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doi 10.18699/letvjgb-2025-11-03

## Original article

## Experimental analysis of polymorphic microsatellite loci in Cladocera: mixed breeding strategy and population dynamics

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**Abstract:** The aim of this work is to identify polymorphic loci in *Daphnia* genomes using genome sequence analysis and experimental analysis of real accessions of samples collected in nature. We use primers predicted by an algorithm for DNA amplification from *Daphnia* samples and capillary electrophoresis to detect polymorphic loci. This was done to detect polymorphic loci in a single experiment, when the presence of polymorphic loci with a frequency sufficient for genetic studies is detected as the appearance of multiple peaks on the electrophoregram. Thus, we evaluate the effectiveness of the capillary electrophoresis method and refine population parameters, including the proportion of sexual reproduction and the effective population size. Studying polymorphic loci becomes especially relevant in the context of climate change and anthropogenic impacts on ecosystems, as these factors can significantly affect genetic diversity and adaptation in populations. Polymorphic loci can serve as indicators of genetic stability and the ability of species to adapt to changing environmental conditions. In the face of global climate change and increasing anthropogenic impacts, such as pollution, urbanization, and landscape changes, populations face new challenges that require rapid adaptation. Polymorphic loci, due to their high variability, can provide valuable information about the genetic structure of populations and their potential for adaptation. In the context of our research, studying polymorphic loci in *Daphnia* becomes particularly important, given their ecological role and sensitivity to environmental changes. *Daphnia*, as key components of freshwater ecosystems, can serve as model organisms for studying genetic adaptation to climate change and anthropogenic impacts. Understanding polymorphic loci in *Daphnia* can provide valuable data for assessing genetic stability and adaptive potential in these populations, which is particularly important in the face of rapidly changing environmental conditions.

**Key words:** polymorphic loci; capillary electrophoresis; agent-based modeling; microsatellites; *Daphnia*; genetic diversity; adaptation; climate change; anthropogenic impacts

**For citation:** Poroshina A.A., Sherbakov D.Yu. Experimental analysis of polymorphic microsatellite loci in Cladocera: mixed breeding strategy and population dynamics. *Pisma v Vavilovskii Zhurnal Genetiki i Seleksii* = *Letters to Vavilov Journal of Genetics and Breeding*. 2025;11(1):12-16. doi 10.18699/letvjgb-2025-11-03

**Funding:** The study was carried out within the framework of the state budget theme No. 0279-2021-0010 "Genetics of Baikal organism communities: genofond structure, conservation strategies".


## Оригинальное исследование


## Экспериментальный анализ полиморфных микросателлитных локусов у кладоцер: стратегия смешанного размножения и динамика популяций

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**Аннотация:** Целью данной работы была идентификация полиморфных локусов в геномах *Daphnia* с помощью анализа последовательности генома и экспериментального анализа реальных образцов, собранных в естественных условиях. Мы использовали праймеры, предсказанные алгоритмом амплификации ДНК из образцов дафний, и капиллярный электрофорез для выявления полиморфных локусов. Это сделано для детекции полиморфных локусов в результате единственного эксперимента, когда наличие полиморфизмов с частотой, достаточной для генетических исследований, детектируется как появление множественных пиков на электрофореграмме. Таким образом, мы оценивали эффективность метода капиллярного электрофореза и уточняли

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параметры популяции, включая долю полового размножения и эффективную численность популяции. Изучение полиморфных локусов становится особенно актуальным в контексте изменения климата и усиления антропогенного воздействия на экосистемы, поскольку эти факторы могут существенно влиять на генетическое разнообразие и адаптацию популяций. Полиморфные локусы, содержащие микросателлитные повторы, могут служить индикаторами генетической стабильности и способности видов адаптироваться к изменяющимся условиям окружающей среды. В условиях глобального изменения климата и усиливающегося антропогенного воздействия, такого как загрязнение окружающей среды, урбанизация и изменения ландшафта, гидробионты сталкиваются с новыми вызовами, требующими быстрой адаптации. Полиморфные локусы, благодаря своей высокой изменчивости, могут предоставить ценную информацию о генетической структуре популяций и их потенциале к адаптации. В контексте наших исследований изучение полиморфных локусов у *Daphnia* приобретает особую важность, учитывая их экологическую роль и чувствительность к изменениям окружающей среды. Представители рода *Daphnia* как ключевые компоненты пресноводных экосистем могут служить модельными организмами для изучения генетической адаптации к изменению климата и антропогенным воздействиям. Понимание полиморфных локусов у *Daphnia* может дать ценные данные для оценки генетической стабильности и адаптивного потенциала этих популяций, что особенно важно в условиях быстро меняющихся условий окружающей среды.

**Ключевые слова:** полиморфные локусы; капиллярный электрофорез; агентное моделирование; микросателлиты; дафнии; генетическое разнообразие; адаптация; изменение климата; антропогенные воздействия

**Финансирование:** Исследование выполнено в рамках темы госзадания № 0279-2021-0010 «Генетика сообществ байкальских организмов: структура генофонда, стратегии консервации».

## Introduction and motivation

Polymorphic loci play a crucial role in studying genetic diversity and evolutionary processes in populations. For cladocerans of the genus *Daphnia* O.F. Müller, 1785, which exhibit a mixed reproductive strategy involving both sexual and asexual reproduction, understanding the polymorphism of loci helps estimate the proportion of sexual reproduction and other population parameters. A previously developed agent-based model allowed us to assess genetic diversity and the disequilibrium parameter ( $D$ ) on simulated data, opening up the possibility of using disequilibrium to estimate the proportion of sexual reproduction in a population (Poroshina, Sherbakov, 2023). This work focuses on identifying polymorphic loci that we are developing to monitor changes in the reproduction strategy, utilizing the previously established agent-based model.

The aim of this work is to identify polymorphic loci in daphniids genomes using genome sequence analysis and experimental analysis of real samples. We use primers predicted by an algorithm for DNA amplification from *Daphnia* samples and capillary electrophoresis to detect polymorphic loci (Thielsch et al., 2012). Thus, we evaluate the effectiveness of the method and refine population parameters, including the proportion of sexual reproduction and the effective population size.

Studying polymorphic loci becomes especially relevant in the context of climate change and anthropogenic impacts on ecosystems, as these factors can significantly affect genetic diversity and adaptation in populations. Polymorphic loci can serve as indicators of genetic stability and the ability of species to adapt to changing environmental conditions. In the face of global climate change and increasing anthropogenic impacts, such as pollution, urbanization, and landscape changes, populations face new challenges that require rapid adaptation. Polymorphic loci, due to their high variability, can provide valuable information about the genetic structure of populations and their potential for adaptation.

Climate change affects ecosystems worldwide, altering temperature regimes, precipitation, and seasonal cycles. These changes can lead to shifts in species ranges, changes in phenology, and even the extinction of some populations. Polymorphic

loci play a key role in the adaptation of species to new climatic conditions (Hoffmann, Sgrò, 2011).

Polymorphism can contribute to the adaptation of species to climate change. Examples where polymorphic loci played a key role in adaptation, such as changes in phenology and physiology in response to climatic changes, highlight the importance of polymorphic loci in evolutionary adaptation and their potential for use in monitoring and managing populations (Franks, Hoffmann, 2012).

The mechanisms of genetic adaptation to environmental changes, including the role of polymorphic loci, are discussed in various studies. Polymorphic loci can influence adaptation through changes in allele frequencies and genetic structure of populations. This emphasizes the importance of studying polymorphic loci for understanding evolutionary processes and developing conservation strategies (Hansen et al., 2012; Yuan et al., 2012; Zhang et al., 2023).

The loss of genetic diversity can affect the survival of species in the face of climate change and anthropogenic impacts. Preserving genetic diversity, including polymorphic loci, is critical for the resilience of species to climate change. This highlights the importance of preserving genetic diversity for the resilience of species to climate change and includes a discussion of polymorphic loci (Kim et al., 2011; Pauls et al., 2013; Engelhardt et al., 2014).

In the context of our research, studying polymorphic loci in *Daphnia* becomes particularly important, given their ecological role and sensitivity to environmental changes. *Daphnia*, as key components of freshwater ecosystems, can serve as model organisms for studying genetic adaptation to climate change and anthropogenic impacts. Understanding polymorphic loci in *Daphnia* can provide valuable data for assessing genetic stability and adaptive potential in these populations, which is particularly important in the face of rapidly changing environmental conditions.

## Materials and methods

As model organisms, we used two species of cladocerans: *Daphnia longispina* O.F. Müller, 1785 and *Simocephalus vetulus*

O.F. Müller, 1786. Previously developed primers from the article “Microsatellite markers for European *Daphnia*” (Brede et al., 2006) were tested only on representatives of the genus *Daphnia*. Our task was to test these primers on their closest relatives from the same family, namely *S. vetulus*.

We previously developed an object-oriented model to obtain theoretical data which was published in the article “A procedure for modeling genetic diversity distortions in populations of organisms with mixed reproductive strategies” (Poroshina, Sherbakov, 2023). The theoretical data were verified using various population metrics such as *D*-linkage disequilibrium parameters and *I<sub>a</sub>*-index association. The R package poppr was used for this analysis (Kamvar et al., 2014). We compared the analysis of microsatellite state tables with real data from the article “Discrimination of hybrid classes using cross-species amplification of microsatellite loci: methodological challenges and solutions in *Daphnia*” (Thielsch et al., 2012). This comparison was necessary to confirm the correctness of our model. The above work analyzed the species *D. cucullata*, *D. galeata*, and *D. longispina*, while we chose *S. vetulus* in addition to *D. longispina*.

*Daphnia longispina* employs cyclical parthenogenesis for reproduction, which means that for the majority of the year, they reproduce asexually without male involvement. During this phase, females generate diploid eggs that develop into females without fertilization. However, under stressful conditions such as temperature fluctuations, food scarcity, or other adverse factors, females start producing haploid eggs that require fertilization by males. This process results in the formation of diapausing eggs, which can endure unfavorable conditions and initiate a new generation when conditions improve.

*Simocephalus vetulus*, on the other hand, also reproduces through cyclical parthenogenesis, similar to *D. longispina*. This means that under favorable conditions, females produce diploid eggs that develop into females without fertilization. However, under stressful conditions, *S. vetulus* can also switch to sexual reproduction, producing haploid eggs that require fertilization by males. Consequently, both *D. longispina* and *S. vetulus* demonstrate cyclical parthenogenesis. These species are ideal for our analysis due to their distinct reproductive strategies. By utilizing the previously developed model and method for assessing the proportion of sexual reproduction based on the states of microsatellite loci (whether homozygous or heterozygous), we can determine the reproductive method of a given population.

*Simocephalus vetulus* specimens were collected in August 2024 in a lake near the village of Bolshye Koty (coordinates 52.92067, 105.07231), which is characterized by a rocky-muddy bottom, an abundance of *Elodea* sp., *Lemna* sp., *Potamogeton lucens* and a diverse fish population. In the same month, samples of *D. longispina* were collected from a reservoir on the Angara River in Irkutsk (coordinates 52.26504, 104.27950), which has a depth of more than 1 m, a pelagic zone and flooded grasses. The samples were collected using a plankton net with a mesh size of 100 µm to ensure the capture of both adult and juvenile individuals.

Approximately 100 individuals per each species were collected for the study. DNA extraction was performed using a classical phenol-chloroform extraction protocol (Abdulla, 2014;

Ye, Lei, 2023). For each species, 10 individuals were pooled to create a mixed sample, resulting in 5 mixed samples per species. The extracted DNA was amplified using previously designed microsatellite primers labeled with four different fluorescent dyes: TAMRA, R6G, ROX, and FAM. The PCR amplification was carried out in a 20 µL reaction volume containing 10 ng of genomic DNA, 1 × PCR buffer, 2 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.2 µM of each primer, and 1 unit of Taq DNA polymerase. The PCR conditions included an initial denaturation step at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at temperatures ranging from 53 to 57 °C for 30 seconds (as specified in the original article by Brede N. et al., 2006), and extension at 72 °C for 45 seconds, with a final extension step at 72 °C for 10 minutes.

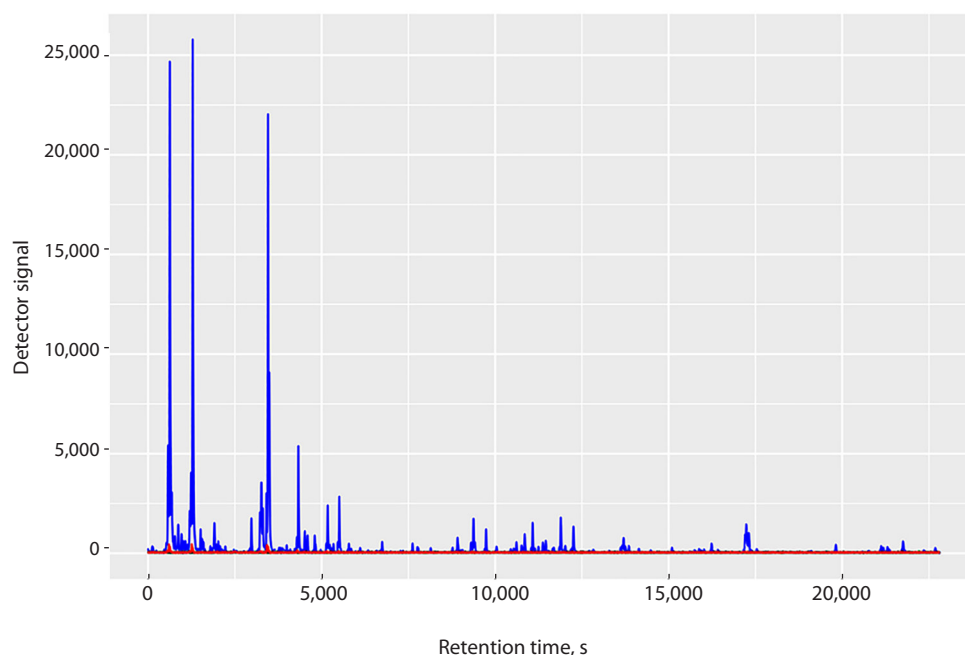
The PCR products were analyzed using the NANOFOR-05 genetic analyzer to identify polymorphic primers (Shewale et al., 2012). The primary objective of this study was to identify primers that exhibit polymorphism in both *D. longispina* and *S. vetulus*. The analysis yielded a set of polymorphic primers, which are listed in the Table below. These primers will be utilized in subsequent experiments to further investigate the genetic diversity and population dynamics of these species.

Below is a Table listing 5 pairs of polymorphic primers for the two species that we will use subsequently. The table presents the results of the polymorphism analysis of 19 primers for two species of cladocerans: *Simocephalus vetulus* (denoted as s1–s5) and *Daphnia longispina* (denoted as d1–d4).

Polymorphic primers for *Daphnia longispina* and *Simocephalus vetulus*

Primer	s1	s2	s3	s4	s5	d1	d2	d3	d4
DaB17/16(R6G)	-	-	-	+	-	-	+	-	-
DaB10/14(TAMRA)	-	-	+	-	-	+	-	-	-
Dgm101(ROX)	-	+	+	+	+	+	-	+	-
Dp281(FAM)	+	-	+	+	-	-	-	-	+
SwiD15(R6G)	+	+	+	?	+	-	-	+	+
SwiD18(TAMRA)	+	+	-	-	-	-	+	-	+
DaB10/15(ROX)	+	-	+	-	-	+	-	+	+
DaB17/17(FAM)	-	?	+	+	-	-	+	+	-
SwiD6(R6G)	-	-	+	-	-	+	-	-	-
SwiD2(TAMRA)	-	+	-	-	+	-	-	-	-
Dgm105(ROX)	+	-	-	+	-	-	-	-	-
SwiD18(FAM)	-	-	-	-	-	-	-	-	-
SwiD14(TMARA)	-	-	-	-	-	+	+	-	-
SwiD12(R6G)	-	-	-	-	-	-	+	-	-
SwiD6(ROX)	+	+	-	-	-	-	-	-	-
Dgm112(R6G)	-	-	-	-	-	-	+	+	-
Dgm109(TAMRA)	-	-	-	-	-	-	+	+	+
Dp519(FAM)	-	-	-	-	-	-	-	-	-
Dp196(ROX)	-	-	-	-	-	-	-	-	-

Note. The “+” sign indicates the presence of polymorphism, the “-” sign indicates its absence, and “?” denotes uncertain results. Primers that are suitable for both species are highlighted in bold.



Electropherogram of primer DaB17/17 obtained using capillary electrophoresis.

The graph shows the dependence of the detector signal on the retention time for the primer DaB17/17. The blue peaks indicate the presence of polymorphic loci, demonstrating differences in the lengths of DNA fragments amplified by this primer. The red line represents the baseline signal level.

## Results

As part of this work, we conducted a comprehensive study to search for polymorphic loci in the genomes of *Daphnia*. As a result of the study, out of 19 previously developed microsatellite markers, 8 were identified as polymorphic in the *Daphnia* genomes. Of these, 5 are polymorphic for both *D. longispina* and *S. vetulus*. These polymorphic markers will be used in the future to determine the breeding strategy within *Daphnia* populations. We analyzed capillary electrophoresis graphs constructed from .fas files using the SeqinR package (Charif, Lobry, 2007) (see the Figure).

## Discussion

Polymorphic markers are crucial indicators of genetic stability and adaptation potential in species, especially under the pressures of global climate change and increasing anthropogenic impacts like pollution and urbanization. These markers, due to their high variability, offer valuable insights into population genetics and adaptive capabilities.

Global climate change is altering ecosystems by modifying temperature patterns, rainfall, and seasonal cycles. Such changes can shift species' geographic ranges, affect life cycle events, and potentially lead to population declines. Polymorphic loci significantly influence a species' ability to adapt to these new conditions (Hoffmann, Sgrò, 2011).

Polymorphic loci play a key role in facilitating phenological and physiological adaptations, as demonstrated in studies showing how genetic variations drive adaptation through allele frequency modifications (Franks, Hoffmann, 2012; Hansen et al.,

2012). Preserving genetic diversity is essential for species resilience against climate change and human activities (Kim et al., 2011; Pauls et al., 2013).

Our study focuses on polymorphic loci in *Daphnia*, given their ecological importance and sensitivity to environmental shifts. As key components of freshwater ecosystems, *Daphnia* serve as model organisms for investigating genetic responses to climate change and anthropogenic influences. Understanding these loci provides critical data on genetic stability and adaptive potential, vital for assessing how *Daphnia* populations will respond to rapidly changing environments.

A limitation of our study is the potential influence of seasonal changes on the genetic diversity of *Daphnia* populations.

## Conclusion

In conclusion, we have identified polymorphic markers suitable for estimation of genetic status and adaptive potential in *Daphnia* populations. These markers provide essential insights into genetic structure and diversity, are crucial for understanding adaptation to changing conditions. Future research using these markers can monitor population dynamics and assess responses in various ecosystems, such as lakes and rivers. This emphasizes the importance of preserving genetic diversity to ensure species resilience in the face of climate change and anthropogenic pressures.

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**Conflict of interest.** The authors declare no conflict of interest.

Received November 2, 2024. Revised December 2, 2024. Accepted December 16, 2024.