Org Divers Evol (2015) 15:285–300
DOI 10.1007/s13127-014-0195-1

ORIGINAL ARTICLE

ORGANISMS DIVERSITY & EVOLUTION

Range of a Palearctic uraniid moth *Eversmannia exornata* (Lepidoptera: Uraniidae: Epipleminae) was split in the Holocene, as evaluated using histone H1 and COI genes with reference to the Beringian disjunction in the genus *Oreta* (Lepidoptera: Drepanidae)

Vladimir I. Solovyev · Vera S. Bogdanova · Vladimir V. Dubatolov · Oleg E. Kosterin

Received: 7 November 2013 / Accepted: 5 December 2014 / Published online: 24 December 2014

Abstract Large-scale climatic cycling during the Pleistocene resulted in repeated split and fusion of species ranges in high northern latitudes. Disjunctions of ranges of some Eurasian species associated with nemoral communities used to be dated to 'glacial time', with existence of their contiguous ranges reconstructed not later than 1 mya, while a recent hypothesis associates them with the Boreal time of the Holocene and reconstructs the contiguous ranges ca 5 thousand years ago. These estimates differing by almost 3 orders of magnitude appealed for their testing via molecular methods. We made such a test for *Eversmannia exornata* (Lepidoptera: Uraniidae: Epipleminae), the only uraniid moth inhabiting Siberia, the range of which is split into three pieces: East European, West Siberian and Far Eastern. Two genes were sequenced in specimens from one population from each geographical isolate: a fragment of the mitochondrial cytochrome *c* oxydase I (COI), frequently used for molecular phylogeny and barcoding, and a nuclear gene encoding histone H1. The COI gene fragment appeared to have two alleles differing by one synonymous substitution; both alleles co-occurring in the European population. The histone H1 gene had two dimorphic synonymous sites, with both variants of one site found in all three isolates. Absence of accumulated difference in both genes and polymorphism for the same synonymous substitution in the H1 gene in all three parts of the range suggests a very recent disjunction which cannot be resolved by coding gene sequences. This well corresponds to the Holocene disjunction hypothesis and rules out the Pliocene/early Pleistocene disjunction hypothesis. The published rate of the COI gene evolution was verified using the Beringian disjunction in the genus *Oreta* (Lepidoptera: Drepaniidae) as comparing two northernmost Asian and one American species which diverged not later than the Pliocene. The rate of substitution accumulation in the histone H1 gene was estimated as 0.48 of that of the COI gene, that is ca 3.6×10^{-9} substitutions per site per year. Four indels were found in the histone H1 gene in the three *Oreta* species studied, differing from each other not less than with two indels.

Keywords Lepidoptera · Disjunctive range · The Holocene · Siberia · Beringian disjunction · Histone H1

Electronic supplementary material The online version of this article (doi:10.1007/s13127-014-0195-1) contains supplementary material, which is available to authorized users.

V. I. Solovyev · V. S. Bogdanova · O. E. Kosterin
Institute of Cytology and Genetics of Siberian Branch of Russian Academy of Sciences, Lavrentyev ave. 10, Novosibirsk 630090, Russia

V. I. Solovyev · O. E. Kosterin (✉)
Novosibirsk State University, Pirogova str. 2, Novosibirsk 630090, Russia
e-mail: kosterin@bionet.nsc.ru

V. V. Dubatolov
Insitute of Systematics and Ecology of Animals of Siberian Branch of Russian Academy of Sciences, Frunze str. 11, Novosibirsk 630091, Russia

Introduction

Isolation plays a key role in speciation events, and climate change is its frequent cause. Long-term changes of temperature and humidity regime result in changes of plant and animal communities, up to alterations in vegetation type. In the Cenosoic, the deepest and fastest climatic changes took place for the last 1.6 mya, that is in the Pleistocene and the

conventional Holocene. There are several superimposed cycles of global temperature oscillations due to astronomic cycles, of which the leading megacycle has a period of ca 100 thousand years (Velichko 1993; Imbrie et al. 1993; Petit et al. 1999; EPICA Community Members 2004). The latest and strongest coolings brought about glaciations in North America and Asia. Because of negligible precipitation, most of North Asia except for its extreme north remained free of ice but had quite severe cold and dry climate, including the so-called tundrosteppe conditions (Belova 1985; Kozhevnikov and Ukraintseva 1992; Velichko 1993; Arkhipov and Volkova 1994; Ukraintseva 1996), the remnant of which can still be found in East Yakutia (Yurtsev 1981). These coolings lead to southwards withdrawal and fragmentation of forest biotas, both the nemoral (broad-leafed) and boreal (taiga) forests. The continuous, latitudinally oriented mountain belt of South Siberia, accompanied from its southern side with vast arid territories, served as barriers for migration of many animal and plant forest species to the south (Bernard et al. 2011), so they survived in refugia mostly situated in Europe and the Far East, with a putative less important refugium reconstructed in the Tian Shan Mts. (Nazarenko 1992; Dubatolov and Kosterin 2000). During the latest glacial time, Siberia missed a continuous forest zone, only its southern regions being occupied by forest-tundra landscapes (Belova 1985; Velichko 1993; Arkhipov and Volkova 1994; Dubatolov and Kosterin 2000), while permafrost spread to North Kazakhstan (Velichko 1993). Nevertheless during interglaciations, the climate was as mild as presently or warmer and the forest zone was restored, up to restoration of the continuous subzone of nemoral forests at the climatic optima. At the same time during interglaciations, the arcto-alpine flora and fauna appeared fragmented and shrunken to the highest mountain regions. Thus, in the course of the Pleistocene-Holocene, North Eurasia served as a kind of 'vibration table' repeatedly splitting and merging main biotic formations.

Noteworthy that the period of 100 thousand years of the leading climatic megacycle in its order of magnitude is close to a minimum time of isolation necessary to initiate speciation (Coyne and Orr 2004). As a result, repeated merger of previously isolated floras and faunas into a continuous belt, thermophilic ones at interglaciations and chionophilic at glaciations, in each particular case had unpredictable consequences. Once isolated, populations of some species may merge into the united one, may appear to be species well isolated reproductively (although not so probable for a single cycle) or may act as 'semispecies' subjected to peripatric speciation through mutual reinforcement of prezygotic and postzygotic isolating mechanisms (Barton et al. 2007). This makes North Eurasia a nice polygon for studying evolutionary phenomena associated with speciation. In this respect, study of the degree of evolutionary divergence of isolates of relatively thermophilic species with presently disjunctive ranges is very promising. Recently, quite a number of phylogeographic studies have been published of boreal species with continuous ranges in North Eurasia (for small reviews, see Bernard et al. 2011; Schmitt and Varga 2012), but only two papers (Suvorov 2011; Bernard et al. 2011) deal with a species which most probably has an amphipalearctic range split into two large parts with a gap in Central Siberia (Kosterin 2002).

Eversmannia exornata (Eversmann, 1837) belongs to the subfamily Epiplemidae (sometimes considered as a family of its own) of the family Uraniidae (superfamily Geometroidea). Most representatives of this subfamily occur in tropics and subtropics, and only few reach as north as the southern forest zone of Eurasia (Dubatolov et al. 1993) and taiga forests of North America (Covell 1984). In Eurasia, just six species penetrate as north as the southern Far East of Russia (Sinev 2008) and only one, *E. exornata*, has a vast range in North Eurasia including the European part of Russia, West and Central Siberia, Far East of Russia (including eastern Transbaikalia), NE China, N Korea and Japan (Dubatolov et al. 1993; Dubatolov et al. 2003; Tshistjakov 2005; Knyazev et al. 2010). However, *E. exornata* belongs to those nemoral (coenotically connected with broad-leafed forests of temperate Eurasia or their derivatives) species of Lepidoptera which have disjunctive ranges. Namely, it is one of few species having ranges of the European-Siberian-Far-Eastern type, that is, split into the isolated European, Siberian and Far-Eastern parts (Dubatolov and Kosterin 2000), as shown in Fig. 1. In spite of thorough search, there are no records of *E. exornata* in the Urals (that is between 50°E and 60°E) and in East Siberia between the East Sayan and Argun River basin (that is between 95°E and 118°E).

Earlier, the origin of disjunctive ranges of nemoral plant and animal species in Eurasia used to be ascribed to the climate cooling of the Pleistocene (e.g. Matyushkin 1976; Belyshev and Haritonov 1981; Polozhiy and Krapivkina 1985; Mikkola 1987; Logunov 1996; for a small review, see Dubatolov and Kosterin 2000), which was thought to bring about fragmentation of the contiguous ranges which existed in 'preglacial times', that is in the Pliocene, not less than 1.6 mya or even earlier. However, it used to be neglected that the Pleistocene climate coolings alternated with warmings with climates comparable to the current one or warmer. (However, Walter and Straka (1970) associated isolates of some European species of herbaceous plants in south-eastern West Siberia with the last intrerlaciation.) This repeated change of warm and cold epochs should have inevitably erase the 'preglacial' pattern of ranges (Belova 1985; Kozhevnikov and Ukraintseva 1992; Ukraintseva 1996; Arkhipov and Volkova 1994) inspiring an alternative hypothesis of a far much later, Holocene dating of range disjunctions of nemoral Lepidoptera species (Dubatolov and Kosterin 2000), as well as some Odonata species (Kosterin 2002). These species were supposed to have spread from east to west following the

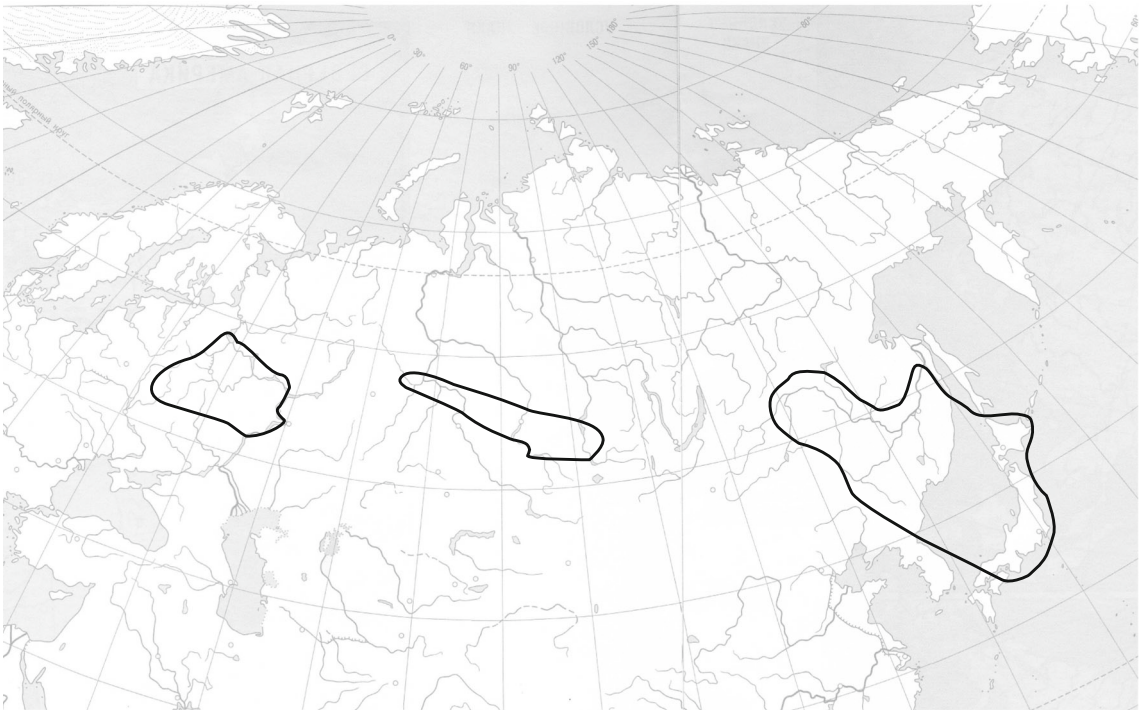

Fig. 1 The presumed range of *E. exornata* (Eversmann, 1837)

Holocene climatic optimum which started in East of Eurasia somewhat earlier that in the West and lasted generally from 8 to 6 thousand years ago (Arkhipov and Volkova 1994). During the Holocene climatic optimum, ranges of those species were most probably continuous but were fragmented during the subsequent small cooling of the so-called Lesser Glacial Period 3–6 thousand years ago, which also started in the east earlier (in the Atlantic time) than in the west (in the subboreal or even Subatlantic time) (Arkhipov and Volkova 1994).

Such a huge, of 3 orders of magnitude (1.6 mya to 6 thousand years ago), range of existing estimates of the time of disjunction of Eurasian nemoral species ranges appeals for a solution using molecular methods. For this reason, we sequenced a fragment of the mitochondrial gene of cytochrome *c* oxydase I, COI, and the nuclear histone H1 gene, in specimens representing the three isolated parts (East European, Siberian and Far Eastern) of the range of *E. exornata*. The COI gene is presently the most popular object of phylogenetic analysis and barcoding of insects (Wilson 2010, 2012).

Genes of histone H1, which is an obligatory component of eukaryotic chromatin (Allan et al. 1986; Bharath et al. 2003) are also quite promising as such objects since, as different from the core histone genes, they exhibit a high rate of variation (Doenecke et al. 1997; Ponte et al. 2003; Happel and Doenecke 2009), in particular in insects (Berdnikov et al. 1993). A large part of histone H1 gene encoding the C-terminal hydrophilic domain, responsible for binding to linker DNA, is characterised by high frequency of deletions and duplications (Hansen et al. 2006; Ponte et al. 2003) which, on one hand, decrease the number of informative sites but, on the other hand, aid in unequivocally resolving the topology of phylogenetic reconstruction by marking certain branches with unique indels. Thus, one of the paralogous genes of histone H1 was successfully applied for phylogenetic reconstruction of the genus *Pisum* L. (peas) even at intraspecies level (Zaytseva et al. 2012). In most insect species, histone H1 is homogenous: among 192 electrophoretically studied Lepidoptera species (Berdnikov et al. 1993), 180 had the only subtype and 12 had two paralogous subtypes (unpubl.). Besides, since histone genes are moderate repeats in insects, e.g. in *Drosophila* (Eirín-López et al. 2009), a degree of heterogeneity of their copies is not excluded which can be overlooked by sequencing products of PCR amplification from genomic DNA. The repeated nature and possible heterogeneity may exert some problems of using histone H1 genes as phylogenetic markers, but they are the same as with the very popular markers associated with rRNA gene cluster (Hovmöller and Johansson 2004).

To estimate the relative evolution rates of the COI and histone H1 gene, we sequenced both in the same representatives of three species of the genus *Oreta* Walker, 1855 found at opposite sides of the Pacific. This genus belongs to the family Drepanidae of the superfamily Drepanoidea (a sister superfamily to Geometroidea). It includes about 60 species mostly from South, South-East and East Asia and Indonesia, with one

species reaching Australia, and the only North American species *Oreta rosea* (Walker, 1855), ranging from boreal forests to subtropics and obviously resulted from a single Beringian invasion into America (Watson 1967). In continental Asia, two species of the same *rosea*-specie group, *Oreta pulchripes* Butler, 1877 and *Oreta paki* Inoue, 1964, occur most far to the north, from North China to Amurland (Zolotukhin and Pugaev 2007; Dubatolov and Dolgikh 2010). We limited our analysis to the three mentioned species, taking the two northernmost Asian species as most probable sister species to the American one. The Beringian land bridge between Eurasia and North America had been existing until about the early Pliocene, that is before 5 mya when the Bering Strait was formed (Marincovich and Gladenkov 1999, 2001) (restoration of the land bridge during the Pleistocene glaciations should not be taken into account in relation to the nemoral fauna because of its tundrous conditions). Flying insects could, however, cross the Bering Strait: for instance, at present, the Lepidoptera fauna associated with tundra is nearly identical at both its sides (Mikkola et al. 1991; Dubatolov 2007). The contact of nemoral Lepidoptera of Eurasia and North America across the Bering Strait could be possible until the mid-Pliocene, that is until 3 mya, when its banks were still covered with broad-leafed forests, but not later when the vegetation changed to boreal forests (Fradkina 1995; Dubatolov and Kosterin 2000). It is indicative that among five invasions of the *Polyommatus* section of blue butterflies from Asia to America through Beringia/Bering Strait which took place from ca 10.7 to 1.0 mya, the three more recent invasions, taking place 1.0–2.4 mya over Bering Strait, involved only cold-adapted taxa, tolerant to low but not high temperatures (Vila et al. 2011). Hence, the American branch of the genus *Oreta* has diverged not less than 3 mya. This gave us an opportunity to apply our markers to a case of the true Pliocene disjunction in Lepidoptera.

Using these estimates, we attempted to evaluate variation for COI and histone H1 genes within and between the three isolates of *E. exornata*. The results confirmed the very recent age of the disjunction, which was not resolved because of the virtually nil divergence in the two studied genes revealed in this species.

Materials and Methods

Material

Specimens of *E. exornata* were collected with the net in their habitat near Novosibirsk Academy Town, Novosibirsk Province, and frozen immediately. Specimens of *O. pulchripes* and *O. paki*, one per each species, were collected by light in the vicinity of Khabarovsk and preserved dry on cotton layers. Dry specimens of *E. exornata* from Jewish Autonomous Province were kindly provided by Alexander N. Streltzov, Amur State Pedagogical University, Blagoveshchensk, those from Tula Province by Lavr V. Bolshakov, Yasnaya Polyana. Specimens of *E. exornata* available for analysis were few because of its being a very rare and elusive species with tiny populations. A specimen of *O. rosea* preserved in alcohol was kindly provided by Kim Mitter, University of Maryland, ATOLep Collection, USA, as legs preserved in pure alcohol. Each *Oreta* species was represented by one specimen. Details of the specimens examined are provided in Table 1.

DNA extraction

Genomic DNA was extracted following Bogdanova et al. (2009), with slight modifications for isolation from individual insects, as follows: dry or frozen moths without abdomen and wings were homogenized in 0.6 ml 0.15 M NaCl. Homogenate was centrifuged (3,300×*g*, 5 min) and supernatant was discarded, then 0.2 ml solution for extraction DNA (0.1 Tris–HCl, pH 8.0; 5 mM EDTA; 0.5 % SDS; 0.1 M NaCl) was added and incubated for 40 min. Then solution was centrifuged (16,100×*g*, 5 min), and supernatant was transferred to fresh tubes. To remove proteins and RNA, LiCl (0.2 ml, 5 M) was added to supernatant solution and incubated on ice for 15 min. Solution was centrifuged (16,100×*g*, 5 min) and supernatant was transferred to fresh tubes. To precipitate DNA, ethanol (1 ml, 96 %) was added and the mixture was incubated on ice for an hour. Then it was centrifuged (16,100×*g*, 10 min) and supernatant discarded. Precipitate was washed with 0.1 ml 75 % ethanol and centrifuged (16,100×*g*, 5 min), then dried at 50 °C for 5 min and dissolved in 50 μl of deionized H_20.

Primer design and sequencing the histone H1 gene and COI gene fragment

To amplify the histone H1 gene, we made use of evolutionary conservation of histone proteins and clustered histone gene organization in insects (Eirín-López et al. 2009). In genomic DNA sequence of *Bombyx mori* (Linnaeus, 1758) available in public databases (AADK01010708), histone genes are found as tandemly repeated units of about 5 kb harbouring these genes in the order H3-H4-H1-H2a-H2b (the presence of H2a being optional). The genomic sequence AC208804 of *Heliconius erato* (Linnaeus, 1764) allows to reconstruct the same gene order of histone genes inside the repeated units. The core histones are moderately (H2a, H2b) or very (H3, H4) conservative while the linker histone H1 has a conservative central nucleosome binding domain. Therefore, it appeared

Table 1 Specimens of Lepidoptera studied

Species	Specimen designation	Locality	Date	Collector
Eversmannia exornata	EeN1–EeN5	Russia, Novosibirsk Province, Novosibirsk Academy Town, a damp forest meadow in the Zyryanka Rivulet valley, 54°49′53″ N 83°07′56″	19 June 2008	Oleg E. Kosterin
Eversmannia exornata	EeT1-EeT7	Russia, Tula Prov., 12 km NW of Yasnogorsk, Shulgino, 54°34.5′ N 37°35.5′ E	25 June 2008	Lavr V. Bolshakov
Eversmannia exornata	EeJ1-EeJ3	Russia, Jewish Autonomous Province, 74 km S of Birakan, Pompeevskiy Mountain Range, the Pompeevka River middle reaches, N48°22′03″ E 131°13′21″	28 June–2 July 2008	A.N. Streltzov, P.E. Osipov
Oreta pulchripes	Opul	Russia, Khabarovskiy Kray, Khabarovsk env., Bychikha village, 48°18′ N 134°49′ E	18–19 June 2010	Vladimir V. Dubatolov
Oreta paki	Opak	Russia, Khabarovskiy Kray, Khabarovsk env., Bychikha village, 48°18′ N 134°49′ E	15–16 July 2008	Vladimir V. Dubatolov
Oreta rosea	Oros	ATOLep tag number CWM-94-0466		Provided by Kim Mitter, University of Maryland, ATOLep Collection. USA

possible to separately sequence the 5′- and 3′ parts of the histone H1 gene within two overlapping amplicons, ca 1 and 1.5–3 kb long, extending from its region coding for the conservative globular domain to the neighbouring genes of histone H4 and H2a, respectively. For this purpose, primers were designed matching the genes of histones H1, H4, H2a of *B. mori* (Online Resource 1: Supplementary Table 1); some primers were also designed to match the histone H1 gene, including flanking non-coding regions of *E. exornata* and *Oreta pulchripes* sequenced in this study.

The position of primers in relation to histone genes is shown in Fig. 2 and their sequences in Table 2. Primers were designed using the Primer3 software (http://frodo.wi.mit.edu/ (Rozen and Skaletsky 2000) and produced by Biosset (Novosibirsk, Russia).

Using primers LH1-f и LH1-2r designed to match the histone H1 gene of *B. mori*, the part of this gene in *E. exornata* coding for the conservative globular domain was amplified and sequenced. Specific primers EEH1g-1r and EEH1g-2r were designed to match this sequence; the use of them and a primer matching the histone H4 gene of *B. mori*, LH4-2f, allowed to amplify and sequence the 5′-terminal part of the gene in *E. exornata.* Using a primer EeH1g-4f matching the globular domain coding part of histone H1 gene in *E. exornata* and a primer HeH2a-2r matching the histone H2a gene of *H. erato*, a 1.6-kb-long DNA fragment was amplified from genome DNA of *E. exornata* and the 3′-terminal part of the histone H1 gene was sequenced from it.

Use of primers LH4-2f и HeH1-r designed to match the histone H4 gene of *B. mori* and the part of the histone H1 gene coding for its globular domain in *H. erato*, respectively, allowed to amplify and sequence the 5′-terminal part of the histone H1 gene in *O. pulchripes* and *O. rosea*. The same in *O. paki* was sequenced using the primer LH4-f instead of LH4-2f. Primer OrH1-f was designed to match this sequence in *O. pulchripes*. Its use in pair with LH2a-r allowed to amplify and sequence the 3’-terminal part of the histone H1 gene in *O. pulchripes* and *O. rosea*. Primers OrH1C-r and OrH1dk-2f were designed to match that sequence in *O. pulchipes*, the same in *O. paki* was sequenced from two

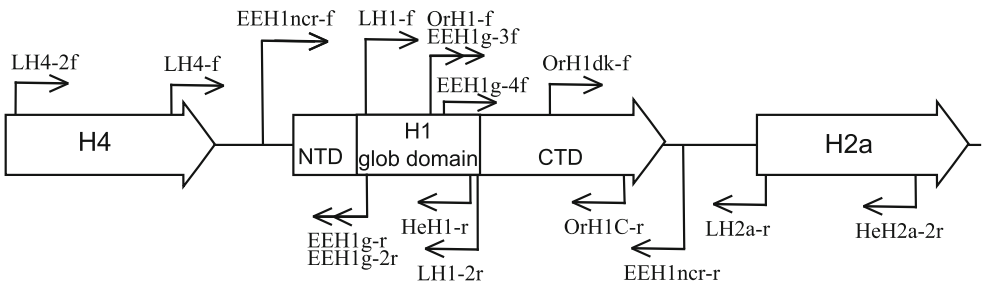

Fig. 2 Positions of primers in a fragment of the histone repeat in Lepidoptera: H4, H1 and H2a—the genes of histones H4, H1 and H2a, respectively; NTD, CTD—the histone H1 gene regions coding for the N-terminal, globular and C-terminal domains, respectively

Table 2 Primers designed to match histone genes of Lepidoptera

Designation	Sequence (5′-3′)	Direction	Matches to	Species
LH1-f	tcaaggagcgcagcggctcatc	Forward	Histone H1 gene, globular domain region	*Bombyx mori*
LH1-2r	gatccggaagctcccttgccttt	Reverse	Histone H1 gene, globular domain region	*B. mori*
EEH1g-1r	cgatgaatttcttgatcgccttc	Reverse	Histone H1 gene, globular domain region	*Eversmannia exornata*
EEH1g-2r.	atgaatttcttgatcgccttcaga	Reverse	Histone H1 gene, globular domain region	*E. exornata*
EEH1g-3f	agtacaaggtcgacgccgag	Forward	Histone H1 gene, globular domain region	*E. exornata*
EEH1g-4f	cagaaaatacttgaagagcgcc	Forward	Histone H1 gene, globular domain region	*E. exornata*
LH4-2f	atgaccggtcgcggtaaaggaggc	Forward	Histone H4	*B. mori*
LH4-f	accctgtacggtttcggcggttaa	Forward	Histone H4	*B. mori*
LH2a-r	ttccgccttttccgcgaccggacat	Reverse	Histone H2a	*B. mori*
HeH2a-2r	atgttgggcagtacacctccctgcg	Reverse	Histone H2a	*Heliconius erato*
HeH1-r	agcgcccttgcccttggtctgtatc	Reverse	Histone H1 gene, globular domain region	*H. erato*
OrH1-f	agtataaagtcgacgccgaa	Forward	Histone H1 gene, globular domain part	*Oreta pulchripes*
OrH1C-r	ttacttctttgacgccgatg	Reverse	C-terminal domain H1	*O. pulchripes*
OrH1dk-2f	agggacaagaaggccgccgc	Forward	C-terminal domain H1	*O. pulchripes*
EeH1ncr-r	gccgcacctatcacctacc	Reverse	5′- noncoding region of H1	*E. exornata*
EeH1ncr-f	atcaactgctagcgccatct	Forward	3′- noncoding region of H1	*E. exornata*

overlapping fragments amplified using primer pairs OrH1-f–OrH1C-r and OrH1dk-2f–LH2a-r.

A 708-bp-long fragment of COI gene was amplified with the published universal insect primers LCO-1490 and HCO-2198 (Folmer et al. 1994).

DNA amplification and sequencing

PCR mixtures (30 μl) contained 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 25 mM KCl, 60 mM TrisHCl (pH 8.5), 10 mM β-mercaptoethanol, 0.1 % Triton X-100, 0.5 μM of each primer, 1 μl of genomic DNA and 1 U of Taq DNA polymerase (Lab. Medigen, Novosibirsk, Russia) or 1 U of Smart-Taq DNA Polymerase (Lab. Medigen). PCR was performed using a thermal cycler MyCycler (Bio-Rad, USA) with the following program: (1) 94 °C—2 min 30 s, 1 cycle; (2) 95 °C-15 s, 55–57 °C (adjusted for specific cases)—30 s, 68 °C—3 min, 35 cycles; (3) 68 °C—4 min, 1 cycle. For amplification of the 3′-terminal part of the histone H1 gene of *O. paki*, another program was used: (1) 95 °C—2 min, 1 cycle; (2) 95 °C—30 s, 59 °C (−0.3 °C per cycle)—30 s, 68 °C—4 min, 16 cycles; (3) 94 °C—30 s, 55 °C—30 s, 68 °C—4 min, 20 cycles; (5) 68 °C—4 min, 1 cycle.

Sanger reaction was conducted in 30 μl volume of mixture containing 1 μl of BigDye Terminator, version 3.1, 200–400 ng of DNA, 5 pmol of primer and 6 μl of buffer solution for BigDye 3.1. MyCycler (Biorad) was used with the following program: 95 °C—45 s, 50 °C—30 s, 60 °C—4 min; 26 cycles. Analyses of the products were carried out at the SB RAS Genomics Core Facility, Novosibirsk.

CAPS analysis

For genotyping specimens of *E. exornata* with respect to nucleotide substitution A-G in the histone H1 gene, CAPS analysis was used (Konieczny and Ausubel 1993). PCR was conducted with primers EeH1ncr-f and EEH1g-2r; 9 μl of the product was added with 1 μl of buffer containing 33 mM Tris-acetate (pH 7.9 at 25 °C), 10 mM Mg $(CH3COO)_2$, 66 mM K $(CH3COO)_2$; 1 mM DTT and 0.5 units of endonuclease *Mbo*II. The mixture was incubated at 37 °C for 2 h, inactivated at 80 °C for 20 min and analysed by electrophoresis in 6 % PAAG. For genotyping of the same specimens for the nucleotide substitution T-A in the COI gene, the endonuclease *Bst*SFI was used in the same procedure but at 60 °C and using the buffer 50 mM Tris–HCl (pH 7.6), 10 mM $MgCl_2$, 100 mM NaCl and 1 mM DTT. The buffers and enzymes were produced by Sibenzim, Novosibirsk, Russia.

Sequence alignment and analysis

Sequence alignments and calculating genetic distances were done using MEGA 5.0 software package (http://www.megasoftware.net) (Tamura et al. 2011), with the regions involved into indels excluded (the complete deletion mode).

While comparing species of *Oreta*, the uncorrected p-distance (the mean number of base differences between sequences per site) was calculated for both the COI and histone H1 gene, and for the latter gene also distances by the Jukes-Cantor model (Jukes and Cantor 1969) and Nei-Gojobori model (Nei and Gojobori 1986). The distance for amino acid

sequences of the histone H1 genes were calculated using the Poisson correction model (Zuckerkandl and Pauling 1965).

To estimate the time of existence of the most recent common ancestor (MRCA) of the studied species of *Oreta* spp., we used sequences of the COI gene fragment obtained in this work along with those of *O. rosea* available in public databases (Sup. mat.). The Bayesian estimation was obtained by BEAST v.1.7.1 software (Drummond et al. 2012). The following options were chosen: the Hasegawa, Kishino and Yano (HKY) model of nucleotide substitution, the empirical base frequences, the strict clock option implying identical substitution rates in different branches and the coalescent model with the constant population size. The value of 7.5×10^{-9} per site per year was assumed as the substitution rate in COI following Quek et al. (2004). The parametres were estimated in three independent Markov chain Monte Carlo (MCMC) runs of 10 million generations with a pre-run burn-in of 1,000,000 generations. Independent runs were combined using Tracer v1.5.0 software available at http://beast.bio.ed.ac.uk/Tracer.

Results

Variation in the mitochondrial COI gene in *E. exornata* and *Oreta* spp.

A 646-bp-long fragment of the COI gene (positions 1526–2171 as to the sequence X03240 of *Drosophila yakuba* Burla, 1954) has been sequenced in three specimens of *E. exornata* representing three different parts of its range (EeJ1, EeN2, EeT3) and in the three studied species of *Oreta*; these sequences were submitted to EMBL (deposited in public databases as accessions HG737848-HG737853). In *E. exornata*, it appeared identical in all the three specimens but one synonymous substitution T–A in position 2031 in the specimen EeT3 from the European isolate. Polymorphism for this position creates the recognition site CTGTAG for restriction endonuclease *Bst*SFI which allowed genotyping the rest of *E. exornata* specimens (Fig. 3). All specimens from Novosibirsk (EeN1-EeN5) and Jewish Autonomous (EeJ2, EeJ3) provinces appeared to have T in this position while those from Tula Province had either T (specimens EeT4, EeT6) or A (specimens EeT1-EeT3, EeT5).

In the studied species of *Oreta*, this COI gene fragment exhibited a considerable variation, which is illustrated by pairwise uncorrected p-distance between them (Table 3). Sixteen sequences of this fragment in *O. rosea* available in public databases (Online Resource 1: Suppl. Table 1) appeared quite variable as well; the maximum p-distance between two individuals is also given in Table 3. The table also provides estimations of the divergence event obtained by direct relation of the p-distances to the substitution rate in COI as 7.5×10^{-9} per site per year following Quek et al. (2004).

Assuming the same substitution rate in COI, we estimated the time of existence of MRCA for *O. rosea*, *O. paki* and *O. pulchripes* using the Bayesian coalescent approach. Its maximum probability corresponds to 8.45 mya and the range of highest probability density embracing 95 % of probability mass (95 % HPD) is 6.88–10.08 mya (Fig. 4). The MRCA of the sequenced representatives of *O. rosea* had the maximum probability to exist 2.45 mya, with 95 % HPD of 1.65–3.34 mya.

Primary structure of the histone H1 gene in *E. exornata* and *Oreta* spp.

The histone H1 gene sequences were obtained from 13 specimens of *E. exornata* and from 1 specimen per each of the three studied species of *Oreta* (deposited as accession HG737854-HG737869). Differences between the derived histone H1 amino sequence of *E. exornata* (specimen EeN2) and *O. pulchripes* are summed up in Table 4. It is 250 amino acid residues long in the former and 239 residues in the latter. These differences resulted from seven deletions and two insertions. There are one deletion and one insertion in the N-terminal hydrophilic domain and six deletions (of them three are one amino acid residue long) and one insertion in the C-terminal domain. There are 38 substitutions among 237 common amino acid positions that comprise 16 %. Most of them (31) are also confined to the most variable C-terminal domain; three are found in the central globular domain and four in the N-terminal domain. In the corresponding nucleotide sequences, there are 176 substitutions in 711 common positions (24.8 %).

Nucleotide sequences of the histone H1 gene have diverged quite substantially in *O. rosea*, *O. pulchripes* and *O. paki* (see nucleotide and amino acid alignments in Online Resource 1: Supplementary Fig. 1). Five synonymous substitutions were found in the gene region coding for the N-terminal domain, 13 synonymous and 1 non-synonymous substitutions in that coding for the globular domain, while the region coding for

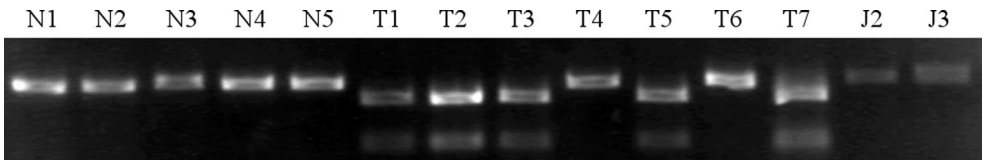

Fig. 3 Polymorphism for presence/absence of restriction site for the *Bst*SFI endonuclease in the COI gene fragment in *E. exornata*

Table 3 Uncorrected p-distances between sequences of the studied COI gene fragment in representatives of the genus *Oreta* and estimation of their divergence time assuming the substitution rate of 7.5×10^{-9} per site per year following Quek et al. (2004). Standard errors are given after ±

Comparison	Uncorrected p-distance	Divergence time estimation (mya)
Most distant representatives of *O. rosea*	0.036±0.007	2.4
O. rosea consensus–*O. pulchripes*	0.105±0.012	7.0
O. rosea consensus–*O. paki*	0.099±0.011	6.6
O. pulchripes–*O. paki*	0.104±0.011	6.9

the C-terminal domain was found to contain 35 substitutions, 10 of which were non-synonymous, and also 4 indels (Table 5). *O. pulchripes* has the histone H1 gene corresponding in length to the consensus sequence for the three studied species, while each of the H1 gene in *O. paki* and *O. rosea* differs from it by two different insertions. The insertions found in *O. paki* starting at consensus positions 361 and 571 are situated in tracts of tandemly repeated triplets and are additional triplet copies at the tract ends (Table 5). The insertions in *O. rosea* are longer. That at position 544 is an insertion of two additional copies of tandemly repeated triplets. The insertion at position 469 is a segment of 12 nucleotides, coding for tetrapeptide GASG, which differs by three synonymous substitutions from a similar segment at consensus position 425, that is 44 nucleotides apart in the 5′ direction (Table 6) and most probably results from duplication of the latter. In *O. pulchripes* and *O. paki*, this supposed matrix of the duplication differs by a non-synonymous substitution and encodes the tetrapeptide GASA; this should be the ancestral state for the studied group of species.

In *E. exornata*, only four variants of the coding sequence differing in two synonymous substitutions were revealed among three sequenced specimens (see below).

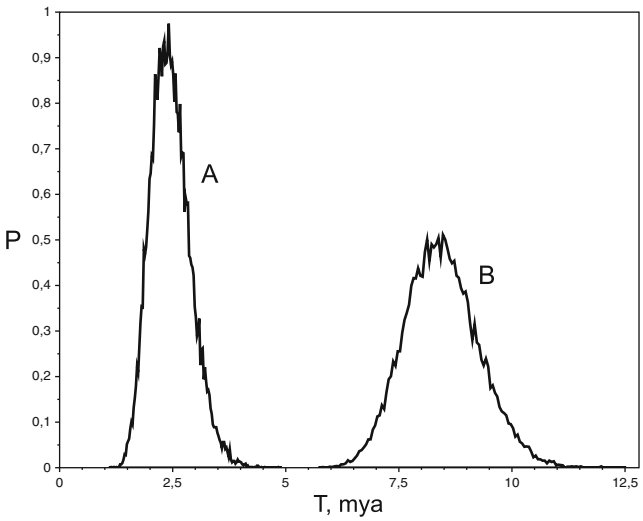

Fig. 4 The probability density P of the Bayesian coalescent estimation of the time of existence of the most recent common ancestors, T, of the recent representatives of *O. rosea* (*A*) and of the three northernmost species of the genus *Oreta* occurring at opposite sides of the Pacific: *O. rosea* (North America), *O. pulchripes* and *O. paki* (East Asia) (*B*)

Genetic distances between nucleotide sequences of the histone H1 gene in *O. rosea*, *O. pulchripes* and *O. paki*

Table 7 presents different measures of evolutionary distances between histone H1 gene sequences in pairs of the studied species of *Oreta*. As soon as there is an independent estimation of the time of divergency (t), such distances (d) allow to estimate the rate of nucleotide substitutions per site per year as $r = d/2t$ (Nei and Kumar 2000). If variation is low, as in our case, it is preferable to take the uncorrected distance p as a measure of d (Ibid.). Unfortunately, we have no independent estimation of the divergence time of the American *O. rosea* and the Asian species of the *rosea*-species group; nevertheless, we have a minimum estimation of this time as 3 mya, as the latest time when nemoral forests still existed at both sides of the Bering Strait (Fradkina 1995; Dubatolov and Kosterin 2000). Hence, we can get an upper estimation of the rate of evolution of the histone H1 gene as 9.5×10^{-9} substitutions per site per year for the pair *O. rosea* vs *O. pulchripes* and 9.3×10^{-9} for the pair *O. rosea* vs *O. paki*, with the mean of 9.4×10^{-9}.

It was more informative to estimate the evolution rate of the histone H1 gene with reference to that of COI as the ratio of nucleotide substitutions accumulated by the respective genes in the same species in their pairwise comparison. The data of Tables 3 and 7 provide the following values of this ratio for the three species pair: 0.54 for *rosea* versus *pulchripes*, 0.57 for *rosea* versus *paki* and 0.38 for *pulchripes* versus *paki*; the linear regression of these values gives the value of 0.48. Using the estimation of the COI evolution rate by Quek et al. (2004), we obtain that of the histone H1 evolution rate as 3.6×10^{-9} per site per year.

Polymorphism for histone H1 gene sequence in *E. exornata*

Coding sequences of the histone H1 gene were obtained from 13 specimens of *E. exornata* (excluding EeJ1 and EeJ3) representing all the three isolated range parts. They appeared to be identical except for two synonymous substitutions in positions 57 and 357 from the coding sequence start. The only specimen EeJ2 had T in position 357, while others sequenced specimens had C there; CAPS analysis revealed C in specimen EeJ3 too. Position 57 was occupied by either A (EeN2-5) or G (EeT1-5, EeT7, EeJ2), or both A and G were revealed in sequences obtained from a fragment amplified from genome DNA (EeN1, EeT6). This substitution is synonymous and

Table 4 Length, the number of substitutions and indels in the histone H1 gene, its protein product and their main parts in *E. exornata* (EeN2) as compared to *O. pulchripes* (Opu)

Histone H1 domain	Length in base pairs (stop-codon included)	Number of synonymous substitutions	Number of non-synonymous substitutions	Number of deletions of certain lengths (base pairs)	Number of insertions of certain length (base pairs)	Protein length in amino acid residues
N-terminal domain	111	21	4	1 and 3 bp	1 and 3 bp	37
Central globular domain	219	34	3	–	–	73
C-terminal domain	EeN2—423 Opu—390	83	31	3 by 3 bp 2 by 6 bp 1 and 15 bp	1 and 3 bp	EeN2—140 Opu—129
Total	EeN2—753 Opu—720	138	38	7, total of 39 bp	2, total of 6 bp	EeN2—250 Opu—239

creates the recognition site GAAGA for the restriction endonuclease *Mbo*II. This allowed to score 15 specimens of *E. exornata* at our disposal for the two possible variants in position 57. CAPS analysis revealed A in specimens EeN2-N5, G in EeT1–T5 and EeT7, and both A and G in EeN1, EeT6 and EeJ1–EeJ3. This well corresponded to the sequencing data except for the specimen EeJ2 which did not show superposition of the A and G peaks in position 57 when sequenced while CAPS analysis revealed this specimen to be heterogeneous. So, most specimens from Tula Province (East European isolate) had G, most specimens from Novosibirsk Province (Siberian isolate) had A but both samples contained specimens heterogeneous for these nucleotides (that was confirmed by using two methods), as all the three specimens from Jewish Autonomous Province (Far Eastern isolate) were (Table 8).

Discussion

Beringian disjunction of nemoral lepidopteran genera as a possible paleogeographical landmark in estimations of gene evolution rates

Since many authors used to non-critically associate formation of large Eurasian range disjunction of nemoral animal and plant species with the Pleistocene climate cooling in general (e.g. Matyushkin 1976; Belyshev and Haritonov 1981; Polozhiy and Krapivkina 1985; Mikkola 1987; Logunov 1996), that is to date it to the Pliocene, we completed our study of such a species, *E. exornata*, by examination of the same molecular markers in three lepidopteran species of the *rosea*-group of the genus *Oreta*, from the same superfamily, involving not younger than a Pliocene divergence. A substantial divergence revealed between them contrasted drastically to the nearly absence of divergence between isolates of *E. exornata*.

There are evolution rate estimations available for popular molecular markers, such as the COI gene. The Beringian disjunctions of Lepidoptera allow their additional verification. For this reason, it is necessary to evaluate the divergence age for a number of pairs of amphiberingian sister taxa using the existing estimations of marker evolution rates. In each pair, the actual divergence could have taken place before the latest possible contacts of nemoral faunas of Lepidoptera of Asia and North America, which is the time of the latest existence of broad-leafed communities at the opposite banks of the Bering Strait, which is not an impermeable barrier for flying insects. The youngest among estimation for the pairs involved should be close to this critical time. Still it can be an overestimation since the divergence of colonising lineage could take place before the actual colonisation. From paleoecological data, we assume the latest time of existence of nemoral communities at the Beringian Strait as close to 3 mya (Fradkina 1995; Dubatolov and Kosterin 2000), so closeness of the estimation obtained by molecular methods to this figure would verify the evolution rate estimations for the markers involved.

Table 5 Indels in the studied *Oreta* species

Species	consensus position of the indel first nucleotide			
	361	469	544	571
O.pulchripes	ACCACCACC---GCC	GCT----------GCC	GCCGCCGCC------GCA	GCCGCC---AAG
O. paki	ACCACCACCACCGCC	GCT----------GCC	GCCGCCGCC------GCT	GCCGCCGCCAAG
O. rosea	ACCACCACC---GCC	GCCGGCGCATCTGGTGCC	GCCGCCGCCGCCGCCGCT	GCCGCC---AAG
amino acid translation	T T T - A	A - - - - A	A A A - - A	A A - K
	T T T T A	A - - - - A	A A A - - A	A A A K
	T T T - A	A G A S G A	A A A A A A	A A - K

Table 6 The part of the histone H1 gene in the studied *Oreta* species, containing the duplication of 12 nucleotides in *O. rosea*, starting fromconsensus position 469, with the corresponding amino acid sequences. The homologous sequences underlined, substitutions in them boldfaced

```
O. pulchripes   AAGGGCGCGTCCGCCTCCGCCGCCGGCAAGGGTGGCAGGAAAGCT-----------GCC
O. paki         AAGGGCGCGTCCGCGTCCGCCGCCGGCAAGGGTGGCAGAAAAGCT-----------GCC
O. rosea        AAGGGCGCGTCCGGGTCCGCCGCCGGTAAGGGTGGCAGGAAAGCCGGCGCATCTGGTGCC
                 K  G  A  S  A  S  A  A  G  K  G  G  R  K  A  -  -  -  -  A
                 K  G  A  S  A  S  A  A  G  K  G  G  R  K  A  -  -  -  -  A
                 K  G  A  S  G  S  A  A  G  K  G  G  R  K  A  G  A  S  G  A
```

As the first step in this program, we evaluated the age of the Beringian disjunction in the *rosea*-group in the genus *Oreta*, to which the three studied species belong. The *rosea*-group of 17 species has its core range extending from the East Himalaya to South and East China and Japan, with one species ranging in Indochina and Indonesia to Sulawesi, two species in North China and Russian Amurland, and one species, *O. rosea*, occupying North America from subtropics to boreal forests (Watson 1967). The two northernmost Eurasian species studied by us, *O. pulchripes* and *O. paki*, range from North China to Amurland (Zolotukhin and Pugaev 2007; Dubatolov and Dolgikh 2010), the former penetrating more northerly to Lower Amurland (51°N) (Dubatolov 2009). By the male genitalia structure, they are the closest relatives to the American *O. rosea*. Of these species, *O. rosea* and *O. pulchripes* exhibit some similarity in the wing pattern, and their ranges extend northernmost, yet the genital morphology (Watson 1967) does not allow to claim any two of the three species to be more closely related. Both Asiatic species are coenotically connected to nemoral, broad-leafed forests while the North American species extends beyond the zone of broad-leafed forests, occupying most of its range, to the boreal, taiga forests (Covell 1984).

Existing estimations of the substitution rate in the COI gene in arthropods vary substantially. In earlier works, that by Brower (1994) of $10.5–11.5\times10^{-9}$ substitutions per site per year was in predominant usage; later works argued for a lower value of 7.5×10^{-9} (Farrel 2001; Quek et al. 2004). These authors noted that in the sequence used by Brower (1994), only 250 bp belonged to the COI gene while most of it belonged to the COII gene which, according to Crozier and Crozier (1993), evolved faster in insects (as shown for Apidae and Drosophilidae). Besides, Brower (1994) supposed that divergence between races of *H. erato* (with uncorrected *p* value of 0.034) resulted from the Pleistocene climate cooling ca 1.6 mya, which restricted the species to refugia. However, the value calculated under this assumption could be only the upper estimation of the substitution rate, since divergence of the gene could take place before the divergence of populations while the latter could take place before the climate cooling.

Basing on the uncorrected *p* value for the studied COI fragment for the two pairs of Asian/American species, *O. pulchripes-O. rosea* and *O. paki-O. rosea* and using the estimation of 7.5×10^{-9} substitutions per site per year by Quek et al. (2004), we estimated the age of the most MRCA of the three studied species of *Oreta* as 6.6–7.0 mya. Application of the Bayesian coalescent approach, which is more adequate for this task, resulted in the interval (95 % HPD) of 6.9–10.1 mya, with the highest probability at 8.5 mya. The 95 % HPD for the age of MRCA for the sequenced recent representatives of *O. rosea* is 1.7–3.3 mya, with the highest probability at 2.5 mya. Thus, we have the range of 2.5–8.5 mya for the estimation of the most probable time of penetration of *Oreta* from Eurasia to North America, which corresponds to our independent upper estimation for it from paleoecological data as 3 mya.

Oreta is a single case of the amphiberingian disjunction of nemoral Lepidoptera, and many more such cases should be investigated. A list of 27 such genera with amphigeringian disjunction is given in (Dubatolov and Kosterin 2000), which can provide the necessary sample of estimations of the disjunction times.

Earlier, dating of an amphiberingian disjunction in Lepidoptera was made for *Limenitis* sensu stricto (Nymphalidae) basing on the nuclear and mitochondrial genes (COI and EF1-α by Mullen 2006; COI, EF1-α, Wg, GPDH,

Table 7 Pairwise evolutionary distances between *O. rosea*, *O. paki* and *O. pulchripis* obtained from histone H1 gene sequences: *p*—uncorrected p-distance; d_{JC}—distance according to Jukes-Cantor nucleotide substitution model; d_{NG}—distance according to the modified Nei-Gojobori method for synonymous substitutions via Jukes-Cantor model; d_{PC}—distances for amino acid substitutions with Poisson corrections. Standard errors are provided after "±". Deletions removed from analysis

Species pairs	*p*	d_{JC}	d_{NG}	d_{PC}
O. rosea–O. pulchripes	0.057±0.009	0.059±0.009	0.224±0.040	0.025±0.010
O. rosea–O. paki	0.056±0.009	0.058±0.009	0.203±0.038	0.034±0.012
O. pulchripes–O. paki	0.039±0.007	0.040±0.008	0.147±0.031	0.017±0.008

Table 8 Genotypes for position 57 of the histone H1 gene, A versus G, in *E. exornata* specimens in the samples from the three isolated parts of its range

Region	Total number of specimens	Specimens with A	Specimens with G	Specimens with both A and G
Tula Province	7	0	6	1
Novosibirsk Province	5	4	0	1
Jewish Autonomous Province	3	0	0	3

IDH, RPS5 by Mullen et al. 2011). The larvae of all but one Eurasian species of this genus feed on plants from family Caprifolicaeae, while those of *Limenitis populi* (Linnaeus, 1758) and the Nearctic species feed on Salicaceae plants. It was supposed that the common ancestor of *L. populi* and the Nearctic species inhabited Eurasia and changed its host plant family from Caprifoliaceae to Salicaceae. This allowed it to occupy the entire forest zone, including boreal forests, and hence to colonise North America. For this reason, representatives of this lineage can no more be considered nemoral and we can expect a later time of colonisation of North America than for nemoral species, up to the Lower Pleistocene that is to ca 1.6 mya. Mullen (2006) estimated this time as 4 mya that is close to the upper limit of our estimation of the Beringian disjunction in *Oreta*. This is hardly surprising since the actual invasion of America by the Salicaceae-feeding *Limenitis* s. str. could have taken its place quite before the boreal forests disappeared from the Bering Strait banks. Later, Mullen et al. (2011) using Bayesian approach estimated the amphyberingian disjunctions for the Nearctic and Palearctic species of *Limenitis* in the range of 9–3 mya; this time range is more consistent with our estimation of the *Oreta* disjunction. Note that invasion across Bering Strait to North America of *Lycaeides* butterflies, which are also widespread throughout the forest zone, was estimated to have taken place in a similar time of 2.4 mya (Vila et al. 2011).

Histone H1 gene as a phylogenetic marker

There is no published estimations of the histone H1 gene evolution rate in insects. Having pairwise compared the three studied species of *Oreta*, we estimated the ratio of the evolution rates of the COI and H1 genes as 0.48. Using the above-mentioned estimation of the former by Quek et al. (2004), the latter can be estimated as 3.6×10^{-9} substitutions per site per year. So, the histone H1 gene is about twice less variable than the COI gene and hence can be used as a phylogenetic marker at a similar time scale. However, the histone H1 gene has an advantage of frequent deletions and insertions in its part coding for the variable C-terminal domain, which uniquely mark some lineages and hence aid in resolution of evolutionary tree topology.

The source of these indels is though to be unique crossing over (mostly for long indels) and, more importantly, slippage mispairing while DNA replication (especially for short indels), which take place in tracts of tandem repeats and less perfect motifs abundant in the region coding for the C-terminal domain (Ponte et al. 2003). Indeed in *Oreta* spp., three of the four indels were found in tracts of tandemly duplicated nucleotide triplets as additions of one more triplet (the two duplications in *O. paki*) or two triplets (one of the two duplications in *O. rosea*). Most probably they resulted from slippage mispairing. The duplication of 12 nucleotides in *O. paki*, with the duplicated stretch situating 30 nucleotides apart from its template, is a less expected event. Such a phenomenon, intragenic duplications from a matrix situated at some distance in the 5′-direction, was earlier found in a histone H1 gene in pulses and was called intragenic conversion (Berdnikov et al. 2003; Trusov et al. 2004).

O. paki differs from its two other congeners by two 3-bp-long duplications while *O. rosea* by two duplications, 6- and 12-bp long. One can conclude that it is *O. pulchripes* which keeps the ancestral histone H1 gene structure, since the two other species differ from it by two indels but by four indels from each other. However, there is a problem of three synonymous substitutions in the 12-bp-long duplication as compared to its presumed matrix. Their comparison provides Nei-Gojobori distances for synonymous substitutions of 0.33, compare to 0.20–0.22 between *O. rosea* and the two other species for the entire gene (Table 7). Of course, as much as three synonymous substitutions in so short sequence could occur after divergence of the *O. rosea* lineage by chance. An alternative supposition could be that the duplication occurred before divergence of the three species, was present in their common ancestor and was accumulating substitutions for quite a long time, but then was lost in the Asian lineage represented by *O. pulchripes* and *O. paki*. However, this explanation demands a further assumption that the duplication was precisely excised at the same positions that seems even more improbable. More *Oreta* species from the *rosea*-group should be involved into the analysis to solve the dilemma. But whatever would be the solution, it seems that we are facing results of some unknown peculiarities of mechanisms generating indels in the sequence coding for the histone H1 C-terminal domain: either high probability of point mutations in a sequence being duplicated or a possibility of a precise ‘reversion of duplication’.

Note that among pairwise evolutionary distances between *Oreta* species calculated by all the methods, those between *O. rosea* and *O. paki* appeared the least among the three

(Table 7). However, the differences were small and all the three distances are comparable.

So, our data, along with a successful attempt of phylogeny reconstruction even at the intraspecies level in peas (Zaytseva et al. 2012), suggest the histone H1 genes have a perspective as an object of molecular phylogenetic studies in different organisms.

Disjunction of the range of *E. exornata*

The histone H1 gene nucleotide sequences appeared identical in the three isolated range parts of *E. exornata* but two polymorphic synonymic positions: one of which was found only in one specimen from the Far Eastern part while the other appeared to be present in all these parts. The studied COI gene fragment sequences appeared identical as well, again with a polymorphic synonymous position but found only in the East European range part. This evidences for a very recent range disjunction of this species. Both genes were tested as a measure of divergence time against a doubtless case of a Pliocenic disjunction in the genus *Oreta* and both showed substantial variation (with uncorrected *p* values of ca 0.10 for the COI gene and 0.04–0.06 for histone H1 genes). Zero divergence found in both studied ca 700-bp-long sequences means that the age of the range disjunction in *E. exornata* is beyond the resolution of the markers chosen and suggests its being not less than 2 orders of magnitude less than the age of disjunction in *Oreta*. This strongly supports the hypothesis of the Holocenic (about 5,000 years ago) dating of disjunction in *E. exornata* (Dubatolov and Kosterin 2000).

A similar result was obtained while comparing the European and Far Eastern populations of a damselfly *Nechalennia speciosa*: in the three studied mitochondrial sequences just three nucleotide substitutions were found in the COII gene, one in 16sRNA–ND1 sequence (Bernard et al. 2011) and two in the COI gene; the nuclear ITS1 region showed no variation (Suvorov 2011). For us, it is important that *N. speciosa* is most probably the only species of Odonata which has a disjunctive amphipalearctic range (Belyshev and Haritonov 1981; Kosterin 2002), alike the range of some nemoral Lepidoptera with Siberian gaps in their ranges (Dubatolov and Kosterin 2000). Although *N. speciosa* inhabits bogs in taiga forests and so reaches as northerly as 60° N in Siberia (Bernard and Kosterin 2010), its range is atypical for boreal species which usually have broad and contiguous distribution over the taiga zone of North Eurasia. Molecular studies of some of such Boreal species (for small reviews see Bernard et al. 2011; Schmitt and Varga 2012) as a rule revealed small DNA variation providing low phylogeographical signal, although not zero as in the case of *E. exornata* or nearly so as in the case of *N. speciosa*. Postglacial recolonisation of Eurasia was reconstructed to take place from a single, either East Asian or European refugium (such examples mostly refer vertebrates, see Babik et al. 2004; Ursenbacher et al. 2006 for evidence of western refugia, and Zink et al. 2002; Oshida et al. 2005; Fedorov et al. 2008; Saitoh et al. 2010 for evidence of eastern refugia), or a simultaneous recolonisation from both western and eastern refugia (see Schmitt and Seitz 2001; Schmitt and Varga 2012 for such a case in Lepidoptera; Jödicke et al. 2004 for a case in Odonata, and Uimaniemi et al. 2000; Hundertmark et al. 2002; Goropashnaya et al. 2004 for cases in Vertebrata). For the first glance, a greater variation and phylogenetic signal in species with broad contiguous ranges as compared to species with vast but disjunctive range looks paradoxical: the latter species look like evolutionary very young while their ecology suggest them to be remnants of an ancient, more thermophilic fauna and their disjunct ranges look like confined to its refugia. This contradiction can be explained by a logical assumption that during the Pleistocene glaciations, the ranges of thermophilic (e.g. nemoral Lepidoptera) or otherwise stenotopic (*N. speciosa* confined to pools in peat moss bogs) species were reduced catastrophically, most probably to single small refugia, so that overwhelming majority of pre-existing genetic variation was lost (Bernard et al. 2011). Disjunctions of ranges of many such species which we observe presently are by no means ancient but manifest the beginning of the new range reduction due to the climate cooling after the Holocene climatic optimum, at the dusk of our current interglaciation and in view of the next glaciation ongoing (the notorious global warming being too short to restore the contiguous ranges again).

Resolution of the histone H1 and COI genes as measures of divergence time is no more than hundreds of thousand years, so they can be applied to events not later than the Middle Pleistocene. More variable markers such as microsatellites could help in dating more recent events and may be attempted for the range disjunction of *E. exornata*.

Polymorphic position 57 in the histone H1 gene in *E. exornata*

Since in insects, histone H1 genes are arranged in the so-called histone repeat which is tandemly repeated to form the histone cluster (Eirín-López et al. 2009), homogeneity of the histone H1 gene sequence amplified from genomic DNA from most specimens of *E. exornata* implies homogeneity of at least overwhelming majority of copies of this gene (the number of which is unknown) in a histone cluster. At the same time, presence in comparable quantities of both variants of position 57 in sequences amplified from genomic DNA of five specimens, EeN1, EeT6, EeJ1–EeJ3, suggest two possible interpretations: either these specimens are heterozygotes, that is have two homogenous but different histone clusters on a pair of homologous chromosomes, or their histone clusters are heterogeneous themselves, at least one of the two allelic ones.

In the case of within-cluster heterogeneity, the proportion of the two variants in a specimen could vary freely, so CAPS analysis would reveal in most specimens both variants with varying relative abundance, that is not the case. In heterozygotes for homogenous clusters, both variants should be present in comparable quantities, which must not be precisely equal because PCR would enhance any fluctuation of their relative abundance. If there were heterozygotes, homozygotes containing only one variant should be present as well. It is rather this picture which we observed.

Note that in heterozygotes for two homogenous histone clusters, meiotic cross-over exchange would lead to heteroheneisation of both clusters, again making homogenous homozygotes rare in populations. However, crossing over is as a rule suppressed in chromosome regions containing tandem repeats to preserve their copy number from uncontrolled variation by unequal crossing over and slippage mispairing. Different mechanisms could be recruited for this suppression: e.g. in yeast, recombination is suppressed in the tandemly repeated rRNA gene cluster by protein Sir2 (Kaeberlein et al. 1999), while histone methylation H3K9 and RNA interference are involved into preservation of the copy number of rRNA genes and other repeated DNA in *Drosophila* (Peng and Karpen 2007).

Still recently, an opinion prevailed that tandem repeats such as the histone cluster undergo the so-called concerted evolution which homogenises the repeat copies. Unequal crossing over and gene conversion were nominated to the role of its mechanisms (Liao 1999). It seemed that the concerted evolution model satisfactory explained behaviour of rRNA gene cluster (Hillis and Dixon 1991; Liao 1999). However, thorough investigations of internal transcribed spacers (ITS) between them reveal their heterogeneity almost in every specimen, as it was in the case of *Leucorrhinia* dragonflies (Hovmöller and Johansson 2004). More recently, the opposite point of view becomes more popular for histone genes, evolution of which is thought to follow a birth-and-death model under strong purifying selection (Nei and Rooney 2005; Eirín-López et al. 2004); this conclusion was based on organisms having non-allelic heterogeneity for histone genes but with tandemly repeated replication-dependent histone genes.

It was unexpected to find both nucleotide versions, A and G, in the variable position 57 in the histone H1 gene in all three range parts of *E. exornata*. This means that neither of them happened to be fixed in any isolate, that as a rule takes place when a population strongly reduces in number ('bottleneck effect'). The result looks especially striking if to take into account that *E. exornata* seems to be very stenotopic and has tiny populations. Thus, the here studied population at Novosibirsk Academy Town occupies just about 100 m of a 3–6 m wide stripe of a humid valley meadow along the small forest rivulet of Zyryanka: in June 1981, 1992, 2007 and 2008 the moth were found strictly in this section of a much longer meadow stripe (Dubatolov et al. 1993; Kosterin and Dubatolov 2007 and unpublished), the factors limiting their occurrence to the section remaining unrevealed. However, such micropopulations of *E. exornata* could be quite densely scattered over the territory (for instance, in 1994 a moth of *E. exornata* came to the light at a site 3 km apart, in the low reaches of the same rivulet), so that some individuals could migrate between populations as it is common in many Lepidoptera species with small local populations. Retaining of two haplotypes of the histone cluster while spreading of tiny local populations over vast territories of North Eurasia looks very improbable, since genetic drift would soon lead to fixation of one of them. From this point of view, preservation of the two versions of the histone H1 gene sequences in cis-position within the same heterogeneous cluster looks more realistic because in this case they would not segregate in meiosis. However, in two of the three studied populations, we found specimens homogenous for one of the H1 gene versions, although in neither population both types of homogeneous specimens were found together. Unfortunately, our samples were small because of scarcity of these moths: no more than 10–15 individuals could be found at once and larger samples could undermine a population.

Whatever be the nature of the heterogeneity for the histone H1 gene in some specimens, preservation of both versions of a substitution in the histone H1 gene in all three isolated range parts of *E. exornata* evidences for a recent, fast and contiguous spread of this species over North Eurasia, with subsequent range fragmentation, that well corresponds to the hypothesis of both events taking place in the Holocene (Dubatolov and Kosterin 2000).

Acknowledgments The work was supported by Integration Complex Projects by Presidium of Siberian Branch of Russian Academy of Sciences No. 10–2 'Nature of Academy Town: results of half a century exploitation', and No.6, 'Dynamics of ecosystems of Academy Town: monitoring and working out recommendations on rational exploitation', by collaborative research grant No. 63 'Phylogeography of insects, birds and mallams of Siberia and the Far East: the history of formation of faunas and contemporary evolutionary trends" by Siberian and Far Eastern Branches of Russian Academy of Sciences, and by the grant 10-04-00230 by Russian Foundation for Fundamental Research. We are grateful to Lavr Bol'shakov and Alexander Streltzov for offering specimens of *E. exornata* and to Kim Mitter for offering a sample of *O. rosea* for our study.

Conflict of Interest There is no conflict of interests.

References

Allan, J., Mitchell, N., Harborne, C., Bohm, C., & Crane-Robinson, C. (1986). Roles of H1 domains in determining higher order chromatin structure and H1 location. *Journal of Molecular Biology, 187*(4), 591–601.

Arkhipov, S. A., & Volkova, V. S. (1994). *Geological history, Pleistocene landscapes and climate in West Siberia*. Novosibirsk: Nauka (in Russian).

Babik, W., Branicki, W., Sandera, M., Litvinchuk, S., Borkin, J. J., Irwin, J. T., & Rafinki, J. (2004). Mitochondrial phylogeography of the moor frog, *Rana arvalis*. *Molecular Ecology, 13*(6), 1469–1480.

Barton, N. H., Briggs, D. E. G., Eisen, J. A., Goldstein, D. B., & Patel, N. H. (2007). *Evolution*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Belova, V. A. (1985). *Vegetation and climate of the Late Cenozoic of the southern Eastern Siberia*. Novosibirsk: Nauka (In Russian).

Belyshev, B. F., & Haritonov, A. Y. (1981). *Geography of Odonata of Boreal Faunistic Kingdom*. Novosibisrk: Nauka (In Russian).

Berdnikov, V. A., Rozov, S. M., Temnykh, S. V., Gorel', F. L., & Kosterin, O. E. (1993). Adaptive nature of interspecies variation of Histone H1 in insects. *Journal of Molecular Evolution, 36*(5), 497–507.

Berdnikov, V. A., Bogdanova, V. S., Gorel, F. L., Kosterin, O. E., & Trusov, Y. A. (2003). Large changes in the structure of the major histone H1 subtype result in small effects on quantitative traits in legumes. *Genetica, 119*(2), 167–182.

Bernard, R., & Kosterin, O. E. (2010). Biogeographical and ecological description of the Odonata of eastern Vasyugan Plain, West Siberia, Russia. *Odonatologica, 39*(1), 1–28.

Bernard, R., Heiser, M., Hochkirch, A., & Schmidt, T. (2011). Genetic homogenity of hedgling *Nechalennia speciosa* (Odonata: Coenagrionidae) indicates a single Würm glacial refugium and trans-Palearctic postglacial expansion. *Journal of Zoological Systematics and Evolutionary Research, 49*(4), 292–297.

Bharath, M. M. S., Chandra, N. R., & Rao, M. R. S. (2003). Molecular modeling of chromatosomal particle. *Nucleic Acids Research, 31*(14), 4264–4274.

Bogdanova, V. S., Galieva, E. R., & Kosterin, O. E. (2009). Genetic analysis of nuclear-cytoplasmic incompatibility in pea associated with cytoplasm of an accession of wild subspecies *Pisum sativum* subsp. *elatius* (Bieb.) Schmahl. *Theoretical and Applied Genetics, 118*(4), 801–809.

Brower, A.V.Z. (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial-DNA evolution. Proceedings of the National Academy of Sciences USA, 91 (14), 6491–6495.

Covell, C. V. (1984). *A field guide to the moths of Eastern North America. The Peterson field guide series*. Boston: Houghton Mifflin Company.

Coyne, J. A., & Orr, H. A. (2004). *Speciation*. Sunderland: Sinauer Associates.

Crozier, R. H., & Crozier, Y. C. (1993). The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. *Genetics, 133*(1), 97–117.

Doenecke, D., Albig, W., Bode, C., Drabent, B., Franke, K., Gavenis, K., & Witt, O. (1997). Histones: genetic diversity and tissue-specific gene expression. *Histochemistry and Cell Biology, 107*(1), 1–10.

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution, 29*(8), 1969–1973.

Dubatolov, V. V. (2007). *Arctiinae (Lepidoptera, Arctiidae) of the Palearctic. D. Sci. thesis*. Novosibirsk: Institute of Systematics and Ecology of Animals, 50 pp.

Dubatolov, V. V. (2009). Macroheterocera excluding Geometridae and Noctuidae s. lat. (Insecta, Lepidoptera) of Lower Amur//Amurian. *Zoological Journal, 1*(3), 221–252.

Dubatolov, V. V., Antonova, E. M., & Kosterin, O. E. (1993). *Eversmannia exornata* (Eversmann, 1837), the only known representative of the Epiplemidae family (Lepidoptera) in West Palearctic. *Actias, 1*, 19–23.

Dubatolov, V. V., & Dolgikh, A. M. (2010). New records of macromoths (Insecta, Lepidoptera, Macroheterocera) in the Bolshekhekhtsyrskii Nature Reserve (Khabarovsk suburbs). *Amurian Zoological Journal, 2*(2), 136–144. color pl. II.

Dubatolov, V. V., & Kosterin, O. E. (2000). Nemoral species of Lepidoptera (Insecta) in Siberia: novel view on their history and timing of their disjunctions. *Entomological Fennica, 11*(3), 141–166.

Dubatolov, V. V., Vasilenko, S. V., & Streltzov, A. N. (2003). New nemoral insect species of Diptera, Coleoptera, Neuroptera, Mecoptera, Lepidoptera from River Agrun basin (Chita Oblast') and their possible zoogeographic significance. *Euroasian Entomological Journal, 2*(3), 167–180. in Russian, with Engl.s.

Eirín-López, J. M., Gonzáles-Tizón, A. M., Martínes, A., & Méndez, J. (2004). Birth-and-death evolution with strong purifying selection in histone H1 multigene family and the origin of orphon H1 genes. *Molecular Biology and Evolution, 21*(10), 1992–2003.

Eirín-López, J. M., González-Romero, R., Dryhurst, D., Méndez, J., & Ausio, J. (2009). Long-term evolution of histone families: old notions and new insights into their mechanisms of diversification across eukaryotes. In P. Pontarotti (Ed.), *Evolutionary biology: concept, modeling, and application* (pp. 139–162). Heidelberg: Springer.

EPICA Community Members. (2004). Eight glacial cycles from an Antarctic ice core. *Nature, 429*(6992), 623–628.

Farrel, B. D. (2001). Evolutionary assembly of the milkweed fauna: cytochrome oxidase I and the age of *Tetraopes* beetles. *Molecular Phylogenetics and Evolution, 18*(3), 467–478.

Fedorov, V. B., Goropashnaya, A. V., Boeskorov, G. G., & Cook, J. A. (2008). Comparative phylogeography and demographic history of the wood lemming (*Myopus schisticolor*): implications for late Quaternary history of the taiga species in Eurasia. *Molecular Ecology, 17*(2), 598–610.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*(5), 294–299.

Fradkina, A. F. (1995). *Palynostratigraphy of Paleogene and Neogene sediments in North-Eastern Russia*. Novosibirsk: United Institute of Geology, Geophysics and Mineralogy Press (In Russian).

Goropashnaya, A. V., Fedorov, V. B., Seifert, B., & Pamilo, P. (2004). Limited phylogeographical structure across Eurasia in two red wood ant species *Formica pratensis* and *F. lugubris* (Hymenoptera, Formicidae). *Molecular Ecology, 13*(7), 1849–1858.

Hansen, J. C., Lu, X., Ross, E. D. & Woody, R. W. (2006) Intrinsic protein disorder, amino acid composition, and histone terminal domains. *Journal of Biological Chemistry, 281*(4), 1853–1856.

Happel, N., & Doenecke, D. (2009). Histone H1 and its isoforms: contribution to chromatin structure and function. *Gene, 431*(1), 1–12.

Hillis, D. M., & Dixon, M. T. (1991). Ribosomal DNA: molecular evolution and phylogenetic inference. *Quarterly Review of Biology, 1991*, 411–453.

Hovmöller, R., & Johansson, F. (2004). A phylogenetic perspective on larval spine morphology in *Leucorrhinia* (Odonata: Libellulidae) based on ITS1, 5.8 s and ITS2 rDNA sequences. *Molecular Phylogenetics and Evolution, 30*(3), 653–662.

Hundertmark, K. J., Shields, G. F., Udina, I. G., Bowyer, R. T., Danilkin, A. A., & Schwartz, C. C. (2002). Mitochondrial phylogeography of moose (*Alces alces*): late Pleistocene divergence and population expansion. *Molecular Phylogenetics and Evolution, 22*(3), 375–387.

Imbrie, J., Berger, A., Boyle, E., Clemens, S., Duffy, A., Howard, W., Kukla, G., Kutzbach, J., Martinson, D., & McIntyre, A. (1993). On the structure and origin of major glaciation cycles. 2. The 100,000-year cycle. *Paleoceanography, 8*(6), 699–735.

Jödicke, R., Langhoff, P., & Misof, B. (2004). The species group taxa in the Holarctic genus *Cordulia*: a study in nomenclature and genetic differentiation (Odonata: Corduliidae). *International Journal of Odonatology, 7*(1), 37–52.

Jukes, T. H., & Cantor, C. R. (1969). Evolution of protein molecules. In H. N. Munro (Ed.), *Mammalian protein metabolism* (pp. 21–132). New York: Academic.

Kaeberlein, M., McVey, M., & Guarente, L. (1999). The SIR2/3/4 complex and SIR2 alone promote longevity in *S. cerevisiae* by two different mechanisms. *Genes & Development, 13*(19), 2570–2580.

Knyazev, S. A., Ponomaryov, K. B., Abramov, V. A., & Sitnikov, P. S. (2010). The new findings of *Eversmannia exornata* (Eversmann, 1837) (Lepidoptera: Epiplemidae) in West Siberia. *Eversmannia, 21–22*, 98 (In Russian).

Konieczny, A., & Ausubel, F. M. (1993). A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. *The Plant Journal, 4*(2), 403–410.

Kosterin, O. E. (2002). Western range limits and isolates of eastern odonate species in Siberia and their putative origins. *Odonatologica, 34*(3), 219–242.

Kosterin, O. E., & Dubatolov, V. V. (2007). Local population of a rare species *Eversmannia exornata* Eversmann, 1837 (Epiplemidae, Lepidoptera) in Academy Town. In I. F. Zhimulev (Ed.), *Nature of Academy Town: 50 years after* (pp. 140–144). Novosibirsk: Izdatelstvo SO RAN (in Russian).

Kozhevnikov, Y. P., & Ukraintseva, V. V. (1992). Some features of vegetation cover of Eurasia in the earliest Holocene. *Ukrainskiy Botanicheskiy Zhurnal, 77*(8), 1–9 (In Russian).

Liao, D. (1999). Concerted evolution: molecular mechanism and biological implications. *The American Journal of Human Genetics, 64*(1), 24–30.

Logunov, D. V. (1996). Preliminary report on the Euro-Siberian faunal commections of jumping spiders (Aranea, Salticidae). *Acta Zoological Fennica, 201*, 71–76.

Marincovich, L., & Gladenkov, A. Y. (1999). Evidence for an early opening of the Bering Strait. *Nature, 397*(6715), 149–151.

Marincovich, L., & Gladenkov, A. Y. (2001). New evidence for the age of the Bering Strait. *Quaternary Science Reviews, 20*(1), 329–335.

Matyushkin, E. N. (1976). European–East Asian break between the ranges of terrestrial vertebrates. *Zoologicheskiy Zhurnal, 55*(9), 1277–1291 (in Russian).

Mikkola, K. (1987) Pattern of noctuid species common between the extremeties of the Palaeacrtic zone: a result of glacial and postglacial movements. Tinea, 12 (supplement), 310–315.

Mikkola, K., Lafontaine, J. D., & Kononenko, V. S. (1991). Zoogeography of the Holarctic species in the Noctuidae (Lepidoptera): importance of the Beringian refuge. *Entomologica Fennica, 1991*, 153–173.

Mullen, S. P. (2006). Wing pattern evolution and the origins of mimicry among North American admiral butterflies (Nymphalidae: Limenitis). *Molecular Phylogenetics and Evolution, 39*(3), 747–758.

Mullen, S. P., Savage, W. K., Wahlberg, N., & Willmott, K. R. (2011). Rapid diversification ant not clade age explains high diversity in Neotropical Adelpha butterflies. *Proc R Soc B Biol Sci, 278*, 1777–1785.

Nazarenko, A.A. (1992) Faunistical cycles: extinction–expansion–extinction… A modern history of the Dendrphilous Ornithofauna of Eastern palaearctic. D. Sci. Thesis. Vladivostok: Institute of Biology and Pedology. (in Russian).

Nei, M., & Kumar, S. (2000). *Molecular evolution and phylogenetics*. New York: Oxford University Press.

Nei, M., & Gojobori, T. (1986). Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Molecular Biology and Evolution, 3*(5), 418–426.

Nei, M., & Rooney, A. P. (2005). Concerted and birth-and-death evolution of multigene families. *Annual Review of Genetics, 39*, 121–152.

Oshida, T., Abramov, A., Yanagawa, H., & Masuda, R. (2005). Phylogeography of the Russian flying squirrel (*Pteromys volans*): implication of refugia theory in arboreal small mammal of Eurasia. *Molecular Ecology, 14*(4), 1191–1196.

Polozhiy, A. V., & Krapivkina, E. D. (1985). *Relics of the tertiary broad-leafed forests in the flora of Siberia*. Tomsk: Tomsk University Press, Tomsk (in Russian).

Ponte, I., Vila, R., & Suau, P. (2003). Sequence complexity of histone H1 subtypes. *Molecular Biology and Evolution, 20*(3), 371–380.

Peng, J. C., & Karpen, G. H. (2007). H3K9 methylation and RNA interference regulate nucleolar organization and repeated DNA stability. *Nature Cell Biology, 9*(1), 25–35.

Petit, J.-R., Jouzel, J., Raynaud, D., Barkov, N., Barnola, J.-M., Basile, I., Bender, M., Chappellaz, J., Davis, M., & Delaygue, G. (1999). Climate and atmospheric history of the past 420, 000 years from the Vostok ice core, Antarctica. *Nature, 399*(6735), 429–436.

Quek, S. P., Davies, S. J., Itino, T., & Pierce, N. E. (2004). Codiversification in an ant-plant mutualism: stem texture and the evolution of host use in *Crematogaster* (Formicidae: myamicinae) inhabitants of Macaranga (Euphorbiaceae). *Evolution, 58*(3), 554–570.

Rozen, S., & Skaletsky, H. J. (2000). Primers on the WWW for general users and for biologist programmers. In S. Krawetz & S. Misener (Eds.), *Bioinformatics methods and protocols: methods in molecular biology*. Totowa: Humana Press.

Saitoh, T., Alström, P., Nishiumi, I., Shigeta, Y., Williams, D., Olsson, U., & Ueda, K. (2010). Old divergences in a boreal bird supports long-term survival through the Ice Ages. *BMC Evolutionary Biology, 10*(1), 35.

Sinev, S. Y. (2008). Uraniidae. In S. Y. Sinev (Ed.), *Catalogue of the Lepidoptera of Russia* (Vol. 336, pp. 189–190). Moscow: KMK Press. in Russian.

Schmitt, T., & Seitz, A. (2001). Intraspecific allozymatic differentiation reveals the glacial refugia and the postglacial expansions of European *Erebia medusa* (Lepidoptera: Nymphalidae). *Biological Journal of the Linnean Society, 74*(4), 429–458.

Schmitt, T., & Varga, Z. (2012). Extra-Mediterranean refugia: the rule and not the exception? *Frontiers in Zoology, 9*(1), 1–12.

Suvorov, A. (2011). Comparative molecular genetics of *Nechalennia speciosa* (Charpentier) from geographically distant populations (Zygoptera: Coenagrionidae). *Odonatologica, 40*(2), 131–136.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution, 28*(10), 2731–2739.

Trusov, Y. A., Bogdanova, V. S., & Berdnikov, V. A. (2004). Evolution of regular zone of histone H1 in Fabaceae plants. *Journal of Molecular Evolution, 59*(4), 546–555.

Tshistjakov, Y.A. (2005) Epiplemidae. In: (ed.) Key to the insects of Russian Far East. Vol. 5. Trichoptera and Lepidoptera. Vladivostok: Dalnauka (in Russian).

Uimaniemi, L., Orell, M., Mönkkönen, M., Huhta, E., Jokimäki, J., & Lumme, J. (2000). Genetic diversity in Siberian Jay *Perisoreus infaustus* in fragmented old-growth forests of Fennoscandia. *Ecography, 23*(6), 669–677.

Ukraintseva, V. V. (1996). Late Pleistocene and Holocene floras of Siberia. *Botanicheskiy Zhurnal, 81*(12), 37–48 (In Rusian).

Ursenbacher, S., Carlsson, M., Helfer, V., Tegelström, H., & Fumagalli, L. (2006). Phylogeography and Pleistocene refugia of the adder (*Vipera berus*) as inferred from mitochondrial DNA sequence data. *Molecular Ecology, 15*(11), 3425–3437.

Velichko, A. A. (1993). *Evolution of landscapes and climates of the Northern Eurasia. Late Pleistocene–Holocene, elements of prognosis. I. Regional Plaeogeography*. Moscow: Nauka. in Russian.

Vila, R., Bell, C. D., Macniven, R., Goldman-Huertas, B., Ree, R. H., Marshall, C. R., Balint, Z., Johnson, K., Benyamini, D., & Pierce, N. E. (2011). Phylogeny and palaeoecology of *Polyommatus* blue butterflies show Beringia was a climate-regulated gateway to the New World. *Proc R Soc B Biol Sci, 278*, 2737–2744.

Walter, H., & Straka, H. (1970). *Arealkunde: floristisch-historische Geobotanik* (p. 478). Stutgart: Ulmer.

Watson, A. (1967). A survey of extra-Ethiopian Oretinae (Lepidoptera: Drepanidae). Bulletin of the British Museum Natural History. *Entomology, 19*, 151–221.

Wilson, J. J. (2010). Assessing of the value of DNA barcodes and other priority gene regions for molecular phylogenetics in Lepidoptera. *PLoS ONE, 5*, e10525.

Wilson, J. J. (2012). DNA barcoding in insects. *Methods in Molecular Biology, 858*, 17–46.

Yurtsev, B. A. (1981). *Relic Steppen Complexes of North-East Asia* (p. 168). Novosibirsk: Nauka. In Russian.

Zaytseva, O. O., Bogdanova, V. S., & Kosterin, O. E. (2012). Phylogenetic reconstruction at the species and intraspecies levels in the genus *Pisum* (L.) (peas) using a histone H1 gene. *Gene, 504*(2), 192–202.

Zink, R. M., Drovetski, S. V., & Rohwer, S. (2002). Phylogeographic patterns in the great spotted woodpecker *Dendrocopos major* across Eurasia. *Journal of Avian Biology, 33*(2), 175–178.

Zolotukhin, V. V., & Pugaev, S. N. (2007). To a study of drepanids (Lepidoptera) of the genus *Oreta* Walker, 1855 of the fauna of Russia. *Eversmannia, 11–12*, 14–19 (in Russian).

Zuckerkandl, E., & Pauling, L. (1965). Evolutionary divergence and convergence in proteins. In V. Bryson & H. J. Vogel (Eds.), *Evolving Genes and Proteins* (pp. 97–166). New York: Academic.