Replication, Pseudoreplication and Model Experiment in the Study of Population Genetics

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Abstract. The paper is dedicated to the problem of true and pseudoreplication of a biological experiment, in particular in the educational process. It was found that this issue is relatively new and actual for the methodology of biological experiments in general. Its solution in science ensures the veracity of the results obtained and the relevancy of the formulated conclusions. In national biology teaching methods at school, the problem of true and pseudoreplication of the experiment was not reflected. The author covers an issue of true replication teaching when setting up a model experiment to study genetic-evolutionary processes in populations. The paper discloses the experience in evolution of a model experiment and its development aimed at formation of ideas about technical and biological replication by the example of study of the genetic structure of an ideal population in generations. For this purpose, there was developed a web page that allows to automatically implement technical and biological experiment replication. There was described an experience of approbation of the proposed variant of the experiment, and its difficulties and advantages were revealed.

1 Introduction

The educational subject «Biology» is a didactically adapted system of scientific biological knowledge. As came about from Aristotle times, the natural sciences, and the biology is undoubtedly one of them, have an experiment as one of the main research methods. It is this that allows, on the basis of the various factual material obtained, to make wide generalizations, to proceed to the establishment of connections, patterns that allow deeper penetration into the essence of the phenomena under study. A lot has already been said about the experiment in biological science, about its types, methods, requirements for organization, limitations and difficulties of application. A huge number of scientific works are dedicated to the issues of experimental method history in biology. We, however, were interested and are interested, at this point in time, in the experimental method from the point of view of the possibilities of its use in biological education. In addition, having narrowed the subject field we are interested in, it is worth pointing out that a model experiment occupies a special place in high school. It allows to create models of real objects and to prototype the processes occurring with them in reality. In previous works, the author highlighted some aspects of this issue [1].

In the process of studying the topic of the display of experimental method at the level of school biological education, transformation of ideas about how it is possible to implement the experimentation with complex biological systems, including those inaccessible to the student for direct study, we initially started out from the following. An educational biological experiment should maximally meet the requirements that are put forward for scientific biological experimentation. These, in particular, are the reliability in essence, the rule of single difference, replication, mass nature. From the mid-XXth century, a lot of attention has been paid to the organization of a school biological experiment, moreover to its various types, differing both in the object of research (botanical, zoological, physiological tests, functional tests, etc.), and in the form of carrying out under the conditions of school laboratory, class (demonstrational, laboratorial, mental) [2-10]. Regardless of the type and form of carrying out, all various types of educational biological experiment must meet the abovementioned requirements in order that the results obtained were maximum consistent.

The biggest difficulties in the educational process are caused by the observance of such requirements as replication and mass nature. In other words, to ensure the veracity of results, the educational experiment should be conducted several times using a sufficiently large number of objects.

It is difficult to implement both the first and the second condition in the educational process due to the following reasons:

- firstly, the temporal limitations of the educational process;
- secondly, due to the inaccessibility of objects for study in the required quantity;
- thirdly, in the principle of inaccessibility of some objects and processes for direct study, primarily due to

their objective specificity: either too small (organic molecules, cells, viral particles), or too large (populations).

Let's turn our attention to these reasons, possible ways of their elimination.

Analysis of scientific literature in regards to the experimentation in biology showed that since the end of the 1980s, one of the actively discussed problems became the problem of pseudoreplication in ecological and biological research. In the classical variation, from the moment of publication of the first paper on this topic, pseudoreplication was considered as a negative experimental practice [11]. Even now, one of the criteria by which reviewers evaluate the submitted paper for a journal indexed in the authoritative international scientometrical Scopus and Web of Science databases is the true and pseudoreplication of the experiments conducted [12]. Please note that at the moment the scientific community is still not so categorical in regards to pseudoreplication of experimental research. Discussions are being conducted on the issue of reality and contrivedness of the problem [13-15].

We proceed from the assumption that the biology teaching methods cannot stay on the sidelines of the problems actively discussed in biological science. Moreover, this question lies in the plane of the science methodology. The mastery of methodological knowledge and the ability to apply them is the basis for the formation of a system of biological knowledge for senior high school students. The author's early works were devoted to this question [16]. So, we consider the question of to what extent in school experimentation in biology it is necessary to take into account the requirements that S. Hurlbert identified as the problem of pseudoreplication of experimental research in science, as definitively solvable in the direction of their observance. At the same time, given that the educational subject still differs from the basic science in that it is a didactically adapted version of it, it is necessary to achieve a double effect in organization of a school biological experiment. The first effect is that the results of the educational experiment should be maximum consistent, obtained by true replication. The second effect is that the use of true replication should be maximum ergonomical. Ergonomic in time, cost and complexity.

The purpose of this paper is to demonstrate the capabilities of the school model experiment in study of the genetic structure of populations in time while meeting the requirements of true technical and biological replication of experimental objects. Or, in another way: to consider the possibilities of solving the problem of pseudoreplication in the school biological experiment aimed at study of the genetic-evolutionary processes in populations.

We consider it logical to state the essence of the declared problem in the sequence of answers to the following questions: what is the essence of replication in biological research? What kind of experimental replication occurs, what goals does it pursue? What is the essence of pseudoreplication in biological research, what is the history of the problem? Is pseudoreplication as scary as it might seem? How to ensure true replication in a model experiment by studying complex ideal and real biological objects in school biology (by the example of genetic structure of the population)?

The answers to the set of the abovementioned questions make sense if we answer one of the most important questions: «Why should we conduct at school a model experiment in study of the genetic structure of the population?» It can be concretized in the following way: what is the purpose of this experiment, if it is possible manage without it, to replace it? What fundamental knowledge and skills do pupils acquire when performing this experiment? Why the model experiment in study of population genetics is considered by us as the most important instrument for the formation of not only genetic-evolutionary concepts, but also of metasubject skills?

2 Technique and methods

Main issue. Why should we conduct at school a model experiment in study of the genetic structure of the population?

Study of the issue of model experimentation with the genetic structure of the population during 2015-2020 convinced us that its goal is the obtention by the pupils of a direct subject and mediated activity result. Subject result – 1) mastery of the essence of the law of genetic balance and the conditions under which it is consistent; 2) understanding of the mechanism of influence of evolutionary factors, such as natural selection, gene drift, gene flow, mutation process on the genetic structure of the population; understanding of the mechanisms forming the basis of micro- and macroevolutionary processes. The fundamental significance of the law of genetic equilibrium (Hardy-Weinberg) is that it is the central law of population genetics, it is based on the application of statistical methods in genetics [17].

Activity result – mastery by pupils of the method of the result achievement maximally approximate to the consistent value. In other words, this refers to the formation of a metasubject ability to plan and set up an experiment methodologically correctly, to collect data, to process them and to formulate reasonable conclusions.

Before the development of methods of the model experiment in study of the genetic structure of the population, we posed two questions:

1. Is it necessary to conduct a model experiment when studying the law of genetic equilibrium and the conditions for its consistency?

2. Can a model experiment be replaced with other educational methods?

The answer to the first question is: no, not necessarily. It is possible to limit to the demonstration of the multimedia presentation and video on this topic.

The answer to the second question is: yes, it is possible. An alternative is familiarization with theoretical material on a printed basis about the factors of change in the genetic structure of a population, overlearning of Hardy-Weinberg equations, teaching of the solution of problems on determination of the genetic structure of a population.

The answers to both questions demonstrate that in the alternative version, at the best, only one result will be achieved – a subject one. Without performing experimental actions, it is extremely difficult to form such elements of methodological knowledge as a variant of experience, replication, sampling. In addition, it has to be considered that the law of genetic equilibrium is a law, the substantial part of which consists of abstract categories not attached to a specific biological object (abstract homozygotes and heterozygotes, dominant and recessive alleles, conditions for the veracity of the law). And the law itself is applicable to some really non-existent ideal object, or, conversely, is not applicable to any really existing object (real population).

The abovementioned reasons are the answer for us to the main question, namely: 1) model experimentation contributes to the mastery by the pupils of abstract biological categories on concrete material objects; 2) allows to visualize the processes in an ideal population non-existent in reality; 3) allows to simulate the changes taking place in real populations over several generations. Thus, for educational purposes, the time frame of the actually occurring processes is condensed; 3) allows to vary the replications and variants of the experiment with minimal material costs; 4) allows to teach true replicates (replications) of the experimental impact; 5) allows to artificially quickly change the conditions (factors) affecting the population, including acting stochastically [18].

Issue No. 2. What is the essence of replication in biological research?

A person possessing a basic level of biological knowledge within the scope of the school curriculum of complete secondary education, the term «replication» is known as a process related to the molecular level of organization of a living being. In the English-language scientific biological literature, the term «replication» is used not only in the meaning of the synthesis of new nucleotide sequences, but also in the meaning of the replicate of experimental attempts. In other words, the principle of replication in experiment is the well-known principle of replication. The last term is more widely used in domestic scientific works.

Replication in biological research can be technical and biological [19].

Technical replicates give us these things:

1. They give us an accurate measurement they give this particular object.
2. If we want to tell more about this object or we do not want to generalize the data and transfer it to the population - a technical experiment is what we need.
3. They will also tell us how accurately we performed the measurements.
4. «If we wanted to publish a paper about how awesome our new method is, we'd use technical replicates» [19].
5. If the experimental technique is transformed, different samples are taken simultaneously from one object, then technical replication will also take place, since they tell us about an individual.

In the biological replicates each measurement comes from different sample that comes from different objects.

Biological replicates give us these things:

1. Biological replicates tell us about a trait that occurs in a group. In biological replicates, each measurement comes from different samples or is obtained differently from one object.
2. You can mix biological and technical replicates, but the wisdom of doing this depends on the type of the experiment. Sometimes you get more bang for your buck if you add more biological replicates and ignore technical replicates.

So, the difference between technical and biological replication is as follows: technical replicates are just repetition of the same experiment on the same person.

1. Biological replicates use different biological sources of samples (i.e. different people, different plants, and different cell lines) [19].

When choosing the type of replication of a biological experiment, it is necessary to proceed from the purpose in view. If it is planned to describe a specific object, whether it be an individual, a population, or to research a method, it is necessary to use technical replication. If the goal is to study a group of objects, it is necessary to choose biological replication.

Issue No. 3. What is the history of the problem of pseudoreplication in biological research?

The problem of pseudoreplication was raised for the first time in 1984 by S. Hurlbert, who published a critical analysis of 156 experimental scientific papers in English-language editions published in 1960-1980. He came to the conclusion that in 27% of cases there was one of two variants: 1) the experimental influence was applied in one replication; 2) the experimental replications were not statistically independent. Such errors were called pseudoreplication by S. Hurlbert. M. Kozlov notes that in Russian academic journals in 1998-2001 the part of papers based on pseudoreplication turned out to be twice as high (47%) than in the English-language periodicals for 1960-1980, i.e. before the publication of S. Hurlbert's paper. This situation was considered as non-normal, at the same time it was pointed out that the reason for the pseudoreplication lies not only in errors in experiment planning, but also in the incorrect application of statistical analysis to the results of a well-planned experiment [20].

After the publication of S. Hurlbert's paper in 1984 during the period from 1987 to 2001, according to M. Kozlov: 1) the term «pseudoreplication» firmly came into the ecological scientific lexicon of foreign authors, the problem of pseudoreplication in foreign ecological studies is actively discussed; 2) the number of foreign publications based on pseudoreplication began to decrease.

Back in 2003, M. Kozlov paid attention to the fact that the concept of pseudoreplication is completely unknown to the overwhelming majority of Russian ecologists. In addition, the author emphasized that S. Hurlbert's work was never cited in Russian-language periodicals, against the background of more than 2000 references (2015 references as of 2001) in English-language publications. M. Kozlov repeatedly published his works on standing up for the position that the problem of pseudoreplication is a problem of the world scientific community, which should be treated with all possible seriousness [20, 14].

The English term «pseudoreplication» does not have a direct analogue in Russian, since it primarily denotes a process – an erroneous choice of replicates for assessment of intragroup variability in statistical analysis [11, 14]. In this regard, direct translation of terminology is difficult enough; the authors provide English equivalents of key concepts. «In medical experiments, where they are designated to as «spurious replication», «trial inflation», or «the unit of analysis problem or error» (Whiting-O'Keefe et al., 1984; Andersen, 1990; Altman, Bland, 1997). Although the concept of «pseudoreplication», which is most adequately translated as «statistical analysis based on pseudoreplication», is not found in all works listed above, and we do not agree with all the conclusions of the indicated authors, all the cited studies are united by a serious approach to the problem» [14].

Issue No. 4. Is pseudoreplication as scary as it might seem?

In Russian-language sources, the attitude to the problem specified by S. Hurlbert and supported by M. Kozlov can be characterized as far-fetched and already well-known and studied (V. Nalimov, A. Lyubishchev, A. Bakanov, N. Plokhinskiy, T. Golikova). The Russian-speaking authors agree that there are two indisputable theses in the ideas of S. Hurlbert:

1. «it is not always correctly to extend the conclusions, obtained in the study of private samplings, to the entire general population;
2. assessment of the degree of factor influence may turn out to be erroneous if the studied effect is not properly localized, and the compared data are taken from insufficiently randomized sources» [13].

The conducted analysis of literary sources [21-27] on the problem allowed us to single out the «pros» and «cons» of the consideration of the problem of pseudoreplication as significant for biological research. The analysis results are presented in Table 1.

Table 1. Pseudoreplication - a real problem in biological research

«Pro» arguments	«Con» arguments
1. Each object in the sampling is a functional part of the whole, and not a separate element of a set. In a number of studies, the results and conclusions obtained for discrete objects apply to the entire population, which does not correspond to one of the requirements for biological experimentation – consistency in essence. 2. During the experiment, there is a multiple determination of reaction of the same organism in the course of sampled counts. As an alternative, the same sampling is studied in different time intervals. In this case, living objects (their populations) are pseudoreplications. 3. Two main problems of pseudoreplication is an insufficient mass nature of experimental objects and their initial incomparability with each other. In the first case, the researcher receives insufficient data for the consistent statistical result. In the second case, the problem has an objective causality due to the initial uniqueness of living objects.	1. Each object in the sampling is discrete and individual. 2. Factors acting independently on the sampling, act on a set of separate biological objects, and not on an integral object. The specificity of a biological experiment lies in the uniqueness of the objects and, in certain cases, in the impossibility of repeating the experiment in an accurate manner. 3. Living objects react to the actions of factors independently on a physical level, and thus they are statistically independent. In a majority of research variants, living objects are true replications. 4. The specificity of living objects in their uniqueness and originality. Some ecological research involves study of the reactions of individuals or parts to the impact. In a number of studies, it is not possible to repeat a unique biological object, whether it be an individual or a population. 5. The problem of pseudoreplication is artificial, since technical and biological replication is distinguished in biology. The attempt to apply the goals and requirements of technical replication to biological is a prime cause of the issue of pseudoreplication in biological experiments. 6. According to one of the points of view, the attention of English-speaking authors to the problem of pseudoreplication is explained by several reasons: - the desire to join the campaign of criticism and to incriminate colleagues in pseudoreplication; - the attempt to divert the stigma of pseudoreplication from their work and the work of colleagues; - as a warning signal to the reviewer that the author is acquainted with the work of S. Hurlbert, therefore there should be no comments on the paper [6].

Issue No. 5. How to ensure true replication in a model experiment in school biology?

In previously published materials [1, 16], we described the method of model experimentation developed for senior high school students to study the supraorganismal levels of life organization, namely, population-specific.

The development of a model experiment methodology aimed at the study of the essence of genetic-evolutionary changes in the population by pupils, and its improvement during 2015-2020, was carried out by us in a staged manner. This was dictated by the objective and subjective difficulties of implementation of a model experiment into teaching practice.

At the first stage, we used only material models of gene alleles, created models of genotypes in a manual way, and, respectively, models of parental and daughter populations in generations. Mathematical calculations were performed without the use of a computer, the participants in the experiment manually calculated the frequencies of genotypes and alleles in populations, and presented the results obtained in the graphical representation.

At the second stage, we combined material modelling and use of the computer. Work with material models consisted of carrying out of the experiment itself, creation of a model of the parental population in manual way, and combination of the gene alleles at random (this is how panmixia was simulated). The participants entered the results of the experiments into a table on the developed web pages. With the help of a computer, the obtained frequencies of alleles and genotypes were automatically calculated. In automatic mode, the results of the experiment were optionally presented in the graphical representation.

But both variants did not allow to work with a large number of experimental objects. That is, it was impossible to comply with the condition of mass nature. The reasons are as follows:

1. It is physically impossible in the course of the educational process to explore a large number of material model objects – homozygous dominant, homozygous recessive and heterozygous individuals. The work was accompanied by the enormous time spent on manual modelling and counting of randomly formed pairs of alleles. Such a calculation had to be carried out both within one generation, and in several replications. Note that in this variant we are talking about the difficulties with the technical replication of the experiment.

2. The use of material models was limited to elementary material costs for the manufacture of model elements. The maximum number of individuals whose genotype models were used in the experiment was equal to 50. In the case of diallelic inheritance of a trait (as the simplest variant of inheritance), the number of alleles was equal to 100. Let's point to the fact that in the classrooms there were carried out parallel experiments on the study of influence of different factors of the dynamics of the population genetic structure, the work was carried out in small groups, each of which worked with a separate set of elements for modelling. There were 5 such groups. The first group studied the genetic structure of an ideal population in generations. The second group studied the effects of gene drift. The third group studied the essence of the gene flow phenomenon. The fourth group studied the influence of natural selection on the genetic structure of the population. The fifth group studied the role of the mutational process in the dynamics of the genetic structure of the population. In total, at least a set of 500 material elements was needed for modelling.

We place the emphasis on the fact that even with 50 simulated members of the population, we obtained results that allowed to illustrate the essence of genetic transformations in populations in the absence of any factors and in their presence.

In work on the improvement of the experimental methodology, we tried to: 1) get closer in school modelling of genetic-evolutionary processes to the real process taking a course in populations; 2) take into account significant differences and commonality between scientific and educational experiment. Particularly, this was expressed in the fact that it was necessary to:

1. Cover by the experiment the maximally large number of individuals. It has been assumed that the hundreds and thousands of individuals could be the experimental objects.

2. Reduce the amount of routine work for pupils on the calculation of the resulting genotypes and alleles in one generation.

3. Simulate a larger number of replications (replicates) of the experiment, which would increase the veracity of results and their closeness to the mathematical formula of Hardy-Weinberg. We also set the task to provide the possibility for technical and biological replication of an experiment on one topic.

3 Results

Taking into account the abovementioned tasks, we have developed a web page http://mybio.education/mod/exp6/en/index.html# (Model experiment 1. Study of the genetic structure of the ideal population (third variant).

Using the tools of this web page, we can conduct an experiment on the modelling of a structure of an ideal population in the absence of such factors as natural selection, gene flow, gene drift, mutations. Note that this is the third variant for conduction of a model experiment on the stated topic. The first two are displayed on the following pages: http://mybio.education/mod/exp1/en/index.html and http://mybio.education/mod/exp2/en/index.html.

What is the difference between the proposed third variant?

First. In two early variants, the number of individuals was limited by the physical ability to manually count the resulting pairs of alleles and the number of material elements for modelling. The studied population in the proposed variant can be very large – several hundreds, thousands, millions of individuals. This contributes to the implementation of the first of the tasks pursued by us – an increase in the number of objects used in the model experiment. And in this case, it can be considered as a step towards the increase in the veracity of the experimental results. And thus, the maximum convergence with the actually occurring genetic and evolutionary transformations in the population. For example, for an experiment, you can take several tens of thousands of individuals and several million (Fig.1, Fig.2).

Model experiment 1. Study of the genetic structure of the ideal population (third option)

1. In column 2 for the parent generation P, we introduce the number of pairs of two-body gene alleles (in other words, the number of individuals).
2. We are determined by the ratio of the dominant (A) and recessive (a) alleles, and we introduce their values in columns 9 and 10 for the parent generation P.
3. Click the "Calculate" button.
4. Click the "Calculate" button opposite the lines F1, F2, F3, F4, F5.
5. Click on the "Show Graphs" button.
6. Based on the analysis of the obtained graphs and diagrams, formulate the conclusions of the plan:
   - Change in the frequency of genotypes in generations;
   - Change in the ratio of gene frequencies in generations;
   - The direction of evolutionary changes in the population.

Table 6. Genetic structure of the ideal population

Generation	Number of individuals	Distribution of genotypes						Gene frequencies		
		AA		Aa		aa		A(p)	a(q)	
1	2	3	4	5	6	7	8	9	10	
P	20000	10910	0.545	6180	0.309	2910	0.145	0.7	0.3	Calculate
F1	20000	10893	0.545	6214	0.311	2893	0.145	0.738	0.380	Calculate
F2	20000	10883	0.544	6234	0.312	2883	0.144	0.738	0.380	Calculate
F3	20000	10890	0.544	6220	0.311	2890	0.144	0.738	0.380	Calculate
F4	20000	10823	0.541	6354	0.318	2823	0.141	0.736	0.376	Calculate
F5	20000	10869	0.543	6262	0.313	2869	0.143	0.737	0.379	Calculate

Show Graphs

Fig.1 Results of a model experiment with a number of 20000 individuals, allele frequencies p (0, 7) and q (0, 3).

Model experiment 1. Study of the genetic structure of the ideal population (third option)

1. In column 2 for the parent generation P, we introduce the number of pairs of two-body gene alleles (in other words, the number of individuals).
2. We are determined by the ratio of the dominant (A) and recessive (a) alleles, and we introduce their values in columns 9 and 10 for the parent generation P.
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   - The direction of evolutionary changes in the population.

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Generation	Number of individuals	Distribution of genotypes						Gene frequencies		
		AA		Aa		aa		A(p)	a(q)	
1	2	3	4	5	6	7	8	9	10	
P	2000 000	1087149	0.544	625702	0.313	287149	0.144	0.7	0.3	Calculate
F1	2000000	1087690	0.544	624620	0.312	287690	0.144	0.737	0.379	Calculate
F2	2000000	1087753	0.544	624494	0.312	287753	0.144	0.737	0.379	Calculate
F3	2000000	1087745	0.544	624510	0.312	287745	0.144	0.737	0.379	Calculate
F4	2000000	1087290	0.544	625420	0.313	287290	0.144	0.737	0.379	Calculate
F5	2000000	1087641	0.544	624718	0.312	287641	0.144	0.737	0.379	Calculate

Show Graphs

Fig.2 Results of a model experiment with a number of 2000000 individuals, allele frequencies p (0, 7) and q (0, 3).

Second. The user (student) can independently enter the initial allele frequencies in the graph for the parental population. In the first two variants, the allele frequencies were calculated automatically after data entering by the manual calculation of the randomly obtained genotypes. This function opens up an opportunity to demonstrate the essence of **biological replication of experiments**. This function is especially remarkable in the lesson during the simultaneous work of several groups of students with different populations in number and frequency of alleles occurrence (Fig.1, Fig.3).

Model experiment 1. Study of the genetic structure of the ideal population (third option)

1. In column 2 for the parent generation P, we introduce the number of pairs of two-body gene alleles (in other words, the number of individuals).
2. We are determined by the ratio of the dominant (A) and recessive (a) alleles, and we introduce their values in columns 9 and 10 for the parent generation P.
3. Click the "Calculate" button.
4. Click the "Calculate" button opposite the lines F1, F2, F3, F4, F5.
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Table 6. Genetic structure of the ideal population

Generation	Number of individuals	Distribution of genotypes						Gene frequencies		
		AA		Aa		aa		A(p)	a(q)	
1	2	3	4	5	6	7	8	9	10	
P	20000	2295	0.115	3410	0.171	14295	0.715	0.2	0.8	Calculate
F1	20000	2261	0.113	3478	0.174	14261	0.713	0.336	0.844	Calculate
F2	20000	2310	0.116	3380	0.169	14310	0.716	0.340	0.846	Calculate
F3	20000	2274	0.114	3452	0.173	14274	0.714	0.337	0.845	Calculate
F4	20000	2221	0.111	3558	0.178	14221	0.711	0.333	0.843	Calculate
F5	20000	2285	0.114	3430	0.172	14285	0.714	0.338	0.845	Calculate

Show Graphs

Fig.3 Results of a model experiment with a number of 200000 individuals, allele frequencies p (0, 2) and q (0, 8).

Third. The number of generations of the population has been increased. In the proposed variant, it is equal to 5. I.e. together with the parental population, the total number of replications of the experiment is equal to 6. In previous variants of the experiment, the number of replications was equal to 3 (one parental generation and two daughter generations). In addition, note that it is technically possible to increase the number of replications by times. This will offer an opportunity, first of all, to quickly get a picture of the genetic structure of the population, without bothering students with mechanical work on mixing and distribution of genotypes, since there is an automatic distribution of genotype frequencies within the limits of the ideal population. Secondly, it contributes to the implementation of one of the tasks pursued by us – an increase in the number of replications of the experiment within the limit of one sample (population). This function opens an opportunity to conduct the **technical replication of experiments**.

The visualized replication results are displayed on the user's screen by clicking the «Show graphs and diagrams» button. Note that in one session the user can only see the results of technical replication, i.e. distribution of alleles and genotypes in generations with initially specified parameters (number of individuals and allele frequencies). The generations of the population will act as technical replications. In order to simulate biological replication, it is necessary to load the page once again without closing the previous one and enter other initial data (the number of individuals, allele frequencies). Within each session, generations of a population in relation to each other will act as technical replications, but in relation to the first population and its generations – biological replications.

In 2019/2020 academic year, the developed web page was tested with the participation of 6 students of the 3rd year of the Institute of Living Systems of Immanuel Kant Baltic Federal University, in specialty «Biology» and 12 students of the 11th form of the Municipal Budgetary General Education Institution General Secondary School «School of the Future» of Guryevsky district of Kaliningrad Region (Russian Federation). The approbation took place within the framework of carrying out by Municipal Budgetary General Education Institution General Secondary School «School of the Future» together with the National Research University Higher School of Economics of the conference «Effective High School» (January 23-25). Within the framework of the conference, there were organized practical classes for pupils of 11th forms on the topic «Modelling of the genetic evolutionary processes in the population». One of the proposed experiments for carrying out was a model experiment «Study of the genetic structure of an ideal population» according to the methodology updated by us without using material objects.

In approbation, the participants were divided into 2 groups (3 students and 6 pupils). One group was asked to start with an experiment at http://mybio.education/mod/exp1/en/index.html#, and then at http://mybio.education/mod/exp6/en/index.html#. Another group was asked to click a link to the web page http://mybio.education/mod/exp6/en/index.html# (Model experiment 1. Study of the genetic structure of the ideal population (third variant) and simulate the genetic structure of a population of any number more than a thousand with an arbitrarily given combination of allele frequencies. It was proposed three times to the participants to carry out model experiment in the third variant with different initial data (number of individuals of the population, allele frequencies). Each of the participants of the approbation both in the first and second groups in carrying out of the third variant of the experiment worked separately. The participants were asked to use the «Show graphs and diagrams» function, and also to formulate conclusions at the end of the experiment.

The goals pursued by us were as follows:

1. To find out the availability of understanding by users of the tasks and results of experiments.
2. To find out the main difficulties faced by users when working with a web page http://mybio.education/mod/exp6/en/index.html#.

During the oral survey of the participants in the experiment, it was found:

1. Participants of the first group, when conducting an experiment with material objects at the beginning of work, hardly understood the essence of the performed similar actions. Only after data entering into the table, calculation of the frequencies of alleles and genotypes, the understanding of the meaning of the uniformity of actions came.
2. Participants of the first group complained about the routine of the performed actions, increased fatigue during their performance. Participants sought to complete the experiment more quickly, which increased the error rate in calculation of the absolute number of genotypes. The latter was displayed at the frequency of genotypes calculated by the program. Thus, the obtained results in several cases were erroneous, the experimental actions had to be performed over again.
3. Participants of the first group, after passage to the second experiment, which, in fact, duplicated the first variant, but did not require manual counting, expressed great approval of the possibility to operate only with numbers.
4. Participants of the second group completed the assigned task more quickly. However, in both groups, there arose questions about the purpose of three-time replicate of the experiment (with different number of population and allele frequencies). Note that practically no questions arose in both groups regarding the advisability of repeating the experiment in generations of the same population. It follows that the essence and necessity of technical replication is recognized and accepted by the participants.

With biological replication, the situation is different. Its objectives were not clear to the participants, most likely due to a lack of methodological awareness of this type of replication.

5. Before the performance of the experiment, we deliberately did not focus the participants' attention on the goals of repeated replicate of experimental actions. This was done in order to find out whether the participants understood the conditions for the veracity of the results of the biological experiment. Since among the examinees there were both students of a biological specialty and pupils of graduating profile chemical and biological classes. We assumed that the participants already possess the necessary methodological tools for planning, conduction of biological experiments and interpretation of the results. The results of approbation showed that teaching the methodology of a biological experiment should be started with distinguishing between technical and biological replication of experimental effects. We can only assume that a lack of understanding of the differences between them (for purposes, methodology) could initiate the spread of the problem of pseudoreplication in biological research in principle. We believe that in order to confirm this assumption, it is necessary to conduct additional studies aimed at a retrospective analysis of biological scientific literature, primarily of scientific papers, conference materials containing a description of the methods and results of experiments. The question is, is it worth doing? Or to accept the fact that even if we consider the problem of pseudoreplication as far-fetched, then the issue of distinguishing between technical and biological replications and teaching this in the secondary school and in higher educational establishment definitely deserves further study.

4 Conclusions

As a result of work on the topic of true replication by means of a school biological experiment, we came to the following conclusions:

1. The question of the artificiality and reality of the problem of true replications in biological science remains open.
2. One of the reasons for the artificiality of the problem may be an implicit distinction between the purpose and methods of technical and biological replication.
3. Model experiment on the study of genetic-evolutionary processes in populations by means of computer modelling is ideal for demonstration of the essence of technical and biological replication.
4. Computer modelling of genetic-evolutionary processes in populations allows to take into account the requirement of mass nature of experimental impact, which is one of the necessary for obtention of consistent results.
5. In the educational model experiment, it is impossible to take into account all the requirements for a scientific biological experiment, therefore, it is necessary to rely only on its essential features: replicativity, mass nature, principle of single difference, veracity in essence.

5 Outlook

Continuation of approbation of the effectiveness of the proposed method for studying the law of genetic equilibrium and the essence of technical and biological replication of the experiment is scheduled to be conducted in 2020/2021 academic year. Elective courses «Population Biology» and «Fundamentals of Theoretical Biology» for students of 3rd and 4th academic years of the Institute of Living Systems of Immanuel Kant Baltic Federal University will be used as an experimental site, as well as the course «Olympiad Biology» for pupils of 10-11 forms of the School of the Future of Guryevsky district of Kaliningrad region.

Further work on studying the possibilities of a model experiment in training of the pupils of 11 forms and students-biologists in true replication, as well as the essence of technical and biological replication, we can see in the following. It is necessary to develop and approbate web pages to model the structure

of a very large population under the influence on its numerous generations of such factors as natural selection, gene flow, gene drift, mutations.

The modelling of the genetic structure should be fully automated. The initial platforms for improvement of methodology will be the existing web pages

http://mybio.education/mod/exp3/en/index.html# (Model experiment 2. Study of the genetic structure of the population under the influence of natural selection), http://mybio.education/mod/exp4/en/index.html# (Model experiment 3. Modelling the effect of gene flow on the genetic structure of the population), http://mybio.education/mod/exp5/en/index.html# (Model experiment 4. Modelling the effect of random processes on the genetic structure of the population, modelling the drift of genes*), providing one of the stages of work with material objects.

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