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Wintering and Cold Hardiness of the Small Tortoiseshell *Aglais urticae* (Linnaeus, 1758) (Nymphalidae, Lepidoptera) in the West and East of the Northern Palearctic

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Abstract: The geographic variability of the cold hardiness of poikilothermic animals is one of the keys to understanding the mechanisms of the formation of their ranges under climate change or anthropogenic introductions. A convenient object is the small tortoiseshell butterfly *Aglais urticae*, which is distributed from the Atlantic Ocean to the Pacific Ocean. On the edges of the distribution range, the difference between the averages of the absolute minimum air temperatures reaches 60 °C. The cold hardiness (supercooling point and lower lethal temperatures) of imago wintering in a supercooled state in the northeast of Russia was assessed in comparison to the previously studied European ones. Despite the huge difference in air temperatures, the mean supercooling points ranges in the east (−23...−29 °C) and the west (−17...−22 °C) differ by only 7 °C; the lower lethal temperatures for this species is near −30 °C. The identified cold hardiness is not enough for overwintering of *A. urticae* on the vast majority part of the species range in natural shelters above the level of snow cover. The inhabiting of *A. urticae* in regions with air temperatures below −30 °C is possible only when wintering under snow. This primitive behavioral adaptation probably does not require physiological changes and may not be unique to Lepidoptera.

Keywords: *Aglais urticae*; small tortoiseshell butterfly; wintering; refuge; low temperatures; cold hardiness; *SCP*; lower lethal temperatures; adaptation

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1. Introduction

One of the important and poorly understood ecological problems is the geographic variability in the cold hardiness of widely distributed species living in different climates. In this field, relatively few works are known, and their results are contradictory [1–7]. Meanwhile, the possibility of expanding the ranges of species in regions with significant, seasonally changing temperature regimes, as well as their connection with a changing climate, also depends on the lability of their cold hardiness [1,5,8–10].

A striking and convenient model for solving this problem is the small tortoiseshell butterfly *Aglais urticae* (Linnaeus, 1758), which is very common in the Palearctic region. It is known to range from the British Isles to Kamchatka and from the Himalayas to the forest tundra [11]. Thus, its range occupies a vast territory with fundamentally different climates. Climate is believed to be the main factor limiting the distribution of this species in Europe [12]. The range of summer conditions necessary for the successful inhabiting of populations of the species in Northern Europe is well-studied, which cannot be said regarding other parts of the geographic range and regarding winter conditions. For example, the *A. urticae* is common in the parks of London, where the average January temperature does not fall below 3.8 °C [13]. At the same time, it was found in a basin of the Indigirka River upper reaches (near the cold pole of the Northern Hemisphere, Oymyakon village) [14], which has average January temperatures of −50.0 °C [15]. Mild winters, which ensure high winter survival, are considered one of the factors contributing to the habitation of the species in Northern Europe [12,16]. For the north and northeast of the Asian part of the range, the concept of mild winters is not applicable; however, the *A. urticae* is also widespread in these areas [11,17,18].

This species overwinters as imago. Meanwhile, we did not find descriptions of their natural wintering places in the literature with the exception of single mentions of findings in caves and hollows of trees far from housing [19–22]; consultations with experts added little information. On the contrary, hibernating *A. urticae* is often found in anthropogenic areas in various buildings: sheds, unheated storage house, bomb shelters, tunnels, basements, abandoned buildings or those only used for summer housing, etc. [20,22–24]. In the spring, they begin to fly early and are therefore noticeable. Thus, if *A. urticae* indeed winter in buildings that are poorly isolated from the external environment, then butterflies should experience the effect of air temperatures, which are only slightly weakened by the walls of unheated rooms or structures; however, this effect has not been quantified in the literature.

The resistance of *A. urticae* to negative temperatures has been studied in Southern England [16] and St. Petersburg [25]. However, the physiological response of *A. urticae* to the difference in winter temperature conditions from England to the continental regions of Northeastern Asia (Seymchan village), the span of which reaches 60 °C (according to the average of the absolute minimum air temperatures—see below) is unknown and unpredictable. The determination of this response is the main goal of this work, the achievement of which will not only reveal important adaptive features of the species in extremely severe climatic conditions, but will also contribute to the solution of the general biological problem of the geographical variability of cold hardiness in widely distributed species.

To achieve this goal, we had to first solve two auxiliary problems. First, intraspecific genetic structure was studied to determine the relationship between individuals of *A. urticae* from some geographically distant populations: Southern England, European Russia (St. Petersburg), and Northeastern Asia (Magadan Oblast). Secondly, we estimated the degree of acceptability of temperature conditions for the wintering of the small tortoiseshell in different geographical locations by comparing the temperatures it tolerates with the minimum air temperatures.

2. Materials and Methods

2.1. Molecular Genetic Analysis

We sequenced a fragment of the mitochondrial cytochrome c oxidase 1 (COI) gene from individuals taken for cold tolerance studies, as well as for additional locations (Figure 1, Table 1).

Figure 1. Locations of the *Aglais urticae* capture for genetic analysis (asterisks) and the determination of cold hardiness (circles). Geographical localities and the number of samples studied in the genetic analysis are indicated in Table 1.

Table 1. Geographical localities of the *Aglais urticae* capture, their coordinates, and the number of individuals studied.

Location	Coordinates	*n*
Chester, United Kingdom	53°12′ N 2°53′ W	1
Oriketo District, Turku, Finland	60°28′ N 22°19′ E	5
Saint Petersburg, Russia	59°59′ N 30°23′ E	4
Novgorod, Russia	58°31′ N 31°16′ E	1
Istra District, Moscow Oblast, Russia	55°55′ N 36°52′ E	1
Pinega Village, Arkhangelsk Oblast, Russia	64°42′ N 43°22′ E	3
Podgornoye Village, Tomsk Oblast, Russia	57°47′ N 82°39′ E	1
Novosibirsk, Russia	55°01′ N 82°55′ E	3
Yakutsk, Sakha Republic, Russia	62°3′ N 129°29′ E	3
Yagodnoye Village, Magadan Oblast, Russia,	62°31′ N 149°37′ E	1
Magadan, Russia	59°34′ N 150°48′ E	3
Seymchan Village, Magadan Oblast, Russia	62°52′ N 152°23′ E	3
Total		29

DNA was extracted from dried or ethanol-fixed specimens using BioSilica columns (Novosibirsk, Russia) as described in Shekhovtsov et al. [26]. Amplification of the COI gene fragment (623 bp long) was performed using the universal primers AU-coi-F (5′-TTTTT-GGAAT-TTGAG-CAGGA-A-3′, this study) and HCO2198 (5′-TAAAC-TTCAG-GGTGA-CCAAA-AAATC-A-3′, [27]) with the following amplification profile: 95 °C for 5 min; 37 cycles of 20 s at 95 °C, 20 s at 54 °C, 1 min at 72 °C; final elongation step of 5 min at 72 °C. Sanger sequencing was performed in the SB RAS Genomics Core Facility (ICBFM SB RAS, Novosibirsk, Russia) using the same primers. The analysis and editing of sequences were conducted in Chromas 2.6.6 software [28]. The obtained sequences were submitted to GenBank under accession numbers OQ121629–OQ121657.

All available *A. urticae* COI accessions were extracted from the GenBank database. We filtered out short sequences, those containing ambiguous positions, as well as several specimens that turned out to belong to other species. The final alignment contained 383 nucleotide positions, including 271 specimens from GenBank and 29 sequences that were obtained in this study. For this dataset, we constructed a haplotype network in Network v.10 [29] using the median-joining algorithm.

2.2. Animal Sampling and Care

Caterpillars of different instars were collected on the narrow-leaved nettle *Urtica angustifolia* Fisch. in two populations: in the vicinity of the city of Magadan and in the vicinity of Seymchan village, samplings consisting of about 500 and 300 individuals, respectively.

In Magadan, the timing of the collection of caterpillars differed in 2018, 2020, and 2022 from mid-June to mid-July due to the variation in spring weather and hence the timing of eggs and caterpillar development. In this regard, the timing of pupation, appearance of butterflies, and their maintenance in the laboratory before wintering also differed. In 2022, caterpillars were collected in Magadan and Seymchan at the same time, in mid-July; they were kept and acclimated in the same regimes and according to the same scheme.

Collected caterpillars were placed in the laboratory in 5 L rearing cages of 30–50 individuals and kept at a temperature of 20–23 °C under a natural change in the light regime, where they were fed with fresh shoots of narrow-leaved nettle. Pupation took place 2–10 days after the collection of caterpillars; the emergence of butterflies took place about a week after pupation. Imago *A. urticae* were kept in large gauze tents in the laboratory during the natural photoperiod. During the day, 60 W incandescent lamps were turned on in one of the corners of the tents, which provided the opportunity for the butterflies to choose the temperature. Bouquets of flowers on which butterflies fed were changed daily in the tents, (rosebay willowherb *Epilobium angustifolium*, white clover *Trifolium repens*, and red clover *T. pratense*), and bowls with sweet water were placed in the tents.

After three weeks of maintaining the above conditions, the butterflies were placed one at a time in paper perforated entomological envelopes, which were placed into 250 mL polyethylene containers, at 25 pieces each. Wet filter paper was placed along two sides of each container to maintain humidity.

In addition, five *A. urticae* were collected in Western Siberia in the vicinity of Podgornoye Village, Tomsk region (57°47′ N 82°39′ E). They were caught a few days before the end of the season of activity, in mid-September; the butterflies were also placed in entomological envelopes and kept at the natural course of temperature until the start of acclimation. The acclimation of all *A. urticae* by stepwise cooling was simultaneously carried out according to the schedule shown in Table 2. After the end of acclimation, the butterflies were transferred to a temperature of −5 °C, in which they were kept for 20 days until the main characteristics of cold hardiness were determined (see below). This temperature was a starting point for determining the supercooling point and lower lethal temperatures.

Table 2. Procedure for acclimation of *Aglais urticae* imago.

Temperature, °C	Duration, Days
16	1
14	1
11	3
10	2
8	3
5	21
0	21

2.3. Determination of Cold Hardiness

2.3.1. Supercooling Point

The supercooling point (*SCP*) is the temperature at which ice formation begins in the body of an animal, accompanied by an abrupt release of heat, reflected as a peak on the thermogram. The *SCP* was measured using manganin–constantan thermocouples (wire diameter 0.12 mm) in a WT-64/75 programmable test chamber (Weiss Umwelttechnik GmbH). The thermocouple junction was fastened by parting the hairs on a butterfly's body so that it directly touched the body. The thermocouple signal was converted using an analog–digital board (ADC LA-TK5) via a DC voltage amplifier and recorded on a computer [30,31]. *A. urticae* were cooled at a rate of 0.5 °C/min until the temperature jump on the thermogram. After they were left at lower temperatures for another 5–30 min (to −35–−45 °C) then heated at a rate of 4 °C/min to 2–4 °C; then, the survival was assessed. The sample size from Magadan was 12–29 individuals; from Seymchan, it was 35, and from Podgornoye, it was 5 individuals.

2.3.2. Lower Lethal Temperatures

Survival after long-term (more than a day) exposure to a variety of negative temperatures and the lower lethal temperature (*LLT*) determination were performed in 2018, 2020, and 2022 in 157 individuals from Magadan and in 44 individuals from Seymchan in 2022. Containers with butterflies in entomological envelopes were placed in a WT-64/75 programmable test chamber, and the temperature was lowered at a rate of 0.5 °C/h, starting from −5 °C to −20, −25, −28, −30, and −35 °C for butterflies from Magadan, and to −28 and −35 °C for butterflies from Seymchan. The exposure time at each of these temperatures was 48 h [32,33]. The butterflies were then heated at 1 °C/h to 2–4 °C. Individuals that began to normally fly and feed were considered to be survivors. The sample size ranged from 20 to 40 individuals for each temperature.

The thermocouples, instrumentation, thermostats, and climatic chambers used, as well as the techniques used in determining *SCP* and *LLT*, have been previously described in detail [30,31,34].

2.3.3. Temperatures in Unheated Buildings and in the Air

The determination of minimum temperatures into unheated buildings, as in typical and well-known wintering places, and in the air was carried out in the vicinity of Kulu Village (Magadan Oblast) on the territory of the Kolymskaya weather station (61°51′ N, 147°39′ E). The area of Kulu Village and the indicated weather station is typical for the basin of the upper reaches of the Kolyma River in terms of minimum air temperatures, which fall below −45 °C every winter. Temperatures were measured using iButton DS1922L temperature loggers (Scientific and Technical Laboratory "ElIn", Moscow, Russia); the measurement accuracy of ±0.5 °C within the temperature range from 65 to −10 °C and ±0.6 °C within the temperature range from −10 to −40 °C. The loggers were calibrated before the start of the experiments at 0 °C. Their individual error was +0.2–+0.3 °C. The temperature was recorded every 3 h.

The loggers were installed in the last ten days of January 2019 in two separate small (6 × 2.5 × 2.4 m) cabins designed for living in the warm season. The premises had glazed windows and tightly fitted doors; the walls were insulated to varying degrees. Temperature loggers were suspended at a height of 0.5 and 2 m from the floor; they operated from January 20 to the end of May. The values of air temperature from autumn 2019 to spring 2020 and the values of the snow depths were obtained from measurements conducted at the weather station.

2.4. Statistical Analysis

Statistical analysis was performed by standard methods using the commercially available software Statistica 10 for Windows. Values are reported as the mean ± SE. A comparison of *SCP* values was performed using the Mann–Whitney U test. The level of significance was set at $p < 0.05$.

3. Results

3.1. Distribution

Aglais urticae is distributed in Northeastern Asia, judging by our collections and observations, from the basins of the upper reaches of the Indigirka and Kolyma rivers to the coast of the Sea of Okhotsk. Here, it is trophically related to the native species—the narrow-leaved nettle (*Urtica angustifolia* Fisch.), which is found in various meadows (in valleys, at sites of fire, etc.). Along the valleys of the Kolyma River tributaries (the rivers Omolon and Maly Anyui), the narrow-leaved nettle reaches Chukotka. In the valley of the Anadyr River, it is known to span in the middle reaches to the mouth of the Belaya River; it is recorded in Koryakia, but it does not enter Kamchatka [35]. In addition, *U. angustifolia* has settled in almost all villages of Northeastern Asia, including Markovo Village in Chukotka, where *A. urticae* was also found (personal comm. of P.Yu. Gorbunov).

3.2. Molecular Genetic Analysis

We found 44 unique COI haplotypes among the 300 COI sequences taken into the analysis (Figure 2). Over two-thirds of the total sample belonged to two haplotypes; two more included 14 and 22 specimens, respectively. The rest of the haplotypes were represented by only one to three specimens.

The haplotype network demonstrated that the majority of sequences represent a tight group. This group included the four abovementioned haplotypes that accounted for 80% of all specimens, as well as several minor satellite haplotypes that differed from the major ones by one or two nucleotide substitutions. The haplotypes of *A. urticae* from Northern Eurasia belonged to this central group, while those from Central and East Asia occupied peripheral positions on the network. All sequences obtained in this study belonged to the two most frequent haplotypes or differed by one substitution.

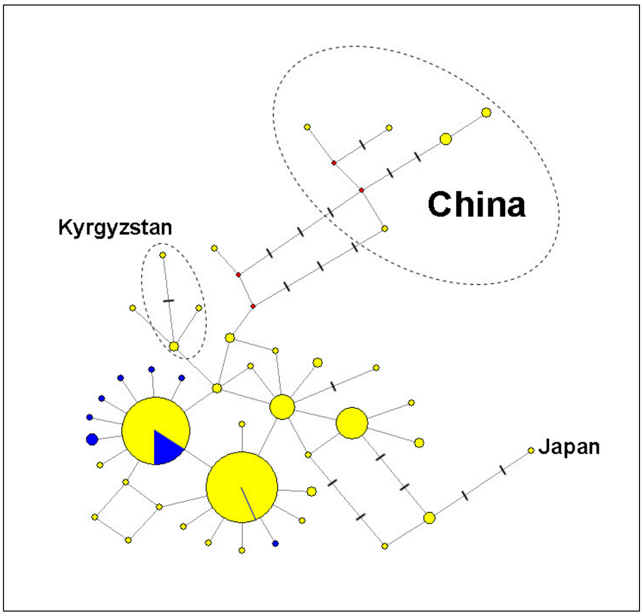

Figure 2. *Aglais urticae* COI haplotype network. Yellow circles: GenBank data; blue circles: sequences obtained in this study. The circle diameter is proportional to the number of individuals; red dots stand for missing haplotypes; hatches indicate mutation steps.

3.3. Cold Hardiness

In the small tortoiseshell, the imago overwinter in a supercooled state; it is believed that they cannot tolerate freezing [16,25]. To verify this statement, we repeatedly made attempts to cool *A. urticae* to a temperature below the supercooling point in different modes and to different temperatures (from −35 to −45 °C). Every time after freezing, the butterflies did not come to life.

The average *SCP* value varied in the same population in different years (Magadan, 2018, 2020, and 2022), forming a continuous series, the extreme values of which differed significantly (U-test, $p \leq 0.05$) (Table 3). The average *SCP* value in the Magadan population for all years was -25.3 ± 0.5 °C; it did not differ from the *SCP* values of *A. urticae* from other populations (Seymchan and Podgornoye). The *SCP* of butterflies from different populations (Magadan, Seymchan, and Podgornoye) collected in the same year (2022) acclimated and cooled according to the same scheme (U-test, $p > 0.05$), and they did not differ either (Table 3, Figure 3).

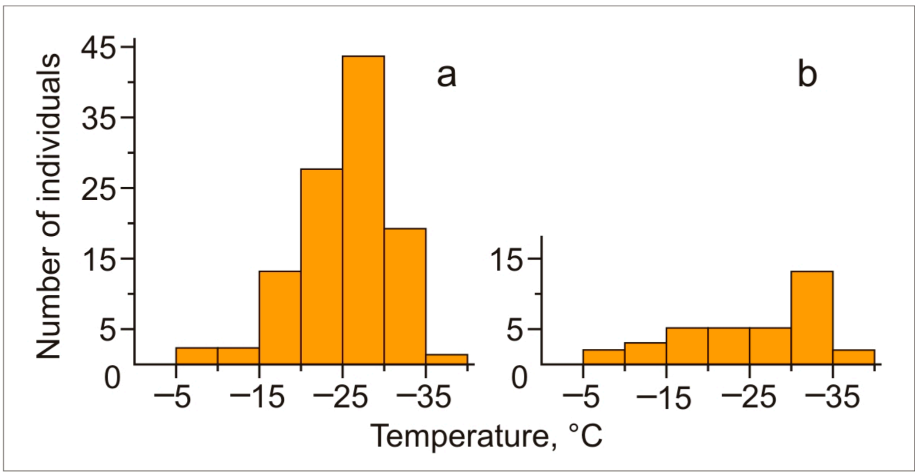

Figure 3. Distribution of the supercooling point values (*SCP*) of the *Aglais urticae* in samples from (**a**) the environs of Magadan (2018, 2020 and 2022) and (**b**) Seymchan (2022).

Table 3. Supercooling point (*SCP*, in °C) of imago *Aglais urticae* in different years in three populations.

Population	Magadan				Seymchan	Podgornoye
Year	2018 *	2018 **	2020 *	2022 *	2022 *	2022 *
n	32	19	29	16	35	5
SCP mean ±SE	−24.0 ± 0.8	−25.0 ± 1.0	−28.1 ± 1.0	−23.1 ± 1.8	−25.3 ± 1.4	−19.4 ± 3.1
SCP min	−34.8	−29.3	−35.2	−32.8	−35.5	−26.1

* starting point for determining the *SCP* was −5 °C; ** starting point for determining the *SCP* was −15 °C.

When determining the lethal temperatures, it was found that 50% mortality ($LT_{50\%}$) in the Magadan sample occurs between −20 and −25 °C; at −28 °C, 33% of individuals survived, and at −30 °C, 10% of individuals survived (4 out of 40 cooled); all individuals died at −35 °C. In the sample of butterflies from Seymchan, after exposure at −28 °C (*n* = 24), 70% of individuals were alive; all butterflies of the sample (*n* = 20) cooled to −35 °C died.

3.4. The Natural Temperature Conditions of Wintering

The natural temperature conditions of wintering of the populations from which the cold hardiness of the small tortoiseshell was determined greatly differ (Table 4). The average January temperatures in London are higher than those in Magadan by almost 22 °C, and they are higher than in Seymchan by 43 °C (and the average of the absolute minimums—by 57 °C!).

Table 4. Air temperature in geographical locations where the cold hardiness of *Aglais urticae* was studied.

Location	Altitude, m	Air Temperature, °C	
		Average, January	Averages of the Absolute Minimum
London [13]	22	3.8	3.3
Saint Petersburg [36]	3	−7.8	−26
Podgornoye [37]	97	−20.6	−44
Magadan [38]	115	−18.2	−32
Seymchan [38]	207	−39.1	−57

Within the part of Northeastern Russia of interest to us, the winter climate significantly changes along a gradient directed from the coast of the Sea of Okhotsk to the north, proceeding inland and creating two steps. The first step is from the coast to the Okhotsk-Kolyma watershed, and the second is from the Kolyma basin to the Indigirka basin. Starting from upper reaches of the rivers of the Pacific basin and the adjacent parts of the Kolyma basin, the differences between the average January temperature are about 10 °C (range of −30–−40 °C). In the upper reaches of the Indigirka River (i.e., in the region of the cold pole of the Northern Hemisphere), it is colder by another 10 °C (Table 5), and the average of the absolute minima drops to −64 °C).

Between the points where butterflies were collected for measuring cold hardiness (the city of Magadan and the village of Seymchan), the distance is about 385 km. The average January temperature along this stretch decreases from −18.2 to −39.1 °C, and the average of the absolute minimums range from −32 to −57 °C.

Table 5. Winter temperature gradient (°C) from the coast of the Sea of Okhotsk to the upper reaches of the basin of the Indigirka River. Location of weather stations illustrated in Figure 4.

No	Location	January Average Air Temperature, °C	Averages of the Absolute Minimum
1	Magadan [38]	−18.2	−32
2	Talon [38]	−29	−49
3	Palatka [38]	−23.4	−40
4	Madaun [38]	−30.6	−52
5	Ust-Omchug [38]	−35.2	−53
6	Kolymskaya [38]	−35.0	−51
7	Yagodnoye [38]	−35.1	−50
8	Seymchan [38]	−39.1	−57
9	Susuman [38]	−39.8	−57
10	Delyankir [15]	−47.6	−61
11	Oymyakon [15]	−50.0	−64
12	Moma [15]	−46.8	−60
13	Yakutsk [15]	−43.2	−57

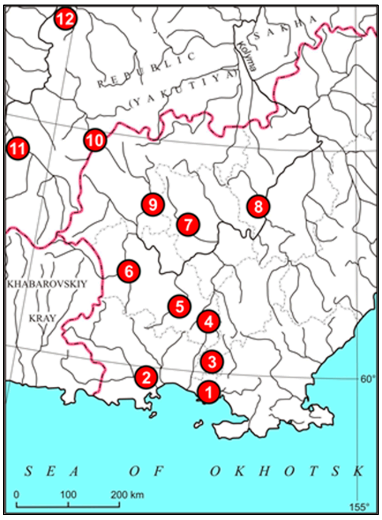

Figure 4. Location of weather stations, of which their data (Table 5) reflect the winter temperature gradient (°C) from the coast of the Sea of Okhotsk to the upper reaches of the basin of the Indigirka River. Yakutsk (No 13 in Table 5) is located off the map.

3.5. Temperatures in Unheated Buildings

The minimum temperatures inside the unheated cabins, the probable wintering grounds for *A. urticae*, were never lower than the air temperatures (Figure 5). They followed the air temperatures with a delay depending on the value and duration of the minimum, as well as on the quality of insulation in the buildings. The maximum difference in the synchronous readings of the temperature of the outside air according to the data of the weather station and the air according to the data of the loggers inside the houses was 6.6 °C (building 1) and 5.2 °C (building 2); more often, it did not exceed 3–4 °C.

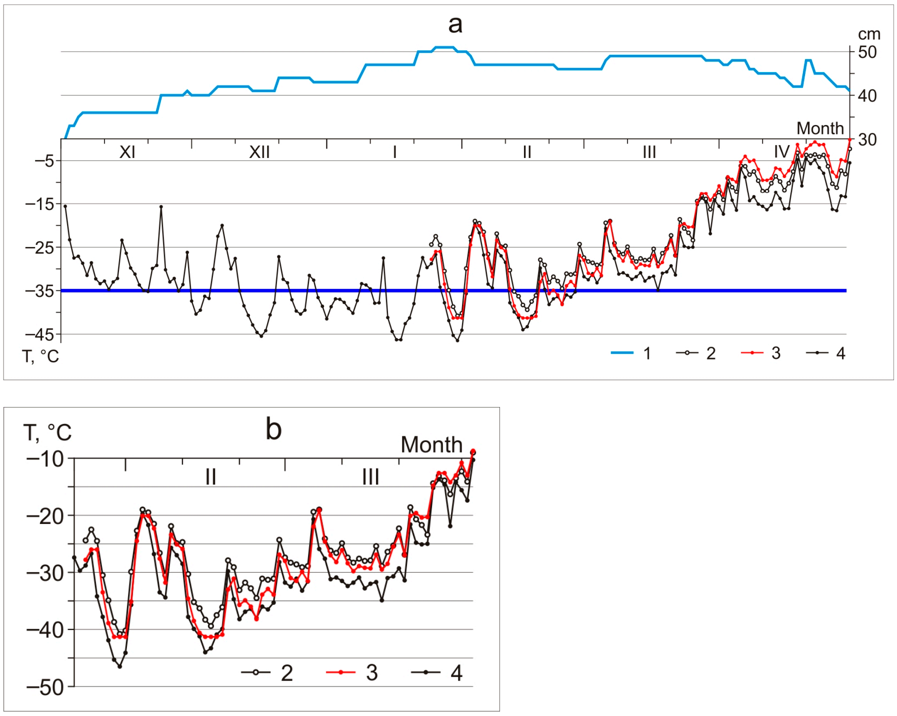

Figure 5. Wintering conditions for the *Aglais urticae* in the basin of the upper reaches of the Kolyma River (on the territory of the Kolymskaya weather station). Dynamics: (**a**) depth of snow (1); minimum temperatures in two unheated cabins (2,3); and daily minimum air temperatures (4). (**b**) Dynamics of the mentioned temperatures on a large scale.

The wintering conditions for the *A. urticae* were studied in the vicinity of Kulu Village on the territory of the Kolymskaya weather station. Here, during the 120 days of the measurement period (from November to February inclusive), the air temperatures dropped below −35 °C sixty times, below −40 °C twenty-four times, and below −45 °C five times, reaching a minimum for the winter of −46.5 °C (Figure 5). The snow cover at the time of the onset of severe frosts (below −30 °C) reached a thickness of about 35 cm in November 2019 and of more than 50 cm by the end of January 2020 (Figure 5).

4. Discussion

A necessary condition for elucidating the geographical variability of the cold hardiness of the small tortoiseshell with a large gradient of winter temperatures from the western edge of the range to the east was the clarification of the taxonomic identity of individuals from the compared populations.

The main difficulty of this part of the work is that the intraspecific structure of *A. urticae* is in the initial stages of study. Meanwhile, this species exhibits significant morphological variability (in color) depending on environmental conditions, primarily on temperature, which was shown in the laboratory [39]. The analysis of the morphological characteristics (coloration, structure of the genitalia) does not provide grounds for distinguishing intraspecific taxa among the populations of *A. urticae* of Northern Eurasia; all of them belong to the nominative subspecies *A. urticae urticae*, perhaps, with the exception of specimens from Sakhalin and the Kuril Islands (personal comm. of P.Yu. Gorbunov).

We attempted to summarize the available COI data and supplement it with our material. As seen in Figure 2, most of the specimens collected throughout Eurasia belong to a closely related group represented by four major haplotypes and several minor satellites. Unfortunately, we could not recover geographical data for many of the GenBank accessions, and thus could not make a proper analysis. However, we found that the haplotypes that do not belong to this core group stem from East (China and Japan) or Central (Kyrgyzstan) Asia. So, it appears that while southern *A. urticae* populations might represent distinct branches (subspecies?) within the species, all populations from Northern Eurasia are genetically close and do not show any phylogeographic differentiation. Similar conclusions were made by Vandewoestijne et al. [40], and they thus support the opinion of P.Yu. Gorbunov.

Thus, the relationship of *A. urticae* from European and northeastern populations (as well as of all butterflies studied by us from Siberia) comprising one taxon opens up the possibility of a correct comparison of their cold hardiness.

Multiple attempts to identify the ability to tolerate freezing in *A. urticae* undertaken by us were unsuccessful; none of the animals survived after freezing, and thus we confirmed the correctness of our predecessors [16,25], who concluded that *A. urticae* can overwinter only in a supercooled state. Therefore, *SCP* and negative temperatures tolerated in a supercooled state reflect the true capabilities of *A. urticae* in relation to winter conditions. The cooling rates at the determination of the *SCP* used by L.K. Lozina-Lozinsky [25], A.S. Pullin and J.S. Bale [16], and our study are the same, so the results are comparable.

Because the *A. urticae* from Magadan were studied in different years, and the duration of their laboratory acclimation until the time *SCP* was determined somewhat differently depending on the timing of the collection of caterpillars, the extended distribution of average *SCP*s (from −23.1 to −28.1 °C) probably indicates a heterogeneity of the physiological state of the individuals before wintering. The average *SCP* value for several years of butterflies from Magadan (−25.3 ± 0.5 °C) did not differ from that of the second population from the northeast, from the village of Seymchan (−25.3 ± 1.4 °C). Therefore, it is reasonable to consider either the indicated range of *SCP* or its average value to be equal to −25.3 °C as a characteristic of the cold hardiness of *A. urticae* from the north of the Far East.

We were surprised by the low variability of *A. urticae* cold hardiness characteristics between the different populations, with a significant variability of the wintering climatic conditions from the west to the east of the range. In southern England, with year-round positive temperatures, the cold hardiness is climatically "unreasonably" high; here, the *SCP* varied under different acclimation regimes in the range of −17–−22 °C [16]. In the vicinity of St. Petersburg, the average January temperature decreases by almost 10 °C compared with England, and the average of the absolute minimums decrease by 30 °C; however, the *SCP*, meanwhile, does not change (−21 °C) [25] (unfortunately, only one *SCP* value is given in the cited article).

L.K. Lozina-Lozinsky [25] and A.S. Pullin and J.S. Bale [16] suggested that the proximity of the *SCP* values in these populations may indicate the absence of an increase in the cold hardiness in the north of the species range. Meanwhile, while remaining at about −19 °C in western Siberia in northeast Asia, the average *SCP* values change to a range of −23.1–−28.7 °C, which does not seem odd against the background of an extreme decrease in air temperatures in the region. However, despite an almost two-fold decrease in the average January temperature (from −18.2 to −32 °C) and average of the absolute minimum the air temperatures (from −39.1 to −57 °C) in Seymchan compared with Magadan, the *SCP* of *A. urticae* in Seymchan did not differ from this indicator in Magadan or Podgornoye (see Table 3). The minimum *SCP* values recorded by us were even more stable. In both the Magadan and Seymchan populations, they were almost the same (−35.2 and −35.5 °C), which is apparently close to the maximum temperature possible for the species. Thus, we can state the closeness of values of supercooling temperatures of the small tortoiseshell in Southern England, St. Petersburg, and Podgornoye on the one hand, and in Podgornoye, Magadan, and Seymchan on the other, differing by only 2–7 °C despite the enormous climatic changes.

The reason for the weak relationship between cold hardiness in *A. urticae* and climate may be determined by different sets of environmental conditions in Europe and Northeast Asia. With an absolute equality of photoperiods in St. Petersburg and Magadan, the temperature regime of autumns (i.e., the regime of preparation for wintering) is extremely different. In the west, autumns are long, with warming weather; in the east, they are short and abruptly terminated by severe frosts.

Unfortunately, lower lethal temperatures for the small tortoiseshell have neither been determined in England nor in St. Petersburg. In the east of its range, *A. urticae* is able to tolerate cooling, probably slightly less than −30 °C, but not lower than −35 °C. Meanwhile, in the area of Kulu Village at the Kolymskaya weather station, as is typical for the region, over the 120 days (from November to February), the air temperatures fell below −35 °C on average every second day, and below −40 °C every sixth day (see Figure 5). The cold hardiness of *A. urticae* is completely insufficient to endure the winter under the bark, in crevices, or in any lightweight constructions. At such temperatures (and even higher ones), low air humidity, even when there is no wind, contributes to the freeze-drying of any moisture-containing objects, except for those with special protection against drying, which is another risk for animals wintering in inadequate shelters. In unheated houses, our measurements showed that background temperatures in December–February were below −35 °C; during deep and prolonged lows, they fell to −40 °C at least 24 times (see Figure 5). All of the aforementioned shelters in the region under discussion are rather deadly traps for the small tortoiseshell.

It is impossible to exclude the possibility of the small tortoiseshell wintering in the attics of residential buildings (near ventilation ducts of houses with central heating and in chimneys in small houses) and even the formation of its anthropogenic foci on this basis. However, the wide distribution of *A. urticae* in northeast Asia is difficult to combine with the rarity of even small settlements here. In addition, findings of nettles with caterpillars are known in areas of Yakutia, where there are no settlements at all [17] (personal comm. of A. Burnasheva). Of course, nettles (and the small tortoiseshell) are more common near settlements than outside anthropogenically modified territories. The foregoing does not mean that in extremely cold regions this butterfly switches to obligatory synanthropy. However, where and how do "wild" *A. urticae* survive the winter in nature? How do they manage to abscond from lethal temperatures?

The only plausible assumption, as it seems to us, may be related to wintering under the snow. The thickness of the snow cover of 35–50 cm, typical for the region in December–February, serves as sufficient insulation for the successful wintering of *A. urticae*. In places with snow covers of 49–50 cm high, the minimum temperatures on the soil surface are very high: −13–−15 °C, whereas on snowless surfaces, they drop to air temperatures, i.e., below −45 °C. In the vast majority of the surveyed biotopes (more than 90%) in the basin of the Kolyma River's upper reaches, the minimum winter temperatures on the soil surface do not fall below −35 °C, and in the coastal areas of the Sea of Okhotsk, temperatures below the above threshold were only noted in 5% of the surveyed biotopes [30,41] (Figure 6). Butterflies can winter under snow cover not only in forest litter on the soil surface, but also in holes or burrows made by animals, as shown for peacock butterflies *Inachis io* [42].

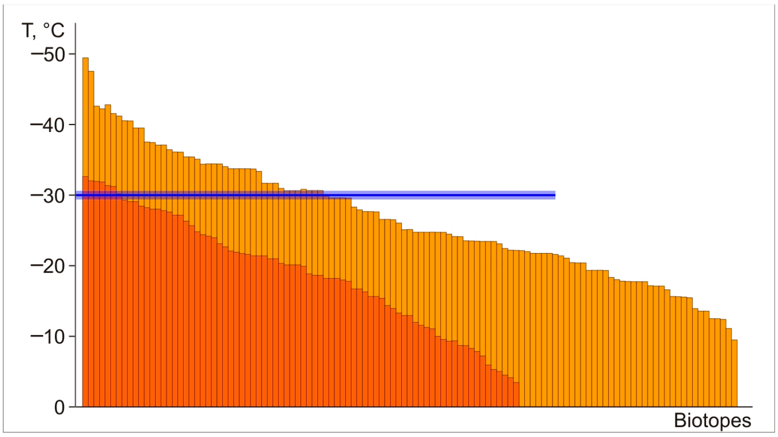

Figure 6. Annual minimum temperatures on the soil surface in 117 biotopes in the basin of the Kolyma upper reaches (yellow) and in 78 biotopes at the coast of the Sea of Okhotsk (orange) (the histogram is based on numerical data from [41]). The solid blue line is the identified threshold of temperatures intolerable by the small tortoiseshell.

Thus, in northeast Asia, the lack of physiological capabilities of *A. urticae* can be compensated for by behavioral adaptation, i.e., wintering under snow. If the small tortoiseshell hibernated over snow cover (i.e., at air temperatures), it would occupy a limited range only in Europe in a temperature range not lower than −30 °C. Its range would not include a significant part of Eastern Europe (the northern half of the Volga basin), Siberia, and the Far East (Figure 7a).

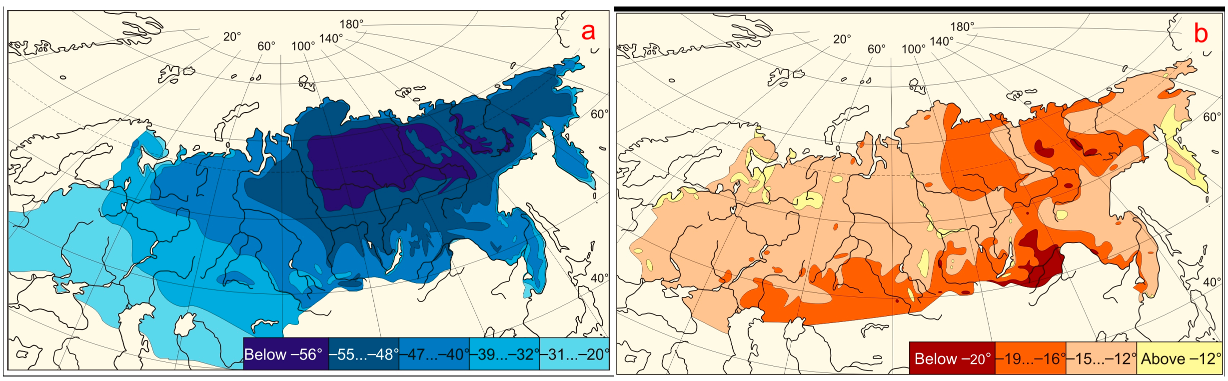

Figure 7. Average of the absolute minima of air temperature (**a**) and annual minima of temperatures at a depth of 2–3 cm of soil under snow (**b**) (according to [43,44]).

Under the snow, in most of the small tortoiseshell range, temperatures are comfortable for it, being not lower than −15 °C, which has allowed it to colonize a vast territory (Figure 7b).

5. Conclusions

Genetic data for 300 COI sequences suggest that *A. urticae* has a shallow genetic structure throughout Northern Eurasia, implying that this species has recently spread through this region. There are thus no deep genetic differences between the populations from the west and east of Northern Eurasia. Therefore, it can be considered correct to compare the parameters of cold hardiness of insects from England, European Russia, Siberia, and northeast Asia.

The cold hardiness of *A. urticae*, which is significant relative to the winter temperatures of Southern England and Western Russia, increases only by 2–7 °C in the ultra-continental climate of northeast Asia. The average and minimum *SCP* values of *A. urticae* from the coldest location where cold hardiness was measured, Seymchan village, were within the ranges of these indicators for the Magadan samples. It follows from this that in the northeast we obtained an estimate that was apparently close to the limiting cryoresistance of the species in the entire range.

Nevertheless, the identified characteristics of *A. urticae* cold hardiness are completely insufficient for wintering in traditionally named places (cracks in the bark of trees, hollows, cavities under the bark, unheated buildings, etc.) not only in northeast Asia, but also in less extreme regions, for example, in the Amur region and Eastern and Western Siberia.

In our opinion, there are only two ways to explain the success of the small tortoiseshell wintering the in the Northeast: either only synanthropic populations exist here (and the finds of butterflies and caterpillars outside the settlements are "migratory" foci, where animals do not survive the winter, but die), or the butterflies hibernate under the snow.

The first way is easier to accept, although it has been extrapolated without proof from the climatically mild European regions to the territories of Siberia with extracontinental climate. However, according to our measurements, the role of abandoned light constructions as shelters in regions that are cold in winter is clearly overestimated; they do not provide the temperature that the butterflies need.

The version regarding wintering under snow "saves the situation" by offering a plausible explanation for such a wide distribution of *A. urticae* in regions that are absolutely unsuitable for its habitat in terms of air temperatures. With a guarantee, snow isolates insects from lethal air temperatures no matter where: in St. Petersburg, with its warm winters, or at the cold pole, with its extremely low temperatures. It is the equalization of temperature conditions under the snow that probably explains the absence of geographic variability in the cold hardiness of *A. urticae*. Moreover, it can be assumed that wintering conditions under snow do not limit the spread of the small tortoiseshell anywhere in the forest zone of the Palearctic (including northern light forests).

A compromising solution cannot be ruled out. The main survival strategy of *A. urticae* at ecologically low temperatures is probably associated with wintering under snow. Additionally, populations in settlements, in the case of cold and snowless weather in late autumn (November), can be an "insurance reserve", from which surviving butterflies settle in subsequent years.

If it is accepted that *A. urticae* winters under snow, it is not a big risk to assume that other widespread species of butterflies may behave in the same way. For extremely cold Yakutia, these can be, for example, *Polygonia c-album* (Linnaeus, 1758), *Nymphalis antiopa* (Linnaeus, 1758), and *N. xanthomelas* (Esper, 1781) (personal comm. of A. Burnasheva). In "milder" regions, the list of species is probably wider. Numerous examples of this are species from various groups of insects that safely winter under snow in the coldest regions [30,45].

Aglais urticae is a convenient model object for solving problems of adaptive strategies for wintering at ecologically low negative temperatures for many species of different groups living in vast areas under harsh conditions. We hope that the work carried out will draw attention to this phenomenon.

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