

3 apple *M. domestica* (Cornille *et al.* 2012) and is consid-
 4 ered to be endangered (Jacques *et al.* 2009). Efforts to
 5 decipher the genetic structure and demographic history
 6 of this tree species are thus timely (i) to mitigate the
 7 effects of climate change and prevent negative effects of
 8 habitat fragmentation on this endangered species and
 9 (ii) to increase the genetic resources available for apple
 10 breeding programs, through the identification of genes
 11 conferring resistance to pathogens or tolerance of
 12 diverse abiotic stress conditions. The genetic structure
 13 of the European crabapple has been studied only in
 14 limited areas (Coart *et al.* 2003; Larsen *et al.* 2006) and
 15 appears to be weak at fine scale, suggesting a high dis-
 16 persal capacity. No study has yet investigated the phy-
 17 logeography of this emblematic wild European species
 18 across its entire distribution range. We therefore used
 19 population genetic analyses and ENM to investigate the
 20 glacial refugia and postglacial recolonization history of
 21 *M. sylvestris*. For genetic analyses, we used a compre-
 22 hensive set of *M. sylvestris* individuals sampled
 23 throughout Europe and 26 microsatellite markers. We
 24 addressed the following questions: (i) Did wild apples
 25 survive the last glacial period in a single refuge area in
 26 Europe or at multiple areas? Did all relict populations
 27 contribute equally to the postglacial recolonization of
 28 Europe by wild apples? (ii) Can we detect the genetic
 29 consequences of successive founder events during post-
 30 glacial colonization, that is, does genetic diversity
 31 decline with increasing distance from refugia? (iii) Can
 32 we detect genetic patterns of isolation-by-distance (IBD)
 33 and obtain information about dispersal capacities? (iv)
 34 Did admixture occur between recolonizing populations
 35 during expansion of the postglacial range of this spe-
 36 cies? (v) Can we reconstruct habitats that were suitable
 37 for European crabapple in the past by ENM methods?

Materials and methods

Sampling

38 We have previously demonstrated the existence of gene
 39 flow from the cultivated apple *M. domestica* to the Euro-
 40 pean wild apple (Cornille *et al.* 2012). We therefore ini-
 41 tially carried out a STRUCTURE 2.3.3 (Pritchard *et al.* 2000)
 42 analysis, including 40 reference *M. domestica* cultivars
 43 previously identified as displaying no introgression
 44 from the European crabapple (i.e. with membership
 45 coefficients >0.9 to the *M. domestica* gene pool), and the
 46 entire *Malus sylvestris* data set comprising 837 individ-
 47 uals. We retained only the 381 individuals assigned with
 48 a value of >0.9 to the *M. sylvestris* gene pool for further
 49 analyses. Leaf material was collected at 37 sites (includ-
 50 ing 25 sites with at least four individuals) across Europe
 51 (Table S1 and Fig. S1, Supporting information), covering

most of the geographical distribution of the European
 crabapple except Spain and Sweden (see Euforgen map
 of the European crabapple [http://www.euforgen.org/
 distribution_maps.html](http://www.euforgen.org/distribution_maps.html)). As the European crabapple is 4
 a scattered species (i.e. 1 individual/ha), we defined
 site as a group of close individuals (<20 km); the geo-
 graphical coordinates for each site were defined as the
 average of geographical coordinates over all individuals
 from that site.

DNA extraction and microsatellite genotyping

DNA was extracted with the NucleoSpin® plant DNA
 extraction kit II (Macherey & Nagel, Düren, Germany).
 PCR amplification was performed with the Multiplex
 PCR kit (Qiagen). We used 26 microsatellites, distrib-
 uted over the 17 chromosomes, typed in 10 different
 multiplex reactions as previously described (Pacocchi
et al. 2009; Cornille *et al.* 2012). We retained only mul-
 tilocus genotypes with fewer than 25% missing data.
 We checked the suitability of the markers for popula-
 tion genetic analysis with ARLEQUIN (Excoffier & Lischer
 2010). None of the 26 microsatellite markers deviated
 significantly from the neutral equilibrium model, as
 shown by the nonsignificant *P*-values obtained in
 Ewens–Watterson tests, and no pair of markers was in
 significant linkage disequilibrium (Raymond & Rousset
 1995; Rousset 2008). The markers used may therefore
 be considered to be unlinked and to be evolving in a
 quasi-neutral manner.

Descriptive statistics

We tested for the occurrence of null alleles with MICRO-
 CHECKER 2.2.3 (Van Oosterhout *et al.* 2004). Allelic rich-
 ness and private allele richness were calculated with
 ADZE (Szpiech *et al.* 2008), for sites (i.e. geographical
 locations) and populations (i.e. clusters inferred by
 TESS analyses including hybrids up to 0.55 member-
 ship coefficient in the given cluster), using sample
 sizes of $N = 12$ (six individuals \times two chromosomes)
 and $N = 200$ (100 individuals \times two chromosomes),
 corresponding to the smallest number of observations
 for sites and populations, respectively. Heterozygosity,
 Weir and Cockerham *F*-statistics and Hardy–Weinberg
 equilibrium were assessed with GENEPOP 4.0 (Raymond
 & Rousset 1995; Rousset 2008). Only sampling sites
 with at least four successfully genotyped specimens
 were included in site-specific computations (25 sites in
 total comprising $N = 344$ individuals) except for ADZE
 analysis in which 22 sites were included (i.e. geogra-
 phical locations with at least four individuals and within
 which at least two individuals were successfully
 genotyped for each marker). The population-specific