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The role of topography in structuring the demographic history of the pine processionary moth, *Thaumetopoea pityocampa* (Lepidoptera: Notodontidae)

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ABSTRACT

Aim We investigated the Quaternary history of the pine processionary moth, *Thaumetopoea pityocampa,* an oligophagous insect currently expanding its range. We tested the potential role played by mountain ranges during the post-glacial recolonization of western Europe.

Location Western Europe, with a focus on the Pyrenees, Massif Central and western Alps.

Methods Maternal genetic structure was investigated using a fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. We analysed 412 individuals from 61 locations and we performed maximum-likelihood and maximum parsimony phylogenetic analyses, hierarchical analysis of molecular variance, and investigated signs of past expansion.

Results A strong phylogeographic pattern was found, with two deeply divergent clades. Surprisingly, these clades were not separated by the Pyrenees but rather were distributed from western to central Iberia and from eastern Iberia to the Italian peninsula, respectively. This latter group consisted of three shallowly divergent lineages that exhibited strong geographic structure and independent population expansions. The three identified lineages occurred: (1) on both sides of the Pyrenean range, with more genetically diverse populations in the east, (2) from eastern Iberia to western France, with a higher genetic diversity in the south, and (3) from the western Massif Central to Italy. Admixture areas were found at the foot of the Pyrenees and Massif Central.

Main conclusions The identified genetic lineages were geographically structured, but surprisingly the unsuitable high elevation areas of the main mountainous ranges were not responsible for the spatial separation of genetic groups. Rather than acting as barriers to dispersal, mountains appear to have served as refugia during the Pleistocene glaciations, and current distributions largely reflect expansion from these bottlenecked refugial populations.

The western and central Iberian clade did not contribute to the northward post-glacial recolonization of Europe. Yet, its northern limit does not correspond to the Pyrenees. The different contributions of the identified refugia to post-glacial expansion might be explained by differences in host plant species richness. For example, the Pyrenean lineage could have been trapped elevationally by tracking montane pines, while the eastern Iberian lineage could have expanded latitudinally by tracking thermophilic lowland pine species.

Keywords: glacial refugia, latitudinal shift, Mediterranean Basin, mitochondrial DNA, mountainous areas, *Pinus*, range expansion, *Thaumetopoea pityocampa*, vertical migration, western Europe.

INTRODUCTION

Quaternary climatic oscillations have produced great changes in species ranges and strongly influenced the present-day geographic distribution of genetic diversity (*e.g.* Hewitt, 1999, 2004; Schmitt, 2007). Ranges of most species shifted latitudinally and/or elevationally as a response to glacial/interglacial cycles, resulting in expansion–contraction phases (Hewitt, 2004; Habel *et al.*, 2005; Schmitt, 2007; Varga & Schmitt, 2008). In general, temperate species have expanded during warm periods and responded to cold phases by local extinctions in northern regions and by survival in southern glacial refugia (Hewitt, 2004). This has commonly resulted in a 'southern richness and northern purity' pattern, in which genetic diversity and divergence are higher at lower latitudes (Hewitt, 1999). Cold-tolerant arctic species exhibit opposite responses, as warm interglacials have caused fragmentation of habitat and range contraction into northernmost locations. Similarly, alpine species have tracked their suitable environment by upslope movements during the warmest periods, and survived the interglacials in limited refugia or 'sky islands' (DeChaine & Martin, 2005; Varga & Schmitt, 2008). More recently, accumulation of phylogeographical data has supported evidence of more complex patterns of response to Quaternary climatic oscillations, both because many species actually have intermediate ecological requirements (Varga & Schmitt, 2008) or habitat-generalist traits (Bhagwat & Willis, 2008), and because the palaeoenvironments were more complex than previously thought (Stewart & Lister, 2001; Hewitt, 2004; Willis & van Andel, 2004; Provan & Bennett, 2008; Médail & Diadema, 2009).

The winter pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1776) (Lepidoptera: Notodontidae), is a phytophagous insect distributed from North Africa to the Balkans. It belongs to a species complex with a wide distribution around the Mediterranean Basin (Simonato *et al.*, 2007; Kerdelhué *et al.*, 2009). The moth's geographic range is constrained by sunshine requirements in winter and susceptibility to both cold winter and high summer temperatures (Huchon & Démolin, 1970; Battisti *et al.*, 2005; see Materials

and Methods). *T. pityocampa* is more restricted geographically than the distribution area of its potential hosts, which include lowland Mediterranean as well as montane or boreal *Pinus* species. In southern Europe and North Africa, *T. pityocampa* occurs from thermo-Mediterranean environments (with hot summers and mild winters) to oro-Mediterranean environments (with milder summers and colder winters). However, the supra-Mediterranean zone (with mild summers and relatively mild winters) could correspond to the optimal ecological niche of this species (Huchon & Démolin, 1970). *T. pityocampa* does not occur in areas under strong continental climates (with both hot summers and cold winters; Huchon & Démolin, 1970). Under Atlantic climates, this species can be found as far north as the 48th parallel (see Fig. 1).

In recent years, the range expansion of *T. pityocampa* to upper latitudes or elevations has been reported in several European countries (Rosenzweig *et al.*, 2007). This distributional change is primarily due to increased winter temperatures and is a consequence of climate warming (Battisti *et al.*, 2005). This rapid response to climatic changes suggests that the past distribution of this species is likely to have been strongly affected by Pleistocene climate changes during both glacial and interglacial episodes. Due to an obligate relationship with its pine hosts (*Pinus* spp.), *T. pityocampa* can have survived only in places where pines persisted. The locations of its refugial areas were thus constrained by those of its hosts, which exhibit different climatic requirements.

A preliminary genetic study in France using microsatellite markers showed that within-population genetic diversity was highest in the eastern Pyrenees (Kerdelhué *et al.*, 2006). This study also suggested that, in spite of its moderate elevation, the Massif Central was an effective barrier to gene flow. Moreover, using mitochondrial DNA and nuclear internal transcribed spacer 1 (ITS1) sequences, Santos *et al.* (2007) showed strong differentiation between Iberian and French populations, although with a limited sample size. Two hypotheses can be proposed to explain both the high genetic diversity observed within the Pyrenees and

the strong genetic differentiation across this moutain range. In the first it is hypothesized that for such a cold-susceptible species with putatively limited dispersal abilities, the Pyrenean range could have acted as a barrier to post-glacial expansion routes from separated refugia. In this case, secondary contact zones should be found either in favourable valleys and/or on western and eastern ends of this mountain range, where the elevation is lower. The high genetic diversity observed in the Pyrenees would then derive from admixture between two strongly differentiated lineages. Such a pattern has already been observed for various European species (Hewitt, 1999, 2004; Habel *et al.*, 2005; Schmitt, 2007). The second hypothesis is that the Pyrenees might have acted as a refugium rather than a barrier. The processionary moth could have survived locally by gradual elevational shifts. In this case, high genetic diversity would mirror ancestral polymorphism rather than being a sign of admixture. A similar scenario has been described for stenotopic montane species that were able to descend or ascend as the climate cooled or warmed, thus surviving glacial oscillations in the same region without major latitudinal shifts (Hewitt, 2004; Varga & Schmitt, 2008).

To test these hypotheses, we sampled *T. pityocampa* throughout western Europe, focusing on mountain ranges. We analysed the distribution of the genetic diversity based on COI partial sequences. Our objectives were: (1) to describe the phylogeographic population structure of *T. pityocampa* over western Europe and particularly to confirm the existence of two deeply divergent clades on both sides of the Pyrenees, and (2) to test if mountain ranges, especially the Pyrenees, Massif Central and Alps, have been effective barriers to gene flow during the Quaternary, and played a strong role in structuring populations.

MATERIALS AND METHODS

Study species – host and climate requirements

The pine processionary moth is a univoltine and semelparous species with very short-lived adults exhibiting sex-biased dispersal, as females may disperse a few kilometres, while males may fly several tens of kilometres. The defoliating and urticating larvae develop in winter, feeding on various native pine and cedar species (*Pinus nigra* Arnold, *Pinus sylvestris* L., *Pinus uncinata* Ramond ex A. DC, *Pinus pinaster* Aiton, *P. pinea* L., *Pinus halepensis* Miller, *Cedrus atlantica* (Endl.) Manetti ex Carrière. The native ranges of these hosts are strongly spatially structured (Barbéro *et al.*, 1998; Kerdelhué *et al.*, 2009). This insect can also attack some exotic conifers [*e.g. Pinus radiata* D. Don, *Cedrus deodara* (Roxb.) G. Don, *Pseudotsuga menziesii* (Mirb.) Franco]. The gregarious larvae spin a silk nest. Pupation takes place in the soil after the typical head-to-tail processions at the end of winter or early spring, and the subterranean survival rate depends on soil moisture (Huchon & Démolin, 1970). Adult emergence and subsequent oviposition take place in summer or autumn depending on latitude and elevation.

The life cycle of the pine processionary moth varies greatly according to climate and is controlled by two major temperature constraints, which also determine distribution area and population dynamics (Huchon & Démolin, 1970; Battisti *et al.*, 2005). The northward and upward limits of the species' range are determined by lower lethal temperatures in winter (-12°C; Huchon & Démolin, 1970), by a minimal number of sunshine hours (isohele of 1800 hours of annual sunshine; Huchon & Démolin, 1970) and by specific temperature requirements necessary for feeding (see Battisti *et al.*, 2005; Robinet *et al.*, 2007). The population dynamics of the species at the southern edge of its distribution are constrained by summer temperatures, as eggs and early-instar larvae are susceptible to high summer temperatures (monthly mean of daily maximum temperatures above 25°C, and maximum temperatures above 32°C; Huchon & Démolin, 1970). Consequently, the highest population densities in France are usually located in sub-Mediterranean mountains and in some areas

under mild oceanic climate. Some plasticity in the timing of sexual reproduction allows the species to adapt to various environments, as the adults emerge later in warmest regions and earlier in places where winters are coldest (Huchon & Démolin, 1970).

Sampling

Sixty-one locations were sampled from 1999 to 2008, and a total of 412 caterpillars were analysed. The number of individuals per site ranged from 4 to 12. They were collected on different native and non-native host tree species (six *Pinus* species and *Pseudotsuga menziesii*). The sampling sites, host tree and year of collection are summarized in Appendix S1 in the Supporting Information, and sampling locations are shown in Fig. 1. The study area covers only the western European part of the distribution range, as populations from North Africa are known to form a distinct lineage (Kerdelhué *et al.*, 2009) and were not included in the present study. The study area includes both the recent expansion areas in northern France and the two southern peninsulas of western Europe (Iberia and Italy). The sampling effort was intentionally highest from north-eastern Spain to north-western Italy to test the hypothesized differentiation of Iberian populations compared to French ones (Santos *et al.*, 2007), to determine the role of the northerly mountainous ranges during post-glacial recolonizations and to locate possible contact zones. The main slopes of the European mountain ranges (French and Italian Alps, western and eastern Massif Central, northern and southern Pyrenees) were sampled. In order to avoid sampling related individuals, only one nest per tree was collected and only one larva per nest was sequenced. Larvae were immediately stored in absolute ethanol and then kept at -20°C until DNA extraction.

DNA extraction and amplification

Genomic DNA extraction, polymerase chain reaction (PCR) amplifications and sequencing of part of the mitochondrial COI gene followed the protocol described in Santos *et al.* (2007). The primers used were C1-J-2183 (Jerry, 5'-CAACATTTATTTTGATTTTTTGG-3') and

TL2-N-3014 (Pat, 5'-TCCAATGCACTAATCTGCCATATTA-3'), respectively located in the gene itself and in its flanking region (tRNA-Leucine gene).

Data analysis

Sequences were aligned in Bioedit 7.05 (Hall, 1999). Haplotypes and their frequencies were calculated with DnaSP 4.5 (Rozas *et al.*, 2003). Pairwise genetic distances between haplotypes were calculated using PAUP* 4.0b10 (Swofford, 1998).

To estimate gene genealogies, a statistical parsimony network was constructed using TCS 1.21 (Clement *et al.*, 2000) allowing a connection between haplotypes of up to 12 steps, to fit the maximal divergence observed in our data set. Maximum likelihood and maximum parsimony inferences were also used to investigate the phylogenetic relationships among the mtDNA haplotypes. Maximum likelihood analyses were based on the best-fit model of sequence evolution estimated using Akaike information criterion (AIC) tests implemented in MODELTEST 3.7 (Posada & Crandall, 1998). For both methods, node support was estimated from 200 bootstrap replicates conducted heuristically using tree bisection–reconnection branch swapping on starting trees generated by five randomly derived stepwise addition sequences. The resulting trees were rooted with a sequence from the sibling species *Thaumetopoea wilkinsoni* Tams (GenBank accession number GU385952). Before following the bootstapping procedure, maximum likelihood heuristic searches were also conducted with and without molecular clock enforced. The molecular clock hypothesis was then tested with a likelihood ratio test (LRT, Felsenstein, 1988), computed in PAUP* 4.0b10, with a homogeneous rate of evolution as the null hypothesis.

The level of genetic polymorphism within sites was assessed by calculating haplotype and nucleotide diversity indices. Gene diversity (h) and within-population mean number of pairwise differences per sequence (k) were computed using ARLEQUIN. Correlations between population parameters (h and k) and latitude were assessed with linear regressions.

The occurrence of a significant phylogeographic structure was inferred by testing if G_{ST} (coefficient of genetic variation over all populations that only considers haplotype identity)

was significantly smaller than N_{ST} (equivalent coefficient taking into account haplotype divergence) by use of 1000 permutations implemented in PERMUT (Pons & Petit, 1996). Population genetic structure was examined by analysis of molecular variance (AMOVA) based on pairwise F_{ST} and computed using ARLEQUIN 3.1 (Excoffier *et al*., 2005). This method was used to partition genetic variance within populations, among populations within groups, and among groups. The populations were grouped either by geographical locations or by host species. Significance was determined by 5000 permutations. Geographical groups were defined on the basis of the distribution area of the lineages identified with phylogenetic and parsimony network analyses. Samples corresponding to putative secondary contact zones between these lineages (*i.e.*, sampling sites containing haplotypes from different phylogenetic lineages) were treated using two options: (1) they were entirely attributed to one of the geographical groups (grouping by regions I); and (2) they were removed from the data set (grouping by regions II). Concerning grouping by hosts, sites where the insect was sampled from more than one *Pinus* species (see Table 1) were split so that each individual was attributed to its actual host group.

Two methods were used to infer the demographic history: mismatch distribution analyses (Rogers & Harpending, 1992) and neutrality tests. For the first approach, the distribution of pairwise nucleotide site differences between haplotypes was calculated and the observed values were compared with the expected values under a sudden expansion model. Demographic expansion parameters (θ_0, θ_1 and τ) were estimated with ARLEQUIN 3.1, and a test of goodness-of-fit based on the sum of square deviations between the observed and expected distributions was performed using 1000 bootstrap replicates. The parameters estimated with ARLEQUIN were used in DNASP to generate mismatch distributions. Unimodal distributions can be related to sudden demographic expansions while multimodal distributions are consistent with stability (Slatkin & Hudson, 1991). We performed Fu's F_S (Fu, 1997) and R_2 tests (Ramos-Onsins & Rozas, 2002) to examine the neutrality of genetic variation. F_S tends to be negative under an excess of recent mutations and a significantly negative value

can be taken as an evidence of population growth and/or selection. The R_2 measure is based on the difference between the number of singleton mutations and the average number of nucleotide differences among sequences within a population sample. The significance of both tests was assessed with 10,000 coalescent simulations implemented in DNASP. These tests were conducted on the whole data set and within each haplogroup.

RESULTS

Haplotype distribution and gene genealogy

The final alignment contains 412 sequences of 802 bp, corresponding to the second half of the COI gene. Fifty polymorphic sites were detected and 46 haplotypes were identified (Appendix S2). Pairwise uncorrected *p*-distances among haplotypes ranged from 0.125 to 2.618 (Appendix S3). Observed haplotype frequencies for each sampled location are given in Table 1. The geographic distribution of the haplotypes is shown in Fig. 1. Haplotype sequences were deposited in GenBank and are available under accession numbers GU385906-GU385951.

The best-fit model of sequence evolution is the transitional model (variable base frequencies and variable transition frequencies; Posada, 2003) with invariant sites and equal substitution rates among sites (TIM+I). The proportion of invariable sites (I) is 80.10%, the base frequencies are π_A=0.3250, π_C=0.1874, π_G=0.1191, π_T=0.3684, and the substitution rate parameters are 95.9003 for A-G and 33.9135 for T-C transitions, 1 for A-C and G-T transversions, and 0 for A-T and C-G transversions. LRT for COI of the TIM+I model with and without the molecular clock enforced does not reject overall rate homogeneity. Consequently, the molecular clock hypothesis was accepted.

Both the maximum likelihood and maximum parsimony phylogenetic trees (Appendix S4) show the existence of two major clades, respectively composed of the haplotypes 1-25, 30-38 (clade A) and the haplotypes 26-29, 39-46 (clade B). Clade A is distributed from eastern

Spain to Italy, while clade B is found in Portugal and western Spain. These clades are very well-supported by bootstrap values (Appendix S4).

The haplotype network shows the existence of four haplogroups (Fig. 2). Three of these (namely A1, A2 and A3) are subdivisions of the previously identified clade A, while the fourth corresponds to clade B. The two clades are separated by 12 mutational steps. Haplogroup A1 (haplotypes 1-13) is distributed from eastern France to Italy (Fig. 1 and Table 1). Haplogroup A2 (haplotypes 14-17, 25, 30-38) is found in eastern Spain and western France, more or less along the Greenwich Meridian. Haplogroup A3 (haplotypes 18-24) is restricted to the Pyrenean range and corresponds to a supported sub-clade in maximum-likelihood and parsimony phylogenetic analyses (Appendix S4). Each of the four haplogroups has a star-shaped topology with one central common haplotype surrounded by rarer but closely allied haplotypes (Fig. 2). The most common haplotype is haplotype 1 for A1 (78.85% of individuals), 14 for A2 (82.19%), 22 for A3 (57.45%), and 28 for B (31.75%). These four common and widely distributed haplotypes are found on several host plants (Table 1, Appendix S5).

Population parameters and genetic diversity

For each sampling location, gene diversity (*h*) and mean number of pairwise differences (*k*) are given in Table 1. Gene diversity ranges from 0 to 0.80 and *k* is between 0 and 8.98. In most sampling locations, we found haplotypes belonging to only one haplogroup (Table 1 and Fig. 1). Yet, two populations contain haplotypes from A1 and A2 groups (sites 26-27), one from A1 and A3 groups (41), three from A2 and A3 groups (44-46), one from A2 and B groups (51), and one from A2, A3 and B group (50). These two latter populations (50-51) exhibit the highest values of k. All these samples were also divided into subsamples, for which *h* and *k* were calculated separately (Appendix S6). Within the haplogroup A2, gene diversity (*h*) and mean number of pairwise differences (*k*) exhibit significant negative

relationship with latitude (P<0.01 and P<0.001 respectively). The relationship between h or k and latitude is not significant in any other haplogroup.

Phylogeographic pattern and population structure

Total gene diversity (H_T) is 0.818 (±0.032), while the average within-population diversity (H_S) is 0.255 (±0.036). The indices of population structure G_{ST} and N_{ST} are 0.689 (±0.038) and 0.880 (±0.036), respectively. The permutation test shows that N_{ST} is significantly greater than G_{ST} (p<0.001) when considering the whole data set. Within clade A, G_{ST} and N_{ST} values are 0.679 (±0.043) and 0.697 (±0.043), respectively, and N_{ST} is not significantly greater than G_{ST}.

Four geographical regions were defined on the basis of the distribution of the four haplogroups for AMOVA: (1) Italy and eastern France, (2) western France and eastern Iberia, (3) Pyrenees, (4) central and western Iberia (Appendix S7). When individuals were grouped by geographical regions, the results always showed that a large and significant proportion of the variance was found among groups (Table 2). Similar results were found when considering only clade A (Table 2). Populations were then grouped by host species. Most of the genetic diversity was then found among populations within groups (Table 2). Nevertheless, a significant part of the variance was found among groups for the whole data set (21.36% of the total variance, P<0.001), but not within clade A (4.58%, P=0.1085).

Demographic history

The mismatch distribution curves are presented in Appendix S8. The parameters estimated under the sudden expansion model and the results of goodness-of-fit and selective neutrality tests are presented in Table 3. For the whole data set, the mismatch distribution exhibits a bimodal curve and the expansion model is rejected (P=0.01). Consistently, the R_2 test does not reject neutrality (R_2=0.053, P=0.211), and only the Fu's F_S shows a significant negative value (F_S=-13.069, P=0.016). Conversely, haplogroups A1 and A2 both exhibit a unimodal

curve and all tests detect a departure from neutrality. The Pyrenean haplogroup A3 also exhibits a unimodal curve, but only the goodness-of-fit test suggests population expansion. Nevertheless, the F_S value is negative but not significant. For the western Iberian haplogroup (B), only the Fu's F_S test indicates a departure from neutrality (F_S=-5.137; P=0.01).

DISCUSSION

Phylogeographic population structure

Two deeply divergent clades in western Europe

Our results demonstrate that the western European populations consist of two deeply divergent clades with a strong geographical structure. One of these clades (A) is widely distributed from eastern Iberia to the Italian peninsula, whereas the second one (B) only occurs in central and western Iberia. A strong phylogeographic pattern is found in the present-day populations as shown by N_{ST} being significantly greater than G_{ST}. This demonstrates that the most related haplotypes tend to co-occur in the same geographic area. The allopatric separation between the two major western European lineages was likely to have been maintained through the Quaternary climatic oscillations, suggesting that even during the most favourable periods, the gene pools remained isolated. The genetic distances found between clades (Appendix S3) are compatible with a recent phylogenetic study in which this divergence was estimated to date back to *ca.* 1.8 Ma (Kerdelhué *et al.*, 2009), *i.e.* the divergence is much older than the last few glacial cycles. Whatever the cause, the contemporary delimitation between the two clades is south–north oriented, and definitely does not correspond to the Pyrenees. These results suggest the existence of a barrier to gene flow between eastern and western Iberia. Interestingly, the distributions of several Iberian endemic plant and animal species suggest a similar east to west polarity, with a trend for the areas of endemism to coincide with the largest mountain ranges (García-Barros *et al.*, 2002). In our

case, the separation between western and eastern Iberia could be due either to the existence of a region where environmental conditions remained unsuitable, or to a gap in host availability. Recent studies suggest that pine hosts were present in at least parts of the distribution areas of clades A and B even during the Last Glacial Maximum (LGM) (Willis *et al.*, 1998; Cheddadi *et al.*, 2006; Gómez & Hunt, 2006; Benito-Garzón, 2007), but pines also repeatedly experienced population fragmentation when the terrain was dominated either by other tree species or by steppe vegetation during the driest (cold or warm) phases (Willis *et al.*, 1998; Suc & Popescu, 2005; Carrión *et al.*, 2009).

Within clade A, both the haplotype network and the AMOVA show the existence of three groups of haplotypes that are spatially structured (Figs 1 & 2). In many species of phytophagous insects, the host plant is expected to play a role in population structure. However, no significant host effect was observed within clade A (Table 2). The three haplogroups had a star-shaped topology, which could be a genetic signature of population growth (Slatkin & Hudson, 1991; Rogers & Harpending, 1992) consistent with post-glacial expansion. Plausible scenarios for each of the three groups are discussed below.

Haplogroup A3, a mountain lineage originating from eastern Pyrenees

Haplogroup A3 is a monophyletic lineage that occurs only in the Pyrenees. It probably differentiated in this area over the most recent glacial cycles. Within this strictly Pyrenean lineage, most of the private haplotypes and the highest diversity parameters are observed in the samples from the eastern Pyrenees (sites 41-42, 44; Table 1, Appendix S6), suggesting the existence of a refugial area. This region, known as a biodiversity hotspot (Médail & Diadema, 2009), probably satisfied both temperature and host requirements during the LGM, in spite of its close proximity to the Pyrenean ice sheet. It could have benefited both from the adiabatic warming of downward air masses (Brown & Lomolino, 1998) and from the sea buffer effect. Moreover, the ice sheets were restricted to mountain systems over 1500 m and to some adjacent valleys (Jalut *et al.*, 1982). Pollen and fossil records support the local continuous

occurrence of pine species despite strong variation in abundance (González-Sampériz *et al.*, 2005; Cheddadi *et al.*, 2006). Molecular data indicate the persistence of montane pine species (Gómez & Hunt, 2006; Afzal-Rafii & Dodd, 2007) and suggest the possible occurrence of relictual and rather coastal populations of the Aleppo pine (Gómez *et al.*, 2005). Continuous host availability and favourable climatic conditions could thus have allowed the pine processionary moth to survive the glaciation in the eastern Pyrenees. Interestingly, a similar hot spot of genetic diversity was found in the same region for other species (Horn *et al.*, 2009).

Haplogroup A2, a lineage occurring from Spain to France and showing a phylogeographical pattern of "southern richness and northern purity"

Within the A2 group, haplotypic and nucleotidic diversities are significantly and negatively correlated with latitude. The highest values of these parameters are found in eastern Iberia, while most of the populations north of the Pyrenees are monomorphic (Table 1, Appendix S6). This is consistent with the "southern richness and northern purity" pattern, well known for numerous temperate taxa (Hewitt, 1999). The southern areas where these species persisted through glaciations would have accumulated and maintained a high genetic diversity that mirrors ancestral diversity, while founder effects during northward post-glacial expansion led to the loss of genetic variation in the recolonized areas (Hewitt, 1999, 2004; Canestrelli *et al.*, 2006). Even during the LGM, eastern Iberia offered spatial and elevational climatic gradients thanks to mountainous and coastal areas. The persistence of the pine processionary moth along the Mediterranean coast of Spain is thus supported by the putative past distribution of several hosts, including Mediterranean native pines (Carrión *et al.*, 2000; Carrión, 2002; Gómez *et al.*, 2005; Gómez & Hunt, 2006).

Haplogroup A1, a non-Iberian lineage possibly with more northerly refugial areas

Haplogroup A1 was the only one of the four major lineages that did not occur in the Iberian Peninsula. More extensive sampling, especially in the Italian and Balkan peninsulas, is needed to elucidate the origin of this lineage and to know whether distinct lineages occur in the unsampled eastern regions. Nevertheless, in the present data set, most of the diversity was found in south-eastern France, from the Massif Central to the Alps. It is worth noting that few diverging private haplotypes were found in one location along the north-western Italian coast, in Ligury (site 5 in Italy), which could reflect the existence of a localized coastal refugium south of the western Alps. This suggests that refugial areas were not confined to the southernmost parts of the peninsula during the LGM. Moreover, several rare and private haplotypes closely related to the most common one were found in eastern France (sites 10-12, 16, 18, 20, 21, 24-25; Table 1, Fig. 1). They could have independently appeared from point mutations during or following range expansion, but, for this shallowly divergent lineage, some of them might also originate from a more northerly and diffuse refugial area, as was hypothesized for other temperate species (Provan & Bennett, 2008; Horn *et al.* 2009; Médail & Diadema, 2009). Two of the private haplotypes occurred in very recent expansion areas where pine afforestation dates back from the 19th century (sites 16, 25), but the southernmost ones occurred in areas where some pine species (*P. nigra* for instance) probably occurred throughout the glacial ages (Afzal-Rafii & Dodd, 2007; Beaudoin *et al.*, 2007), allowing the persistence of associated insect species. It was recently suggested that palaeoenvironments in southern France were more complex than previously thought (Blondel & Aaronson, 1999; Médail & Diadema, 2009) and might have permitted the local survival of populations of the pine processionary moth.

To summarize, clade A exhibits at least three main refugial areas located along the Mediterranean coast: (1) along the Spanish shore from the Betic to the Iberian Chain, (2) in the eastern Pyrenees, and (3) probably near the Massif Central and the Alps and possibly in the unsampled eastern range of the pine processionary moth. *P. nigra* and/or *P. sylvestris*

probably persisted in all the glacial refugia identified for clade A (Cheddadi *et al.*, 2006; Gómez & Hunt, 2006; Afzal-Rafii & Dodd, 2007). These pines occur at present mainly from the meso- to the mountain-Mediterranean belt, and from the supra- to the oro-Mediterranean belt respectively, and probably largely predominated in the Pyrenean refugial areas of the pine processionary moth. On the other hand, eastern and south-eastern Iberia were major refugia for *P. halepensis* and/or *P. pinaster* (Gómez *et al.*, 2005), which occur at present from the thermo- to the meso-Mediterranean belt. We can thus hypothesize that refugial populations of *T. pityocampa* mostly survived the ice ages on *Pinus nigra*, which is nowadays the preferred host for egg-laying (Huchon & Démolin, 1970; Montoya, 1981). Yet, haplogroup A2 may also have survived the glaciations on Mediterranean pines, and could exhibit different adaptation to pine hosts. Concerning clade B, the available sampling did not permit us to clearly describe the patterns of distribution of genetic diversity and to identify the regions of endemism. A better sampling all over the Iberian peninsula will probably allow the identification of additional refugial areas.

Role of mountainous areas in structuring populations

No detectable role of physical barrier to dispersal …

Based on previous studies (Kerdelhué *et al.*, 2006; Santos *et al.*, 2007), it was so far hypothesized that the Pyrenees, the Massif Central, and maybe all mountain ranges could have posed a barrier to dispersal during the post-glacial expansion of this cold-sensitive species with short-range dispersal. It was thus expected that the favourable low-elevation habitats on each slope of the main ranges were colonized by different lineages, still separated by unsuitable high elevation areas (Italian *vs.* French Alps, eastern *vs.* western Massif Central, and southern *vs.* northern Pyrenees). Secondary contact zones with higher genetic diversity were expected to occur where favourable habitats connect the two sides. The present study, based on a much more extensive sampling, now rules out this hypothesis, as we show that the same haplogroup occurs on all slopes of any given mountain range.

Lineage A1 occurs from southern Italy to eastern France, showing that the Alps do not separate lineages originating from different refugial areas as known for several other taxa (Hewitt, 1999, 2004; Schmitt, 2007). In France, we hypothesized that the higher elevation areas of the Massif Central, which separate a wide western and a more abrupt eastern side, contributed to strongly structure the populations, as was suggested in a preliminary study using microsatellite markers (Kerdelhué *et al.*, 2006). Our results using mitochondrial sequences confirmed this east–west differentiation, but showed that the two lineages are not separated by the high elevation areas of the south-eastern Massif Central. On the contrary, lineage A1 occupied all suitable areas from eastern France up to the western side of the Massif Central, and the A1/A2 contact zone is located there in lowlands at the foot of this mountain range (Fig. 1, site 27), where there is no obvious physical barrier to dispersal. However, this secondary contact zone might be of very recent origin, because the native forests of *P. pinaster* (in the west) and *P. sylvestris* (in the Massif Central) have been connected by artificial plantations.

One of our major questions was to determine whether the high genetic diversity observed in the eastern Pyrenees resulted from admixture (secondary contact of diverged lineages) or from retention of ancestral variation (reflecting a glacial refugium imprint). We identified that one of the genetic lineages, namely haplogroup A3, managed to survive the glaciations *in situ*. Nevertheless, the four identified lineages A1, A2, A3 and B appeared to be in contact near the southern rim of the Pyrenees. North-eastern Spain was thus both an admixture area and a centre of differentiation, which means that the role of the Pyrenees in structuring populations was more complex than merely posing a physical barrier to dispersal. Lineages did not all respond in the same way to the climatic oscillations. While lineage A3 probably colonized both sides of the Pyrenean range in an upward and westward movement after the ice sheet retreat, but did not contribute to northward recolonization of newly suitable environments, lineage A2 expanded mainly latitudinally and colonized the south-western French lowlands,

bypassing the Pyrenees to the west. Artificial plantations and, more recently, climate change further allowed colonization of northern France. A more limited spatial expansion and a gradual upslope movement could thus account for the contradictory results of the expansion tests for the haplogroup A3 contrary to A2 (Table 2).

… but a possible role via the elevational distribution of the host species

Maternal lineages A2 and A3 show very different responses to past climatic oscillations that might be explained by contrasting responses of their host species to the post-glacial warming. Haplogroup A3 probably originated from a glacial refugium located in the eastern Pyrenees and did not extend much geographically. Our results rather suggest that it responded to glacial/interglacial cycles by limited upslope movements and was "trapped" within a mountainous zone. In this region, the montane pine species were probably the main continuously available hosts (González-Sampériz *et al.*, 2005), but these species did not contribute to post-glacial recolonization of northern Europe because of *in situ* persistence and vertical migrations throughout climatic pulses (Robledo-Arnuncio *et al.*, 2005; Cheddadi *et al.*, 2006; Afzal-Rafii & Dodd, 2007). We hypothesize that lineage A3 tracked the early recolonization of the Pyrenean range by the largely dominant pine species and was consequently trapped by vertical migration. On the contrary, lineage A2 most probably survived the ice ages in refugia located along the eastern coast of Spain, where the Mediterranean pines *P. pinaster* and *P. halepensis* could also have persisted (Gómez *et al.*, 2005). During warming periods, these thermophilic lowland pine species could have made possible the expansion to the north and thus the moth could have reached the lowlands of western France from the eastern Iberian Chain. Interestingly, this expansion pathway corresponds to one of the migration routes suggested for *P. pinaster* (Salvador *et al.*, 2000), which would be consistent with the moth following the migration route of one of its main host.

Sampling the entire range would allow one to test whether all the Mediterranean refugia of montane pines, especially *P. nigra*, correspond to differentiation centres of the moth, and if the major dispersal centres are associated with expansions of the lowland pines. Rather than showing that mountains acted as physical barriers to dispersal, our results suggest that topography played a major role in shaping the distribution of maternal lineages through the demographic history of its main host plants. Most mid-elevation regions served as glacial refugia, and the moth later expanded into lowlands from these bottlenecked populations, following its relatively thermophilic pine hosts. Mountains offered suitable environmental conditions along the slopes that permitted the persistence of this oligophagous insect during the glacial and interglacial periods. The rest of the species' range could be recurrently recolonized by spatial expansions from these refugia.

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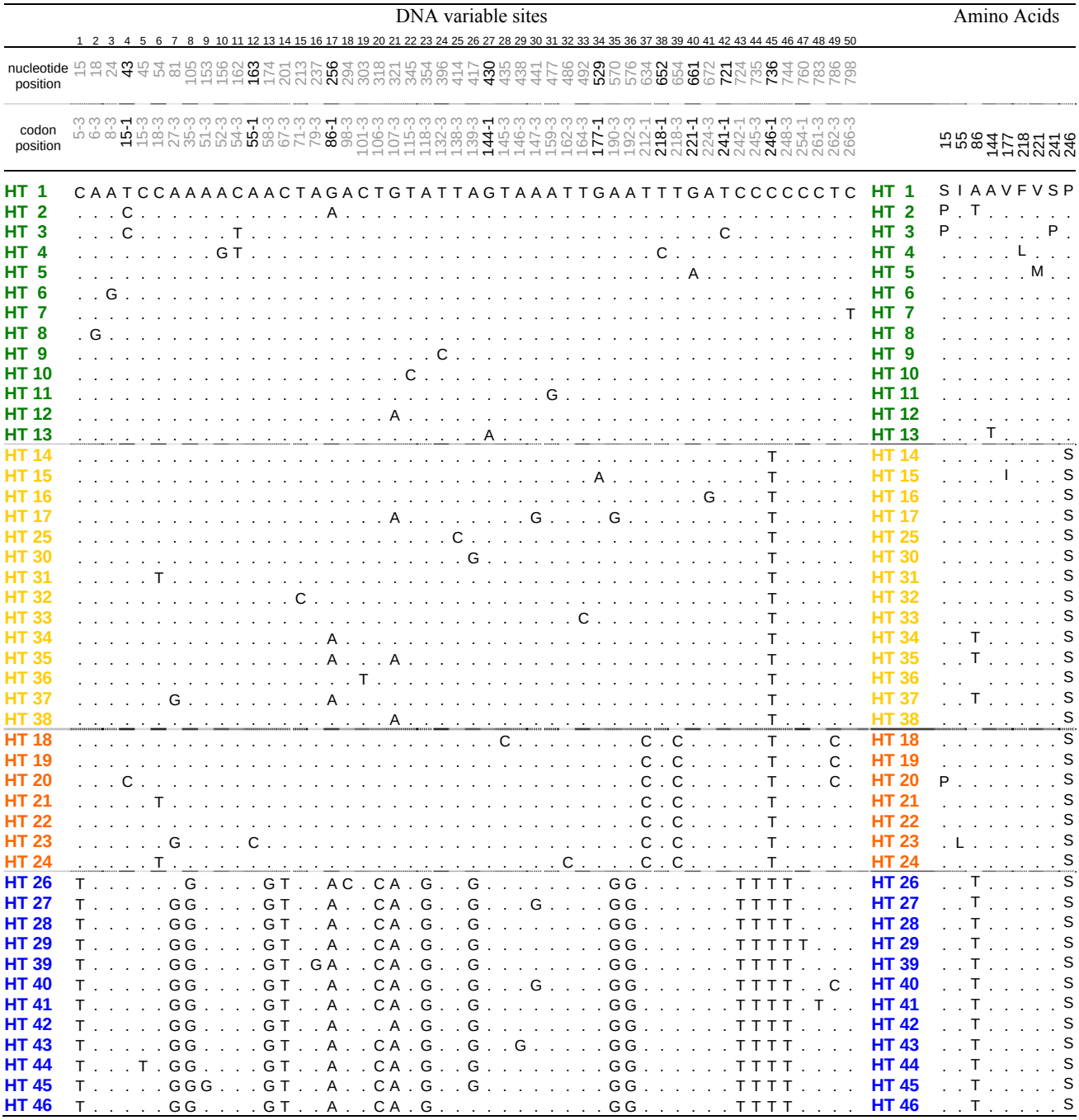
Appendix S1 Geographical location of the 61 population samples of *Thaumetopoea pityocampa* collected in western Europe. The first column corresponds to the code used to identify the sampling sites in Fig. 1.

Code	Site of collection	Region, Country	*N*	Host species	Alt. m	Latitude d°mm'	Longitude d°mm'	Generation
1	Calbarina	Padua, Veneto, Italy	5	*Pinus nigra*	136	45°16' N	11°43' E	1999-2000
2	Bari	Bari, Apulia, Italy	5	*Pinus halepensis*	20	41°07' N	16°50' E	2003-2004
3	Monte San Michele	Siena, Tuscany, Italy	4	*Pinus nigra*	890	43°31' N	11°24' E	1999-2000
4	Massimino	Savona, Liguria, Italy	5	*Pinus sylvestris*	600	44°25' N	8°26' E	1999-2000
5	Rollo	Savona, Liguria, Italy	5	*Pinus halepensis*	250	43°57' N	8°08' E	1999-2000
6	Germagnano	Turin, Piemont, Italy	5	*Pinus nigra*	500	45°16' N	7°28' E	1999-2000
7	Ruines Verrès	Aosta, Aosta valley, Italy	8	*Pinus sylvestris*	1000	45°39' N	7°41' E	1999-2000
8	Susa	Turin, Piemont, Italy	9	*Pinus sylvestris*	786	45°09' N	7°03' E	2004-2005
	Oulx				1082	45°03' N	6°51' E	2004-2005
9	Tende	Alpes Maritimes, France	5	*Pinus sylvestris*	1212	44°08' N	7°31' E	2001-2002
10	Excenevex	Haute-Savoie, France	5	*Pinus sylvestris*	386	46°21' N	6°21' E	2003-2004
11	Prunières	Hautes-Alpes, France	5	*Pinus nigra* (11a: 1) *Pinus sylvestris* (11b: 4)	816	44°32' N	6°19' E	2003-2004
12	Montagny	Savoie, France	8	*Pinus sylvestris*	883	45°28' N	6°34' E	2003-2004
13	Beaune	Côte d'Or, France	5	*Pinus nigra*	350	47°03' N	4°49' E	2001-2002
14	Leynes	Saône-et-Loire, France	5	*Pinus nigra*	414	46°16' N	4°44' E	2004-2005
15	Chaniat	Haute-Loire, France	7	*Pinus sylvestris*	579	45°18' N	3°29' E	2002-2003
16	Briennon	Loire, France	5	*Pinus nigra*	257	46°09' N	4°05' E	2004-2005
17	Bourg-Argental	Loire, France	5	*Pinus sylvestris*	473	45°17' N	4°36' E	2001-2002
18	La Seyne-sur-Mer	Var, France	5	*Pinus halepensis*	100	43°03' N	5°51' E	2002-2003
19	Tarascon	Bouches-du-Rhône, France	5	*Pinus halepensis*	130	43°50' N	4°42' E	2001-2002
20	Frontignan	Hérault, France	5	*Pinus halepensis*	106	43°27' N	3°43' E	2002-2003
21	Bédarieux	Hérault, France	5	*Pinus nigra* (21a: 1) *Pinus sylvestris* (21b: 4)	305	43°37' N	3°11' E	2006-2007
22	Saint-Affrique	Aveyron, France	5	*Pinus nigra*	344	43°57' N	2°54' E	2006-2007
23	Marcillac-Vallon	Aveyron, France	8	*Pinus nigra*	418	44°29' N	2°28' E	2001-2002
24	Fabrezan	Aude, France	10	*Pinus pinaster* (24a: 5) *Pinus halepensis* (24b: 5)	136	43°07' N	2°44' E	2001-2002
25	Toury-sur-Jour	Nièvre, France	10	*Pinus nigra*	245	46°43' N	3°15' E	2001-2002
26	Lapan	Cher, France	10	*Pinus nigra*	147	46°55' N	2°18' E	2005-2006
27	Lavercantière	Lot, France	10	*Pinus nigra* (27a: 5) *Pseudotsuga menziesii* (27b: 5)	280	44°38' N	1°21' E	2002-2003
28	Mainvilliers	Eure-et-Loir, France	5	*Pinus nigra*	162	48°27' N	1°26' E	2005-2006
29	Lorris	Loiret, France	5	*Pinus sylvestris*	146	47°50' N	2°29' E	2002-2003
30	Fondettes	Indre-et-Loire, France	5	*Pinus nigra*	93	47°24' N	0°37' E	2004-2005
31	Vierzon	Cher, France	10	*Pinus nigra*	127	47°15' N	2°03' E	2006-2007
32	Ploubalay	Côtes d'Armor, France	5	*Pinus nigra*	18	48°35' N	2°08' W	2005-2006
33	Plouharnel	Morbihan, France	9	*Pinus nigra*	1	47°34' N	3°08' W	2001-2002
34	Vouillé	Vienne, France	5	*Pinus nigra*	141	46°36' N	0°10' E	2001-2002
35	Les Portes-en-Ré	Charente-Maritime, France	5	*Pinus nigra*	9	46°15' N	1°31' W	2001-2002
36	Rioux-Martin	Charente, France	9	*Pinus nigra* (36a: 5) *Pinus pinaster* (36b: 4)	73	45°14' N	0°01' W	2001-2002
37	Cestas	Gironde, France	10	*Pinus pinaster*	59	44°44' N	0°47' W	2002-2003
38	Réaup-Lisse	Lot-et-Garonne, France	10	*Pinus pinaster*	111	44°07' N	0°13' E	2004-2005
39	Saint-Jory	Haute-Garonne, France	5	*Pinus nigra*	114	43°46' N	1°22' E	2006-2007
40	Hasparren	Pyrénées-Atlantiques, France	5	*Pinus pinaster*	163	43°24' N	1°21' W	2002-2003
41	Cerbère	Pyrénées-Orientales, France	12	*Pinus pinaster* (41a: 4) *Pinus halepensis* (41b: 4) *Pinus pinea* (41c: 4)	225	42°27' N	3°08' E	2002-2003
42	Osséja	Pyrénées-Orientales, France	10	*Pinus sylvestris*	1514	42°24' N	2°00' E	2002-2003
43	Gajan	Ariège, France	10	*Pinus sylvestris*	567	43°03' N	1°08' E	2003-2004
44	Vilaller	Lleida, Catalonia, Spain	8	*Pinus sylvestris* (44a: 7) *Pinus uncinata* (44b: 1)	1000	42°27' N	0°43' E	2006-2007
45	Santa Maria d'Oló	Barcelona, Catalonia, Spain	10	*Pinus nigra*	649	41°53' N	2°04' E	2003-2004
46	Boltaña	Huesca, Aragón, Spain	7	*Pinus sylvestris*	682	42°26' N	0°02' E	2003-2004
47	Argente	Teruel, Aragón, Spain	8	*Pinus nigra*	1270	40°42 N	1°11' W	2007-2008
48	Xeraco	Comunidad Valenciana, Spain	8	*Pinus halepensis*	50	39°02' N	0°14' W	2007-2008
49	Vélez Blanco	Almería, Andalucía, Spain	8	*Pinus nigra*	1140	37°42' N	2°07 W'	2007-2008
50	Undiano	Pamplona, Navarra, Spain	10	*Pinus nigra*	700	42°45' N	1°47'W	2003-2004
51	Zuera	Zaragoza, Aragón, Spain	10	*Pinus nigra*	390	41°51' N	0°39' W	2003-2004
52	Ariza	Zaragoza, Aragón, Spain	4	*Pinus nigra*	725	41°18' N	2°05' W	2007-2008
53	Collado Mediano	Madrid, Spain	5	*Pinus nigra*	1050	40°42' N	4°02' W	2003-2004
54	Otívar	Granada, Andalucía, Spain	8	*Pinus pinaster*	1200	36°50' N	3°43' W	2003-2004
55	Gibraltar	Gibraltar	4	*Pinus pinea* *Pinus halepensis*	40	36°08' N	5°21' W	2003-2004
56	Alcacer	Setúbal, Portugal	5	*Pinus pinaster*	40	38°23' N	8°31' W	2002-2003
57	Apostiça	Setúbal, Portugal	5	*Pinus pinaster*	60	38°34' N	9°08' W	2002-2003
58	Leiria	Leiria, Portugal	5	*Pinus pinaster*	50	39°50' N	8°57' W	2002-2003
59	Viseu	Viseu, Portugal	7	*Pinus pinaster*	460	40°40 N	7°54' W	2002-2003
60	Varges	Bragança, Portugal	5	*Pinus pinaster*	840	41°52' N	6°40' W	2002-2003
61	Sevivas	Vila Real, Portugal	5	*Pinus pinaster*	650	41°31' N	7°30' W	2002-2003

N: sample size; Alt.: altitude in metres; Generation: eggs in year n – adults in year n+1

Appendix S2 Polymorphic sites of the 46 haplotypes found in the 412 cytochrome *c* oxidase subunit I sequences of *Thaumetopoea pityocampa* from 61 population samples in western Europe (doted lines separate the four haplogroups described in Fig. 2: Italy and eastern France; western France and eastern Spain; Pyrenees; western Spain and Portugal). The 802 pb long DNA sequences were translated into 267 AA long peptides using Mega4 (Tamura *et al.*, 2007*) and the invertebrate mitochondrial code (DNA variable sites on left, amino-acids variable sites on right , conserved sites not shown, dots represent identity with the first sequence; DNA synonymous substitutions in grey, nonsynonymous substitutions and corresponding expected amino-acid change in black)



* Tamura, K., Dudley., J, Nei., M. & Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**, 1596-1599.

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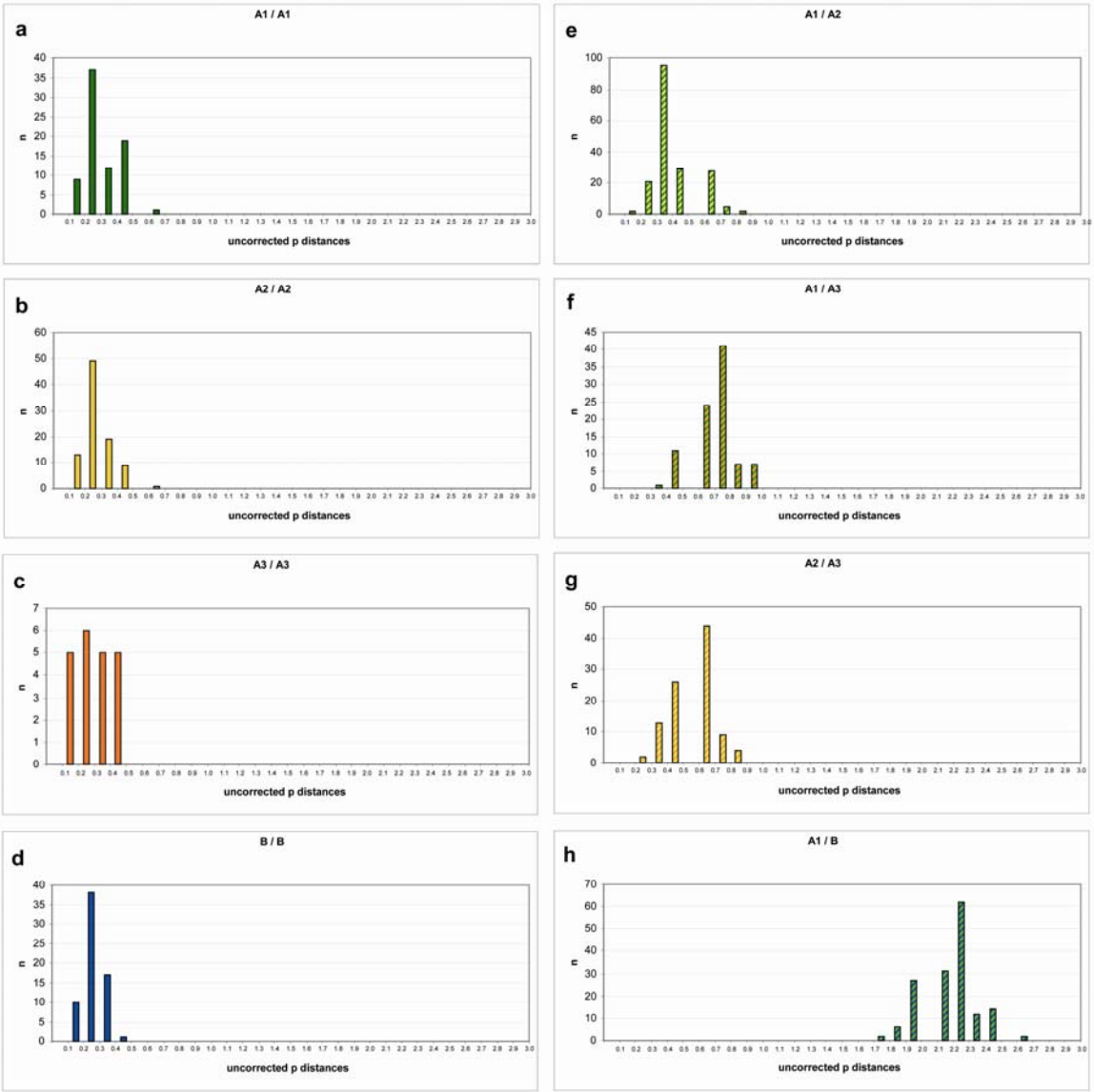
Rousselet, J., Zhao, R., Argal, D., Simonato, M., Battisti, A., Roques, A., Kerdelhue, C. (Auteur de correspondance) (2010). The role of topography in structuring the demographic history of the pine processionary moth Thaumetopoea pityocampa (Lepidoptera: Notodontidae). Journal of Biogeography, 37 (8), 1478–1490. DOI : 10.1111/j.1365-2699.2010.02289.x

		Haplogroup A1 (Italy and eastern France)													Haplogroup A2 (western France and eastern Spain)													
		HT 1	HT 2	HT 3	HT 4	HT 5	HT 6	HT 7	HT 8	HT 9	HT 10	HT 11	HT 12	HT 13	HT 14	HT 15	HT 16	HT 17	HT 25	HT 30	HT 31	HT 32	HT 33	HT 34	HT 35	HT 36	HT 37	HT 38
A1	HT 1		0.252	0.384	0.382	0.125	0.124	0.125	0.124	0.125	0.125	0.124	0.125	0.125	0.125	0.252	0.252	0.510	0.253	0.252	0.254	0.253	0.253	0.252	0.382	0.254	0.381	0.252
	HT 2	0.249		0.383	0.650	0.381	0.380	0.383	0.380	0.383	0.383	0.380	0.381	0.381	0.383	0.515	0.514	0.781	0.517	0.514	0.517	0.517	0.517	0.254	0.384	0.517	0.383	0.515
	HT 3	0.374	0.374		0.516	0.517	0.516	0.519	0.516	0.519	0.519	0.516	0.517	0.517	0.519	0.654	0.653	0.927	0.657	0.653	0.657	0.657	0.657	0.654	0.791	0.657	0.790	0.654
	HT 4	0.374	0.623	0.499		0.514	0.513	0.516	0.513	0.516	0.516	0.513	0.514	0.514	0.516	0.650	0.649	0.921	0.653	0.649	0.653	0.653	0.653	0.650	0.786	0.653	0.785	0.650
	HT 5	0.125	0.374	0.499	0.499		0.251	0.252	0.251	0.252	0.252	0.251	0.251	0.251	0.252	0.382	0.381	0.643	0.383	0.381	0.383	0.383	0.383	0.382	0.513	0.383	0.512	0.382
	HT 6	0.125	0.374	0.499	0.499	0.249		0.252	0.250	0.252	0.252	0.250	0.251	0.251	0.252	0.381	0.380	0.642	0.382	0.380	0.382	0.382	0.382	0.381	0.512	0.382	0.510	0.381
	HT 7	0.125	0.374	0.499	0.499	0.249	0.249		0.252	0.253	0.253	0.252	0.252	0.252	0.254	0.383	0.382	0.646	0.385	0.382	0.385	0.385	0.385	0.383	0.515	0.385	0.514	0.383
	HT 8	0.125	0.374	0.499	0.499	0.249	0.249	0.249		0.252	0.252	0.250	0.251	0.251	0.252	0.381	0.380	0.642	0.382	0.380	0.382	0.382	0.382	0.381	0.512	0.382	0.510	0.381
	HT 9	0.125	0.374	0.499	0.499	0.249	0.249	0.249	0.249		0.253	0.252	0.252	0.252	0.253	0.383	0.382	0.646	0.384	0.382	0.385	0.384	0.384	0.383	0.515	0.385	0.514	0.383
	HT 10	0.125	0.374	0.499	0.499	0.249	0.249	0.249	0.249	0.249		0.252	0.252	0.252	0.253	0.383	0.382	0.646	0.384	0.382	0.385	0.384	0.384	0.383	0.515	0.385	0.514	0.383
	HT 11	0.125	0.374	0.499	0.499	0.249	0.249	0.249	0.249	0.249	0.249		0.251	0.251	0.252	0.381	0.380	0.642	0.382	0.380	0.382	0.382	0.382	0.381	0.512	0.382	0.510	0.381
	HT 12	0.125	0.374	0.499	0.499	0.249	0.249	0.249	0.249	0.249	0.249	0.249		0.251	0.252	0.382	0.381	0.381	0.383	0.381	0.383	0.383	0.383	0.382	0.253	0.383	0.512	0.126
	HT 13	0.125	0.374	0.499	0.499	0.249	0.249	0.249	0.249	0.249	0.249	0.249	0.249		0.252	0.382	0.381	0.643	0.383	0.381	0.383	0.383	0.383	0.382	0.513	0.383	0.512	0.382
A2	HT 14	0.125	0.374	0.499	0.499	0.249	0.249	0.249	0.249	0.249	0.249	0.249	0.249	0.249		0.125	0.125	0.379	0.125	0.125	0.125	0.125	0.125	0.125	0.251	0.125	0.251	0.125
	HT 15	0.249	0.499	0.623	0.623	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.125		0.251	0.508	0.252	0.251	0.253	0.252	0.252	0.251	0.380	0.253	0.379	0.251
	HT 16	0.249	0.499	0.623	0.623	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.125	0.249		0.507	0.252	0.250	0.252	0.252	0.252	0.251	0.379	0.252	0.379	0.251
	HT 17	0.499	0.748	0.873	0.873	0.623	0.623	0.623	0.623	0.623	0.623	0.623	0.374	0.623	0.374	0.499	0.499		0.510	0.507	0.511	0.510	0.510	0.508	0.379	0.511	0.639	0.251
	HT 25	0.249	0.499	0.623	0.623	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.125	0.249	0.249	0.499		0.252	0.254	0.253	0.253	0.252	0.382	0.254	0.381	0.252
	HT 30	0.249	0.499	0.623	0.623	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.125	0.249	0.249	0.499	0.249		0.252	0.252	0.252	0.251	0.379	0.252	0.379	0.251
	HT 31	0.249	0.499	0.623	0.623	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.125	0.249	0.249	0.499	0.249	0.249		0.254	0.254	0.253	0.382	0.254	0.381	0.253
	HT 32	0.249	0.499	0.623	0.623	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.125	0.249	0.249	0.499	0.249	0.249	0.249		0.253	0.252	0.382	0.254	0.381	0.252
	HT 33	0.249	0.499	0.623	0.623	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.125	0.249	0.249	0.499	0.249	0.249	0.249	0.249		0.252	0.382	0.254	0.381	0.252
	HT 34	0.249	0.249	0.623	0.623	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.125	0.249	0.249	0.499	0.249	0.249	0.249	0.249	0.249		0.125	0.253	0.125	0.251
	HT 35	0.374	0.374	0.748	0.748	0.499	0.499	0.499	0.499	0.499	0.499	0.499	0.249	0.499	0.249	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.125		0.382	0.251	0.125
	HT 36	0.249	0.499	0.623	0.623	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.125	0.249	0.249	0.499	0.249	0.249	0.249	0.249	0.249	0.249	0.374		0.381	0.253
	HT 37	0.374	0.374	0.748	0.748	0.499	0.499	0.499	0.499	0.499	0.499	0.499	0.499	0.499	0.249	0.374	0.374	0.623	0.374	0.374	0.374	0.374	0.374	0.125	0.249	0.374		0.379
	HT 38	0.249	0.499	0.623	0.623	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.125	0.374	0.125	0.249	0.249	0.249	0.249	0.249	0.249	0.249	0.249	0.249	0.125	0.249	0.374	
A3	HT 18	0.623	0.873	0.998	0.998	0.748	0.748	0.748	0.748	0.748	0.748	0.748	0.748	0.748	0.499	0.623	0.623	0.873	0.623	0.623	0.623	0.623	0.623	0.623	0.748	0.623	0.748	0.623
	HT 19	0.499	0.748	0.873	0.873	0.623	0.623	0.623	0.623	0.623	0.623	0.623	0.623	0.623	0.374	0.499	0.499	0.748	0.499	0.499	0.499	0.499	0.499	0.499	0.623	0.499	0.623	0.499
	HT 20	0.623	0.623	0.748	0.998	0.748	0.748	0.748	0.748	0.748	0.748	0.748	0.748	0.748	0.499	0.623	0.623	0.873	0.623	0.623	0.623	0.623	0.623	0.623	0.748	0.623	0.748	0.623
	HT 21	0.499	0.748	0.873	0.873	0.623	0.623	0.623	0.623	0.623	0.623	0.623	0.623	0.623	0.374	0.499	0.499	0.748	0.499	0.499	0.249	0.499	0.499	0.499	0.623	0.499	0.623	0.499
	HT 22	0.374	0.623	0.748	0.748	0.499	0.499	0.499	0.499	0.499	0.499	0.499	0.499	0.499	0.249	0.374	0.374	0.623	0.374	0.374	0.374	0.374	0.374	0.374	0.499	0.374	0.499	0.374
	HT 23	0.623	0.873	0.998	0.998	0.748	0.748	0.748	0.748	0.748	0.748	0.748	0.748	0.748	0.499	0.623	0.623	0.873	0.623	0.623	0.623	0.623	0.623	0.623	0.748	0.623	0.499	0.623
	HT 24	0.623	0.873	0.998	0.998	0.748	0.748	0.748	0.748	0.748	0.748	0.748	0.748	0.748	0.499	0.623	0.623	0.873	0.623	0.623	0.374	0.623	0.623	0.623	0.748	0.623	0.748	0.623
B	HT 26	1.995	1.995	2.369	2.369	2.120	2.120	2.120	2.120	2.120	2.120	2.120	1.870	2.120	1.870	1.995	1.995	1.746	1.995	1.746	1.995	1.995	1.995	1.746	1.621	1.995	1.870	1.746
	HT 27	2.120	2.120	2.494	2.494	2.244	2.244	2.244	2.244	2.244	2.244	2.244	1.995	2.244	1.995	2.120	2.120	1.621	2.120	1.870	2.120	2.120	2.120	1.870	1.746	2.120	1.746	1.870
	HT 28	1.995	1.995	2.369	2.369	2.120	2.120	2.120	2.120	2.120	2.120	2.120	1.870	2.120	1.870	1.995	1.995	1.746	1.995	1.746	1.995	1.995	1.995	1.746	1.621	1.995	1.621	1.746
	HT 29	2.120	2.120	2.494	2.494	2.244	2.244	2.244	2.244	2.244	2.244	2.244	1.995	2.244	1.995	2.120	2.120	1.870	2.120	1.870	2.120	2.120	2.120	1.870	1.746	2.120	1.746	1.870
	HT 39	2.120	2.120	2.494	2.494	2.244	2.244	2.244	2.244	2.244	2.244	2.244	1.995	2.244	1.995	2.120	2.120	1.870	2.120	1.870	2.120	2.120	2.120	1.870	1.746	2.120	1.746	1.870
	HT 40	2.244	2.244	2.618	2.618	2.369	2.369	2.369	2.369	2.369	2.369	2.369	2.120	2.369	2.120	2.244	2.244	1.746	2.244	1.995	2.244	2.244	2.244	1.995	1.870	2.244	1.870	1.995
	HT 41	2.120	2.120	2.494	2.494	2.244	2.244	2.244	2.244	2.244	2.244	2.244	1.995	2.244	1.995	2.120	2.120	1.870	2.120	1.870	2.120	2.120	2.120	1.870	1.746	2.120	1.746	1.870
	HT 42	1.870	1.870	2.244	2.244	1.995	1.995	1.995	1.995	1.995	1.995	1.995	1.746	1.995	1.746	1.870	1.870	1.621	1.870	1.621	1.870	1.870	1.870	1.621	1.496	1.870	1.496	1.621
	HT 43	2.120	2.120	2.494	2.494	2.244	2.244	2.244	2.244	2.244	2.244	2.244	1.995	2.244	1.995	2.120	2.120	1.870	2.120	1.870	2.120	2.120	2.120	1.870	1.746	2.120	1.746	1.870
	HT 44	2.120	2.120	2.494	2.494	2.244	2.244	2.244	2.244	2.244	2.244	2.244	1.995	2.244	1.995	2.120	2.120	1.870	2.120	1.870	2.120	2.120	2.120	1.870	1.746	2.120	1.746	1.870
	HT 45	2.120	2.120	2.494	2.494	2.244	2.244	2.244	2.244	2.244	2.244	2.244	1.995	2.244	1.995	2.120	2.120	1.870	2.120	1.870	2.120	2.120	2.120	1.870	1.746	2.120	1.746	1.870
	HT 46	1.870	1.870	2.244	2.244	1.995	1.995	1.995	1.995	1.995	1.995	1.995	1.746	1.995	1.746	1.870	1.870	1.621	1.870	1.870	1.870	1.870	1.870	1.621	1.496	1.870	1.496	1.621
outgroup	Thwil	8.853	8.853	8.978	8.978	8.728	8.978	8.728	8.978	8.978	8.728	8.978	8.728	8.978	8.728	8.853	8.853	8.603	8.853	8.603	8.603	8.853	8.853	8.603	8.479	8.728	8.728	8.603

		Haplogroup A3 (Pyrenees)							Haplogroup B (western Spain and Portugal)											
		HT 18	HT 19	HT 20	HT 21	HT 22	HT 23	HT 24	HT 26	HT 27	HT 28	HT 29	HT 39	HT 40	HT 41	HT 42	HT 43	HT 44	HT 45	HT 46
A1	HT 1	0.656	0.519	0.656	0.519	0.384	0.652	0.657	2.314	2.455	2.296	2.477	2.455	2.637	2.477	2.123	2.455	2.477	2.455	2.140
	HT 2	0.938	0.794	0.653	0.795	0.654	0.931	0.939	2.333	2.475	2.315	2.498	2.475	2.659	2.498	2.140	2.475	2.498	2.475	2.157
	HT 3	1.092	0.943	0.797	0.944	0.798	1.084	1.093	2.877	3.022	2.852	3.052	3.022	3.223	3.052	2.662	3.022	3.052	3.022	2.685
	HT 4	1.084	0.937	1.084	0.937	0.793	1.076	1.085	2.851	2.995	2.827	3.025	2.995	3.193	3.025	2.639	2.995	3.025	2.995	2.662
	HT 5	0.794	0.654	0.794	0.654	0.517	0.788	0.795	2.479	2.620	2.460	2.645	2.620	2.807	2.645	2.283	2.620	2.645	2.620	2.301
	HT 6	0.792	0.652	0.792	0.653	0.516	0.787	0.793	2.474	2.615	2.455	2.639	2.615	2.800	2.639	2.278	2.615	2.639	2.615	2.296
	HT 7	0.798	0.657	0.798	0.657	0.519	0.792	0.799	2.497	2.639	2.477	2.664	2.639	2.827	2.664	2.298	2.639	2.664	2.639	2.317
	HT 8	0.792	0.652	0.792	0.653	0.516	0.787	0.793	2.474	2.615	2.455	2.639	2.615	2.800	2.639	2.278	2.615	2.639	2.615	2.296
	HT 9	0.797	0.656	0.797	0.657	0.519	0.792	0.798	2.494	2.637	2.475	2.662	2.637	2.825	2.662	2.296	2.637	2.662	2.637	2.315
	HT 10	0.797	0.656	0.797	0.657	0.519	0.792	0.798	2.494	2.637	2.475	2.662	2.637	2.825	2.662	2.296	2.637	2.662	2.637	2.315
	HT 11	0.792	0.652	0.792	0.653	0.516	0.787	0.793	2.474	2.615	2.455	2.639	2.615	2.800	2.639	2.278	2.615	2.639	2.615	2.296
	HT 12	0.794	0.654	0.794	0.654	0.517	0.788	0.795	2.155	2.296	2.140	2.317	2.296	2.475	2.317	1.970	2.296	2.317	2.296	1.985
	HT 13	0.794	0.654	0.794	0.654	0.517	0.788	0.795	2.479	2.620	2.460	2.645	2.620	2.807	2.645	2.283	2.620	2.645	2.620	2.301
A2	HT 14	0.519	0.384	0.519	0.385	0.253	0.515	0.519	2.138	2.278	2.123	2.298	2.278	2.455	2.298	1.955	2.278	2.298	2.278	1.970
	HT 15	0.654	0.517	0.654	0.517	0.383	0.649	0.654	2.300	2.440	2.283	2.462	2.440	2.620	2.462	2.111	2.440	2.462	2.440	2.128
	HT 16	0.652	0.516	0.652	0.516	0.382	0.648	0.653	2.295	2.435	2.278	2.457	2.435	2.615	2.457	2.107	2.435	2.457	2.435	2.123
	HT 17	0.926	0.784	0.926	0.785	0.646	0.919	0.927	1.979	1.810	1.965	2.137	2.118	1.976	2.137	1.801	2.118	2.137	2.118	1.814
	HT 25	0.656	0.519	0.656	0.519	0.384	0.652	0.657	2.314	2.455	2.296	2.477	2.455	2.637	2.477	2.123	2.455	2.477	2.455	2.140
	HT 30	0.652	0.516	0.652	0.516	0.382	0.648	0.653	1.979	2.118	1.965	2.137	2.118	2.291	2.137	1.801	2.118	2.137	2.118	2.123
	HT 31	0.657	0.519	0.657	0.254	0.385	0.652	0.385	2.316	2.457	2.298	2.479	2.457	2.639	2.479	2.125	2.457	2.479	2.457	2.141
	HT 32	0.656	0.519	0.656	0.519	0.384	0.652	0.657	2.314	2.455	2.296	2.477	2.455	2.637	2.477	2.123	2.455	2.477	2.455	2.140
	HT 33	0.656	0.519	0.656	0.519	0.384	0.652	0.657	2.314	2.455	2.296	2.477	2.455	2.637	2.477	2.123	2.455	2.477	2.455	2.140
	HT 34	0.654	0.517	0.654	0.517	0.383	0.649	0.654	1.984	2.123	1.970	2.141	2.123	2.296	2.141	1.804	2.123	2.141	2.123	1.818
	HT 35	0.791	0.651	0.791	0.651	0.515	0.785	0.791	1.830	1.970	1.818	1.986	1.970	2.140	1.986	1.656	1.970	1.986	1.970	1.668
	HT 36	0.657	0.519	0.657	0.519	0.385	0.652	0.657	2.316	2.457	2.298	2.479	2.457	2.639	2.479	2.125	2.457	2.479	2.457	2.141
	HT 37	0.789	0.650	0.789	0.650	0.514	0.515	0.790	2.138	1.965	1.814	1.982	1.965	2.135	1.982	1.652	1.965	1.982	1.965	1.664
	HT 38	0.654	0.517	0.654	0.517	0.383	0.649	0.654	1.984	2.123	1.970	2.141	2.123	2.296	2.141	1.804	2.123	2.141	2.123	1.818
A3	HT 18		0.125	0.253	0.384	0.253	0.515	0.518	2.874	3.019	2.850	3.049	3.019	2.822	3.049	2.659	3.019	3.049	3.019	2.682
	HT 19	0.125		0.125	0.253	0.125	0.381	0.384	2.681	2.825	2.659	2.852	2.825	2.634	2.852	2.475	2.825	2.852	2.825	2.496
	HT 20	0.249	0.125		0.384	0.253	0.515	0.518	2.874	3.019	2.850	3.049	3.019	2.822	3.049	2.659	3.019	3.049	3.019	2.682
	HT 21	0.374	0.249	0.374		0.125	0.382	0.125	2.683	2.827	2.662	2.855	2.827	3.022	2.855	2.477	2.827	2.855	2.827	2.498
	HT 22	0.249	0.125	0.249	0.125		0.251	0.253	2.494	2.637	2.475	2.662	2.637	2.825	2.662	2.296	2.637	2.662	2.637	2.315
	HT 23	0.499	0.374	0.499	0.374	0.249		0.515	2.847	2.652	2.489	2.677	2.652	2.842	2.677	2.308	2.652	2.677	2.652	2.327
	HT 24	0.499	0.374	0.499	0.125	0.249	0.499		2.877	3.022	2.852	3.052	3.022	3.223	3.052	2.662	3.022	3.052	3.022	2.685
B	HT 26	2.369	2.244	2.369	2.244	2.120	2.369	2.369		0.378	0.250	0.380	0.378	0.509	0.380	0.380	0.378	0.380	0.378	0.378
	HT 27	2.494	2.369	2.494	2.369	2.244	2.244	2.494	0.374		0.124	0.250	0.249	0.124	0.250	0.250	0.249	0.250	0.249	0.249
	HT 28	2.369	2.244	2.369	2.244	2.120	2.120	2.369	0.249	0.125		0.124	0.124	0.250	0.124	0.124	0.124	0.124	0.124	0.124
	HT 29	2.494	2.369	2.494	2.369	2.244	2.244	2.494	0.374	0.249	0.125		0.250	0.380	0.252	0.252	0.250	0.252	0.250	0.251
	HT 39	2.494	2.369	2.494	2.369	2.244	2.244	2.494	0.374	0.249	0.125	0.249		0.377	0.250	0.250	0.249	0.250	0.249	0.249
	HT 40	2.369	2.244	2.369	2.494	2.369	2.369	2.618	0.499	0.125	0.249	0.374	0.374		0.380	0.380	0.377	0.380	0.377	0.378
	HT 41	2.494	2.369	2.494	2.369	2.244	2.244	2.494	0.374	0.249	0.125	0.249	0.249	0.374		0.252	0.250	0.252	0.250	0.251
	HT 42	2.244	2.120	2.244	2.120	1.995	1.995	2.244	0.374	0.249	0.125	0.249	0.249	0.374	0.249		0.250	0.252	0.250	0.251
	HT 43	2.494	2.369	2.494	2.369	2.244	2.244	2.494	0.374	0.249	0.125	0.249	0.249	0.374	0.249	0.249		0.250	0.249	0.249
	HT 44	2.494	2.369	2.494	2.369	2.244	2.244	2.494	0.374	0.249	0.125	0.249	0.249	0.374	0.249	0.249	0.249		0.250	0.251
	HT 45	2.494	2.369	2.494	2.369	2.244	2.244	2.494	0.374	0.249	0.125	0.249	0.249	0.374	0.249	0.249	0.249	0.249		0.249
	HT 46	2.244	2.120	2.244	2.120	1.995	1.995	2.244	0.374	0.249	0.125	0.249	0.249	0.374	0.249	0.249	0.249	0.249	0.249	
outgroup	Thwil	9.227	9.102	9.227	8.853	8.978	9.227	8.728	8.728	8.603	8.728	8.728	8.853	8.728	8.603	8.853	8.603	8.603	8.853	8.853

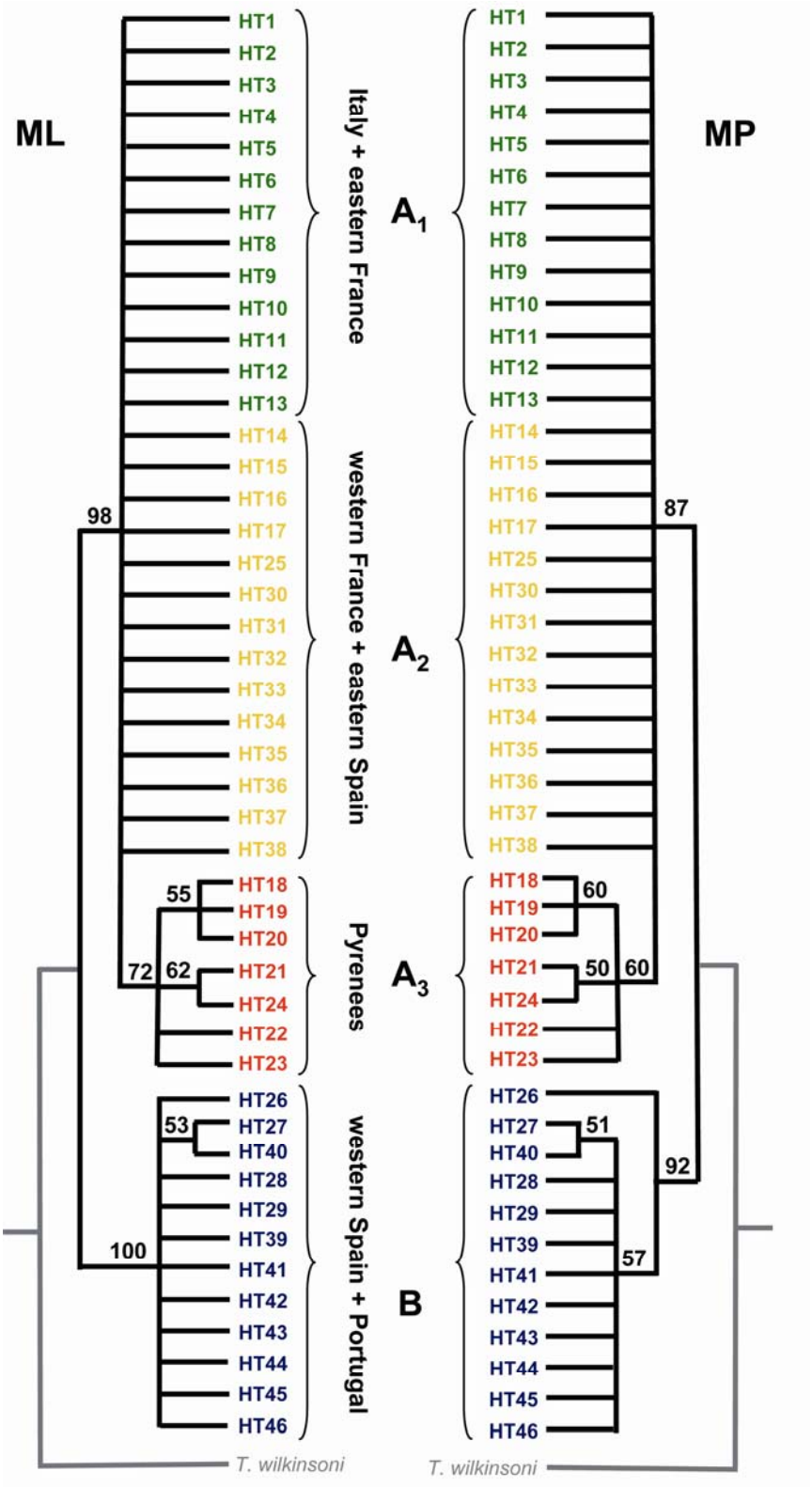
Appendix S3 (a) Pairwise distance matrix (%) between the 46 cytochrome *c* oxidase subunit I haplotypes of *Thaumetopoea pityocampa* found in western Europe (lower-left matrix: uncorrected p-distances; upper-right matrix: distances based on the substitution model used for Maximum-Likelihood analysis). A_1, A_2, A_3 and B are the haplogroups described in Fig. 2 (Italy and eastern France; western France and eastern Spain; Pyrenees; western Spain and Portugal).



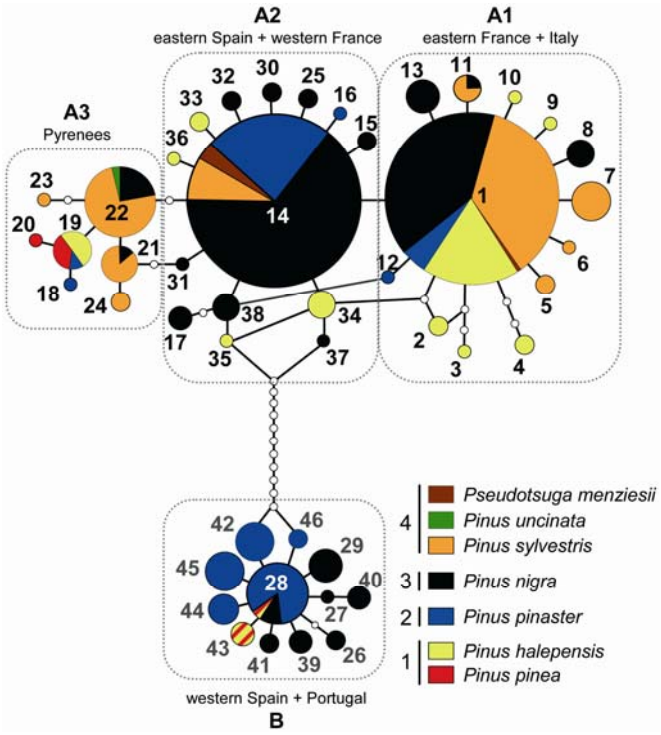
Appendix S3 (b) Histograms of pairwise uncorrected *p* distance (%) between the cytochrome *c* oxidase subunit I haplotypes of *Thaumetopoea pityocampa* found in western Europe

(a) between the 13 haplotypes forming A_1 group (*n*=78)
(b) between the 14 haplotypes forming A_2 group (*n*=91)
(c) between the 7 haplotypes forming A_3 group (*n*=21)
(d) between the 12 haplotypes forming B group (*n*=66)
(e) between haplotypes of A_1 and B groups (*n*=182)
(f) between haplotypes of A_1 and A_3 groups (*n*=91)
(g) between haplotypes of A_2 and A_3 groups (*n*=156)
(h) between haplotypes of A_1 and B groups (*n*=98)
(i) between haplotypes of A_2 and B groups (*n*=168)
(j) between haplotypes of A_3 and B groups (*n*=84).

A_1, A_2, A_3 and B are the haplogroups described in Fig. 2: A_1, Italy and eastern France (haplotypes 1-13); A_2, western France and eastern Iberia (haplotypes 14-17, 25, 30-38); A_3, Pyrenees (haplotypes 18-24); B, western Spain and Portugal (haplotypes 26-29, 39-46).
Distance classes (in abscissa): 0.1%; *n*: number of pairwise comparisons.



Appendix S4 Phylogenetic relationships among the 46 cytochrome *c* oxidase subunit I haplotypes of *Thaumetopoea pityocampa* derived from Maximum Likelihood (ML) and from Maximum Parsimony (MP) analyses. ML analysis was performed using the substitution model TIM+I and with molecular clock enforced. The ML and MP trees presented are rooted with a sequence from the sibling species *Thaumetopoea wilkinsoni* and correspond to the bootstrap 50% majority-rule consensus tree (of respectively 17422 and 1346 bootstrapped trees resulting from 200 bootstrap replicates conducted heuristically using tree bisection-reconnection branch swapping on starting trees generated by 5 randomly derived stepwise addition sequences). Bootstrap values (≥50%) are shown beside nodes (while nodes with less than 50% support were collapsed into polytomies). Haplogroups defined in Fig. 2 and discussed in the text are indicated by capital letters (A_1, A_2, A_3 and B) and their geographic distribution is denoted beside



Appendix S5 Host plant species mapped onto the cytochrome *c* oxidase subunit I haplotype network of *Thaumetopoea pityocampa* (see Fig. 2). For each haplotype, pie-charts represent the proportion of individuals found on each host plant sampled (see Table 1 for details). The four groups used for the analysis of molecular variance (AMOVA, Table 2) are indicated in the legend (numbers 1-4). For the sampling location 55 (Gibraltar, see Fig. 1), the distribution of the individuals (bearing haplotypes 28 and 43) between *Pinus halepensis* and *P. pinea* is unknown

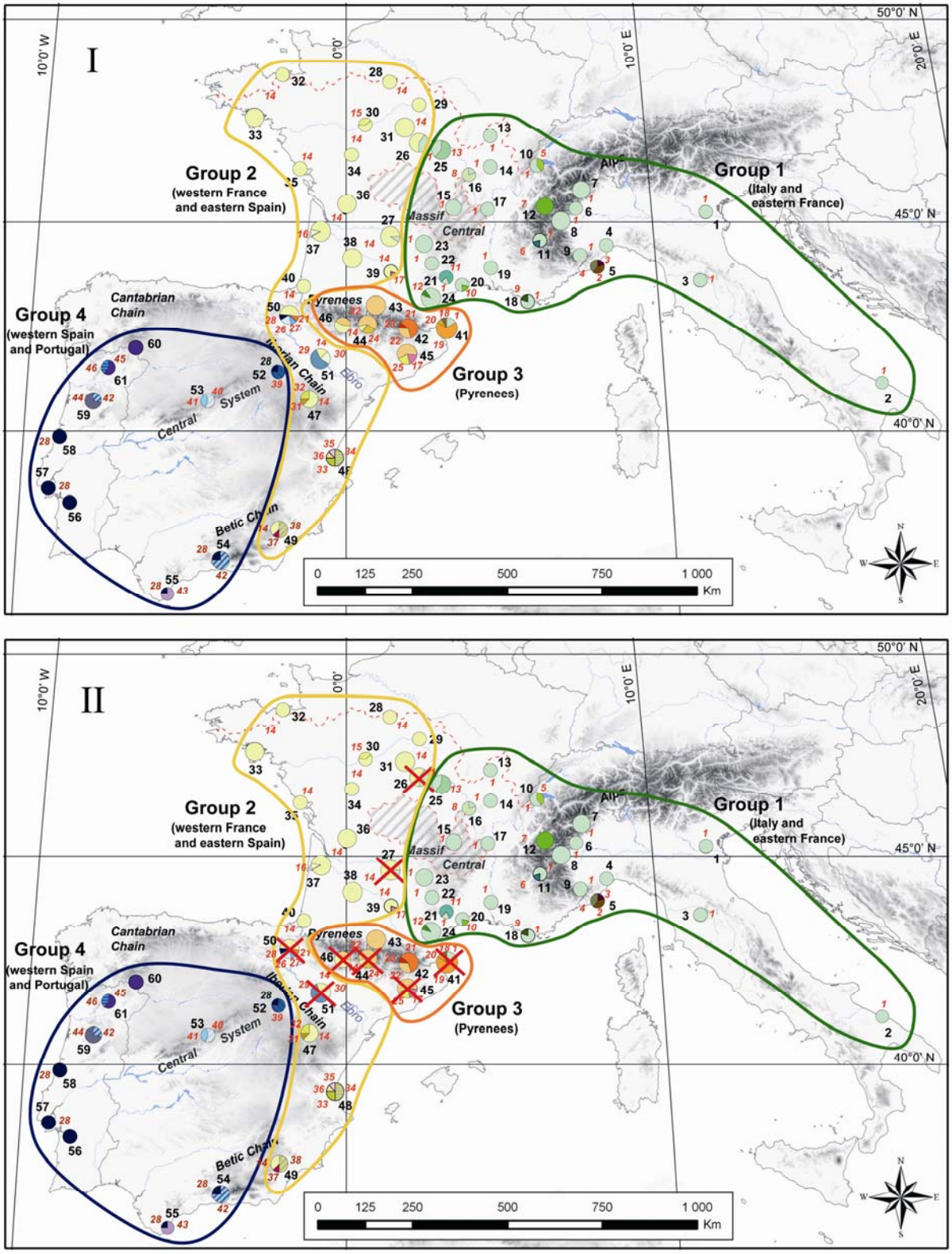
Appendix S6 Cytochrome *c* oxidase subunit I haplotypes found in each population sample of *Thaumetopoea pityocampa* collected in western Europe and population parameters for each haplogroup considered separately. Samples containing haplotypes from different haplogroups were split into subsamples under the admixture hypothesis and the parameters were recalculated within each haplogroup. The corresponding values, modified in comparison with Table 1, are indicated in red.

Site of collection	Whole data set *				Haplogroup A_1		Haplogroup A_2		Haplogroup A_3		Haplogroup B	
	n	N_{HT}	*h*	*k*	*h*	*k*	*h*	*k*	*h*	*k*	*h*	*k*
1 Calbarina	5	1	0.00	0.00	0.00	0.00	-	-	-	-	-	-
2 Bari	5	1	0.00	0.00	0.00	0.00	-	-	-	-	-	-
3 Mt San Michele	4	1	0.00	0.00	0.00	0.00	-	-	-	-	-	-
4 Massimino	5	1	0.00	0.00	0.00	0.00	-	-	-	-	-	-
5 Rollo	5	3	0.80	3.40	0.80	3.40	-	-	-	-	-	-
6 Germagnano	8	1	0.00	0.00	0.00	0.00	-	-	-	-	-	-
7 Ruines Verrès	9	1	0.00	0.00	0.00	0.00	-	-	-	-	-	-
8 Susa - Oulx	5	1	0.00	0.00	0.00	0.00	-	-	-	-	-	-
9 Tende	5	1	0.00	0.00	0.00	0.00	-	-	-	-	-	-
10 Excenevex	5	2	0.60	0.60	0.60	0.60	-	-	-	-	-	-
11 Prunières	5	2	0.40	0.40	0.40	0.40	-	-	-	-	-	-
12 Montagny	8	1	0.00	0.00	0.00	0.00	-	-	-	-	-	-
13 Beaune	5	1	0.00	0.00	0.00	0.00	-	-	-	-	-	-
14 Leynes	5	1	0.00	0.00	0.00	0.00	-	-	-	-	-	-
15 Chaniat	7	1	0.00	0.00	0.00	0.00	-	-	-	-	-	-
16 Briennon	5	2	0.40	0.40	0.40	0.40	-	-	-	-	-	-
17 Bourg-Argental	5	1	0.00	0.00	0.00	0.00	-	-	-	-	-	-
18 La Seyne-sur-Mer	5	2	0.40	0.40	0.40	0.40	-	-	-	-	-	-
19 Tarascon	5	1	0.00	0.00	0.00	0.00	-	-	-	-	-	-
20 Frontignan	5	2	0.40	0.40	0.40	0.40	-	-	-	-	-	-
21 Bédarieux	5	2	0.40	0.40	0.40	0.40	-	-	-	-	-	-
22 Saint-Affrique	5	1	0.00	0.00	0.00	0.00	-	-	-	-	-	-
23 Marcillac-Vallon	8	1	0.00	0.00	0.00	0.00	-	-	-	-	-	-
24 Fabrezan	10	2	0.20	0.20	0.20	0.20	-	-	-	-	-	-
25 Toury-sur-Jour	10	2	0.53	0.53	0.53	0.53	-	-	-	-	-	-
26 Lapan	10	2	0.53	0.53	0.00	0.00	0.00	0.00	-	-	-	-
27 Lavercantière	10	2	0.20	0.20	0.00	0.00	0.00	0.00	-	-	-	-
28 Mainvilliers	5	1	0.00	0.00	-	-	0.00	0.00	-	-	-	-
29 Lorris	5	1	0.00	0.00	-	-	0.00	0.00	-	-	-	-
30 Fondettes	5	2	0.60	0.60	-	-	0.60	0.60	-	-	-	-
31 Vierzon	10	1	0.00	0.00	-	-	0.00	0.00	-	-	-	-
32 Ploubalay	5	1	0.00	0.00	-	-	0.00	0.00	-	-	-	-
33 Plouharnel	9	1	0.00	0.00	-	-	0.00	0.00	-	-	-	-
34 Vouillé	5	1	0.00	0.00	-	-	0.00	0.00	-	-	-	-
35 Les Portes-en-Ré	5	1	0.00	0.00	-	-	0.00	0.00	-	-	-	-
36 Rioux-Martin	9	1	0.00	0.00	-	-	0.00	0.00	-	-	-	-
37 Cestas	10	2	0.20	0.20	-	-	0.20	0.20	-	-	-	-
38 Réaup-Lisse	10	1	0.00	0.00	-	-	0.00	0.00	-	-	-	-
39 Saint-Jory	5	2	0.40	1.20	-	-	0.40	0.60	-	-	-	-
40 Hasparren	5	1	0.00	0.00	-	-	0.00	0.00	-	-	-	-
41 Cerbère	12	4	0.56	1.55	0.00	0.00	-	-	0.38	0.40	-	-
42 Osséja	10	3	0.60	0.93	-	-	-	-	0.60	0.93	-	-
43 Gajan	10	1	0.00	0.00	-	-	-	-	0.00	0.00	-	-
44 Vilaller	8	3	0.61	1.36	-	-	0.00	0.00	0.48	0.95	-	-
45 Santa Maria d'Oló	10	3	0.62	2.49	-	-	0.67	2.67	0.00	0.00	-	-
46 Boltaña	7	2	0.57	1.14	-	-	0.00	0.00	0.00	0.00	-	-
47 Argente	8	3	0.61	0.68	-	-	0.61	0.60	-	-	-	-
48 Xeraco	8	4	0.75	1.46	-	-	0.75	1.46	-	-	-	-
49 Vélez Blanco	8	3	0.68	1.07	-	-	0.68	1.07	-	-	-	-
50 Undiano	10	5	0.76	8.98	-	-	0.00	0.00	0.00	0.00	0.83	1.83
51 Zuera	10	3	0.62	8.36	-	-	0.67	0.67	-	-	0.00	0.00
52 Ariza	4	2	0.50	0.50	-	-	-	-	-	-	0.50	0.50
53 Collado Mediano	5	2	0.60	1.80	-	-	-	-	-	-	0.60	1.80
54 Otívar	8	2	0.43	0.43	-	-	-	-	-	-	0.43	0.43
55 Gibraltar	4	2	0.50	0.50	-	-	-	-	-	-	0.50	0.50
56 Alcacer	5	1	0.00	0.00	-	-	-	-	-	-	0.00	0.00
57 Apostiça	5	1	0.00	0.00	-	-	-	-	-	-	0.00	0.00
58 Leiria	5	1	0.00	0.00	-	-	-	-	-	-	0.00	0.00
59 Viseu	7	2	0.48	0.95	-	-	-	-	-	-	0.48	0.95
60 Varges	5	1	0.00	0.00	-	-	-	-	-	-	0.00	0.00
61 Sevivas	5	2	0.60	1.20	-	-	-	-	-	-	0.60	1.20

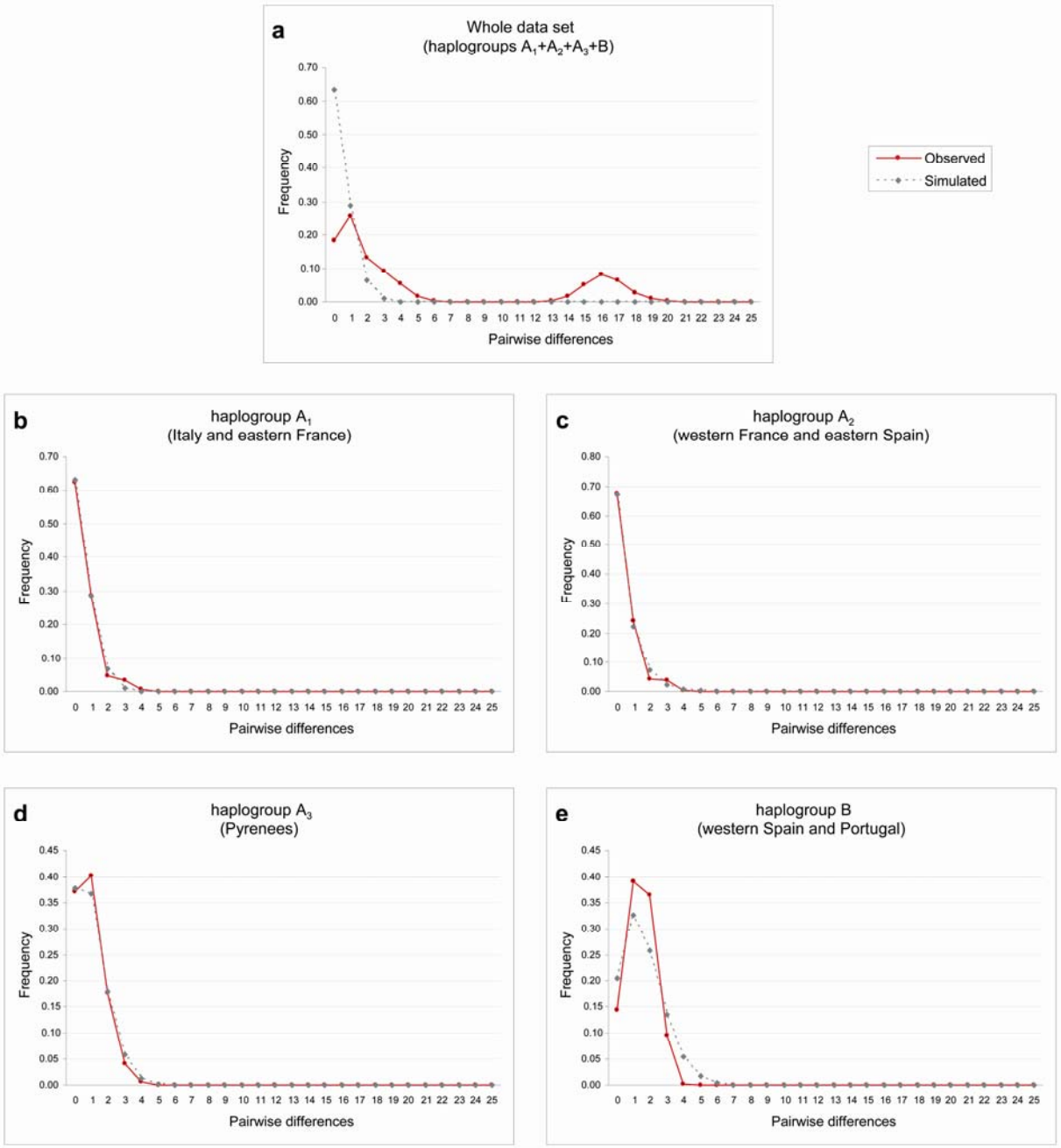
A_1, haplotypes 1-13 (Italy and eastern France); A_2, haplotypes 14-17, 25, 30-38 (western France and eastern Spain); A_3, haplotypes 18-24 (Pyrenees); B, haplotypes 26-29, 39-46 (western Spain and Portugal) (see Fig. 2)

n, sample size; N_{HT}, total number of haplotypes for each sampling location; *h*, gene diversity; *k*, mean number of pairwise differences per sequence.

* also presented in Table 1



Appendix S7 Geographical groupings of population samples used in the analyses of molecular variance (AMOVA) among populations of *Thaumetopoea pityocampa* from western Europe (see Table 2). I. All the samples are attributed to one of the four geographical regions defined; II. The samples from the putative contact zones are removed from the analysis (indicated by the red crosses). The black numbers correspond to the sampling sites and the red numbers correspond to the cytochrome *c* oxidase subunit I haplotype codes (see Table 1 and Fig. 2). The total area of each circle is proportional to the sample size and haplotype frequencies are represented by the area of the circle occupied (colour codes refer to the colour used in the haplotype network; see Fig. 2).



Appendix S8 Mismatch distribution of the number of pairwise nucleotide differences among cytochrome *c* oxidase subunit I sequences of *Thaumetopoea pityocampa* from western Europe (a) for the whole data set (b, c, d, e) for each haplogroup (as defined in Fig. 2: A1, haplotypes 1-13 ; A2, haplotypes 14-17, 25, 30-38; A3, haplotypes 18-24; B, haplotypes 26-29, 39-46). The expected distribution as predicted by the sudden population expansion model is represented by a dotted grey line and the observed distribution is represented by a solid red line.