential survival (62.7)</u>	**6. <u>Differential survival (74.2)</u>**	**6. Biotic potential (65)**
7. <u>Existence of variation (54.4)</u>	**7. <u>Existence of variation (67.0)</u>**	7. <u>Variation inheritable (61.5)</u>
8. <u>Variation inheritable (49.3)</u>	**8. <u>Variation inheritable (66.0)</u>**	8. Origin of species (50.0)
8. Origin of species (49.3)	**9. Origin of species (61.3)**	9. <u>Origin of variation (46.5)</u>
10. Change in a population (27.8)	**10. Change in a population (37.6)**	**10. Change in a population (40.0)**

*Nehm & Schonfeld, 2008.

Table 5: Average score of various student samples for the 20 questions of the CINS.

Student sample	Mean score
Flemish high level biology secondary graduates	12.5
Dutch high level biology secondary graduates	14.4
US non-majors (Anderson et al. 2002)	8.2
US non-majors (Anderson et al. 2002)	10.4
US first year biology majors (Nehm & Schonfeld, 2008)	12.6
US first year students enrolled in introductory biology course (Fiedler et al., 2017)	15.5
Canadian biology majors - no evolution course attended (Frasier & Roderick, 2011)	12.0
Canadian biology majors - evolution course attended (Frasier & Roderick, 2011)	14.6
Greek non-majors – biology course attended (Athanasiou & Mavrikaki, 2014)	9.6
Greek biology majors of first, second and third year (Athanasiou & Mavrikaki, 2014)	11.6
Greek biology majors of fourth year (Athanasiou & Mavrikaki, 2014)	15.1
Greek postgraduate students (Athanasiou & Mavrikaki, 2014)	14.2
German pre-service biology teachers (Grosschedl et al., 2014)	14.0

Table A1. This table shows an example of one of the three reading passages/contexts of the CINS, in particular about Galapagos finches, and two of the eight items related to this particular context. Item 6 is one of the two items representing the CC *origin of variation*, and item 8 is one of the two items representing the CC *variation inheritable*. The correct answer is indicated in bold. For each distractor, the number of the specific alternative conception is indicated between parentheses.

Conceptual Inventory of Natural Selection

Your answer to these questions will assess your understanding of the Theory of Natural Selection. Please choose the answer that best reflects how a biologist would think about each question.

Galapagos finches

Scientists have long believed that the 14 species of finches on the Galapagos Islands evolved from a single species of finch that migrated to the islands one to five million years ago (Lack, 1940). Recent DNA analyses support the conclusion that all of the Galapagos finches evolved from the warbler finch (Grant, Grant & Petren, 2001; Petren, Grant & Grant, 1999). Different species live on different islands. For example, the medium ground finch and the cactus finch live on one island. The large cactus finch occupies another island. One of the major changes in finches is in their beak sizes and shapes.

Choose the one answer that best reflects how an evolutionary biologist would answer.

6. How did the different beak types <u>first</u> arise in the Galapagos finches?

a) The changes in the finches' beak size and shape occurred because of their need to be able to eat different kinds of food to survive. (# 2)
b) Changes in the finches' beaks occurred by chance, and when there was a good match between beak structure and available food, those birds had more offspring.
c) The changes in the finches' beaks occurred because the environment induced the desired genetic changes. (# 6)
d) The finches' beaks changed a little bit in size and shape with each successive generation, some getting larger and some getting smaller. (# 2)

7. What type of variation in finches is passed to the offspring?

a) Any behaviors that were learned during a finch's lifetime. (# 18)
b) Only characteristics that were beneficial during a finch's lifetime. (# 12)
c) All characteristics that are genetically determined.
d) Any characteristics that were positively influenced by the environment during a finch's lifetime. (# 15)

Table A2. The number and percentage of the concepts of natural selection extracted from the CINS from Flemish (FL, N=275) and Dutch (DU, N=97) secondary graduates.

CINS concept	Description of concept with CINS question numbers	Responses per question		Total Responses		% correct responses	
		FL	DU	FL	DU	FL	DU
Origin of variation	Random mutations and sexual reproduction produce variations: while many are harmful or of no consequence, a few are beneficial in some environments (6B, 19C)	189, 170	70, 79	359	149	65.3	76.8
Existence of variation	Individuals of a population vary extensively in their characteristics (9D, 16C)	120, 179	63, 67	299	130	54.4	67.0
Variation inheritable	Much variation is heritable (7C, 17D)	195, 76	85, 43	271	128	49.3	66.0
Limited survival	Production of more individuals than the environment can support leads to a struggle for existence among individuals of a population, with only a fraction surviving each generation (5D, 15D)	159, 213	91, 90	372	181	67,6	93.3
Biotic potential	All species have such great potential fertility that their population size would increase exponentially if all individuals that are born would again reproduce successfully (1C, 11B)	188, 189	70, 75	377	145	68.5	74.7
Natural resources	Natural resources are limited; nutrients, water, oxygen, etc. necessary for living organisms are limited in supply at any given time (2A, 14D)	225, 165	96, 49	390	145	70.9	74.7
Differential survival	Survival in the struggle for existence is not random, but depends in part on the hereditary constitution of the surviving individuals. Those individuals whose surviving characteristics fit them best to their environment are likely to leave more offspring than less fit individuals (10C, 18B)	197, 148	82, 62	345	144	62,7	74.2
Change in a population	The unequal ability of individuals to survive and reproduce will lead to gradual change in a population, with the proportion of individuals with favorable characteristics accumulating over the generations (4B, 13B)	55, 98	31, 42	153	73	27.8	37.6

Population stability	Most populations are normally stable in size except for seasonal fluctuations (3B, 12A)	250, 193	93, 80	443	173	80.5	89.2
Origin of species	An isolated population may change so much over time that it becomes a new species (8A, 20B)	129, 142	50, 69	271	119	49.3	61.3

See text and Table 5 for details on percentage calculations and ranking.

Table A3. The number, percentage and rank of 23 alternative conceptions extracted from the CINS from Flemish (FL) and Dutch (DU) secondary graduates.

Misconception Number *	Misconception (with CINS item number)	Responses per item		Total Responses		Frequencies		Rank	
		FL	DU	FL	DU	FL	DU	FL	DU
1	Organisms can intentionally become new species over time (an organism tries, wants, or needs to become a new species) (8C, 8D, 20A, 20D),	114, 30, 31, 95	38, 7, 6, 22	270	73	12.2	12.7	1	1
2	Mutations are intentional: an organism tries, needs, or wants to change genetically (6A, 6D, 19A, 19B)	40, 14, 66, 2	7, 6, 11, 0	122	24	5.5	4.2	10	11
3	Organisms can always obtain what they need to survive (2B, 2C, 2D, 14A, 14B,14C)	39, 2, 9, 5, 99, 6	1, 0, 0, 1, 46, 1	160	49	7.2	8.5	6	5
4	Populations level off (1B, 11D, 1D)	24, 52, 53	7, 15, 20	129	42	5.8	7.3	8	8
5	Mutations occur to meet the needs of the population (4D, 13D)	120, 97	31, 29	217	60	9.8	10.4	2	2
6	Mutations are adaptive responses to specific environmental agents (6C, 15C, 19D)	42, 43, 37	14, 4, 7	112	25	5.1	4.3	11	10
7	Learned behaviours are inherited (4C, 13C)	89, 63	28, 18	152	46	6.9	8.0	7	6
8	Changes in a population occur through a gradual change in all members of a population (4A, 13A)	11, 17	7, 8	28	15	1.3	2.6	19	13
9	Variations only affect outward appearance, do not influence survival (9B, 9C, 16B)	69, 75, 59	22, 12 , 15	203	49	9.2	8.5	3	4
10	Populations always fluctuate widely/randomly (3C,12D)	6, 54	3, 13	60	16	2.7	2.8	12	12
11	Fitness is equated with strength, speed, intelligence or longevity (10A, 10B, 18A, 18C, 18D)	27, 17, 25, 22, 80	1, 8, 6, 5, 24	171	44	7.7	7.6	5	7
12	When a trait (organ) is no longer beneficial for survival, the offspring will not inherit the trait (7B)	45	8	45	8	2.0	1.4	13	15
13	There is often physical fighting among one species (or among different species) and the strongest ones win (5B, 15B)	10, 19	3, 3	29	6	1.3	1.0	18	17
14	Populations decrease (3D, 12C)	97, 26	29, 4	123	33	5.6	5.7	9	9
15	Traits that are positively influenced by the environment will be inherited by offspring (7D, 17B, 17C)	28, 84, 87	2, 18, 33	199	53	9.0	9.2	4	3
16	Organisms work together (cooperate) and do not compete (5A, 5C, 15A)	1, 19, 0	0, 3, 0	20	3	0.9	0.5	20	20
17	All members of a population are nearly identical (9A, 16A)	11, 31	0, 12	42	12	1.9	2.1	14	14
18	Traits acquired during an organism's lifetime will be inherited by offspring (7A, 17A)	7, 28	2, 3	35	5	1.6	0.9	16	19

19	All populations grow in size over time (3A, 12B)	16, 2	1, 0	18	1	0.8	0.2	21	22
20	Organisms with many mates are biologically fit (10D)	34	6	34	6	1.5	1.0	16	17
21	Not all organisms can achieve exponential population growth (11C)	8	2	8	2	0.4	0.3	21	22
22	Organisms only replace themselves (1A, 11A)	9, 26	0, 5	35	5	1.6	0.9	18	15
23	Organisms in a population share no characteristics with others (16D)	0	0	0	0	0	0	0	0
		Total		2212	577				

See text for details on percentage calculations. * number corresponds to the rank in USA biology majors (see Nehm & Schonfeld, 2008).