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Chromosomal polymorphism of *Belgica antarctica* populations: possible links on ecology and geography

Abstract. Chromosomal inversion polymorphism is an important evolutionary mechanism for many species, particularly insects. *Belgica antarctica* (Chironomidae) is the only endemic insect of the Antarctic Peninsula and is currently considered a model organism for studying adaptations of living organisms to climate change in the extreme terrestrial ecosystems of Antarctica. This polar midge has salivary gland polytene chromosomes, making it convenient for investigating various chromosomal aberrations and analysing their potential relationship with geographical and ecological factors. In this study, chromosomal polymorphism was analysed in the salivary glands of 99 fourth-instar larvae of *Belgica antarctica*, collected in four different localities in the West Antarctic Peninsula region in 2022. Six of the seven previously described heritable heterozygous inversions for this species were found in the newly tested populations. Notably, the precise location of a large inversion in chromosome I, containing the nucleolar organizer region, was identified for the first time since its discovery in 1962. Additionally, the data on chromosomal inversion frequencies were combined with all currently available data — together from 1436 *Belgica antarctica* individuals across 26 populations, from 1962 to 2022, to determine potential links to specific ecological (ecological complexity, ornithogenic influence, and whether samples were collected from nesting or non-nesting material) and geographical (longitude and latitude) factors. It was found that, overall, the ecological factors do not affect the frequency of the studied inversions. However, slight trends were observed in the frequency of four heterozygous rearrangements with latitude, as well as the frequency of specimens with at least one non-sex-linked heterozygous inversion with longitude.

Keywords: chromosomal aberration frequency, chromosome variability, endemic species, polar insect, salivary gland chromosomes

1 Introduction

Chromosomal inversion polymorphism is a common occurrence for numerous species in different groups of organisms (Hoffmann et al., 2004; Naseeb et al., 2016; Wellenreuther & Bernatchez, 2018; Berdan et al., 2023), including numerous insect species in different orders (Ayala et al., 2013; Wellenreuther & Bernatchez, 2018; Christmas et al., 2019; Esteve et al., 2020; Berdan et al., 2021; Michailova et al., 2023). It is now known that there is a significant reduction in recombination within and around inversions and between inverted and non-inverted homologous regions of chromosomes; however, gene flux can occur *via* mechanisms such as gene conversion and double crossovers. As a result, inversions contribute to the chromosomally-specific substructure of populations (reviewed by Wellenreuther & Bernatchez, 2018). Recent studies show that chromosomal inversions usually play a considerable role in eco-evolutionary processes: mating systems and reproductive organization (e.g., the emergence of alternative reproductive strategies), the origin and further evolution of sex chromosomes, adaptive divergence within species, reproductive isolation, and speciation (Wellenreuther & Bernatchez, 2018; Berdan et al., 2023). In general, chromosomal inversions have been considered for a long time the driving forces of evolutionary change (Wellenreuther & Bernatchez, 2018). Considering this, it is particularly interesting to investigate chromosomal polymorphism in populations of species inhabiting relatively isolated yet dynamic ecosystems.

Polytene chromosomes hold a prominent place in chromosome research, particularly in the study of chromosomal inversions in Diptera. After over a century of investigation, they continue to be a powerful tool for analysis (reviewed by Stormo & Fox, 2017). In particular, unlike species for which molecular markers of inversions are known (e.g., *Drosophila melanogaster* Meigen, 1830 (Drosophilidae), where such studies are currently conducted using whole-genome sequences (Kapun et al., 2014)), in species where molecular markers of inversions have not yet been developed, such studies can only be conducted on cytological preparations of polytene chromosomes (Michailova et al., 2023).

Since the fragmentation of Gondwana, the Antarctic region has been biologically isolated for many millions of years, allowing it to develop unique ecosystems (Chown et al., 2015; Fraser et al., 2018; Bargagli, 2020). However, in recent decades, this once pristine environment has undergone significant transformations. The Antarctic Peninsula, in particular, has experienced rapid, significant climate shifts (Turner et al., 2014; Convey & Peck, 2019; Bargagli, 2020; Devlin et al., 2022). Additionally, human activities, including increased tourism, have further impacted the region (Bargagli, 2020; McCarthy et al., 2022), contributing to the introduction and spread of nonnative species (Convey & Peck, 2019; Chwedorzewska et al., 2020; Hughes et al., 2020; McCarthy et al., 2022; Leihy et al., 2023) and compromising the isolation of this area. In addition, abiotic and biotic samples from various locations in Antarctica are characterized by high concentrations of heavy metals caused by both human activity and natural processes (Chu et al., 2019). As a result, the study of how native species adapt to these challenges, particularly at the molecular, genetic, and chromosomal levels, is becoming increasingly important and offers extensive opportunities for research.

Belgica antarctica Jacobs, 1900 (Chironomidae: Orthocladiinae) is an endemic insect of the Antarctic Peninsula and adjoining islands (Usher & Edwards, 1984; Convey & Block, 1996; Chown & Convey, 2016; Kovalenko et al., 2021). This Antarctic midge is a nonselective feeder (Baust & Edwards, 1979), occurs in bird nests, moist habitats with mosses, algal mats, flowering plants (especially in the rhizosphere), cyanobacterial mats, and shallow freshwater lakes with algae (Gressitt, 1967; Wirth & Gressitt, 1967; Peckham, 1971; Richard et al., 1994; Convey & Block, 1996; Potts et al., 2020; Kovalenko et al., 2021; Devlin et al., 2022). Considering the availability of the whole-genome sequence (Kelley et al., 2014), the biological and ecological features of *B. antarctica*, and its usage in numerous scientific studies (e.g., Rinehart et al., 2006; Benoit et al., 2009; Harada et al., 2014; Kawarasaki et al., 2019; Potts et al., 2020; Teets et al., 2020; Ajayi et al., 2021; Devlin et al., 2022; Yoshida & Goto, 2023), this species serves as a model organism for research on adaptations to terrestrial Antarctic ecosystems (Kozeretska et al., 2022).

The *B. antarctica* karyotype was first described by Martin (1962). It is known that the diploid set of chromosomes of this midge is 2n = 6 (Martin, 1962; Atchley & Davis, 1979; Michailova et al., 2021; 2023), which is common for some representatives of the Orthocladiinae subfamily (Michailova, 1980; 2022). A recent study showed that chromosomes I and III of *B. antarctica* are submetacentric, while chromosome II is metacentric (Michailova et al., 2023). Additionally, Michailova et al. (2023) provided the standard polytene chromosome map for this species, which greatly facilitates more accurate identification and localisation of chromosomal rearrangements.

Chromosomal polymorphism in *B. antarctica* has been studied exclusively in salivary glands of polytene chromosomes across four works. First, Martin (1962) described the chromosome inversions for chromosomes I and III, later Atchley and Davis (1979) analysed 1182 individuals of 18 populations and described five inversions for all three chromosomes. In recent years, Michailova et al. (2021; 2023) studied chromosome polymorphism of *B. antarctica.* In total, two new additional inherited heterozygous inversions, four previous, and 24 somatic aberrations (the authors classified as "somatic" those that were found only in one or a few cells and as "inherited" those present in all cells of both salivary glands of a particular individual) were identified and localized. Thus, to date, seven inherited heterozygous inversions have been recorded in this species: one large inversion in chromosome I, three inversions in chromosome II, and three inversions in chromosome III (one of which is sex-linked). However, in these two latest works, the large heterozygous inversion in chromosome I, reported by Martin (1962) and Atchley and Davis (1979), was not detected. Accordingly, its precise location of the chromosome has not been determined yet, and it remains unclear whether this inversion still exists in *B. antarctica* populations today.

Moreover, the factors contributing to the persistence of several large chromosome inversions in *B. antarctica* over at least several decades remain unclear. Notably, in other Chironomidae members, various environmental stress factors can cause particular inherited or somatic rearrangements in polytene chromosomes (Michailova et al., 1998; 2009) (e.g., heavy metals and radionuclides pollution (Michailova & Petrova, 2015), heat shock (Morcillo et al., 1988), galactose (Díez et al., 1990), etc.).

Another reason to research chromosomal polymorphism in *B. antarctica* is to check for potential geographic clines for inversion frequencies. For other Diptera, particularly the Drosophilidae family (e.g., *Drosophila subobscura* Collin, 1936, *D. melanogaster*), it was shown that the frequencies of several inversions depend on latitude or altitude (Knibb, 1982; Balanyá et al., 2006; Kapun et al., 2016; Durmaz et al., 2018; Simões & Pascual, 2018). Among the chironomids, geographic clines for polytene chromosome inversions were recorded for the genus *Axarus* from the Connecticut River (USA) (Werle et al., 2004).

This research aimed to study chromosomal polymorphism in different populations of *B. antarctica* by analysing inherited heterozygous inversions. In particular, to establish potential relationships between the frequency of distinct heterozygous chromosome inversions and geographical (longitude and latitude) and some environmental factors (ecological complexity after Atchley and Davis (1979) and ornithogenic influence) using all available chromosome polymorphism data for this species, including literature (Martin, 1962; Atchley & Davis, 1979; Michailova et al., 2021; 2023).

2 Materials and methods

2.1 Material collection

Altogether ninety-nine alive *B. antarctica* 4th-instar larvae were collected between January and February 2022 from four locations in the Maritime Antarctic (Figure 1, Table 1). All sampling sites were located in low-altitude coastal areas (< 20 m a. s. l.). The temperature during sample collection at all locations was approximately 0 °C.

Specimens from locations B1105 and B1039 were collected from the moss *Sanionia georgicouncinata* (Müll. Hal.) Ochyra & Hedenäs, 1998 (Scorpidiaceae) carpet and mat subformations with low ornithogenic impact. In contrast, two other samples were collected from nesting material, specifically from the nests of kelp gulls (*Larus dominicanus* Lichtenstein, 1823 (Laridae)) and snowy sheathbills (*Chionis albus* (J. F. Gmelin, 1789) (Chionidae)) containing, in addition to *S. georgicouncinata*, other living mosses (e.g., *Warnstorfia fontinaliopsis* (Müll. Hal.) Ochyra, 2001 (Calliergonaceae), *Polytrichum strictum* Menzies ex Brid., 1801 (Polytrichaceae), *Bryum pseudotriquetrum* (Hedw.) G. Gaertn., B. Mey. & Scherb., 1802

(Bryaceae), *Ceratodon purpureus* (Hedw.) Brid., 1826 (Ditrichaceae)), plants (*Deschampsia antarctica* É. Desv., 1854 (Poaceae) and *Colobanthus quitensis* (Kunth) Bartl., 1831 (Caryophyllaceae)), fragments of green algae (*Prasiola crispa* (Lightf.) Kütz., 1843 (Prasiolaceae)), lichens, and dead vegetation. It should be noted that the single *C. albus* nest had not been used for an extended period (medium ornithogenic impact), while the nest of *L. dominicanus* was part of a group from which the fledgling had just left (high ornithogenic influence).

The specimens were fixed in a 3:1 solution of 96% ethanol and glacial acetic acid, with the fixative being changed five times over 15 minutes. The samples were then kept at −20 °C until laboratory analysis.

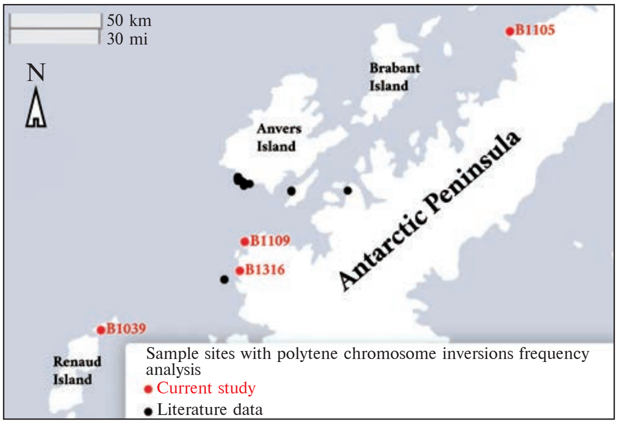

Figure 1. Map showing *Belgica antarctica* populations used for chromosome polymorphism analysis

2.2 Cytogenetic analysis

The salivary glands of each larva were cytogenetically analysed using the acetic-orcein method following Michailova (1989). Preparations were examined under a Zeiss Axio Scope A1 microscope (Germany), with images captured using a Jenoptik Progress MF Cool camera (Jenoptik, Germany) and processed with Pros Res Capture Pro v.2.8.8 software (Jenoptik, Germany). Inherited chromosomal inversions were identified according to Atchley and Davis (1979) and Michailova et al. (2023), with their localization determined following Michailova et al. (2023). The inherited index (H) was calculated as described by Sella et al. (2004).

2.3 Statistical analysis

Confidence intervals of inversion frequencies in the four newly tested populations were calculated

Table 1. Sampling sites

Code	Location	Coordinates	Collection date	Sample size	Short description
B1105	Cape Sterneck, Chavdar Peninsula, Graham Land	64.047706°S, 61.014187°W	21 Jan 2022	25	Bryophyte carpet and mat subformation: *Sanionia georgicouncinata*. Low ornithogenic influence, 5 m a. s. l.
B1109	Port Charcot, Booth Island, Wilhelm Archipelago	65.066675°S, 64.014096°W	26 Jan 2022	20	*Larus dominicanus* nest: *Warnstorfia fontinaliopsis*, *Sanionia georgicouncinata*, *Polytrichum strictum*, *Deschampsia antarctica*, *Colobanthus quitensis*, fragments of *Prasiola crispa*, lichens, feathers, and limpet shells. High ornithogenic influence; 17 m a. s. l.
B1039	Pickwick Island, Pitt Islands, Biscoe Islands	65.484316°S, 65.642274°W	29 Jan 2022	25	Bryophyte carpet and mat subformation: *Sanionia georgicouncinata*. Low ornithogenic influence; 5 m a. s. l.
B1316	Moot Point, Kyiv Peninsula, Graham Land	65.203826°S, 64.075375°W	10 Feb 2022	29	Old abandoned *Chionis albus* nest: *Bryum pseudotriquetrum*, *Ceratodon purpureus*, *Sanionia georgicouncinata*, *Warnstorfia fontinaliopsis*. Medium ornithogenic influence; 3 m a. s. l.

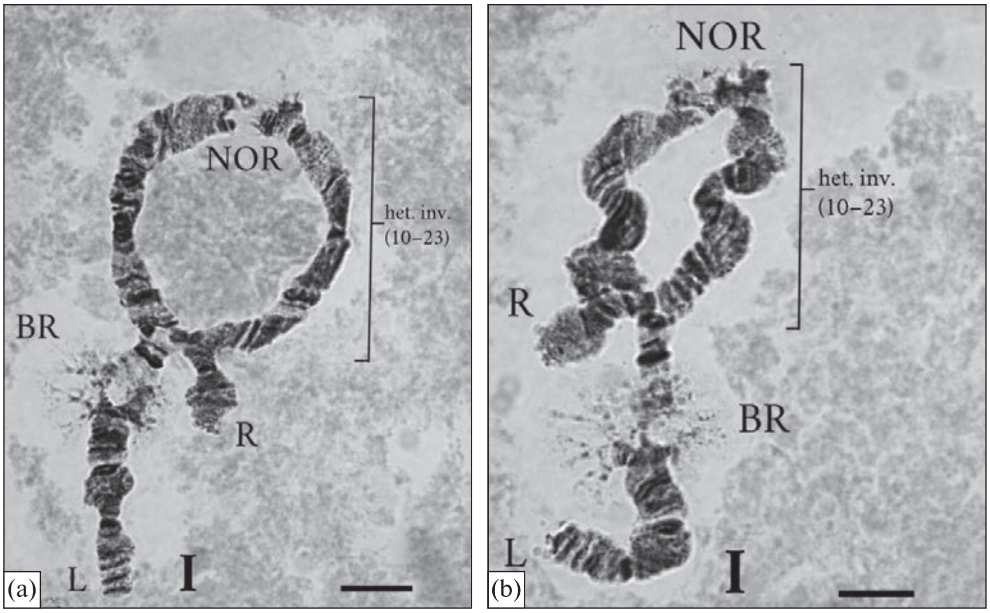

Figure 2. Polytene chromosome I of *Belgica antarctica* with heterozygous inversion (het. inv.) in sections 10—23. (a) — in a specimen from Pickwick Island, Pitt Islands (B1039). (b) — in a specimen from Port Charcot (Booth Island), Wilhelm Archipelago (B1109). NOR — nuclear organizer region, BR — Balbiani Ring, L — left chromosome arm, R — right chromosome arm; scale bar — 10 μm

using the Clopper-Pearson exact test at a confidence level of 0.95 (Clopper & Pearson, 1934).

In addition to the newly tested *B. antarctica* populations, the data from previous studies on 22 different localities (Martin, 1962; Atchley & Davis, 1979; Michailova et al., 2021; 2023) (Figure 1; Appendix Tables 1, 2) were processed and included in the statistical analysis.

Statistical analysis was performed using the RStudio (version 2024.04.2+764) software (available at https://posit.co/download/rstudio-desktop/). Potential differences in the H index values, frequencies of non-sex-linked heterozygous inversions, as well as the proportion of heterozygous individuals (those with at least one non-sex-linked heterozygous inversion on any chromosome, on chromosome II, and chromosome III) among 26 localities were assessed using Fisher's exact test with Monte Carlo simulations (Valz & Thompson, 1994). Differences in inversion and heterozygote frequencies among localities grouped by the factors of ecological complexity depending on the type and condition of the substrate where the larvae were collected (as determined in Atchley and Davis (1979)), ornithogenic influence, and nesting material were evaluated using the Kruskal-Wallis *H* test (Kruskal & Wallis, 1952). Differences in inversion and heterozygote frequencies across localities grouped by the collection year (1962 and 1974/1975 versus 2020 and 2022) were assessed using the Mann-Whitney *U* test (Mann & Whitney, 1947). The same test was applied to compare inversion and heterozygote frequencies based on the presence or absence of nesting material after excluding localities with missing data for this factor. The Spearman's ranked-order correlation coefficient (r_s) was calculated separately for the frequency of heterozygous individuals and the frequency of heterozygous chromosomal inversions in relation to the geographical variables (latitude and longitude), followed by an interpretation of the correlation levels as described by Prion and Haerling (2014). For the dependencies with non-negligible values, a logistic regression analysis was conducted using the generalized linear model (*glm*) function with the binomial argument (Venables & Ripley, 2002; R Core Team, 2024).

The map was prepared using the package "leaflet" in RStudio.

3 Results

3.1 Chromosomal polymorphism in the newly tested *Belgica antarctica* populations

In all newly examined localities, populations of *B. antarctica* exhibited inherited heterozygous chromosome inversions. Six of the seven previously reported inversions for the species were identified, with the exception of the rare heterozygous inversion in sections 9–11 of chromosome II (Table 2).

In chromosome I, only one large heterozygous inversion was identified (Figure 2). It carries the nuclear organizer region (NOR) and was observed in two newly studied localities: Port Charcot and Pickwick Island. The precise location of this inversion was determined for the first time, extending from section 10, near the Balbiani Ring (BR), to section 23, near the end of the R arm of the chromosome.

Two inherited heterozygous inversions in chromosome II – A (sections 22–28) and B (sections 3–10) were present in all studied localities. Some individuals exhibited both inversions simultaneously: 7 out of 25 in locality B1105; 1/20 in B1109; 10/25 – B1039; and 3/29 – B1316.

All previously reported heterozygous inversions for chromosome III were identified, but the sex-linked inversion D (sections 11–12) and heterozygous inversion D1 (sections 2–10) were found across all localities, while inversion C (sections 21–24) was observed only on Cape Sterneck and Pickwick Island.

No new heterozygous inversions large enough to be identified in the preparations were discovered.

3.2 Locality, geographical, temporal, and ecological factor-dependent variations in *Belgica antarctica* inherited non-sex-linked heterozygous inversions

Analysing inversion frequencies in *B. antarctica* across all four newly and twenty-two previously tested localities, differences were identified in the

Table 2. Inherited heterozygous inversions in the newly examined *Belgica antarctica* populations

Inversion (sections of the chromosome)	Frequency in population, % proportion confidence interval in parentheses			
	B1105	B1109	B1039	B1316
Chromosome I: inversion (10–23)	0 (0–13.72)	15 (3.21–37.89)	12 (2.55–31.22)	0 (0–11.94)
Chromosome II: inversion A (22–28)	68 (46.5–85.05	20 (5.73–43.66)	72 (50.61–87.93)	48.28 (29.45–67.47)
Chromosome II: inversion B (3–10)	32 (14.95–53.5)	40 (19.12–63.95)	52 (31.31–72.2)	17.24 (5.85–35.77)
Chromosome III: inversion C (21–24)	12 (2.55–31.22)	0 (0–16.84)	4 (0.1–20.35)	0 (0–11.94)
Chromosome III: inversion D1 (2–10)	28 (12.07–49.39)	30 (11.89–54.28)	32 (14.95–53.5)	41.38 (23.52–61.06)
Chromosome III: sex-linked inversion D (11–12)	40 (21.13–61.33)	30 (11.89–54.28)	40 (21.13–61.33)	48.28 (29.45–67.47)
Inherited index (H)	0.20	0.25	0.24	0.138

occurrence of individuals with at least one non-sex-linked heterozygous inversion among all chromosomes, the presence of at least one inversion on chromosome II, the presence of at least one non-sex-linked heterozygous inversion on chromosome III, and all non-sex-linked heterozygous inversions (*P* = 9.999e-05; Fisher's exact test with Monte Carlo simulation, B = 10 000). The one exception was the inversion in sections 9—11 of chromosome II (*P* = 0.1003; Fisher's exact test with Monte Carlo simulation, B = 10 000) (Appendix Table 2). In addition, no variations were detected in the H index within the studied populations (*P* = 1; Fisher's exact test with Monte Carlo simulation, B = 10 000).

No differences were found in the frequencies of all non-sex-linked heterozygous inversions among the six localities with known ornithogenic influence (B1039, B1105, B1109, B1316, Gal1, and Gal2), grouped by this factor. Similarly, no differences were observed in the frequencies of the examined variables among the seven localities, depending on whether the larvae were collected from nest material or not (B1039, B1105, B1109, B1316, Gal1, Gal2, and WI2020) (see Appendix Tables 1 and 2).

Table 3. Correlation between the frequencies of heterozygous individuals and the frequencies of non-sex-linked heterozygous inversions, and the sites' geographical coordinates. Negligible values are not highlighted

Inversion	r_s coefficient	
	Latitude	Longitude
Frequency of individuals with at least one inherited heterozygous inversion		
at least 1 inherited non-sex-linked in any of the chromosomes	0.04	**−0.21**
in chromosome II	0.09	−0.05
non-sex-linked, in chromosome III	−0.18	**−0.29**
Frequency of inversion		
sections 10—23 on chromosome I	**−0.30**	0.01
A on chromosome II	**0.32**	0.03
B on chromosome II	−0.05	−0.03
C on chromosome III	**0.40**	0.03
D1 on chromosome III	**−0.52**	**−0.23**

No differences were detected in the frequencies of the examined heterozygous inversions between the two temporal groups (first — all samples from 1962 and 1974/1975, second — all collections from 2020 and 2022), except for inversion D1 (chromosome III), which showed a shift (*P* = 9.735e-06, *U* test, Appendix Table 2), and for specimens with at least one non-sex-linked inversion on chromosome III (*P* = 0.00503, *U* test, Appendix Table 2).

Additionally, a difference was found in the frequency of inversion D1 on chromosome III among 25 different localities grouped by the factor of ecological complexity (*P* = 0.0287; Kruskal-Wallis test).

A correlation analysis of the frequency of heterozygous inversions (which were statistically different among localities) with latitude revealed a moderate correlation only for inversion D1 on chromosome III. For three other inversions (the large heterozygous inversion in sections 10—23 of chromosome I, inversion A on chromosome II, and inversion C on chromosome III), weak correlations were found, while the relationships for the remaining variables were negligible (Table 3). A similar analysis for longitude showed negligible correlations for all inversions except D1 on chromosome III, which had a weak negative correlation. Additionally, a weak negative correlation was found between longitude and the proportion of individuals with at least one inherited non-sex-linked heterozygous inversion on any chromosome and at least one inherited non-sex-linked heterozygous inversion on chromosome III (Table 3).

Further analysis of the relationship (in cases where the correlation level was higher than negligible) between the frequencies of chromosomal inversions and the proportion of heterozygous individuals, in relation to longitude and latitude, revealed that statistically significant dependencies existed only for five cases:

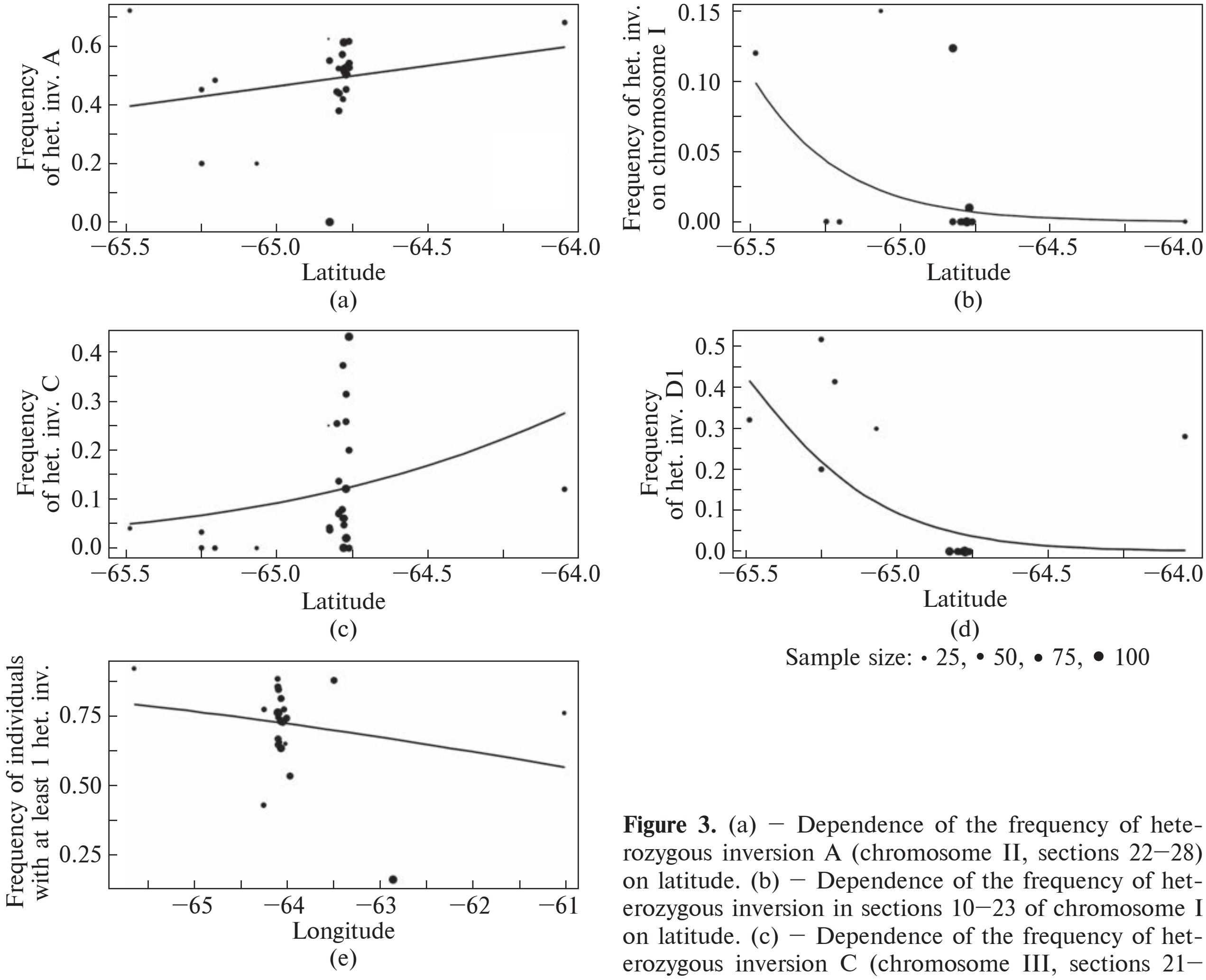

Figure 3. (a) — Dependence of the frequency of heterozygous inversion A (chromosome II, sections 22—28) on latitude. (b) — Dependence of the frequency of heterozygous inversion in sections 10—23 of chromosome I on latitude. (c) — Dependence of the frequency of heterozygous inversion C (chromosome III, sections 21—24) on latitude. (d) — Dependence of the frequency of heterozygous inversion D1 (chromosome III, sections 2—10) on latitude. (e) — Dependence of the frequency of individuals with at least one heterozygous inversion in any of the three chromosomes in relation to longitude. Het. inv. — heterozygous inversion

1) the frequency of heterozygous inversion D1 with respect to latitude (p < 2e-16, GLM, family = “binomial”, slope = −5.7505);

2) the frequency of heterozygous inversion C with respect to latitude (p = 0.000605, GLM, family = “binomial”, slope = 1.5975);

3) the frequency of heterozygous inversion A with respect to latitude (p = 0.0183, GLM, family = “binomial”, slope = 0.7157);

4) the frequency of heterozygous inversion on chromosome I (sections 10—23) with respect to latitude (p = 0.000158, GLM, family = “binomial”, slope = −3.4861);

5) the frequency of individuals with at least one non-sex-linked heterozygous inversion with respect to longitude (p = 1.73e-07, GLM, family = “binomial”, slope = −0.5647) (Figure 3; Appendix Data).

4 Discussion

The results from the analysis of the polymorphism of polytene chromosomes in *B. antarctica* larvae revealed that each of the newly studied populations of this species is characterized by the presence of at least four inherited heterozygous inversions. Notably, inversions A and B on chromosome II and the sex-linked heterozygous inversion on chromosome III were found in all studied populations at high frequencies – up to 72% for inversion A in the population from Pickwick Island (B1039). Interestingly, this high frequency has been observed for the first time, despite the proportion of heterozygotes exceeding 50% in 13 of the 18 studied localities in Atchley and Davis (1979).

Another interesting finding is the presence of the only known inversion on chromosome I, which encompasses more than half the length of the entire chromosome (sections 10–23) and contains the NOR. It is worth noting that this is also the only apparently the same inversion described in the XX century (Martin, 1962; Atchley & Davis, 1979) that was not detected in two recent studies (Michailova et al., 2021; 2023). Overall, six out of seven inherited heterozygous chromosome aberrations (the large inversion in sections 10–23 of chromosome I, inversions A and B in chromosome II, inversions C, D1, and sex-linked D in chromosome III) are present in more than one of the 26 studied localities, and in most of them were observed with considerable frequency.

The fact that the presence of all these inversions, except for D1, in *B. antarctica* populations has been known for over 50 years supports the hypothesis of Michailova et al. (2021) regarding their adaptive role which is known for other species. Fixation and maintenance of chromosomal polymorphism in *B. antarctica* populations could be the result from balancing selection (e.g., overdominance, epistasis, associate overdominance) (Faria et al., 2019). In addition, the level of chromosome inversion polymorphism can also result from gene flow between separate populations (Faria et al., 2019). *Belgica antarctica* imagoes are wingless, and the larvae constantly remain within the substrate. However, they can be transferred by birds, with nesting material (Peckham, 1971; Parnikoza et al., 2018; Ivanets et al., 2022), or by ocean currents, as evidenced by the frequent migrations of this insect between different islands (Edgington et al., 2023).

It should be noted that chromosomal inversions, as well as other genetic aberrations, could be induced by heavy metals (Michailova et al., 2009; 2012; 2018; Szarek-Gwiazda et al., 2013; Michailova & Petrova, 2015; Soulivongsa et al., 2020). Since *B. antarctica* larvae develop continuously within substrates, they are potentially exposed to heavy metals in Antarctic terrestrial ecosystems (Chu et al., 2019; Darham et al., 2023). In addition, the birds could play a role in transporting trace metals (e.g., Cu, Pb, Zn, Hg) from the ocean to Antarctic soils within organic fractions (Castro et al., 2021; Soares et al., 2024). Therefore, the interaction of birds with *B. antarctica* can hypothetically induce new chromosomal rearrangements in the midge. The analysis revealed no significant association with the ornithogenic factor. However, it is important to note that this result is based solely on six localities with approximately established levels of this factor, as well as seven localities with known data regarding whether larvae inhabited the nesting material. Moreover, since these organisms could have lived in a substrate with high concentrations of heavy metals for many generations, they may have developed increased resistance to such conditions. Finally, in *B. antarctica*, it is quite possible that the frequency of functional alternations and somatic rather than hereditary aberrations is dependent on the heavy metal content in the environment, similar to *Chironomus riparius* Meigen, 1804 (Chironomidae) (Sella et al., 2004). Indirectly, this is evidenced by the absence of statistically meaningful differences in the H index among individuals from the studied sites.

The results of examining potential differences among various *B. antarctica* populations, considering a larger dataset (including findings from the past few years), indicate that the factor of ecological complexity does not have a significant impact on the frequency of chromosomal rearrangements.

It is consistent with the findings of Atchley and Davis (1979).

There was a slight trend in the frequency of four chromosomal inversions based on latitude. Inversion D1 and the large inversion in sections 10–23 of chromosome I grew more frequent towards the south. On the contrary, inversions A and C grew more frequent towards the north. The former may provide a slight advantage in colder temperatures, while the latter may offer an advantage in warmer conditions. At the same time, the frequency of individuals with at least one heterozygous inversion shows a weak correlation with longitude, likely induced by the influence of a factor that exhibits a similar trend.

There are chromosomal inversions that have temperature, latitude, altitude, and longitude clines in other Diptera (including members of families Chironomidae and Drosophildae) (Knibb, 1982; Werle et al., 2004; Balanyá et al., 2006; Durmaz et al., 2018; Simões & Pascual, 2018). For some of them, the frequency varies over time (Balanyá et al., 2006; Rezende et al., 2010), while others remain stable despite the presence of a latitudinal gradient (Werle et al., 2004). However, the lack of re-sampling studies and the gap of 60 years between the earliest and latest collections significantly limit a confident interpretation of the results regarding the possible geographical or temporal gradients.

Therefore, further research on chromosomal polymorphism in *B. antarctica* is necessary. Such research should be conducted both in previously studied locations after a certain period and in new ones with different ecological conditions (e.g., substrate types, levels of ornithogenic and anthropogenic influence, concentrations of heavy metals, organic nitrogen, pH, temperature values; etc.). To understand the dynamics of evolutionary processes at the chromosomal level, long-term monitoring should be conducted every 20 years (corresponding to the complete turnover of 10 generations, considering the two-year life cycle of *B. antarctica* (Sugg et al., 1983)).

It is important to emphasize that (as highlighted in Michailova et al. (2023)), the fixed and transported material of this species is quite challenging to analyse due to the merging of bands and the different compactness of chromosomes. This significantly complicates a more thorough analysis of the chromosomes (especially in detecting homozygous, small heterozygous somatic and inherited inversions, and somatic functional alterations). The poor condition of chromosomes was noted by Martin (1962) and is also evident in the figures of Michailova et al. (2021). Given this, using live specimens may be more effective for the analysis of the polytene chromosomes of *B. antarctica*. Additionally, for further chromosomal analysis in this chironomid, it is important to provide gene mapping to determine the localization of specific genes on the chromosome.

Besides, *B. antarctica* has two phylogenetically close chironomid species (Allegrucci et al., 2012; Kozeretska et al., 2022): *Belgica albipes* (Séguy, 1965) – an endemic under-researched species of sub-Antarctic Crozet Islands (Serra-Tosio, 1982; Hullé et al., 2018; Hullé & Vernon, 2021), and *Eretmoptera murphyi* Schaeffer, 1914 – a midge native to sub-Antarctic South Georgia Island, which has now become invasive on Antarctic Signy Island (Bartlett et al., 2020; 2023). To date, there are no studies focusing on their karyological characteristics. Thus, investigating the chromosomes and their potential polymorphism in *B. albipes* and *E. murphyi* from a variety of sites, especially in comparison to *B. antarctica*, may open new avenues for a deeper understanding of evolutionary processes and pathways of speciation in these taxa.

Data availability. The data is within the current article and the Appendix section.

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Хромосомний поліморфізм різних популяцій *Belgica antarctica*: можливі зв'язки з екологічними та географічними факторами

Анотація. Поліморфізм хромосомних інверсій є важливим еволюційним механізмом для багатьох видів, зокрема комах. *Belgica antarctica* (Chironomidae) — єдиний ендемічний вид комах Антарктичного півострова. Сьогодні цей вид вважається модельним об'єктом для дослідження пристосувань живих організмів до змін клімату в екстремальних умовах наземних екосистем Антарктики. Цей полярний комар-дзвінець характеризується наявністю політенних хромосом, що робить його зручним для дослідження різноманітних хромосомних аберацій та аналізу їхнього потенційного зв'язку з географічними та екологічними факторами. У цій роботі було проаналізовано хромосомний поліморфізм слинних залоз 99 личинок *Belgica antarctica* четвертого віку, зібраних із чотирьох різних локалітетів регіону західної частини Антарктичного півострова у 2022 році. Зразки були зібрані з місць із різними екологічними умовами, що дає змогу дослідити можливий вплив середовища існування на розповсюдження хромосомних перебудов. У нових популяціях було виявлено шість із семи раніше описаних для цього виду спадкових гетерозиготних інверсій. Зокрема, було визначено точну локалізацію великої інверсії в хромосомі I, що містить ядерцевий організатор, вперше з часу її виявлення у 1962 році. Також, отримані результати щодо частот виявлених хромосомних інверсій було об'єднано з усіма наявними даними — разом від 1436 особин *Belgica antarctica* з 26 популяцій, охоплюючи період з 1962 по 2022 рік для визначення потенційного зв'язку між окремими екологічними (екологічна складність, орнітогенний вплив та чи з гніздового матеріалу були зібрані зразки) і географічними (довгота і широта) факторами. Було виявлено, що загалом обрані екологічні фактори не впливають на частоту досліджуваних інверсій. Разом із тим, встановлено незначні тренди в залежності частоти чотирьох гетерозиготних перебудов від широти, а також частоти особин із наявністю хоча б однієї не зчепленої зі статтю гетерозиготної інверсії та довготи. Зазначені тренди можуть свідчити про потенційні механізми природного відбору, які діють на досліджувані популяції *Belgica antarctica*.

Ключові слова: вид-ендемік, полярна комаха, хромосоми слинних залоз, хромосомна мінливість, частота хромосомних аберацій

APPENDIX

Table 1. Data on the localities of *Belgica antarctica* collections in which chromosomal polymorphism was studied. Newly tested localities are highlighted in bold. Locality codes Gal1 and Gal2 correspond to sampling locations 1 and 2 from Galindez Island, investigated by Michailova et al. (2023). The locality code GVB1962 represents the abbreviation for "González Videla Base" with the addition of the publication year of the study provided by Martin (1962). WI2020 is the abbreviation for Wiencke Island, with the addition of the year the sample was collected, as performed by Michailova et al. (2021). All other collection years are from 1974/1975, as reported in the research by Atchley and Davis (1979). The coordinates of the localities in the works of Martin (1962) and Atchley and Davis (1979) are approximated based on the data and figures presented in their studies

Locality code	Locality	Collection year	Latitude	Longitude	Sample size	Ornithogenic influence	Ecological complexity	Nest
B1105	**Cape Sterneck, Chavdar Peninsula**	**2022**	**−64.047706**	**−61.014187**	**25**	**1**	**4**	**0**
B1109	**Port Charcot, Booth Island**	**2022**	**−65.066675**	**−64.014096**	**20**	**3**	**6**	**1**
B1039	**Pickwick Island, Pitt Islands**	**2022**	**−65.484316**	**−65.642274**	**25**	**1**	**4**	**0**
B1316	**Moot Point, Kyiv Peninsula**	**2022**	**−65.203826**	**−64.075375**	**29**	**2**	**6**	**1**
Gal1	Galindez Island	2022	−65.247278	−64.250111	35	1	4	0
Gal2	Galindez Island	2022	−65.247861	−64.243500	31	3	4	0
GVB1962	González Videla Base, Danco Coast	1962	−64.823954	−62.857077	81	NA	NA	NA
AA1	Cormorant Island	1974/1975	−64.793763	−63.966527	58	NA	3	NA
AC1	Limitrophe Island	1974/1975	−64.794019	−64.001519	66	NA	3	NA
AE1	Hermit Island	1974/1975	−64.799923	−64.025653	63	NA	1	NA
AF1	Ikes Island (Christine Is.)	1974/1975	−64.794388	−64.031863	44	NA	3	NA
AG1	Shortcut Island	1974/1975	−64.783146	−64.042521	49	NA	1	NA
AG2	Shortcut Island	1974/1975	−64.783146	−64.042521	77	NA	1	NA
AJ1	Bonaparte Point	1974/1975	−64.777182	−64.061910	93	NA	4	NA
AJ3	Bonaparte Point	1974/1975	−64.777182	−64.061910	82	NA	4	NA
AJ4	Bonaparte Point	1974/1975	−64.777182	−64.061910	64	NA	3	NA
AM1	Norsal Point	1974/1975	−64.760340	−64.091179	55	NA	1	NA
AM5	Norsal Point	1974/1975	−64.760340	−64.091179	72	NA	5	NA
AM6	Norsal Point	1974/1975	−64.760340	−64.091179	68	NA	4	NA
AO1	Litchfield Island	1974/1975	−64.770497	−64.092880	70	NA	3	NA
AO4	Litchfield Island	1974/1975	−64.770497	−64.092880	66	NA	4	NA
AO5	Litchfield Island	1974/1975	−64.770497	−64.092880	62	NA	2	NA
AO6	Litchfield Island	1974/1975	−64.770497	−64.092880	101	NA	3	NA
AP1	Strandtmann Island	1974/1975	−64.779984	−64.098849	43	NA	2	NA
EA1	Port Lockroy, Goudier Island	1974/1975	−64.825087	−63.494142	49	NA	2	NA
WI2020	Wiencke Island	2020	−64.829070	−63.488680	8	NA	4	0

Table 2. Data on the frequencies of inherited heterozygous inversions and the proportions of heterozygotes with at least one inversion on any chromosome, specifically on chromosomes II and III for each of the studied localities. The proportion of heterozygotes with at least one inversion on chromosome I is not presented, as it is equal to the frequency of heterozygous inversion in sections 10–23 of chromosome I. The identification of the locality where Atchley and Davis (1979) found the single individual with this inversion in their study was performed analytically by comparing the proportions of different combinations of chromosomal inversions and the frequency of individuals with at least one chromosomal rearrangement according to their data. The frequency of the sex-linked heterozygous inversion D (chromosome III) is not presented, as it was excluded from the analysis due to the absent of data for all 18 localities in the study by Atchley and Davis (1979). Furthermore, the authors reported no significant differences in the frequency of this inversion among the studied localities. Newly tested localities are highlighted in bold. Statistically significant *P*-values of comparison between localities are marked in bold and italic

Locality code	Individuals with at least 1 inherited heterozygous non-sex-linked inversion	Individuals with at least 1 heterozygous inversion in chromosome II	Individuals with at least 1 non-sex-linked heterozygous inversion in chromosome III	Inversion in chromosome I (sections 10–23)	Inversion A (chromosome II)	Inversion B (chromosome II)	Inversion in chromosome II (sections 9–11)	Inversion C (chromosome III)	Inversion D1 (chromosome III)
B1105	**0.760**	**0.72**	**0.40**	**0**	**0.68**	**0.32**	**0**	**0.12**	**0.28**
B1109	**0.650**	**0.55**	**0.30**	**0.15**	**0.20**	**0.4**	**0**	**0**	**0.30**
B1039	**0.920**	**0.84**	**0.36**	**0.12**	**0.72**	**0.52**	**0**	**0.04**	**0.32**
B1316	**0.6552**	**0.5517**	**0.4138**	**0**	**0.4828**	**0.1724**	**0**	**0**	**0.4138**
Gal1	0.4286	0.3143	0.20	0	0.20	0.1143	0	0	0.20
Gal2	0.7742	0.6452	0.5484	0	0.4516	0.2581	0.0323	0.0323	0.5161
GVB1962	0.1605	0	0.037	0.1235	0	0	0	0.037	0
AA1	0.534	0.482	0.069	0	0.379	0.224	0	0.069	0
AC1	0.742	0.727	0.073	0	0.439	0.394	0	0.073	0
AE1	0.730	0.65	0.254	0	0.444	0.492	0	0.254	0
AF1	0.773	0.749	0.136	0	0.523	0.409	0	0.136	0
AG1	0.735	0.734	0.061	0	0.571	0.367	0	0.061	0
AG2	0.727	0.727	0.078	0	0.571	0.377	0	0.078	0
AJ1	0.731	0.731	0	0	0.613	0.247	0	0	0
AJ3	0.634	0.622	0.061	0	0.524	0.317	0	0.061	0
AJ4	0.813	0.781	0.047	0	0.513	0.438	0	0.047	0
AM1	0.745	0.709	0.20	0	0.618	0.20	0	0.20	0
AM5	0.847	0.667	0.431	0	0.542	0.306	0	0.431	0
AM6	0.647	0.646	0	0	0.529	0.235	0	0	0
AO1	0.757	0.685	0.314	0	0.514	0.443	0	0.314	0
AO4	0.667	0.636	0.121	0	0.53	0.288	0	0.121	0
AO5	0.855	0.743	0.258	0	0.452	0.5	0	0.258	0
AO6	0.762	0.753	0.02	0.0099	0.505	0.535	0	0.02	0
AP1	0.884	0.744	0.372	0	0.419	0.512	0	0.372	0
EA1	0.878	0.837	0.041	0	0.551	0.592	0	0.041	0
WI2020	0.875	0.75	0.25	0	0.625	0.5	0	0.25	0

End of Table 2

Locality code	Individuals with at least 1 inherited heterozygous non-sex-linked inversion	Individuals with at least 1 heterozygous inversion in chromosome II	Individuals with at least 1 non-sex-linked heterozygous inversion in chromosome III	Inversion in chromosome I (sections 10–23)	Inversion A (chromosome II)	Inversion B (chromosome II)	Inversion in chromosome II (sections 9–11)	Inversion C (chromosome III)	Inversion D1 (chromosome III)
***P*-value (Fisher's exact test with Monte Carlo simulation, B = 10000)**	***9.999e-05***	***9.999e-05***	***9.999e-05***	***9.999e-05***	***9.999e-05***	***9.999e-05***	0.1003	***9.999e-05***	***9.999e-05***
***P*-value Kruskal-Wallis *H* test (grouped by 'Ornithogenic influence') df = 2**	0.9535	0.8071	0.5013	0.643	0.5004	0.6671	0.3679	0.4586	0.3041
***P*-value Kruskal-Wallis *H* test (grouped by 'Ecological complexity') df = 5**	0.1081	0.1804	0.3033	0.507	0.1789	0.1463	0.879	0.05634	***0.0287***
***P*-value Kruskal-Wallis *H* test (grouped by 'Nest') df = 1**	0.2453	0.2453	0.6985	0.334	0.3286	0.6985	0.5271	0.1079	0.4386
***P*-value Mann-Whitney *U* test (grouped by 'Nest')**	0.381	0.381	0.8571	0.4687	0.4344	0.8571	0.7518	0.1595	0.5714
***P*-value Mann-Whitney *U* test (grouped by 'Collection year', two groups: 1st is 1962 and 1974/1975, 2nd is 2020 and 2022)**	0.8214	0.4523	***0.00503***	0.25	0.8623	0.6646	0.1186	0.0721	***9.735e-06***

Appendix Data

Generalized linear model (GLM) results for chromosomal inversion frequencies and heterozygotes in *Belgica antarctica* natural populations using latitude and longitude as predictors in R Software

1. Results of the GLM analysis for the dependence of the proportion of individuals with heterozygous inversion A on latitude.

```
> summary(glm_model_latitude_Ch2_A)
Call:
glm(formula = cbind(round(Belgica_antarctica_chromosome_polymorphism$Freq_Ch2_A *
    Belgica_antarctica_chromosome_polymorphism$Sample_size),
    round((1 - Belgica_antarctica_chromosome_polymorphism$Freq_Ch2_A) *
        Belgica_antarctica_chromosome_polymorphism$Sample_size)) ~
    Latitude, family = binomial(link = “logit”), data = Belgica_antarctica_chromosome_polymorphism)
Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  46.3083    19.6561   2.356   0.0185 *
Latitude      0.7157     0.3033   2.360   0.0183 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Dispersion parameter for binomial family taken to be 1)
    Null deviance: 159.72  on 25  degrees of freedom
Residual deviance: 154.02  on 24  degrees of freedom
AIC: 264.77
Number of Fisher Scoring iterations: 4
```

2. Results of the GLM analysis for the dependence of the proportion of individuals with heterozygous inversion C on latitude.

```
> summary(glm_model_latitude_Ch3_C)
Call:
glm(formula = cbind(round(Belgica_antarctica_chromosome_polymorphism$Freq_Ch3_C *
    Belgica_antarctica_chromosome_polymorphism$Sample_size),
    round((1 - Belgica_antarctica_chromosome_polymorphism$Freq_Ch3_C) *
        Belgica_antarctica_chromosome_polymorphism$Sample_size)) ~
    Latitude, family = binomial(link = “logit”), data = Belgica_antarctica_chromosome_polymorphism)
Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept) 101.4752    30.1775   3.363 0.000772 ***
Latitude      1.5975     0.4658   3.429 0.000605 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Dispersion parameter for binomial family taken to be 1)
    Null deviance: 207.25  on 25  degrees of freedom
Residual deviance: 195.53  on 24  degrees of freedom
AIC: 269.68
Number of Fisher Scoring iterations: 5
```

3. Results of the GLM analysis for the dependence of the proportion of individuals with heterozygous inversion D1 on latitude.

```
> summary(glm_model_latitude_Ch3_D1)
Call:
glm(formula = cbind(round(Belgica_antarctica_chromosome_polymorphism$Freq_Ch3_D1 *
    Belgica_antarctica_chromosome_polymorphism$Sample_size),
    round((1 - Belgica_antarctica_chromosome_polymorphism$Freq_Ch3_D1) *
```

```
        Belgica_antarctica_chromosome_polymorphism$Sample_size)) ~
    Latitude, family = binomial(link = “logit”), data = Belgica_antarctica_chromosome_polymorphism)
Coefficients:
             Estimate Std. Error z value Pr(>|z|)
(Intercept) -376.5384    35.8173  -10.51   <2e-16 ***
Latitude      -5.7505     0.5507  -10.44   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Dispersion parameter for binomial family taken to be 1)
    Null deviance: 270.41  on 25  degrees of freedom
Residual deviance: 166.51  on 24  degrees of freedom
AIC: 192.15
Number of Fisher Scoring iterations: 7
```

4. Results of the GLM analysis for the dependence of the proportion of individuals with heterozygous inversion in sections 10–23 of chromosome I on latitude.

```
> summary(glm_model_latitude_Freq_Ch1_inv)
Call:
glm(formula = cbind(round(Belgica_antarctica_chromosome_polymorphism$Freq_Ch1_inv *
    Belgica_antarctica_chromosome_polymorphism$Sample_size),
    round((1 - Belgica_antarctica_chromosome_polymorphism$Freq_Ch1_inv) *
        Belgica_antarctica_chromosome_polymorphism$Sample_size)) ~
    Latitude, family = binomial(link = “logit”), data = Belgica_antarctica_chromosome_polymorphism)
Coefficients:
             Estimate Std. Error z value Pr(>|z|)
(Intercept) -230.6151    59.9557  -3.846 0.000120 ***
Latitude      -3.4861     0.9228  -3.778 0.000158 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Dispersion parameter for binomial family taken to be 1)
    Null deviance: 77.612  on 25  degrees of freedom
Residual deviance: 66.467  on 24  degrees of freedom
AIC: 82.178
Number of Fisher Scoring iterations: 6
```

5. Results of the GLM analysis for the dependence of the proportion of individuals with heterozygous inversion D1 on longitude.

```
> summary(glm_model_longitude_Ch3_D1)
Call:
glm(formula = cbind(round(Belgica_antarctica_chromosome_polymorphism$Freq_Ch3_D1 *
    Belgica_antarctica_chromosome_polymorphism$Sample_size),
    round((1 - Belgica_antarctica_chromosome_polymorphism$Freq_Ch3_D1) *
        Belgica_antarctica_chromosome_polymorphism$Sample_size)) ~
    Longitude, family = binomial(link = “logit”), data = Belgica_antarctica_chromosome_polymorphism)
Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept) -9.35506   17.21097  -0.544    0.587
Longitude   -0.09615    0.26900  -0.357    0.721
(Dispersion parameter for binomial family taken to be 1)
    Null deviance: 270.41  on 25  degrees of freedom
Residual deviance: 270.28  on 24  degrees of freedom
AIC: 295.91
Number of Fisher Scoring iterations: 6
```

6. Results of the GLM analysis for the dependence of the proportion of individuals with at least one non-sexlinked heterozygous inversion on longitude.

```
> summary(glm_model_longitude_Freq_atleast_1_noSL_het_inv)
Call:
glm(formula = cbind(round(Belgica_antarctica_chromosome_polymorphism$Freq_atleast_1_noSL_het_inv *
    Belgica_antarctica_chromosome_polymorphism$Sample_size),
    round((1 - Belgica_antarctica_chromosome_polymorphism$Freq_atleast_1_noSL_het_inv) *
        Belgica_antarctica_chromosome_polymorphism$Sample_size)) ~
    Longitude, family = binomial(link = “logit”), data = Belgica_antarctica_chromosome_polymorphism)
Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept) -35.2381     6.9083  -5.101 3.38e-07 ***
Longitude    -0.5647     0.1081  -5.226 1.73e-07 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Dispersion parameter for binomial family taken to be 1)
    Null deviance: 177.30  on 25  degrees of freedom
Residual deviance: 148.05  on 24  degrees of freedom
AIC: 255.61
Number of Fisher Scoring iterations: 4
```

7. Results of the GLM analysis for the dependence of the proportion of individuals with at least one heterozygous inversion on chromosome III (excluding sex-linked inversions) on longitude.

```
> summary(glm_model_longitude_Freq_atleast_1_noSL_het_inv_Ch3)
Call:
glm(formula = cbind(round(Belgica_antarctica_chromosome_polymorphism$Freq_atleast_1_noSL_het_inv_Ch3 *
    Belgica_antarctica_chromosome_polymorphism$Sample_size),
    round((1 - Belgica_antarctica_chromosome_polymorphism$Freq_atleast_1_noSL_het_inv_Ch3) *
        Belgica_antarctica_chromosome_polymorphism$Sample_size)) ~
    Longitude, family = binomial(link = “logit”), data = Belgica_antarctica_chromosome_polymorphism)
Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept) -11.3572     9.3640  -1.213    0.225
Longitude    -0.1510     0.1463  -1.032    0.302
(Dispersion parameter for binomial family taken to be 1)
    Null deviance: 243.35  on 25  degrees of freedom
Residual deviance: 242.23  on 24  degrees of freedom
AIC: 331.43
Number of Fisher Scoring iterations: 5
```