

Phylogeography of the threatened butterfly, the woodland brown *Lopinga achine* (Nymphalidae: Satyrinae): implications for conservation

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Abstract We have studied the phylogeography of the red-listed Palearctic butterfly *Lopinga achine* (Nymphalidae: Satyrinae) based on 1,450 base pairs of mitochondrial DNA sequences from 86 individuals representing 12 populations. Our results indicate a strong structuring of genetic variation, with among-population differences accounting for ca. 67% of the variation and almost all populations being significantly differentiated from each other. We surmise that the insular nature of populations as well as the low dispersal ability of the species has given rise to such a pattern. The genetic diversity within populations is low compared to that in other butterflies. Our results point to a scenario where the species originated in the Eastern Palearctic and expanded into Europe. Based on the analyses, we suggest that the Czech population merits the highest conservation priority. The two Swedish populations represent a

distinct evolutionary lineage, and hence merit high conservation attention. The Estonian and Asian populations had the highest genetic diversity, and although we do not consider them to be under immediate threat, their genetic diversity should be conserved in the long term.

Keywords *Lopinga achine* · Phylogeography · Conservation · Red-list

Introduction

The woodland brown, *Lopinga achine*, Scopoli 1763, is a Palearctic butterfly ranging from Japan and Korea through North and Central Asia, southern Fennoscandia, Central Europe and the northernmost part of Spain (Tolman and Lewington 1997; Tuzov 2000). Despite the wide distribution of the species, populations are highly localized and sporadically distributed across its range, with large distances between them (Bergman 1999; Kudrna 2002). The species is restricted to woodland habitats with partially open canopies (Kralicek and Gottwald 1984; Bergman 1999, 2000; Konvicka et al. 2008) and threatened in many parts of its distribution (Heath 1981; van Helsdingen et al. 1996). The species range has shrunk in recent decades and the overall decline in Europe is estimated at 20–50% between 1970 and 1995. It is now placed under the Vulnerable category in the Red Data Book (van Swaay et al. 2009) and red listed in 18 countries (Bergman 1999). As in many other open woodland species, the reason for decline was abandonment of some historically widespread forest uses, such as forest pastorage and coppicing, plus afforestation of formerly deciduous forests by conifers, collectively resulting in canopy closure and changes to forest ground vegetation.

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Appendix 1 describes the habitats of some populations, with information from our first hand observations as well as from literature. Regional populations of this species exhibit specific habitat requirements and hence the pressures leading to the decline of the range of this species in Europe are likely to be specific to populations. Such population-specific habitat requirements are likely to be a result of local adaptations over time. In turn, the geographic structure of genetically based among-population differences is affected by historical patterns of colonization of the species and subsequent gene flow between populations. Information on the population genetics and phylogeography of the species is hence essential to understand how such habitat preferences have evolved in this species and for conservation planning.

The aims of this study were to infer the phylogeographic history of this species, estimate patterns of gene flow between and genetic variation within populations. Given the presumed low dispersal ability of the species and the insular nature of local populations, we predicted strong genetic differentiation among populations.

Materials and methods

Samples from a total of 86 individuals representing 12 populations of *L. achine* were used in this study (Fig 1, Appendix 2). Additionally, three individuals of the congeneric *L. deidamia*, Eversmann 1851, which occurs in the eastern Palearctic, were included as an outgroup. Seven populations (Gotland, Linköping, Czech republic, Finland, mainland Estonia, the Estonian island of Saaremaa, Kazakhstan, and Buryatia in eastern Russia) were represented by nine to 11 individuals, while four populations (Spain; and Irkutsk, Chelyabinsk and Primorsky regions of Russia) had less than five individuals each. DNA from samples was preserved either by drying or placing two legs in alcohol. The QIAGEN DNeasy tissue extraction kit (Hilden, Germany) was used to extract genomic DNA from two legs using the manufacturer's protocol. The mitochondrial gene COI (cytochrome oxidase subunit I) was amplified using primers and protocols described in Kodandaramaiah and Wahlberg (2007). Amplified PCR products were purified, and sequenced through a commercial sequencing company (Macrogen Inc, Seoul, South Korea). Resulting chromatograms were aligned by eye in Bioedit (Hall 1999).

A 37 bp region with missing data in several samples was excluded from the analysis; thus the final dataset consisted of 1,430 bp. TCS 1.1 (Clement et al. 2000) was used to identify unique haplotypes and construct a statistical parsimony network. The program was also used to estimate outgroup probability for each haplotype, which correlates with haplotype age (Donnelly and Tavaré 1986).

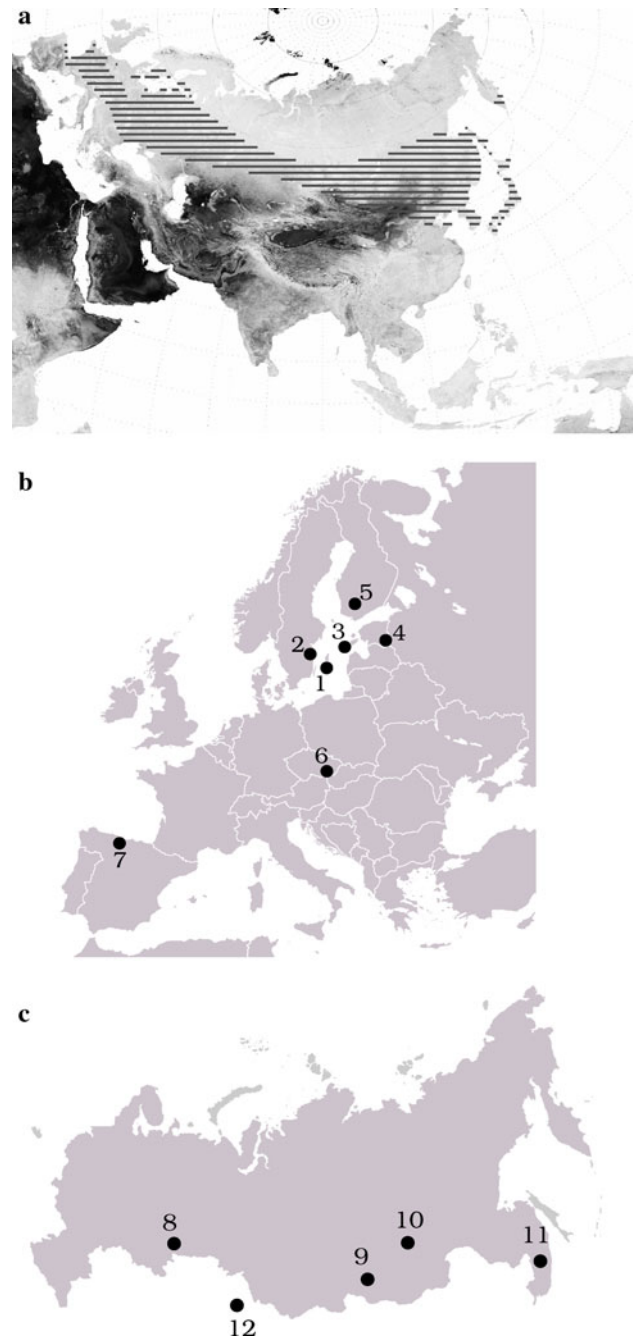


Fig. 1 a Distribution of *Lopinga achine*. b Populations sampled within Europe for this study: 1 Gotland, 2 Linköping, 3 Saaremaa, 4 mainland Estonia, 5 Finland, 6 Czech Republic, 7 Spain. c Map of Russia showing populations sampled within Asia; 8 Chelyabinsk, 9 Irkutsk, 10 Buryatia, 11 Primorsky, 12 Kazakhstan

The distribution of genetic variation in *L. achine* samples was analyzed using Arlequin 3.11 (Excoffier et al. 2005). Populations with less than five individuals were excluded from these analyses. Three indices—total number of haplotypes, haplotype diversity (H ; Nei 1987) and nucleotide diversity (π ; Tajima 1983; Nei 1987)—were

used to assess within population genetic variation. An AMOVA (Analysis of Molecular Variance; Excoffier et al. 1992) with 10,000 permutations was performed to infer the geographic structuring of genetic variation between and among populations. Exact tests of pairwise population differentiation (Raymond and Rousset 1995; Goudet et al. 1996) were conducted with a Markov Chain of 100,000 steps. Between-population pairwise Φ_{st} values were calculated based on haplotype frequencies.

Results

A total of 15 unique *L. achine* haplotypes were identified. The total haplotypic diversity and nucleotide diversity within *L. achine* were 0.841 (± 0.022) and 0.001 (± 0.0001) respectively. *L. achine* and *deidamia* haplotypes formed respective clusters in the statistical parsimony network (Fig. 2), and were also reciprocally monophyletic in the phylogenetic analysis (Fig. 2). In the haplotype network, the number of mutations between the two clusters was higher than the 95% parsimony limit and hence the program was unable to connect them together unequivocally. The network of *L. achine* including four missing haplotypes is depicted in Fig. 2, with names given by us to facilitate discussion. Two central haplotypes ‘Widespread1’ and ‘Widespread2’ were the most ubiquitous haplotypes in the studied samples. *Widespread2* had the highest outgroup

probability within *L. achine*. The three Swedish haplotypes grouped together in the haplotype network, but did not form a clade in the phylogeny. *Czech* and *Spain* were sister to each other in the phylogeny, whereas *Spain* was a terminal haplotype most closely related either to *Czech* or *Widespread1*. *Kazakhstan2* and *Kazakhstan3* formed a clade and this grouping was corroborated in the network where *Kazakhstan2* was present in a terminal position connected to *Kazakhstan3* and the latter connected to *Widespread1*. Four unique Saaremaa and Estonian haplotypes were connected to *Widespread2* as terminal haplotypes.

The Kazakhstani and Estonian populations had the highest genetic diversity (Table 1). The Linköping, Finland and Czech populations had a single haplotype each and the lowest values for *H* and π . The total Φ_{st} , an estimate of the proportion of variation among populations, was 0.67 (Table 2). Almost all populations were significantly differentiated from each other based both on exact permutation tests and pairwise Φ_{st} values calculated from haplotype frequencies (Table 3). The exceptions were Estonia, Finland and Saaremaa, which were not differentiated among each other.

Discussion

The genetic diversity in *L. achine* is low in comparison to that in other mtDNA phylogeographic studies of Palearctic

Fig. 2 Statistical parsimony network of the 14 *Lopinga achine* haplotypes reconstructed in TCS. Each circle represents one haplotype, and the diameter of the circle is approximately proportional to the number of samples the haplotype was present in. Each haplotype is named to facilitate discussion in the text, and the number of individuals with the haplotype is shown in brackets

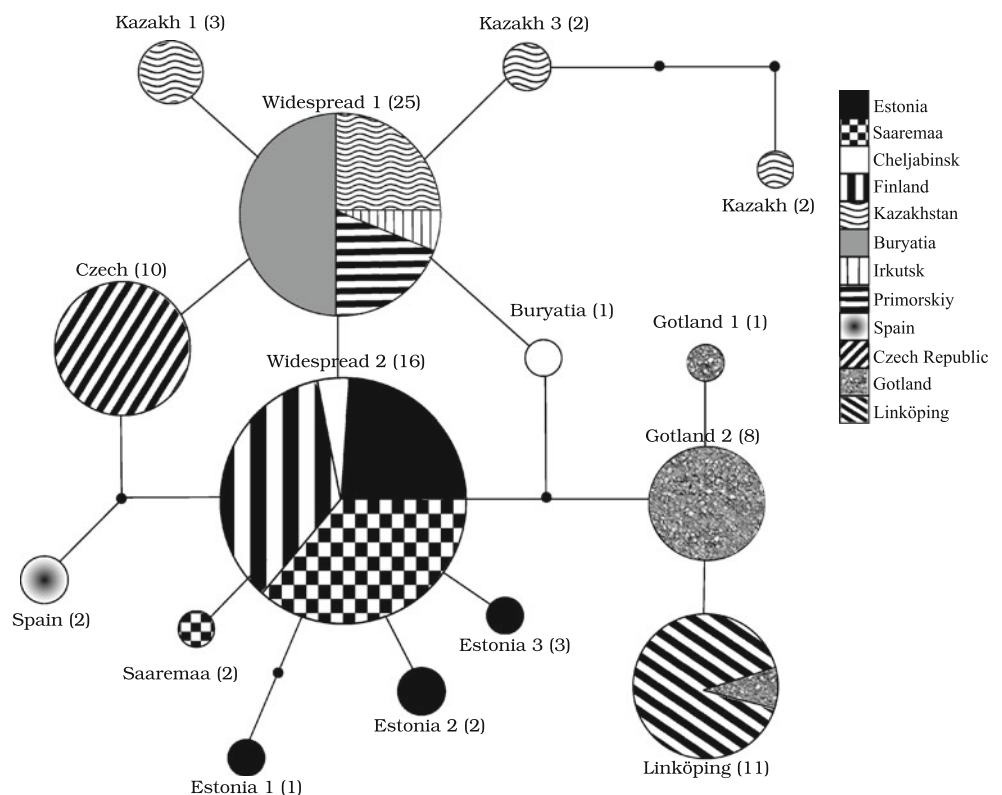


Table 1 Estimates of within population genetic diversity in *Lopinga achine*

	No of haplotypes (no of samples)	Haplotype diversity (H)	Nucleotide diversity (π_n)
Estonia	4 (10)	0.6444 \pm 0.1518	0.000668 \pm 0.000561
Linköping	1 (10)	0.0000 \pm 0.0000	0.0000 \pm 0.0000
Gotland	3 (10)	0.3778 \pm 0.1813	0.000280 \pm 0.000319
Kazakh	4 (10)	0.7778 \pm 0.0907	0.001072 \pm 0.000791
Czech	1 (10)	0.0000 \pm 0.0000	0.0000 \pm 0.0000
Buryatia	2 (9)	0.2222 \pm 0.1662	0.000155 \pm 0.000228
Saaremaa	2 (11)	0.3273 \pm 0.1533	0.000229 \pm 0.000280
Finland	1 (9)	0.0000 \pm 0.0000	0.0000 \pm 0.0000
Chelyabinsk	1 (1)	N/A	N/A
Primorsky	1 (5)	N/A	N/A
Irkutsk	1 (1)	N/A	N/A
Spain	1 (2)	N/A	N/A

Total haplotype diversity = 0.8411 \pm 0.0222 and total nucleotide diversity (π_n) = 0.001404 \pm 0.000886

Table 2 Results of the AMOVA analysis of 8 populations of *Lopinga achine*

Source of variation	df	Sum of squares	% of variation
Among populations	7	22.349	67.32
Within populations	71	10.625	32.68
Total	78	32.975	

Total Φ_{st} = 0.6732, $p < 0.0001$

butterflies (Appendix 3). Among population differences account for much (ca. 67%) of this variation. As expected from the insular nature of the populations and the low dispersal ability of the species, most populations are significantly differentiated from each other. The geographic proximity of Saaremaa and Finland to mainland Estonia may explain the lack of significant differentiation between the latter and the former two populations. In contrast, the

two Swedish populations are surprisingly strongly differentiated from each other (Φ_{st} = 0.79) despite the short distance separating the two. The Linköping population in particular is very highly differentiated from all other populations (Table 3).

Phylogeography

The Asian haplotype *Widespread2* had the highest out-group probability in the haplotype network. This suggests a scenario where the extant populations descended from one or more ancestral populations in Central/East Asia. This explanation is further strengthened by the fact that the Kazakhstani population had the highest genetic diversity. Recolonization from glacial refugia within Europe is the predominant pattern reported in other European butterflies (Dennis and Schmitt 2009). It is known that the Pleistocene glaciations were more extensive in Europe than in Asia (Svendsen et al. 2004) and, moreover, taxa in Asia had access to more refugia during this period (Tiffney 1985). It is possible that an ancestral population (or populations) of *L. achine* survived the glaciations in one or more Asian refugia and colonized Europe after the most recent glacial period. Although our results do not rule out European refugia, it is worth noting that the Spanish haplotype is terminal, which does not fit with a scenario where the species dispersed out of the Iberian refugium.

The low genetic diversity in Finland suggests it was colonized very recently, either across the Baltic from central Europe or overland from Russia. Given the position of the Gotland and Linköping haplotypes, the most likely explanation for the origins of the Swedish populations is a colonization event across the Baltic into Gotland, followed by invasion of the mainland. The affinities of the contemporary Czech population remain unclear, and more sampling from Central Europe would be necessary to clarify it; but it is likely that it is a remnant population of a past expansion from the East. The Spanish population is the westernmost limit of the species and its position in the haplotype network

Table 3 Results of the pairwise population comparisons of 8 populations of *Lopinga achine*

	Estonia	Linköping	Gotland	Buryatia	Czech	Kazakh	Saaremaa
Linköping	0.68						
Gotland	0.49	0.79					
Buryatia	0.56	0.89	0.70				
Czech	0.68	1.00	0.81	0.89			
Kazakh	0.29	0.61	0.42	0.21	0.61		
Saaremaa	0.05	0.83	0.65	0.72	0.83	0.45	
Finland	0.18	1.00	0.80	0.89	1.00	0.60	0.07

Numbers reported are Φ_{st} values between corresponding populations. Φ_{st} values that were significant at $p = 0.05$ are in bold. All pairwise comparisons for which Φ_{st} were significant were also significant in the exact permutation tests

corroborates dispersal into Spain from the East. However, we cannot completely rule out a scenario where the Spanish population is an ice age lineage that did not undergo post-glacial expansion.

Implications for conservation

It is widely recognized that maintenance of genetic variation is of utmost importance for the long term conservation prospects of species and populations (Frankham et al. 2002; Hansson and Westerberg 2002). Impoverished genetic variation has been implicated in higher rates of extinction due to decreased viability and adaptability to changing environments (see Schmitt and Hewitt 2004 and references therein). In the current study, the Spanish, Finnish, Czech and Linköping populations have the lowest genetic diversity. Of these, we are of the opinion that the Czech population deserves the highest level of conservation effort. This population is a remnant unit that has survived large scale extinctions in the region; we surmise that the impoverished genetic variability in past Czech populations could have resulted from overall loss of populations in the wide area, perhaps coupled with bottleneck effects due to population size fluctuations. Although the population is numerous at present (about 10,000 individuals in 2007; Konvicka et al. 2008), its low genetic variation, plus occurrence in a single woodland, may threaten its survival in the near future. Thus, we recommend that the Czech population needs to be given high conservation priority.

We were only able to procure two Spanish individuals for this study and thus are unable to reliably estimate the standing genetic variation within the population. Although the Finnish population has very low diversity, it is genetically indistinct from the Estonian populations. In contrast, the two Swedish populations together form an evolutionarily distinct lineage that deserves a higher level of conservation priority. The greatest genetic diversity is found in the Estonian and Asian populations, and although we do not consider them to be under immediate threat, their genetic diversity should be conserved in the long term.

Our results show that the species is genetically differentiated among different parts of Europe, and any reintroduction attempt should take such phylogeographic information into account. In the case a reintroduction programme in central Europe is considered we recommend an additional, geographically more fine-scaled study with a more comprehensive sampling. Results here would serve as a baseline for such a detailed study.

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Appendix 1

Description of habitats of some *Lopinga achine* populations, based both on our personal observations and that obtained from literature

The presence of a ground layer of its larval host plants, fine-leaved sedges (*Carex* spp.), was found to be a prerequisite for patch occupancy in Sweden, Czech republic and Finland (Bergman 2000; Konvicka et al. 2008). Nevertheless, the species has been recorded on other larval host plants including various species in the genera *Lolium*, *Triticum*, *Aryrpyron*, *Dactylis* and *Melica* (all Poaceae grasses; Tolman and Lewington 1997; Tuzov 2000).

Sweden

The Swedish mainland population (Linköping) is found in grazed oak (*Quercus robur*) forests interspersed with hazel shrubs (*Corylus avellana*). The population on Gotland occurs in a quite different habitat of pine forests (*Pinus sylvestris*) on calcareous soils that are opened up by clearings, bogs and small dirt roads, with a shrub layer of Juniper (*Juniperus communis*), Swedish Whitebeam (*Sorbus intermedia*) and Glossy buckthorn (*Frangula alnus*). According to a recent inventory of the area, the species is found abundantly in pine forests of 30–40 years of age as well as older and denser forests. However, the species was largely absent from clear cuts and more recently planted pine forests (Kullingsjö 2006). Apparently suitable habitats are found in several other parts of Sweden that are not inhabited by *L. achine* (Bergman 1999).

Czech Republic

The only surviving population inhabits 40 km² lowland woods surrounded by intensive farmlands, preserved due to bedrock unsuitable for farming (partly Quaternary sands, partly boggy alluvium). Originally dominated by oak (*Quercus robur*), a half of the wood area now consist of pine (*Pinus sylvestris*) plantations. *L. achine* is restricted to ca 6 km² central section, located on sandy hummocks alternating with boggy depressions, and there the distribution is patchy (map: Konvicka et al. 2008). It occurs in mature and

sparse (canopy closure ca 80%) oak-dominated growths with trees of uneven age, located on base-rich but nitrogen poor sandy soils, with sparse shrub layer (mainly *Tilia* spp., *Crataegus* spp.) and rich ground layer with a high representation of forbs and sedges, including locally used host plants *Carex fritschii* and *C. michelii*. These growths were historically grazed and coppiced, which is still evident on trees growth forms, and also utilised for litter raking and animal fodder harvest. After cessation of these uses (ca. 1950 s), the canopy is closing, whereas ground layer succumbs to eutrophication caused by litter accumulation. Apart from further conifer plantation and clear-felling, completely detrimental for *L. achine* but hopefully prevented in the central part by Natura 2000 status, future risks include spontaneous canopy closure and continuing eutrophication.

Estonia

The typical habitat of *L. achine* in Eastern Estonia is moist woodland with grassy openings, crossed by minor roads, and with *Alnus incana* as the dominant tree species. The butterflies are typically found at forest edges, and flying along forest roads. On Saaremaa—which differs in having limestone as the bedrock—the diversity of available habitats is higher, with *L. achine* also being found in more arid conditions like wooded meadows with *Corylus avellana* as the dominant woody plant.

Finland

The occurrence of *L. achine* in Finland has recently been reviewed (Piirainen et al. 2009). The species was first recorded in 1900 in southeastern Finland. It was documented to spread northwestwards to Tavastia until the 1960s, when the populations suddenly crashed. It survived the next 30 years in a few isolated populations in SE Finland and Tavastia, but began expanding again in the 1990s and is still expanding. In Finland, *L. achine* is found in spruce dominated wet forests on the edges of mires, or in spruce dominated mires. Characteristic of these forests is a patchy structure, with open sunny spots and shady spots. Larvae are known to feed on *Carex* sp. and *Poa* sp., both of which are common in all types of forests of Finland, and are thus not restricting the occurrence of the butterfly.

Germany

The species is known to be associated with coppice forests (Weidemann 1995), whereas it occurs in wooded savannahs in the Carpathian mountains (Kralicek and Gottwald 1984) and open evergreen forests in the Alps (Lepidopterologen-Arbeitsgruppe 1987).

Appendix 2

See Table 4.

Table 4 List of samples used in this study along with their collection locality, Genbank accession numbers and their haplotypes as identified in this table

Species	Voucher	Collection locality	Haplotype	Genbank
deidamia	UK4-20	Russia, Buryatia Republic, Mondy, Upper Irkut River	N/A	JQ671339
deidamia	UK12-20	Russia, Irkutsk region, Sludyanka distr.	N/A	JQ671340
deidamia	UK12-19	China, Quinhai prov. Lajishan Mts. Sinin.	N/A	JQ671341
achine	UK12-1	Czech Republic, Hodonin env. Hodoninska Doubrava wood, popn 2	Czech	JQ671396
achine	UK12-2	Czech Republic, Hodonin env. Hodoninska Doubrava wood, popn 2	Czech	JQ671397
achine	UK12-3	Czech Republic, Hodonin env. Hodoninska Doubrava wood, popn 2	Czech	JQ671398
achine	UK12-4	Czech Republic, Hodonin env. Hodoninska Doubrava wood, popn 2	Czech	JQ671399
achine	UK12-5	Czech Republic, Hodonin env. Hodoninska Doubrava wood, popn 2	Czech	JQ671400
achine	UK12-6	Czech Republic, Hodonin env. Hodoninska Doubrava wood, popn 3	Czech	JQ671401
achine	UK12-7	Czech Republic, Hodonin env. Hodoninska Doubrava wood, popn 3	Czech	JQ671402
achine	UK12-8	Czech Republic, Hodonin env. Hodoninska Doubrava wood, popn 3	Czech	JQ671403
achine	UK12-9	Czech Republic, Hodonin env. Hodoninska Doubrava wood, popn 3	Czech	JQ671404
achine	UK12-10	Czech Republic, Hodonin env. Hodoninska Doubrava wood, popn 3	Czech	JQ671405
achine	UK13-12	Estonia, Saaremaa, Mustjala	Widespread1	JQ671417
achine	UK13-13	Estonia, Saaremaa, Mustjala	Widespread1	JQ671418
achine	UK13-6	Estonia, Saaremaa, Konnu	Widespread1	JQ671411
achine	UK13-7	Estonia, Saaremaa, Konnu	Widespread1	JQ671412
achine	UK13-8	Estonia, Saaremaa, Konnu	Widespread1	JQ671413

Table 4 continued

Species	Voucher	Collection locality	Haplotype	Genbank
achine	UK13-9	Estonia, Saaremaa, Lõetsa	<i>Saaremaa</i>	JQ671414
achine	UK13-3	Estonia, Saaremaa, Tagamoisa	<i>Widespread1</i>	JQ671408
achine	UK13-4	Estonia, Saaremaa, Tagamoisa	<i>Widespread1</i>	JQ671409
achine	UK13-5	Estonia, Saaremaa, Tagamoisa	<i>Widespread1</i>	JQ671410
achine	UK13-10	Estonia, Saaremaa, Viiumae	<i>Widespread1</i>	JQ671415
achine	UK13-11	Estonia, Saaremaa, Viiumae	<i>Saaremaa</i>	JQ671416
achine	UK15-14	Estonia, Tartu, Karilatsi	<i>Widespread1</i>	JQ671356
achine	UK15-15	Estonia, Tartu, Karilatsi	<i>Estonia1</i>	JQ671357
achine	UK15-16	Estonia, Tartu, Karilatsi	<i>Widespread1</i>	JQ671358
achine	UK15-17	Estonia, Tartu, Karilatsi	<i>Estonia3</i>	JQ671359
achine	UK15-18	Estonia, Tartu, Karilatsi	<i>Widespread1</i>	JQ671360
achine	UK11-1	Estonia, Tartu, Laeva	<i>Estonia2</i>	JQ671354
achine	UK11-2	Estonia, Tartu, Laeva	<i>Widespread1</i>	JQ671355
achine	UK13-1	Estonia, Tartu, Laeva	<i>Widespread1</i>	JQ671352
achine	UK13-2	Estonia, Tartu, Laeva	<i>Estonia2</i>	JQ671353
achine	UK15-19	Estonia, Tartu, Laeva	<i>Widespread1</i>	JQ671361
achine	NW177-1	Finland, Hämeenlinna, Kalvola	<i>Widespread1</i>	JQ671419
achine	NW177-2	Finland, Hämeenlinna, Kalvola	<i>Widespread1</i>	JQ671420
achine	NW177-3	Finland, Hämeenlinna, Kalvola	<i>Widespread1</i>	JQ671421
achine	NW177-9	Finland, Hämeenlinna, Kalvola	<i>Widespread1</i>	JQ671427
achine	NW177-10	Finland, Hämeenlinna, Kalvola	<i>Widespread1</i>	JQ671428
achine	NW177-4	Finland, Hattula	<i>Widespread1</i>	JQ671422
achine	NW177-5	Finland, Hattula	<i>Widespread1</i>	JQ671423
achine	NW177-6	Finland, Hattula	<i>Widespread1</i>	JQ671424
achine	NW177-7	Finland, Hattula	<i>Widespread1</i>	JQ671425
achine	JL13-10	Kazakhstan, Altai Mts. Uryl	<i>Widespread2</i>	JQ671348
achine	JL13-24	Kazakhstan, Altai Mts. Uryl	<i>Widespread2</i>	JQ671349
achine	UK12-11	Kazakhstan, Bukombai Mt. Range, N. Zaisan Lake	<i>Kazakh1</i>	JQ671342
achine	UK12-15	Kazakhstan, S. Altai, Narymsky Mt. Range, Novoberezhovskoe	<i>Kazakh2</i>	JQ671344
achine	UK12-16	Kazakhstan, S. Altai, Narymsky Mt. Range, Novoberezhovskoe	<i>Kazakh3</i>	JQ671345
achine	UK12-17	Kazakhstan, S. Altai, Narymsky Mt. Range, Novoberezhovskoe	<i>Kazakh3</i>	JQ671346
achine	UK12-18	Kazakhstan, S. Altai, Narymsky Mt. Range, Novoberezhovskoe	<i>Widespread2</i>	JQ671347
achine	UK15-3	Kazakhstan, S. Altai, Narymsky Mt. Range, Novoberezhovskoe	<i>Widespread2</i>	JQ671350
achine	UK15-4	Kazakhstan, S. Altai, Narymsky Mt. Range, Novoberezhovskoe	<i>Kazakh1</i>	JQ671351
achine	UK12-12	Kazakhstan, Saur Mt. Range, Kendyrlik river	<i>Kazakh1</i>	JQ671343
achine	UK15-10	Russia, Primorsky Krai, Vladivostok Botanical Garden	<i>Widespread2</i>	JQ671384
achine	UK15-5	Russia, Buryatia republic, Dalchai village	<i>Widespread2</i>	JQ671389
achine	UK15-6	Russia, Buryatia republic, Dalchai village	<i>Widespread2</i>	JQ671390
achine	UK15-7	Russia, Buryatia republic, Dalchai village	<i>Widespread2</i>	JQ671391
achine	UK15-8	Russia, Buryatia republic, Dalchai village	<i>Widespread2</i>	JQ671392
achine	UK4-21	Russia, Buryatia republic, Dalchai village	<i>Widespread2</i>	JQ671388
achine	UK4-24	Russia, Buryatia republic, Dalchai village	<i>Widespread2</i>	JQ671387
achine	UK15-11	Russia, Buryatia republic, Kultuk st.	<i>Widespread2</i>	JQ671393
achine	UK15-12	Russia, Buryatia republic, Kultuk st.	<i>Widespread2</i>	JQ671394
achine	UK15-13	Russia, Buryatia republic, Kultuk st.	<i>Buryatia</i>	JQ671395
achine	UK12-22	Russia, Chelyabinsk reg., Snezhinsk env.	<i>Widespread1</i>	JQ671386
achine	UK12-14	Russia, Irkutsk region, Sludyanka distr.	<i>Widespread2</i>	JQ671382
achine	UK15-9	Russia, Primorskiy Krai, Vladivostok Botanical Garden	<i>Widespread2</i>	JQ671383

Table 4 continued

Species	Voucher	Collection locality	Haplotype	Genbank
achine	UK4-22	Russia, Primorskiy Krai, Vladivostok Botanical Garden	<i>Widespread2</i>	JQ671385
achine	UK12-23	Spain, Picos de Europa, León	<i>Spain</i>	JQ671406
achine	UK12-24	Spain, Picos de Europa, León	<i>Spain</i>	JQ671407
achine	UK11-6	Sweden, Gotland	<i>Gotland2</i>	JQ671377
achine	UK11-7	Sweden, Gotland	<i>Gotland2</i>	JQ671378
achine	UK11-8	Sweden, Gotland	<i>Gotland2</i>	JQ671379
achine	UK13-14	Sweden, Gotland	<i>Gotland2</i>	JQ671372
achine	UK13-15	Sweden, Gotland	<i>Linköping</i>	JQ671373
achine	UK13-17	Sweden, Gotland	<i>Gotland2</i>	JQ671374
achine	UK13-18	Sweden, Gotland	<i>Gotland2</i>	JQ671375
achine	UK13-19	Sweden, Gotland	<i>Gotland2</i>	JQ671376
achine	UK15-20	Sweden, Gotland	<i>Gotland1</i>	JQ671380
achine	UK15-21	Sweden, Gotland	<i>Gotland2</i>	JQ671381
achine	UK11-3	Sweden, Linköping	<i>Linköping</i>	JQ671369
achine	UK11-4	Sweden, Linköping	<i>Linköping</i>	JQ671370
achine	UK11-5	Sweden, Linköping	<i>Linköping</i>	JQ671371
achine	UK13-20	Sweden, Linköping	<i>Linköping</i>	JQ671365
achine	UK13-21	Sweden, Linköping	<i>Linköping</i>	JQ671366
achine	UK13-22	Sweden, Linköping	<i>Linköping</i>	JQ671367
achine	UK13-24	Sweden, Linköping	<i>Linköping</i>	JQ671368
achine	UK15-22	Sweden, Linköping	<i>Linköping</i>	JQ671362
achine	UK15-23	Sweden, Linköping	<i>Linköping</i>	JQ671363
achine	UK15-24	Sweden, Linköping	<i>Linköping</i>	JQ671364

Appendix 3

See Table 5.

Table 5 Comparison of haplotype diversity (H) and nucleotide diversity (π) values reported in phylogeographic studies on European butterfly species based on mtDNA

Species	Reference	Gene	Haplotype diversity (H)	Nucleotide diversity (π)
<i>Aglais urticae</i>	Vandewoestijne et al. (2004)	COI	0.97	0.01
<i>Melitaea cinxia</i>	Wahlberg and Saccheri (2007)	COI	0.95	0.02
<i>Euphydryas aurinia</i>	Joyce and Pullin (2001)	Cytochrome <i>b</i>	N/A	0
<i>Lopinga achine</i>	Current study	COI	0.84	0

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