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Diversification of the cold-adapted butterfly genus *Oeneis* related to Holarctic biogeography and climatic niche shifts ☆

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ABSTRACT

Both geographical and ecological speciation interact during the evolution of a clade, but the relative contribution of these processes is rarely assessed for cold-dwelling biota. Here, we investigate the role of biogeography and the evolution of ecological traits on the diversification of the Holarctic arcto-alpine butterfly genus *Oeneis* (Lepidoptera: Satyrinae). We reconstructed the molecular phylogeny of the genus based on one mitochondrial (*COI*) and three nuclear (*GAPDH*, *RpS5*, *wingless*) genes. We inferred the biogeographical scenario and the ancestral state reconstructions of climatic and habitat requirements. Within the genus, we detected five main species groups corresponding to the taxonomic division and further paraphyletic position of *Neominois* (syn. n.). Next, we transferred *O. aktashi* from the *hora* to the *polixenes* species group on the bases of molecular relationships. We found that the genus originated in the dry grasslands of the mountains of Central Asia and dispersed over the Beringian Land Bridges to North America several times independently. Holarctic mountains, in particular the Asian Altai Mts. and Sayan Mts., host the oldest lineages and most of the species diversity. Arctic species are more recent, with Pliocene or Pleistocene origin. We detected a strong phylogenetic signal for the climatic niche, where one lineage diversified towards colder conditions. Altogether, our results indicate that both dispersal across geographical areas and occupation of distinct climatic niches promoted the diversification of the *Oeneis* genus.

1. Introduction

Although allopatric and ecological speciation processes interact in evolution (Rundell and Price, 2009; Bennett and O'Grady, 2012), they are rarely studied together, especially in older radiations (cf. Vila et al., 2011; Condamine et al., 2012; Bentley et al., 2014). A clade is rarely the pure product of allopatry (Muller and Beharegaray, 2010; Imada et al., 2011). Geographical speciation can be coupled with ecological speciation (Hall, 2005; Willmott et al., 2001; Matos-Maravi et al., 2013) involving the differentiation of species lineages by changes in habitat use or behaviour (Jiggins, 2008; Chamberlain et al., 2009). Diversification along climatic gradients, such as moisture (Rieseberg et al., 1999; Gee, 2004) or temperature (Keller and Seehausen, 2012) has been documented in several clades. However, the signal of ecological speciation might be blurred by non-adaptive ecological speciation (Rundell and Price, 2009). In that case, speciation via geographic isolation precedes significant ecological diversification and reproductive isolation (Swensson, 2012). Geographical and ecological speciation processes probably frequently interacted in rugged environments with unstable climates such as in the Holarctic region, especially in the diversification of cold-dwelling biota inhabiting mountains.

Distributions of Holarctic organisms were shaped by climatic changes during the past 15 million years (Vila et al., 2011), the effect of which interacted with regional topographies (Todisco et al., 2012). Cold periods interconnected biota across the Holarctic by promoting lowland dispersal events (Schmitt and Haubrich, 2008; Todisco et al., 2012) and movements of Arctic assemblages to southern latitudes (Päckert et al., 2012; Eidesen et al., 2013) followed by in-situ speciation (Vila et al., 2011). This connectivity was probably associated to species' ecological

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preferences (Vila et al., 2011). For instance, dry-adapted alpine species widely dispersed to lowlands (Schmitt and Hewitt, 2004), which had mainly an arid character, whereas alpine species not tolerant of dryness were restricted to more humid areas (e.g. along rivers) around the glaciated high mountain systems (Schmitt et al., 2006). The interactions of ecological preferences and geographical speciation processes in montane and Arctic regions are relatively well studied (Todisco et al., 2012; Eidesen et al., 2013; Yannic et al., 2014), but mainly using plants and birds as models (e.g. Drovetski, 2003; Kadereit and Baldwin, 2012; Fjeldsa, 2013). The understanding of the speciation in cold-dwelling butterflies is still limited (Kodandaramaiah and Wahlberg, 2009; Simonsen et al., 2010; Todisco et al., 2012).

Butterflies represent a model group of organisms to study the mechanisms of speciation in relation to their ecology and geographic distribution. Butterflies show conservatism in their habitat affiliations (van Swaay et al., 2006) and thermal niches (cf. Vila et al., 2011; Kleckova et al., 2014), but this remains to be tested in the evolution of cold-adapted butterfly lineages. The butterfly genus *Oeneis*, Hübner, 1819 (Lepidoptera: Satyrinae) arose in the Miocene (Peña et al., 2011) and diversified into 30 species across the mountains and Arctic of the Holarctic region. Here, we investigated the Holarctic diversification of this cold-dwelling genus in relation to shifts in ecological traits and geographic range. We reconstructed the biogeographic history and evolution of habitat affiliations as well as climatic requirements as drivers of diversification. Specifically, we asked: (1) Where was the origin of the distribution and radiation of this genus? (2) What are the relative contributions of geographical and ecological speciation in the evolution of the genus *Oeneis*? (3) Is a traditional taxonomical division of the genus *Oeneis* and classification of closely related genera consistent with molecular phylogeny?

2. Methods

2.1. Study organisms and molecular data

We studied the phylogenetic relationships within the genera *Oeneis*, *Paroeneis* Moore, 1893 and *Neominois* Scudder, 1875 (Lepidoptera: Satyrinae, Satyrina). More than half of the *Oeneis* species occur in the mountainous areas of Central Asia, five species are distributed in Arctic Eurasia and roughly ten species in the Arctic and mountain regions of North America (Tuzov et al., 1997; Layberry et al., 1998; Gorbunov, 2001). Seven species are distributed in both Eurasia and North America (Tuzov et al., 1997, Table S1). Individual *Oeneis* species occur in steppic or alpine grasslands, tundra, taiga, mountain woodlands and screes (Tuzov et al., 1997; Layberry et al., 1998; Gorbunov, 2001). Larvae feed on various grasses (Layberry et al., 1998), hence diversification should be primarily driven by other factors than host plant shifts (cf. Schweiger et al., 2008). A phylogeny of the genus has been lacking to understand the drivers of diversification and solve the currently problematic taxonomy of the genus (Pelham, unpublished). The representatives of the genus *Oeneis* were traditionally sorted into two subgenera, *Protoeneis* Gorbunov, 2001 and *Oeneis* Hübner, [1819] s. str., and then to morphologically defined species groups (Table S1, Lukhtanov and Lukhtanov, 1994a; Gorbunov, 2001; Pelham, unpublished), but the internal taxonomy of the genus has not been established and differs between publications (Tuzov et al., 1997; Layberry et al., 1998; Gorbunov, 2001).

The genera *Paroeneis* and *Neominois* share similar appearance, ecology and bionomy as the genus *Oeneis*. The genus *Paroeneis* includes about six species, all inhabiting the mountains of Central Asia (Gorbunov, 2001). The genus *Neominois* includes 2–3 species living in the sparsely vegetated grasslands of North America, from southern Alberta to northern Mexico (Warren et al., 2008).

For our study, we used 19 species (43 specimens), representing all recognised species groups of the genus *Oeneis* (Gorbunov, 2001, Table S2), plus *Paroeneis palaearcticus* (Staudinger, 1889), *P. pumilus* (C. & R. Felder, [1867]) and two (sub)species of the *Neominois ridingsii ridingsii* (Edwards, 1865) and *Neominois ridingsii wyomingo* (Scott, 1998), frequently considered as distinct species (Opler and Wright, 1999). We were able to extract DNA from 14 species, represented by at least two specimens from different populations; 9 species were represented only by 1 specimen. As outgroup taxa, we used the related Satyrinae butterflies *Melanargia galathea* (Linnaeus, 1758), *Hipparchia statilinus* (Hufnagel, 1766), *Karanasa pamira* (Staudinger, 1887), and *Karanasa bolorica* (Grum-Grshimailo, 1888).

DNA was extracted from two legs or part of the thorax of dry specimens using DNEasy extraction kit (QIAGEN). DNA was amplified by Polymerase Chain Reactions (PCR) for one mitochondrial (*COI* – 1487 bp) and three nuclear genes (*GAPDH* – 691 bp, *RpS5* – 617 bp, *wingless* – 400 bp), using forward-reverse primer pairs described in Wahlberg and Wheat (2008). In total, the genetic information contained 3207 bp. For *COI* synthesis, we used two primer pairs, LCO–HCO for the first half and Jerry–Pat for the second half, for *GAPDH* primer pair Frigga-Burre, for *RpS5* pair rpS5degF–rpS5degR and for *wingless* LepWG1–LepWG2. PCR run in 20 µl volume by using PPP mastermix (TOP-BIO, following a standard protocol). PCR thermal program 95 °C for 5 min, 40 cycles at 94 °C for 30 s, annealing temperature 50 °C for 30 s (*COI*, *wingless*) resp. 55 °C for 30 s (*GAPDH*, *RpS5*), 72 °C for 1 min 30 s and final extension period at 72 °C for 10 min. The amplified genes were sequenced commercially by the Macrogen Company (dna.macrogen.com) using an ABI 3730XL DNA analyser. Sequences were aligned manually in the program BIOEDIT v 7.0.5.3 (Hall, 1999). Sequences of outgroup taxa as well as of *Neominois r. ridingsii* were downloaded from GenBank (Peña et al., 2011, http://www.ncbi.nlm.nih.gov/, Table S2).

2.2. Phylogenetic analyses

Maximum parsimony analysis was performed in software TNT 1.1 (Goloboff et al., 2008) using *Melanargia galathea* as outgroup. All characters (with gaps as the fifth state) were treated as unordered and equally weighted. We performed heuristic searches, using the New Technology Search algorithms with a level of search 15, followed by branch swapping of resulting trees. The strict consensus tree was built from 10 final trees.

Two Bayesian analyses were performed using MrBayes v 3.1.2 (Ronquist and Huelsenbeck, 2003) and Beast v 1.8.0 (Drummond and Rambaut, 2007) with *Melanargia galathea* as outgroup. First, we performed the model tests of substitution rates. The best fit of the model of evolution was determined by using the FindModel tool of the Los Alamos HIV databases and compendia (www.hiv.lanl.gov), which implements Modeltest script (Posada and Crandall, 2001) calculating AIC scores, and the program Weighbor (Bruno et al., 2000) generating the tree based on Jukes-Cantor distances.

In the MrBayes analysis, we defined variable evolution rates, which were independent for each partition, representing one gene. The most general GTR+G substitution model was applied to all partitions. Markov Chain Monte Carlo Metropolis–Hastings (MCMC) algorithm with 4 chains was run for 10 million generations. Chains were sampled every 1000 generations. The convergence of two runs was checked visually according to the log likelihood of both runs. The first 2 million trees were discarded as burn-in.