Antarctic Science 35(5), 328–344 (2023) © The Author(s), 2023. Published by Cambridge University Press on behalf of Antarctic Science Ltd
doi:10.1017/S0954102023000202

A chromosome map of *Belgica antarctica* Jacobs (Diptera: Chironomidae) from Antarctica, including chromosome variability

PARASKEVA MICHAILOVA [1], PAVLO A. KOVALENKO [2,3], SVITLANA SERGA [3,4,5], IVAN PARNIKOZA [3,6], IRYNA KOZERETSKA [3] and PETER CONVEY [7,8,9]

[1]*Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences, Sofia, 1000, Bulgaria*
[2]*Institute for Evolutionary Ecology of the National Academy of Sciences of Ukraine, Kyiv, 03143, Ukraine*
[3]*National Antarctic Scientific Center of Ukraine, Kyiv, 01601, Ukraine*
[4]*Taras Shevchenko National University of Kyiv, Kyiv, 01601, Ukraine*
[5]*CBGP, INRAE, CIRAD, IRD, SupAgro, University Montpellier, Montpellier, 34980, France*
[6]*Institute of Molecular Biology and Genetics of National Academy of Science of Ukraine, Kyiv, 03143, Ukraine*
[7]*British Antarctic Survey, NERC, Cambridge, CB3 0ET, UK*
[8]*Department of Zoology, University of Johannesburg, Auckland Park, Johannesburg, 2006, South Africa*
[9]*Millennium Institute Biodiversity of Antarctic and Sub-Antarctic Ecosystems, Santiago, Chile*
pavlo.kovalenko97@yahoo.com

Abstract: *Belgica antarctica* Jacobs (Diptera: Chironomidae) is the only endemic insect found in the Antarctic Peninsula region and has stimulated considerable research interest. Due to recent rapid changes in regional climate in Antarctica, there is growing interest in studying the responses of this species to environmental changes, in particular at the chromosomal level. Chromosomal inversions are known to play an important role in speciation and adaptation in many insect species, but their frequencies in natural populations are poorly understood. In the current study, we provide the first standard polytene chromosome map for *B. antarctica*, which will enable the precise location of chromosomal abnormalities in future studies. We further analysed chromosomal polymorphisms in fourth-instar larvae collected from two different locations on Galindez Island, Argentine Islands, western coast of the Antarctic Peninsula. We found four previously reported and two new inherited inversions, and we discuss their possible adaptive role in response to environmental stressors in the Antarctic Peninsula region. Our data provide a foundation for future studies exploring the potential role of *B. antarctica* chromosomal polymorphisms in adaptation to the changing environment.

Received 31 March 2023, accepted 18 June 2023

Key words: Antarctic midge, chromosome aberrations, polytene chromosomes

Introduction

Belgica antarctica (Diptera: Chironomidae) is the only endemic insect occurring on the Antarctic Peninsula and, with *Parochlus steinenii* (Diptera: Chironomidae) on the adjacent South Shetland Islands, native to this region (Usher & Edwards 1984, Richard *et al.* 1994, Convey & Block 1996, Chown & Convey 2016, Kozeretska *et al.* 2022). It has stimulated considerable research interest across disciplines: from genomics and phylogeography to biochemistry, physiology and ecology (Kozeretska *et al.* 2022). Of particular interest are its possible responses to climate change, as has been occurring in the Antarctic Peninsula region in recent decades (Turner *et al.* 2014, Convey & Peck 2019, Bargagli 2020). However, to date, few studies of either *B. antarctica* or Antarctic microarthropods more generally have directly addressed climate change (Devlin *et al.* 2022, Matheson & McGaughran 2023). *Belgica antarctica*'s karyotype was briefly described 60 years ago on the basis of the salivary gland chromosomes (Martin 1962). The diploid set of chromosomes is $2n = 6$ (Martin 1962, Atchley & Davis 1979, Michailova *et al.* 2021). However, to date, a cytological map of the polytene chromosomes of this species has not been compiled. *Belgica antarctica* has one of the smallest insect genomes yet sequenced, although other members of the Chironomidae (including the subfamily Orthocladiinae to which *B. antarctica* belongs) also have small genomes, and the number of functional genes is comparable to that in other Diptera species (Kelley *et al.* 2014, Cornette *et al.* 2015, Kaiser *et al.* 2016).

Chromosomal indels are common in many species of insects representing multiple different groups, including Diptera, Orthoptera, Coleoptera, Hemiptera, Lepidoptera and Dermaptera (Da Cunha 1960, John & Lewis 1966, Hoffmann *et al.* 2004). They can contribute > 20 Mb of the genome in some insect species (e.g. 22 Mb in *Anopheles gambiae*; White *et al.* 2007). It is known that chromosomal inversions often occur as balance

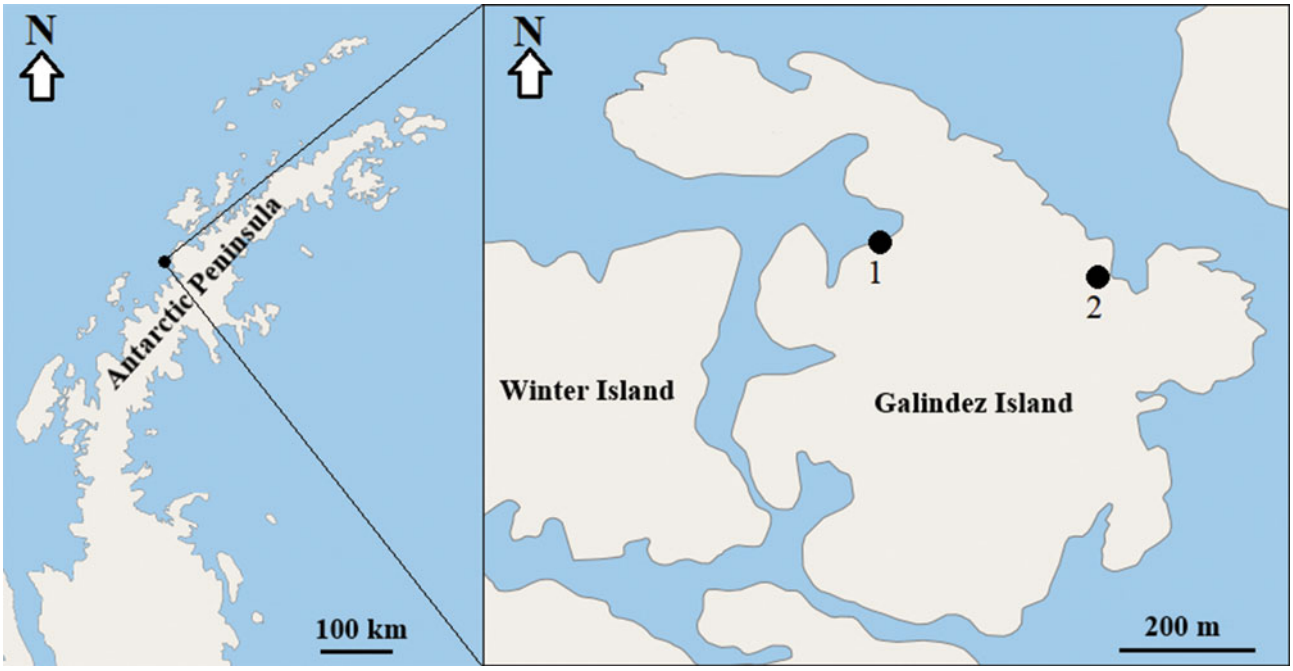

Fig. 1. Map indicating the sampling locations (1 and 2) for *Belgica antarctica* larvae on Galindez Island (Argentine Islands) used in this study.

polymorphisms and potentially can play an important role in speciation (Noor *et al.* 2001, Fuller *et al.* 2018, Wellenreuther & Bernatchez 2018). However, it remains unknown which factors evoke changes in the frequencies of chromosomal inversions in natural populations (Kirkpatrick 2010). Inversions are thought to perform adaptive functions by suppressing recombination in the heterozygous state, when heterozygous individuals have an advantage in stabilizing selection (Wellenreuther & Bernatchez 2018). Pool-seq technology, which has recently been used to study the genomes of many species, allows the study of inversion events but cannot be used to obtain inversion frequencies in species whose genomes have not been sufficiently studied (Kapun *et al.* 2014). In such cases, cytological studies of polytene chromosomes provide a useful tool. Although the *B. antarctica* genome has been sequenced (Kelley *et al.* 2014), a set of diagnostic indel markers has yet to be determined (Michailova *et al.* 2021).

Martin (1962) described the presence of inversions in two of the three chromosomes of *B. antarctica*: an inversion in chromosome III and a large inversion in chromosome I. Atchley & Davis (1979) described five inversions: one large heterozygous inversion in chromosome I, apparently the same as that described by Martin (1962), two further inversions in chromosome II (annotated as inversions A and B), one inversion in chromosome III (a small inversion, inversion C) and also one further inversion in chromosome III was sex-linked (D) and was always heterozygous in males. In the most recent research (Michailova *et al.* 2021), the presence of inversions A and B in chromosome II was confirmed, as well as that of the small inversion near the telomere in chromosome III. However, the inversion reported in chromosome I and the sex-linked inversion in chromosome III were not detected. Michailova *et al.* (2021) also first noted the presence of a dark band in chromosomes in a few cells. However, they only examined a small sample of *B. antarctica* individuals from a single location. To provide greater understanding of chromosomal variability in *B. antarctica*, material from a wider proportion of the range of the species needs to be examined, and, to enable such research, a chromosome map of the species is required allowing precise locating of chromosome abnormalities from all sampling locations. Comparative analyses of the presence and frequencies of chromosomal indels from different locations can also then be performed.

In this study, we provide the first standard polytene chromosome map for *B. antarctica*. In addition, we analysed chromosomal variability in sample material collected from two separate locations on Galindez Island (Argentine Islands, western coast of the Antarctic Peninsula). We also performed a comparative chromosomal analysis of material obtained from these two locations and, using statistical analyses, identified differences in the occurrence of common chromosomal abnormalities of the populations present at these sampling locations and others reported by Martin (1962), Atchley & Davis (1979) and Michailova *et al.* (2021).

https://doi.org/10.1017/S0954102023000202 Published online by Cambridge University Press

Table I. Description of sampling locations on Galindez Island (Argentine Islands, western coast of the Antarctic Peninsula) used in this study.

Code	Location	Coordinates	Altitude (m above sea level)	Date of collection	Description
1	Green Grotto, Galindez Island	-65.247278°, -64.250111°	2	10 February 2022	Protected rock grotto Ornithogenic influence - low Short moss turf and cushion subformation (Ochyra *et al.* 2008): *Cratoneuropsis relaxa* as dominant; additionally, *Bryum pseudotriquetrum*, *Pohlia cruda*, *Sanionia georgicouncinata*
2	Roztochchia Ridge, Galindez Island	-65.247861°, -64.243500°	4	7 February 2022	Open area Ornithogenic influence - high Bryophyte carpet and mat subformation (Ochyra *et al.* 2008): *Sanionia georgicouncinata*; very close to the new nesting area of gentoo penguins (*Pygoscelis papua*; since 2019); close to *Deschampsia antarctica* and *Colobanthus quitenis* stands

Material and methods

Study sites

Samples were obtained during the 26th Ukrainian Antarctic expedition 2021–2022. *Belgica antarctica* fourth-instar larvae were collected from moss cushions from two separate locations on Galindez Island, Argentine Islands, western coast of the Antarctic Peninsula (Fig. 1 & Table I) and immediately fixed in ethanol acetic acid (3:1) for cytogenetic analysis. The fixative was changed several times between February and May 2022.

Cytogenetic analyses

The polytene chromosomes of *B. antarctica* were studied in the fourth-instar larvae. To develop the chromosome map, we used living larval collections returned from the Antarctic (contained and transported within their native substrate in moist sterile boxes at a temperature +4°C) in order to obtain well-spread polytene chromosomes. Chromosome preparations were performed as described by Michailova (1989). To localize the centromeric region, we used the 'C' banding method (Michailova 1994). Preparations are archived at the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences and at the National Antarctic Scientific Center of Ukraine.

For the preparation of the standard chromosome map, each chromosome is divided into sections, indicated by numbers, with the chromosomes numbered one (I), two (II) and three (III). The notation used for inversions follows Atchley & Davis (1979). We used the standard chromosome map for the accurate location of different puffs, as well as those of somatic and inherited aberrations. It is necessary to underline the number of cells in every salivary gland. There are two round salivary glands in the studied species, and after fixation specific for cytogenetic analysis of the larva, the cells are located peripherally. Their number in each gland is ~16. We considered an inversion to be inherited when it affected all cells of both salivary glands of the same individual, although we did not have the opportunity to confirm inheritance through generations. Somatic structural aberrations are typically located in only a few cells of the salivary glands (Sella *et al.* 2004). Their locations and those of inherited aberrations were established by detailed comparative analysis against the standard chromosome map of the species.

Statistical analyses

Percentages of different inherited and somatic aberrations in the salivary gland cells in larvae from each sampling location were calculated. A somatic index (S) was calculated for each location as the ratio of the number of different somatic aberrations relative to the number of individuals sampled from that location. The inherited index (H) was calculated as the ratio between the number of individuals with inherited aberrations at each location and the number of individuals sampled from that location (Sella *et al.* 2004).

The frequencies of common hereditary aberrations in the polytene chromosomes of larvae sampled from both locations as well as those from our previous study (Michailova *et al.* 2021) were compared using Fisher's exact test (Fisher 1922). The frequencies of inherited aberrations recorded in previous studies (Martin 1962,

Table II. The number of individual *Belgica antarctica* larvae studied and indices calculated.

Sampling location code	Number of specimens studied	Inherited index (H)	Somatic index (S)
1	35	0.11	0.34
2	31	0.23	0.52

https://doi.org/10.1017/S0954102023000202 Published online by Cambridge University Press

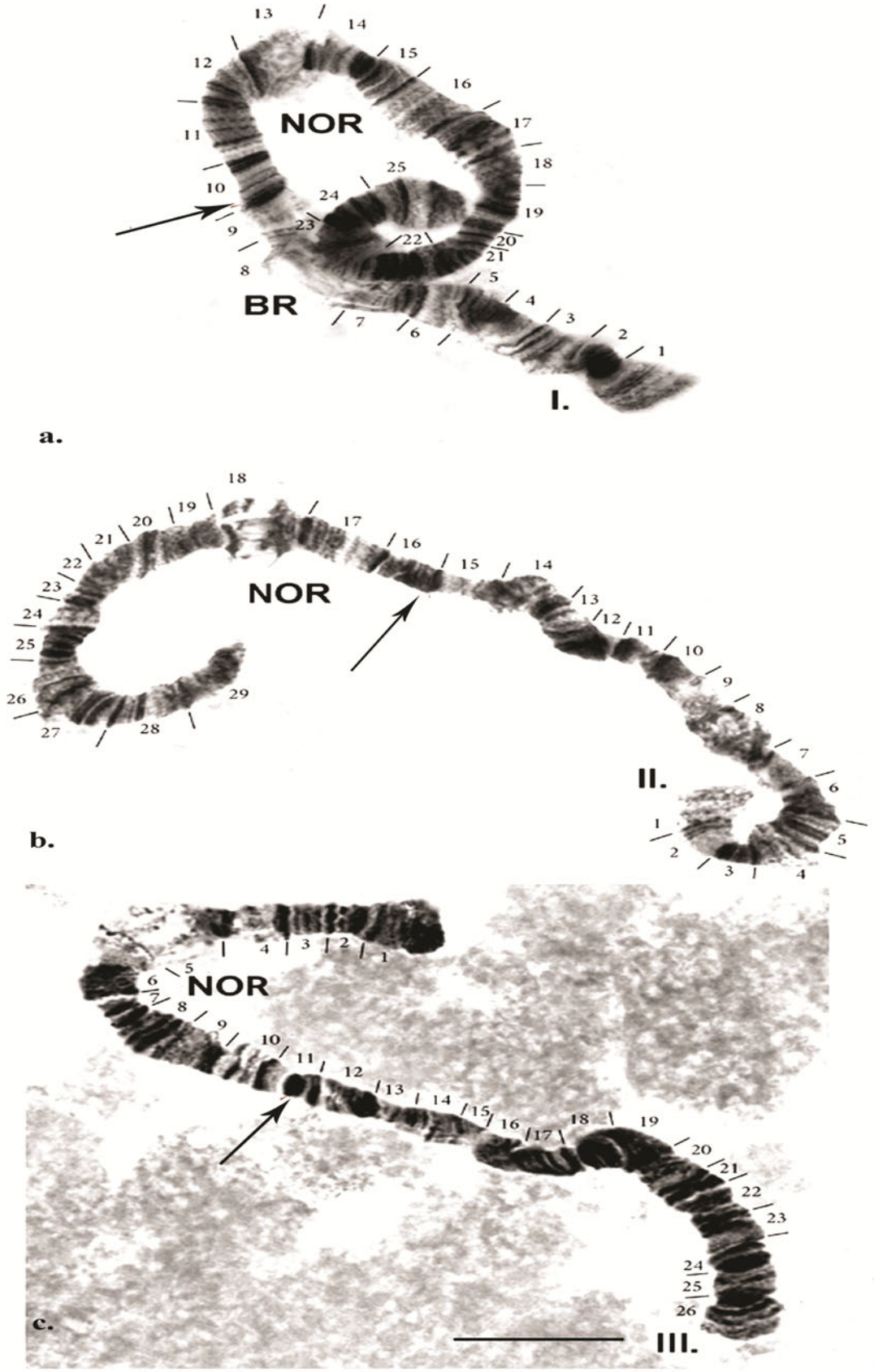

Fig. 2. Chromosome map of the polytene chromosomes of *Belgica antarctica*: **a.** chromosome I; **b.** chromosome II; **c.** chromosome III. Scale bar = 10 μm. BR = Balbiani ring; NOR = nuclear organizer region.

https://doi.org/10.1017/S0954102023000202 Published online by Cambridge University Press

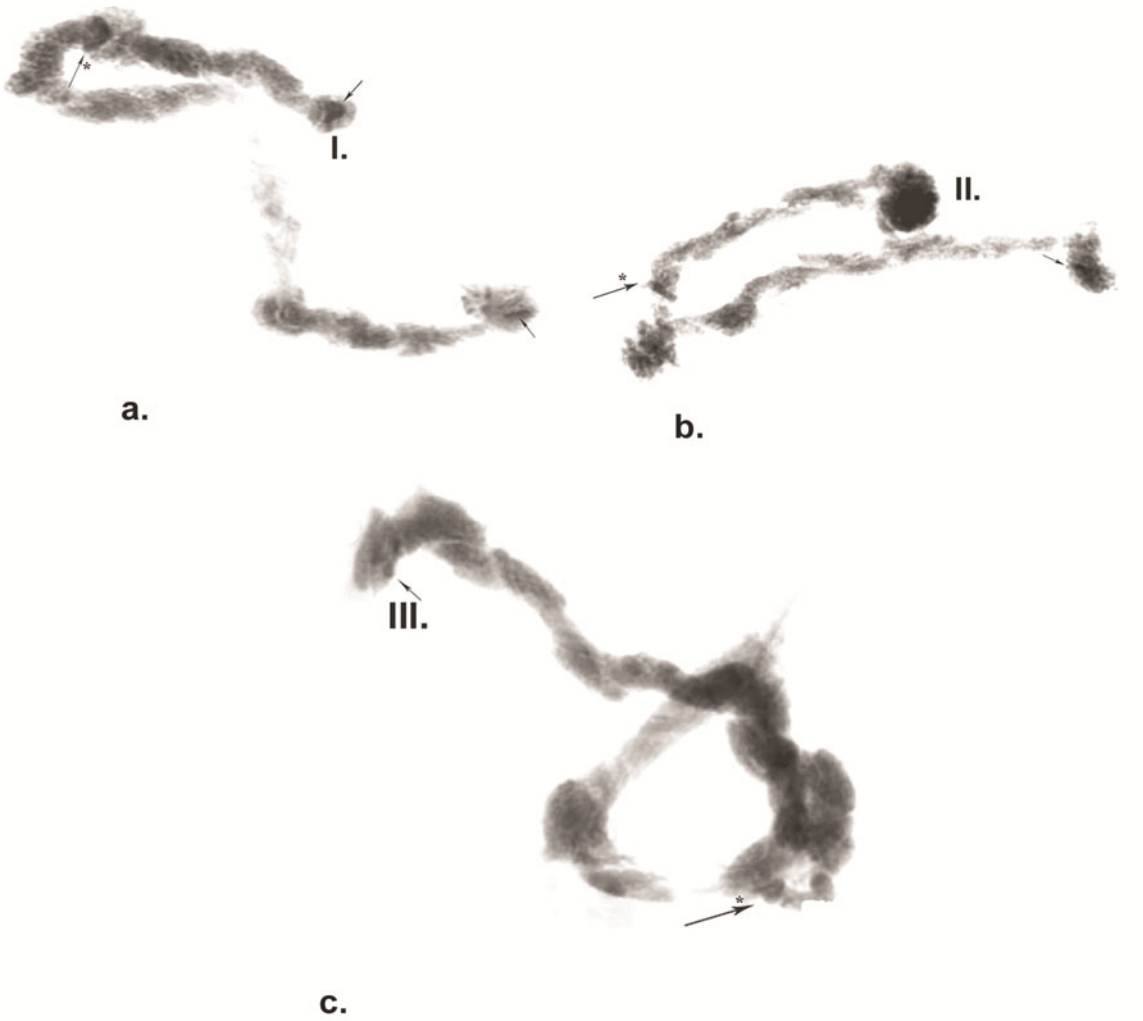

Fig. 3. 'C' banding in polytene chromosomes of *Belgica antarctica*. **a.** 'C' banding of chromosome I; **b.** 'C' banding of chromosome II; **c.** 'C' banding of chromosome III. Scale bar = 10 μm. Large arrows with asterisks indicate centromere regions, small arrows indicate telomere heterochromatin.

Atchley & Davis 1979, Michailova *et al*. 2021) were compared using the Kruskal-Wallis test (Kruskal & Wallis 1952). Confidence intervals were calculated using the Clopper-Pearson exact method (at a confidence level of 0.95; Clopper & Pearson 1934).

Results

The numbers of larvae analysed from each location and the indices calculated are given in Table II.

Chromosome map of B. antarctica *and functional activity along its polytene chromosomes*

In living larval material of *B. antarctica*, the salivary polytene chromosomes show a good band-like structure. However, in fixed material, the bands of each chromosome are often of different compactness and can become fused, complicating analysis of the chromosomes. In addition, chromosomes in salivary glands with varying degrees of polytene can be seen in the same individual. By applying the 'C' banding method, the precise location of the centromeric regions of the chromosomes can be determined. The

https://doi.org/10.1017/S0954102023000202 Published online by Cambridge University Press

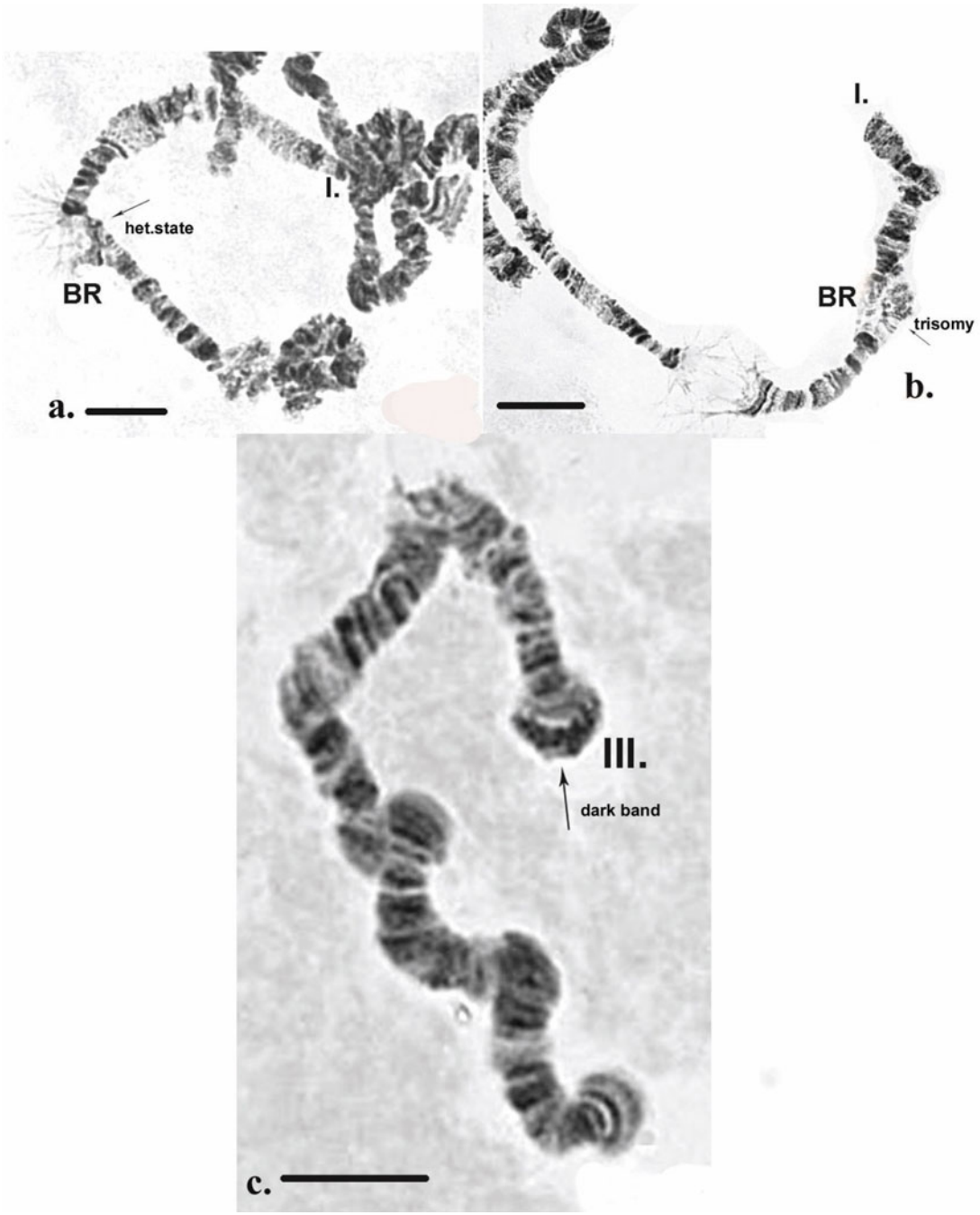

Fig. 4. Polytene chromosomes of *Belgica antarctica* with functional chromosome alterations and structure aberrations. **a.** Chromosome I: Balbiani ring (BR) in a heterozygous state; **b.** chromosome I: BR with a trisomy; **c.** chromosome III: dark band at telomere. Scale bar = 10 μm.

centromeric region is a dark band where structural heterochromatin is localized. Polytene chromosomes, after the specific processing characteristic of the 'C' banding method, lose their typical disc-shaped structure. Despite that, the bands are light and can be used for chromosome identification. Only certain regions of the chromosome that contain the structural heterochromatin remain darkly stained (these are the centromeres and telomere regions). This method reveals structural heterochromatin containing satellite DNA, most often located in the centromeric regions of the chromosome. The resulting original chromosome map of *B. antarctica* is shown in Fig. 2a–c.

Chromosome I (Fig. 2a). The chromosome is submetacentric - the centromere is localized between sections 9 and 10 (Fig. 3a). The first chromosome is

https://doi.org/10.1017/S0954102023000202 Published online by Cambridge University Press

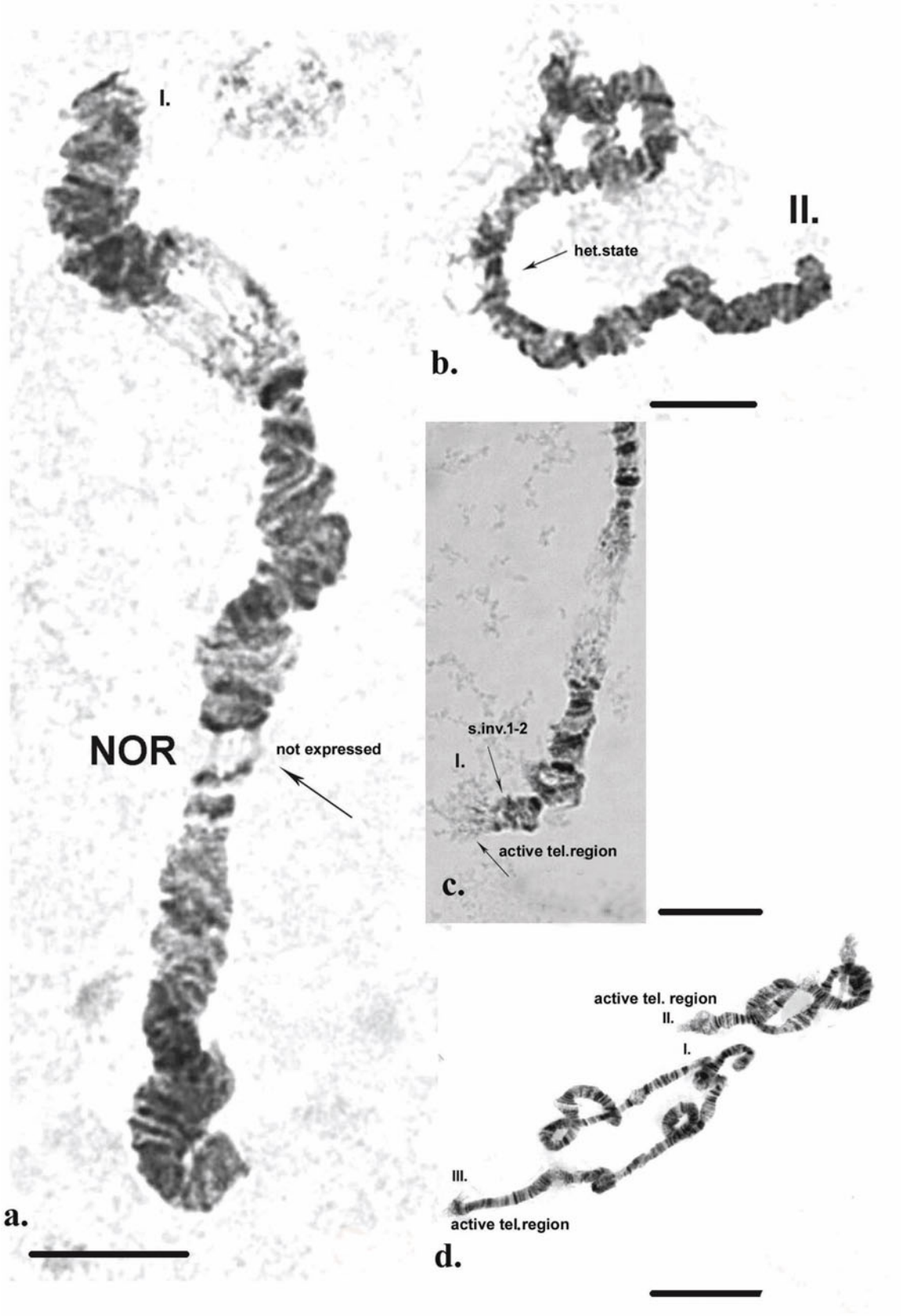

Fig. 5. Polytene chromosomes of *Belgica antarctica* with functional **c**hromosome alterations and structure aberrations. **a.** Chromosome I: nuclear organizer region (NOR) is not expressed; **b.** chromosome II: NOR in a heterozygous state, heterozygous inversion A; **c.** chromosome I: an active telomere region, a somatic inversion 1–2; **d.** chromosomes II and III: an active telomere region. Scale bar = 10 μm.

https://doi.org/10.1017/S0954102023000202 Published online by Cambridge University Press

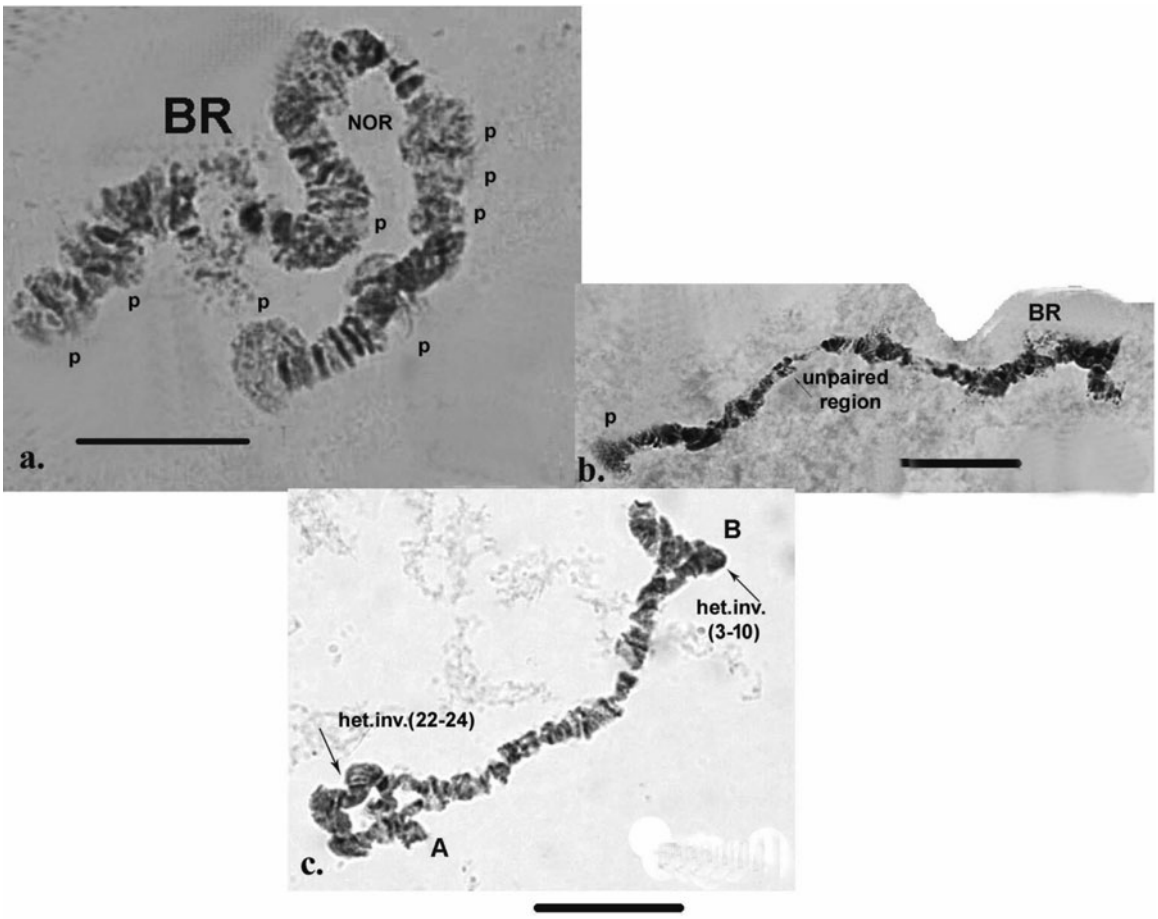

Fig. 6. Polytene chromosomes of *Belgica antarctica* with functional chromosome alterations and structure aberrations. **a.** Many puffs (p) along chromosome I; **b.** puff in section 25 of chromosome I, unpaired region; **c.** heterozygous inversions in chromosome II: A (sections 22–28) + B (sections 3–10). Scale bar = 10 μm. BR = Balbiani ring; NOR = nuclear organizer region.

provisionally divided into 25 sections. Atchley & Davis (1979) designated the arm near the Balbiani ring (BR) as the left arm (L) and that on the opposite side as the right arm (R). In *Chironomus tentans*, the BR contains genes encoding 75 rRNA (Kirov *et al.* 1991). It is a matrix for the synthesis of the high-molecular-weight proteins of the salivary gland, and they are used to build 'houses' for the larvae, where the development of the larvae takes place. In section 2, three fused pairs of dark discs are often present. Using 'C' differential staining in zone 1, a strong heterochromatin disc is always detected. From section 3 to section 7, alternating pairs of dark and light discs are apparent. The BR is located in section 8 and is a very well-expressed marker of this chromosome.

It is notable that in some cells in certain individuals the BR was in a heterozygous state or in trisomy (Fig. 4a,b). The dark disc located between sections 9 and 10 represents the centromere region and is a very well-defined section with 'C' banding staining. As diagnostic features of this chromosome, the dark bands of the centromere region, those in section 10 and the two lighter discs located between them were used. The bands next to the nuclear organizer region (NOR), located in section 13, were not always well-expressed. The NOR is a functionally active sector of the chromosome with genes responsible for the synthesis of 18s and 28s RNA (Gunderina *et al.* 2015). It is a giant puff that functions through all stages of larval development, and material of transcriptional activity is visible around it (using phase-contrast microscopy). However, the NOR is surrounded by two dark bands, providing a good chromosome marker. In some cases, the NOR was expressed in sections 16, 17 or 18. The group of dark bands in sections 14, 17 and 18 is diagnostic for this chromosome. The embroidery-like pattern of discs between zones 15 and 18 is also a chromosome marker, as are the group of dark bands in sections 21, 22 and 24. The chromosome terminates with a few bands, before which an active zone is often observed. It is notable that, using 'C' differential staining, these bands appear as separate dots, with a well-defined dark disc in front of them (Fig. 3a–c).

https://doi.org/10.1017/S0954102023000202 Published online by Cambridge University Press

Chromosome II (Fig. 2b). The second chromosome is metacentric, with the centromere located in sections 15–16 (Fig. 3b), and it is provisionally divided into 29 sections. The chromosome begins with a slight expansion, and the telomere part of the chromosome contains well-expressed heterochromatin (Fig. 3b), which is well revealed by 'C' banding. Atchley & Davis (1979) referred to this part of the chromosome as the left arm (L) and the opposite part as the right arm (R). There is a narrowing in section 3, in which three pairs of dark bands are located. A faint extension is apparent in section 4, being diagnostic for the chromosome. The dense dark discs delimiting each of sections 7, 8, 10, 11, 12, 13, 14, 15, 16 and 17 provide good diagnostic markers for this chromosome. The NOR, located in section 18, is also a chromosome marker, along with dark bands in sections 20, 23, 26 and 27. Section 28 has a weak extension and is bounded by dark discs. The telomere part of the chromosome is often weakly active. It is notable that, using 'C' differential staining, these bands appear as separate dots, with a well-defined dark disc in front of them (Fig. 3a–c).

Chromosome III (Fig. 2c). The third chromosome is submetacentric, with the centromere region included in a heterozygous inversion in section 11 (Fig. 3c). The chromosome is divided into 26 sections. A characteristic feature of the chromosome is that the NOR is not far from the telomere, in section 5, forming part of the left arm (L) of this chromosome, and the opposite part is the right arm (R; Atchley & Davis 1979). A large dark band was frequently present in the telomere of this chromosome, expressed by a telomere dark disc. In section 6, delimiting the NOR, a group of dark discs was present, providing a marker for this chromosome. Following this, a series of solid dark bands was present up to section 8. The centromere is located in section 11, which is clearly shown by 'C' differential staining (Fig. 3c). Discs between the centromere region and section 19 were not always well defined. Sections 21, 22, 23, 24 and 26 contained well-defined dark discs, providing markers for this chromosome. Using 'C' differential staining, several dark spots were detected in the telomere of this chromosome (Fig. 3c).

A telomere dark disc (Fig. 4c) was observed on all chromosomes of individuals from the different sampling locations, in some cases in a heterozygous state. Different expressions of the NORs of the polytene chromosomes were often observed in different cells, both in the same and different individuals (Fig. 5a). In some cells, the NOR occurred in a heterozygous state (Fig. 5b); in a heterozygous state, one homologue is active, expressed by the formation of a puff and reflecting transcriptional activity, and the other homologue is with the corresponding discs.

High functional activity characterized the specimens analysed, as indicated by the presence of numerous puffs along the length of the chromosomes, as well as at their telomeres (Figs 5c,d & 6a). Functional variability in the telomeres of the chromosome (appearance of puffs) can be observed in species living in conditions to which they are not well-adapted (e.g. polluted areas).

The telomere activity of all chromosomes from specimens from each of the sampling locations was notable. The telomere heterochromatin often changed from clearly visible heterochromatin bands with a granular structure to very active regions with the formation of puffs (Figs 5c,d & 6a,b). In some cases, individual bands were missing or appeared as a large dark band (Fig. 4c).

Chromosome aberrations in B. antarctica

The markers characteristic of each chromosome, combined with the standard chromosome map, allowed different aberrations along the polytene chromosomes to be identified and comparisons made between these aberrations from the different sampling locations. Two types of aberrations were present in the cytogenetically examined samples: hereditary and somatic.

Inherited aberrations from both sampling locations

Inherited aberrations were represented by paracentric heterozygous inversions distributed in the second and third chromosomes with varying frequencies (Table III). An inversion is a structural chromosomal aberration in which a chromosomal segment is rotated 180°. It is paracentric when it occurs in one arm of the chromosome only. In the heterozygous state, this inversion covers only one homologue, and to preserve the conjugation of the chromosomes a loop is formed, which is observed when this type of aberration occurs. In samples from location 1, the heterozygous inversions A and B in the second chromosome were present in separate specimens, whereas in sampling location 2, the inversions in the second chromosome were found in both the same and separate individuals (Fig. 6c). There was a significant difference in the frequency of inversion A (inv. A and A + B; Table III) between the two sampled locations (Fisher's exact test, $P = 0.036$), but not of inversion B (inv. B and A + B; Table III; Fisher's exact test, $P = 0.201$; Table S1). A heterozygous inversion (D1) and a sex-linked inversion (D) in the third chromosome were detected in samples from both locations (Fig. 7a & Table III). We also observed a heterozygous deficiency in chromosome I (Fig. 7b). Inversion D1 occurred at significantly different frequencies at the two sampling locations (Fisher's exact test, $P = 0.0099$). The small inversion (C) in the third chromosome (Fig. 8a) was found only in sampling location 2 (Table III). There was no significant difference in the frequencies of sex-linked inversion D (Fisher's exact test, $P = 0.3536$).

https://doi.org/10.1017/S0954102023000202 Published online by Cambridge University Press

Table III. Inherited aberrations and their chromosomal locations in *Belgica antarctica* larvae from the studied locations.

Inherited aberrations	Localization	Number of individuals with aberrations		Frequency (%), proportion confidence interval in parentheses		*P*-value
		Location 1	Location 2	Location 1	Location 2	
Heterozygous inversion	Chromosome II (inversion B): (3–10)	4	5	11.43 (3.20–26.74)	16.13 (5.45–33.73)	0.7240
Heterozygous inversion	Chromosome II: 9–11	0	1	0 (0–10)	3.23 (0.08–16.70)	0.4697
Heterozygous inversion	Chromosome II (inversion A): (22–28)	7	11	20 (8.44–36.94)	35.48 (19.23–54.63)	0.1784
Heterozygous inversion	Chromosome II (A + B): A (22–28); B (3–10)	0	3	0 (0–10)	9.68 (2.00–25.75)	0.0982
Heterozygous inversion	Chromosome III (inversion D1): 2–10	7	16	20 (8.44–36.94)	51.61 (33.06–69.85)	0.0099
Heterozygous inversion	Chromosome III (inversion C): (21–24)	0	1	0 (0–10)	3.23 (0.08–16.70)	0.4697
Sex-linked inversion	Chromosome III (inversion D): (11–12)	5	8	14.29 (4.81–30.26)	25.81 (11.86–44.61)	0.3536

Comparison of the frequency of common inversions found in the current study with previous studies

We did not identify a significant difference in the general patterns of frequencies of inversions A, B and C between the data obtained in our study and those of Atchley & Davis (1979) and Michailova *et al.* (2021; Kruskal-Wallis test, $P = 0.062$ for inversion A, $P = 0.127$ for inversion B, $P = 0.185$ for inversion C; Table S2).

The frequencies of heterozygous inversion A in the current study and those reported by Michailova *et al.* (2021) were significantly different for sampling location 1 (Fisher's exact test, $P = 0.028$) but not for sampling location 2 (Fisher's exact test, $P = 0.451$). The same result was obtained for heterozygous inversion B in chromosome II: the frequency differed significantly only for sampling location 1 (Fisher's exact test, $P = 0.028$; Table III & Table S1).

No significant difference was observed in the frequency of the large dark band in the telomere of the third chromosome in samples from location 2 compared with that reported by Michailova *et al.* (2021; Fisher's exact test, $P = 0.1011$), while a significant difference was detected in the comparison of Michailova *et al.*'s (2021) data and the current samples from location 1 (Fisher's exact test, $P = 0.031$).

In samples from location 2, we found a heterozygous inversion (C) in the third chromosome (Table IV) in the same chromosomal section in which an inversion was reported by Martin (1962). There was no difference in the frequency of this aberration between the two studies (Fisher's exact test, $P = 1$).

Somatic aberrations from both sampling locations

A wide spectrum of somatic chromosome rearrangements (paracentric heterozygous inversions, deficiencies, trisomy (trisomy is a numerical change in the chromosome in which three parts of the chromosome are visible) and a large dark band) was observed in individuals sampled from both sampling locations in the current study (Figs 7a,b, 8b,c & 9a–c & Table IV). Such rearrangements occurred in a small region, at a low frequency and only in a few cells of the salivary gland. The frequency of the presence of the dark band at the telomeres of the three chromosomes showed a significant difference only in the third chromosome (Fisher's exact test, chromosome I: $P = 0.4501$; chromosome II: $P = 1$; chromosome III: $P = 0.0001$).

Discussion

This study provides the first polytene chromosome map of *B. antarctica*, an important contribution enabling analysis of chromosomal instability in this species, which is

https://doi.org/10.1017/S0954102023000202 Published online by Cambridge University Press

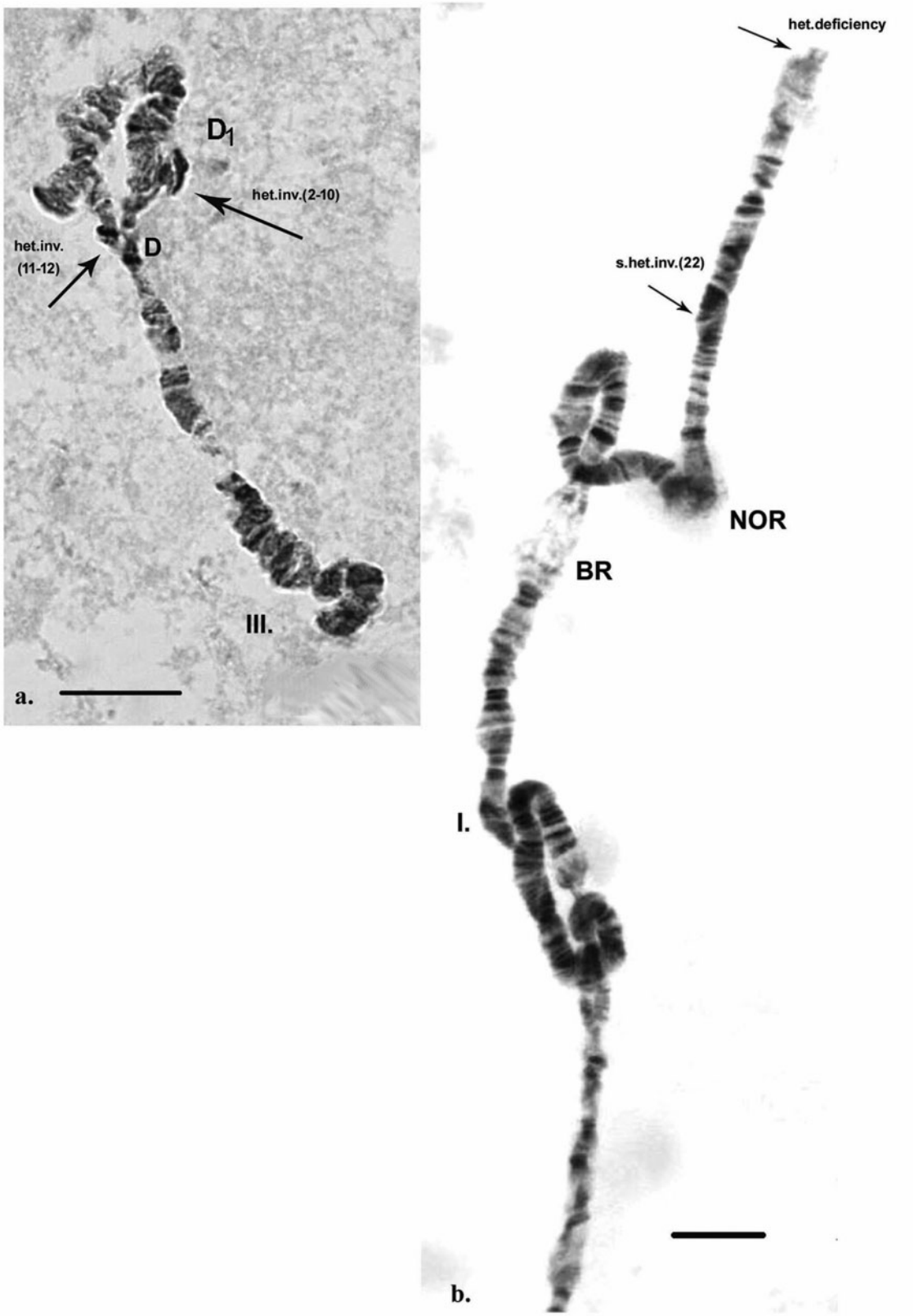

Fig. 7. Polytene chromosomes of *Belgica antarctica* showing structural aberrations. **a.** Heterozygous inversion in chromosome III: D1 (sections 2–10), sex-linked inversion D (11–12); **b.** chromosome I: somatic heterozygous deficiency, somatic heterozygous inversion (section 22). Scale bar = 10 μm. BR = Balbiani ring; NOR = nuclear organizer region.

https://doi.org/10.1017/S0954102023000202 Published online by Cambridge University Press

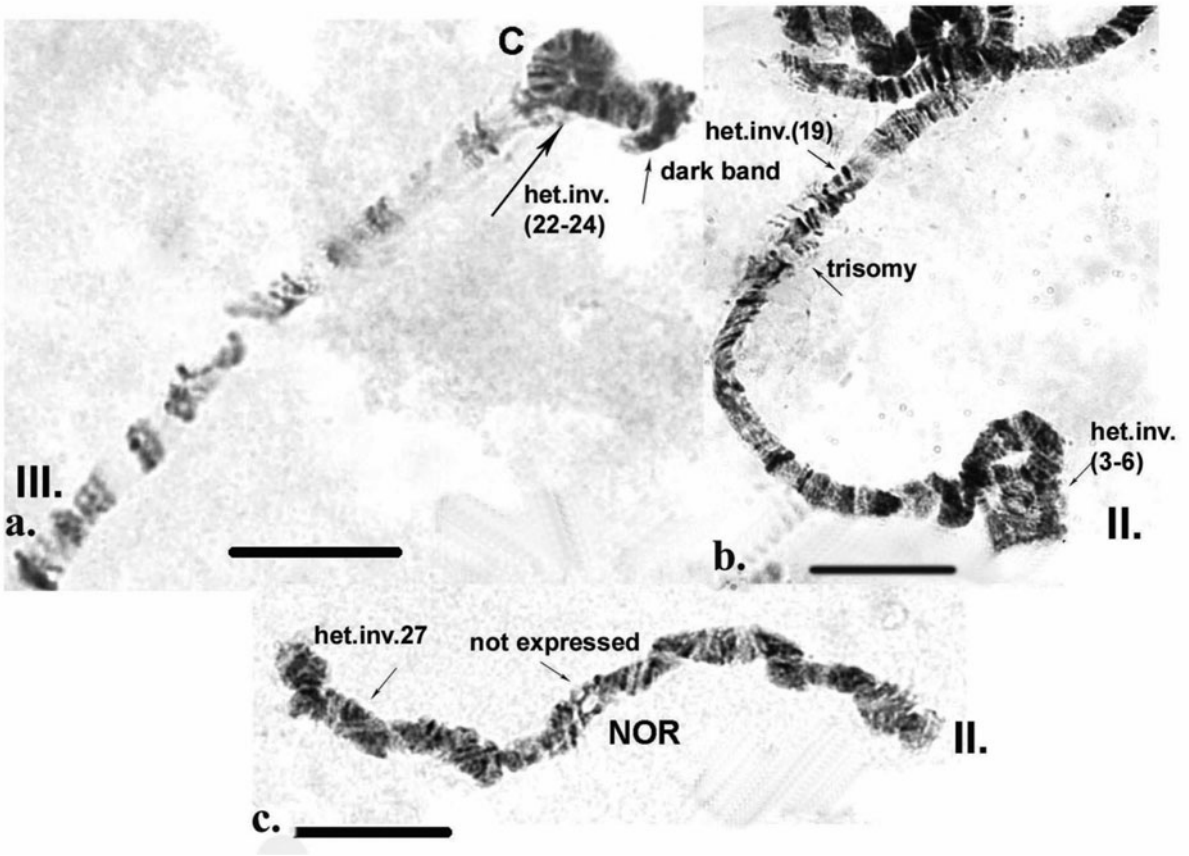

Fig. 8. Polytene chromosomes of *Belgica antarctica* showing structural aberrations and functional chromosome alterations. **a.** Chromosome III: heterozygous inversion C (22–24); **b.** chromosome II: somatic trisomy, somatic heterozygous inversion (sections 3–6), somatic heterozygous inversion (section 19); **c.** chromosome II: nuclear organizer region (NOR) is not expressed, somatic heterozygous inversion (section 27). Scale bar = 10 μm.

important for enabling the use of this species as a model (Kozeretska *et al.* 2022). This adds to the maps available for various chironomid genera (Keyl 1962, Michailova 1989, Kiknadze *et al.* 2016). These maps contribute to studies in taxonomy, cytogenetics and chromosomal polymorphism in natural populations and help us to identify pathways of species divergence.

The chromosome maps of Keyl (1962) provided a detailed analysis of the structural-chromosomal aberrations and primarily homozygous inversions involved in the divergence of members of the genus *Chironomus*, which are otherwise characterized by great variation in external morphology and poor efficacy of separation using morphological characters. Michailova (1989) indicated on chromosome maps of species representing different genera of the family the band marker patterns by which separate species can be reliably determined. Kiknadze *et al.* (2016) used the chromosome maps of each species to clearly indicate the locations of fixed homozygous inversions characteristic of species in this genus. Michailova (1989) reported other chromosomal aberrations in standard chromosome maps of species of the genus *Cricotopus* (subfamily Orthocladiinae) that were involved in the divergence of these species, in particular tandem translocations. The chromosomal map of *Chironomus thummi* (Kiknadze *et al.* 2016) has proved to have great application in analysing chromosome variability in this species caused by environmental pollution (Michailova *et al.* 2021). Standard chromosome maps of other chironomid species have also been used to analyse chromosomal changes due to exposure to various environmental pollutants (Ilkova *et al.* 2017). Chromosome maps are particularly widely used in the analysis of chromosomal polymorphism within populations of species under different environmental conditions and from geographically distinct populations (e.g. Kiknadze *et al.* 2016).

The map of polytene chromosomes of *B. antarctica* we created has the potential to inform knowledge on the paths of speciation in this taxon, provided that corresponding maps become available in closely related species (notably *Belgica albipes* and *Eretmoptera murphyi*; Allegrucci *et al.*

https://doi.org/10.1017/S0954102023000202 Published online by Cambridge University Press

Table IV. Somatic aberrations and their chromosomal location in *Belgica antarctica* larvae from both sampling locations in the current study.

Somatic aberrations	Specific location	Number of individuals with aberrations		Frequency (%), proportion confidence interval in parentheses		Number of cells	
		Location 1	Location 2	Location 1	Location 2	Location 1	Location 2
Heterozygous inversion	Chromosome I: sections 1–2	0	1	0 (0–10)	3.23 (0.08–16.70)	0	1
Heterozygous inversion	Chromosome I: section 3	1	0	2.86 (0.07–14.92)	0 (0–11.22)	1	0
Heterozygous inversion	Chromosome I: section 12	0	1	0 (0–10)	3.23 (0.08–16.70)	0	1
Heterozygous inversion	Chromosome I: sections 13–14	1	0	2.86 (0.07–14.92)	0 (0–11.22)	1	0
Heterozygous inversion	Chromosome I: section 22	2	1	5.71 (0.70–19.16)	3.23 (0.08–16.70)	1	1
Trisomy	Chromosome I: BR	0	1	0 (0–10)	3.23 (0.08–16.70)	0	1
Heterozygous dark band	Chromosome I	1	0	2.86 (0.07–14.92)	0 (0–11.22)	2	0
Heterozygous dark band	Chromosome III	2	0	5.71 (0.70–19.16)	0 (0–11.22)	1	0
Heterozygous inversion	Chromosome II: sections 3–6	0	1	0 (0–10)	3.23 (0.08–16.70)	0	1
Heterozygous inversion	Chromosome II: sections 4–5	0	1	0 (0–10)	3.23 (0.08–16.70)	0	2
Heterozygous inversion	Chromosome II: sections 11–12	1	0	2.86 (0.07–14.92)	0 (0–11.22)	1	0
Heterozygous inversion	Chromosome II: section 19	0	1	0 (0–10)	3.23 (0.08–16.70)	0	1
Heterozygous inversion	Chromosome II: section 27	0	1	0 (0–10)	3.23 (0.08–16.70)	0	1
Trisomy	Chromosome II: NOR	0	1	0 (0–10)	3.23 (0.08–16.70)	0	1
Heterozygous inversion	Chromosome III: section 7	1	0	2.86 (0.07–14.92)	0 (0–11.22)	1	0
Heterozygous inversion	Chromosome III: section 10	0	1	0 (0–10)	3.23 (0.08–16.70)	0	1
Heterozygous inversion	Chromosome III: section 12	0	1	0 (0–10)	3.23 (0.08–16.70)	0	1
Heterozygous inversion	Chromosome III: sections 12–13	0	1	0 (0–10)	3.23 (0.08–16.70)	0	1
Heterozygous inversion	Chromosome III: sections 14–15	0	1	0 (0–10)	3.23 (0.08–16.70)	0	1
Heterozygous inversion	Chromosome III: section 22	1	0	2.86 (0.07–14.92)	0 (0–11.22)	1	0
Deficiency	Chromosome I: section 26	1	0	2.86 (0.07–14.92)	0 (0–11.22)	1	0
Dark band	Chromosome I: section 1	20	21	57.14 (39.35–73.68)	67.74 (48.63–83.32)	27	9
Dark band	Chromosome II: sections 1 and 29	10	8	28.57 (14.64–46.30)	25.81 (11.86–44.61)	8	6
Dark band	Chromosome III: section 1	28	9	80 (63.08–91.56)	29.03 (14.22–48.04)	26	6

BR = Balbiani ring; NOR = nuclear organizer region.

2012, Kozeretska *et al.* 2022). Inversions are the result of damage being caused to DNA molecules followed by their repair. Such damage can include errors in the repair system and can be caused by external factors such as ionizing radiation and other sources of oxidative damage (Theodorakis 2008). The inversions can become fixed in the population through both selection and random drift. Many inversions, particularly small ones in intergenic regions, are likely to evolve neutrally by drift alone. Some characteristics of inversions can influence selection processes. Inversions that generate structural problems in the process of meiosis can disrupt open reading frames or alter gene expression in deleterious manners, but they can also be drivers of adaptive shifts and speciation (Hoffmann & Rieseberg 2008, Kirkpatrick 2010). The established inherited aberrations observed in specimens from the two sampling locations here were first observed several decades ago (Martin 1962, Atchley & Davis 1979). It is plausible that the inherited inversions A, B and C, which appear at high frequency, have an adaptive value and hence enhance individual survival potential, possibly by playing a role in adaptation to the extreme conditions of Antarctica. Further detailed functional genomic analyses would be required to investigate this hypothesis further.

Atchley & Davis (1979) proposed that the Antarctic terrestrial environment is inherently very variable, and therefore genetic systems may be highly buffered through selection supporting those mechanisms that preserve heterozygosity and reduce recombination (Otto & Barton 1997). More generally, Peck *et al.* (2006) also emphasized that, while Antarctic environmental conditions are correctly considered to be extreme, in reality the terrestrial biota in particular can also face highly variable conditions. Our data illustrate that *B. antarctica* provides a good example of a species characterized by highly heterozygous chromosomal inversions, consistent with evolutionary processes in a highly variable environment.

A large spectrum of somatic aberrations was observed in this study. The appearance of the dark band at the telomeres of the chromosomes is particularly notable. A dark disc called a 'dark knob' has been reported in

https://doi.org/10.1017/S0954102023000202 Published online by Cambridge University Press

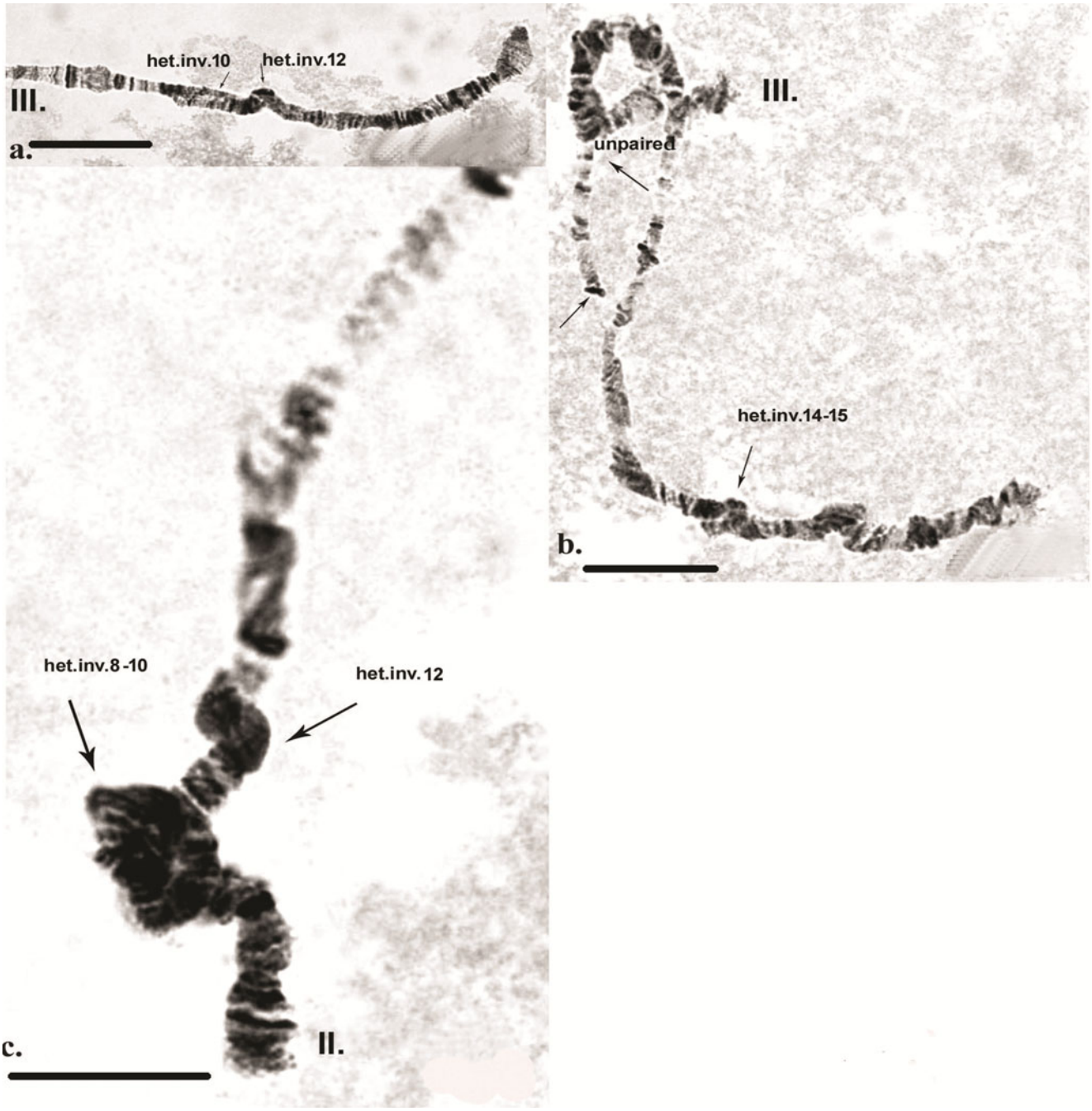

Fig. 9. Polytene chromosomes of *Belgica antarctica* showing structural aberrations. **a.** Chromosome III: somatic heterozygous inversions in section 10 and section 12; **b.** chromosome II: unpairing homologous, somatic heterozygous inversion (sections 14–15); **c.** chromosome II: somatic heterozygous inversion (sections 8–10), somatic heterozygous inversion (section 12). Scale bar = 10 µm.

Chironomus nuditarsis populations from Switzerland (Fischer 1978) and in a Siberian population (Kiknadze *et al.* 2016), probably resulting from amplification events. The somatic alterations observed in chironomid larvae have been proposed to be useful biomarkers for the action of genotoxic agents (Michailova *et al.* 2012). Their sensitivity may be indicated by the S index calculated from the samples obtained at the two study locations. The high number of somatic aberrations observed in the genome of *B. antarctica* suggests that the polytene chromosomes of this species may be particularly sensitive to breakages that occur under the environmental conditions of the studied locations (Parnikoza *et al.* 2016). Such structural-chromosomal aberrations have been observed in many species of the family Chironomidae: *Chironomus riparius*, *Chironomus piger*,

https://doi.org/10.1017/S0954102023000202 Published online by Cambridge University Press

Chironomus plumosus, *Glyptotendipes barbipes* and *Glyptotendipea glaucus* (Sella *et al.* 2004, Michailova *et al.* 2012).

Larval development of *B. antarctica* often takes place in mosses, which could potentially provide a source of contaminants. Contaminants in Antarctic terrestrial ecosystems can include trace elements originating both from the natural weathering of source minerals and from anthropogenic activities (Chu *et al.* 2019, Magesh *et al.* 2021). The spectrum of somatic aberrations we documented in *B. antarctica* is similar to that noted in other chironomid species as a result of trace element exposure (Michailova *et al.* 2012). Trace elements are known to affect not only the structure of chromosomes but also the transcriptional activity of genes. In particular, it is notable that the transcriptional activity of important polytene chromosome structures of *B. antarctica*, such as the NOR of the three chromosomes, is reduced by such exposure, as has also been noted in other chironomids (Michailova *et al.* 2012). A heterozygous state of the BR was also found in specimens in this study, along with high functional activity along the length of the chromosomes, as indicated through the presence of multiple puffs. The functional activity of the polytene chromosomes observed here may be a result of the environmental conditions experienced in the habitats sampled or during transport from Antarctica. Of particular interest is the formation of puffs in the telomeric part of chromosomes, which depends on the specific telomere structure. For instance, some *Chironomus* species (e.g. *C. thummi* and *C. piger*) are capable of producing heat shock proteins, an activity indicated by puffs at the telomeres (Martinez *et al.* 2001). These proteins are permanently expressed at high levels by *B. antarctica* larvae (Rinehart *et al.* 2006). However, it is also important to recognize that the precise locations of specific genes on the chromosomes are not currently known. We hypothesize that these genes may be located in the telomere regions, which should be addressed in future research.

In conclusion, we suggest that the range of aberrations documented for the first time in this study may indicate damage as a result of the intense environmental conditions that characterize Antarctic terrestrial habitats. They may, therefore, serve as an important bioindicator of Antarctic environmental conditions (Bickham & Smolen 1994), and in particular provide information regarding the occurrence of changes in the structure and functioning of these ecosystems.

Acknowledgements

We are sincerely grateful to Ina Aneva, Anna Ganeva, Snezhana Grozeva, Peter Ostoich, Petar Zhelev, Roumiana Metcheva and Mila Ihtimanska (Bulgarian Academy of Sciences) for their support and assistance. We also thank the participants of the 26th Ukrainian Antarctic expedition for technical and logistical support in the organization of the sample collection. We certify that this work was made possible thanks to brave Ukrainian soldiers and the support of the entire progressive world in countering Russian aggression. The authors thank the reviewers and the editorial board for the recommendations that helped to improve the text and make it more accessible to readers.

Financial support

This study was performed within the framework of the Ukrainian State Special-Purpose Research Program in Antarctica for 2011–2023. PAK is supported by a scientific project of the National Academy of Sciences of Ukraine to research laboratories/groups of young scientists (Code 6541230) 'The impact of global climate change on the populations of individual plants and animals world' (No. of state registration 0122U002153). SS is supported by PAUSE-ANR Gandhi-Ukraine Program. PC is supported by Natural Environment Research Council (NERC) core funding to the British Antarctic Survey (BAS) 'Biodiversity, Evolution and Adaptation' Team.

Author contributions

PM: conceived and designed the study, performed experiments, data analysis and interpretation, initial drafting and subsequent revision of the manuscript. PAK: sample collection, performed experiments, data analysis and interpretation, initial drafting and subsequent revision of the manuscript. SS: data analysis, manuscript revision. IP: sample collection, manuscript revision. IK: project administration, study conception and design, interpretation, initial drafting and revision of the manuscript. PC: data interpretation, manuscript revision, critical review. All authors have agreed to the final version of the manuscript.

Supplemental material

A supplemental material section containing two supplemental tables will be found at https://doi.org/10.1017/S0954102023000202.

References

Allegrucci, G., Carchini, G., Convey, P. & Sbordoni, V. 2012. Evolutionary geographic relationships among orthocladine chironomid midges from maritime Antarctic and sub-Antarctic islands. *Biological Journal of the Linnean Society*, **106**, 258–274.

ATCHLEY, W.R. & DAVIS, B.L. 1979. Chromosomal variability in the Antarctic insect, *Belgica antarctica* (Diptera: Chironomidae). *Annals of the Entomological Society of America*, **72**, 246–252.

BARGAGLI, R. 2020. Terrestrial ecosystems of the Antarctic Peninsula and their responses to climate change and anthropogenic impacts. *Ukrainian Antarctic Journal*, **2**, 84–97.

BICKHAM, J.W. & SMOLEN, M.J. 1994. Somatic and heritable effects of environmental genotoxins and the emergence of evolutionary toxicology. *Environmental Health Perspectives*, **102**, 25–28.

CHOWN, S.L. & CONVEY, P. 2016. Antarctic entomology. *Annual Review of Entomology*, **61**, 119–137.

CHU, W.-L., DANG, N.-L., KOK, Y.-Y., IVAN YAP, K.-S., PHANG, S.-M. & CONVEY, P. 2019. Heavy metal pollution in Antarctica and its potential impacts on algae. *Polar Science*, **20**, 75–83.

CLOPPER, C.J. & PEARSON, E.S. 1934. The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika*, **26**, 404–413.

CONVEY, P. & BLOCK, W. 1996. Antarctic Diptera: ecology, physiology and distribution. *European Journal of Entomology*, **93**, 1–13.

CONVEY, P. & PECK, L.S. 2019. Antarctic environmental change and biological responses. *Science Advances*, **5**, eaaz0888.

CORNETTE, R., GUSEV, O., NAKAHARA, Y., SHIMURA, S., KIKAWADA, T. & OKUDA, T. 2015. Chironomid midges (Diptera, Chironomidae) show extremely small genome sizes. *Zoological Science*, **32**, 248–254.

DA CUNHA, A.B. 1960. Chromosomal variation and adaptation in insects. *Annual Review of Entomology*, **5**, 85–110.

DEVLIN, J.J., UNFRIED, L., LECHETA, M.C., MCCABE, E.A., GANTZ, J.D., KAWARASAKI, Y., *et al*. 2022. Simulated winter warming negatively impacts survival of Antarctica's only endemic insect. *Functional Ecology*, **36**, 1949–1960.

FISCHER, J. 1978. Zum problem der chromosomen - Evolution durch translokationen bei *Chironomus* (Diptera). *Archiv für Genetik*, **51**, 73–98. [In German]

FISHER, R.A. 1922. On the interpretation of χ^2 from contingency tables, and the calculation of P. *Journal of the Royal Statistical Society*, **85**, 87–94.

FULLER, Z.L., LEONARD, C.J., YOUNG, R.E., SCHAEFFER, S.W. & PHADNIS, N. 2018. Ancestral polymorphisms explain the role of chromosomal inversions in speciation. *PLoS Genetics*, **14**, e1007526.

GUNDERINA, L., GOLYGINA, V. & BROSHKOV, A. 2015. Chromosomal organization of the ribosomal RNA genes in the genus *Chironomus* (Diptera, Chironomidae). *Comparative Cytogenetics*, **9**, 201–220.

HOFFMANN, A.A. & RIESEBERG, L.H. 2008. Revisiting the impact of inversions in evolution: from population genetic markers to drivers of adaptive shifts and speciation? *Annual Review of Ecology, Evolution, and Systematics*, **39**, 21–42.

HOFFMANN, A.A., SGRÒ, C.M. & WEEKS, A.R. 2004. Chromosomal inversion polymorphisms and adaptation. *Trends in Ecology and Evolution*, **19**, 482–488.

ILKOVA, J., MICHAILOVA, P., SZAREK-GWIAZDA, E., KOWNACKI, A. & CISZEWSKI, D. 2017. The response of genome of the Chironomidae (Diptera) to heavy metal pollution in two rivers of southern Poland. *Acta Zoologica Bulgarica* (*Supplement*), **8**, 9–15.

JOHN, B. & LEWIS, K.R. 1966. Chromosome variability and geographic distribution in insects. *Science*, **152**, 711–721.

KAISER, T.S., POEHN, B., SZKIBA, D., PREUSSNER, M., SEDLAZECK, F.J., ZRIM, A., *et al*. 2016. The genomic basis of circadian and circalunar timing adaptations in a midge. *Nature*, **540**, 69–73.

KAPUN, M., VAN SCHALKWYK, H., MCALLISTER, B., FLATT, T. & SCHLÖTTERER, C. 2014. Inference of chromosomal inversion dynamics from Pool-seq data in natural and laboratory populations of *Drosophila melanogaster*. *Molecular Ecology*, **23**, 1813–1827.

KELLEY, J.L., PEYTON, J.T., FISTON-LAVIER, A.S., TEETS, N.M., YEE, M.C., JOHNSTON, J.S., *et al*. 2014. Compact genome of the Antarctic midge is likely an adaptation to an extreme environment. *Nature Communications*, **5**, 4611.

KEYL, H.G. 1962. Chromosomenevolution bei *Chironomus*. *Chromosoma*, **13**, 464–514. [In German]

KIKNADZE, I., ISTOMINA, A., GOLYGINA, V. & GUNDERINA, L. 2016. *Karyotypes of Palearctic and Holarctic species of the genus* Chironomus. Novosibirsk: Academic Publishing House 'Geo', 489 pp.

KIRKPATRICK, M. 2010. How and why chromosome inversions evolve. *PLoS Biology*, **8**, e1000501.

KIROV, N., WURTZ, T. & DANEHOLT, B. 1991. The complexity of 75S premessenger RNA in Balbiani ring granules studied by a new RNA band retardation assay. *Nucleic Acids Research*, **19**, 3377–3382.

KOZERETSKA, I., SERGA, S., KOVALENKO, P., GOROBCHYSHYN, V. & CONVEY, P. 2022. *Belgica antarctica* (Diptera: Chironomidae): a natural model organism for extreme environments. *Insect Science*, **29**, 2–20.

KRUSKAL, W.H. & WALLIS, W.A. 1952. Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association*, **47**, 583–621.

MAGESH, N.S., TIWARI, A., BOTSA, S.M. & DA LIMA LEITAO, T. 2021. Hazardous heavy metals in the pristine lacustrine systems of Antarctica: insights from PMF model and ERA techniques. *Journal of Hazardous Materials*, **412**, 125263.

MARTIN, J. 1962. Inversion polymorphism in an Antarctic species living in a simple environment. *The American Naturalist*, **96**, 317–318.

MARTINEZ, J.L., SANCHEZ-ELSNER, T., MORCILLO, G. & DIEZ, J.L. 2001. Heat shock regulatory elements are present in telomeric repeats of *Chironomus thummi*. *Nucleic Acid Research*, **29**, 4760–4766.

MATHESON, P. & MCGAUGHRAN, A. 2023. How might climate change affect adaptive responses of polar arthropods? *Diversity*, **15**, 47.

MICHAILOVA, P.V. 1989. The polytene chromosomes and their significance to the systematics of the family Chironomidae, Diptera. *Acta Zoologica Fennica*, **186**, 1–107.

MICHAILOVA, P.V. 1994. The role of heterochromatin in the speciation of family Chironomidae, Diptera. *Folia Biologica*, **42**, 79–87.

MICHAILOVA, P.V., SELLA, G. & PETROVA, N. 2012. Chironomids (Diptera) and their salivary gland chromosomes as indicators of trace-metal genotoxicity. *Italian Journal of Zoology*, **79**, 218–230.

MICHAILOVA, P.V., ILKOVA, J., KOVALENKO, P., DZHULAI, A & KOZERETSKA, I. 2021. Long-term retainment of some chromosomal inversions in a local population of *Belgica antarctica* Jacobs (Diptera, Chironomidae). *Czech Polar Reports*, **11**, 16–24.

NOOR, M.A.F., GRAMS, K.L., BERTUCCI, L.A. & REILAND, J. 2001. Chromosomal inversions and the reproductive isolation of species. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 12084–12088.

OCHYRA, R., SMITH, R.I.L. & BEDNAREK-OCHYRA, H. 2008. *The illustrated moss flora of Antarctica*. Cambridge: Cambridge University Press, 704 pp.

OTTO, S.P. & BARTON, N.H. 1997. The evolution of recombination: removing the limits to natural selection. *Genetics*, **147**, 879–906.

PARNIKOZA, I., ABAKUMOV, E., KORSUN, S., KLYMENKO, I., NETSYK, M., KUDINOV, A., *et al*. 2016. Soils of the Argentine Islands, Antarctica: diversity and characteristics. *Polarforschung*, **86**, 83–96.

PECK, L.S., CONVEY, P. & BARNES, D.K.A. 2006. Environmental constraints on life histories in Antarctic ecosystems: tempos, timings and predictability. *Biological Reviews*, **81**, 75–109.

RICHARD, K.J., CONVEY, P. & BLOCK, W. 1994. The terrestrial arthropod fauna of the Byers Peninsula, Livingston Island, South Shetland Islands. *Polar Biology*, **14**, 371–379.

RINEHART, J.P., HAYWARD, S.A.L., ELNITSKY, M.A., SANDRO, L.H., LEE JR, R.E. & DENLINGER, D.L. 2006. Continuous up-regulation of heat shock proteins in larvae, but not adults, of a polar insect. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 14223–14227.

https://doi.org/10.1017/S0954102023000202 Published online by Cambridge University Press

Sella, G., Bovero, S., Ginepro, M., Michailova, P., Petrova, N., Robotti, C.A., *et al*. 2004. Inherited and somatic cytogenetic variability in Palearctic populations of *Chironomus riparius* Meigen 1804 (Diptera, Chironomidae). *Genome*, **47**, 332–344.

Theodorakis, C.W. 2008. Mutagenesis. *In* Jørgensen, S.E. & Fath, B.D., *eds*, *Encyclopedia of ecology*. Oxford: Academic Press, 2475–2484.

Turner, J., Barrand, N., Bracegirdle, T., Convey, P., Hodgson, D., Jarvis, M., *et al*. 2014. Antarctic climate change and the environment: an update. *Polar Record*, **50**, 237–259.

Usher, M.B. & Edwards, M. 1984. A dipteran from south of the Antarctic Circle: *Belgica antarctica* (Chironomidae), with a description of its larvae. *Biological Journal of the Linnean Society*, **23**, 19–31.

Wellenreuther, M. & Bernatchez, L. 2018. Eco-evolutionary genomics of chromosomal inversions. *Trends in Ecology and Evolution*, **33**, 427–440.

White, B.J., Hahn, M.W., Pombi, M., Cassone, B.J., Lobo, N.F., Simard, F. & Besansky, N.J. 2007. Localization of candidate regions maintaining a common polymorphic inversion (2La) in *Anopheles gambiae*. *PLoS Genetics*, **3**, e217.

https://doi.org/10.1017/S0954102023000202 Published online by Cambridge University Press