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True Service-tree (*Sorbus domestica*, Rosaceae) in the British Isles: Rare but diverse

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True Service-tree (*Sorbus domestica* L.) is an extremely rare and only spontaneously occurring tree species in the British Isles. Its populations are characterised by low census size and strong fragmentation. Uni- and bi-parentally inherited markers were used to study the regional genetic make-up of the species. We analysed 31 samples from six different sites across south Wales and south-west England, spanning the whole putative natural occurrence in Britain. Genetic diversity in the entire British population (all six sites) was unexpectedly high, with 2.912 alleles per locus (nuclear DNA) and three different maternal lines (chloroplast DNA). In contrast, diversity within sites was low as we found identical multilocus microsatellite genotypes in five out of the six sampled locations. Finally, comparison with a pan-European dataset of *S. domestica* revealed a close association between British and French populations rather than with populations from the Mediterranean area or Balkan Peninsula. The putative native status of *S. domestica* in the British Isles is discussed based on our population genetic results as well as on ecological and historical aspects.

Keywords: chloroplast DNA, genetic diversity, microsatellites, minisatellites, native status, nuclear DNA, phylogeography

Introduction

Sorbus domestica L. (True Service-tree) is a temperate, insect-pollinated, thermophile broadleaved tree species occurring in central, south-western and south-eastern Europe. Within its distribution, the species is characterised by a scattered occurrence at low density (Kutzelnigg, 1995; Rotach, 2003). Today it is considered to be a critically endangered species in the British Isles (Cheffings & Farrell, 2005). In 1983, *S. domestica* was unexpectedly found for the first time in the British Isles growing at two sea-cliff sites in southern Wales (Hampton & Kay, 1995). Further records followed, mainly along sea- and river-sites located in southern Wales and south-western England. To date, there are approximately 60 records of *S. domestica* in the British Isles, but the actual census population size is still unknown because some trees might have been recorded more than once by different voluntary recorders. Also it is not clear, whether single trees or coppice is the product of sexual or vegetative reproduction, i.e. root suckering and underground growth. However, this has an important impact on the effective population size, because individuals that originate from vegetative reproduction are genetically identical. Besides the recent findings that are close to the sea- or river-sites, there was also one old individual growing in the Wyre Forest in Worcestershire, which had been known since 1677 (Green, 2009). Although this individual was destroyed by fire in 1862, there are some descendants left in the University of Oxford Botanic Garden. Nevertheless, this individual and its derivatives are still of unknown origin. Recently, there have been discussions (e.g. Hampton & Kay, 1995; Paton, 1967) on whether the species is native in the British Isles or has been introduced, e.g. by the Romans. Both hypotheses seem to have some support. On the one hand, it seems unlikely that trees (apart from the individual in Wyre Forest) have been planted, because they are growing at almost inaccessible locations (steep slopes, directly in the cliffs), which has resulted in small, wind-stunted phenotypes. On the other hand, it can be argued that a considerable portion of the trees are found close to water routes which might have had historic economic importance, and therefore be associated with man. There are numerous conceivable intermediate scenarios, for instance a mixture of native individuals and those introduced from remote regions as has been suggested for white oaks in the British Isles (Cottrell *et al.*, 2002).

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Table 1 List of studied sites across south-western Britain.

Site	Habitat	Latitude	Longitude	*N*
Porthilly	Riverside	50.54146	−4.91310	4
Fontygary	Seaside	51.38403	−3.36935	3
Sedbury	Riverside	51.64071	−2.63834	2
Lavernock	Seaside	51.40260	−3.18268	3
Porthkerry	Seaside	51.39269	−3.31181	14
Shirehampton	Riverside	51.48704	−2.65929	5

N, number of sampled individuals.

Our objectives in this study were to provide a molecular basis for ongoing discussions rather than to test the different hypotheses in detail, since this would require a statistical framework that is impossible to achieve based on the available population sample. We focused on the genetic diversity within and among sites of *S. domestica* in the south-west of Britain using biparentally inherited molecular markers (nuclear DNA). Furthermore, we also used a uniparentally inherited marker (chloroplast DNA) to provide data on extant maternal lines. This information is essential, especially if a single introduction event (with seeds originating from only one or few trees) is assumed. Finally, we compared the British dataset with pan-European data of *S. domestica* that was published recently (George *et al.*, 2015) in order to assign British samples to the most likely European cluster. Our assumption is that if *S. domestica* had been recently introduced to the British Isles from a remote region (e.g. Balkan Peninsula, Mediterranean area) this might be traceable in its present genetic makeup.

Material and methods

Sampling, DNA extraction and genotyping

Six sites in south-western Great Britain were sampled in September 2014 and a second time in October 2015. These included Porthilly, Shirehampton and Sedbury in England and Lavernock, Porthkerry and Fontygary in southern Wales (Fig. 1 and Table 1). One or two leaflets of a pinnate leaf per tree were collected either by hand or, where it was necessary, by using a self-built fishing rod-like cutting device. Samples were stored in silica gel until DNA extraction. In addition to the 13 samples collected wild from Porthkerry, one individual was included from the garden of Fonmon Castle, which is approximately 2 km away. This tree originated from the wild Porthkerry site, and was collected in 1998 from the western side of the population as a small plant and then nurtured in the garden. Sampling was conducted with approval from local authorities and conservation offices in all cases.

DNA was extracted using a DNeasy plant mini kit (Qiagen, USA) with slight modifications (George *et al.*, 2015) of the manufacturer's extraction protocol. We analysed seven nuclear microsatellites and one chloroplast minisatellite which had already been developed for *S. domestica* (George *et al.*, 2015; Kamm *et al.*, 2009). All seven microsatellites are dinucleotide repeats whereas the cpDNA-minisatellite is a 22 bp repeat which is located in the rps16 intron gene (Oxelman *et al.*, 1997). PCR conditions were the same as described in George *et al.* (2015). Analysis of fragment lengths was conducted on an eight-capillary CEQ 8000 fragment analyser (Beckmann-Coulter, USA). Five genotypes previously analysed (George *et al.*, 2015) were re-analysed and used as calibration standards.

Statistical analysis

Genetic diversity indices were calculated per locus and as means and standard errors over all loci using GenAlEx6.5 (Peakall & Smouse, 2012). For nuclear co-dominant microsatellites, this included the total number of alleles (N_a), effective number of alleles (N_e), the information index I (equivalent to Shannon-Weaver index), observed (H_o) and expected heterozygosity (H_e) as well as the fixation index F (values significantly different from zero indicate deviations from random mating). For cpDNA, we calculated N_a, N_e and unbiased haploid genetic diversity (uh) which is equivalent to Simpson's index of diversity but accounts for unequal sample sizes. Even though *S. domestica* also occurs close to the Avon Gorge, which is one of the most investigated hybridisation hotspots of the genus *Sorbus* in Europe (Rich *et al.*, 2010), there is no evidence of gene exchange with other congeners (Nelson-Jones *et al.*, 2002; Robertson *et al.*, 2010). Thus, we can exclude a potential bias through introgression in our data.

To assess the genetic affinity between British and other European populations, we performed three different analyses based on nuclear data. First, we calculated pairwise Euclidean distances among all available populations following the method of Gregorius (1984), where distance is simply the absolute value of differences in allele frequencies averaged across all loci and standardised to the value of one, i.e. a value of zero means, that populations are identical and a value of one means that populations are completely different. The resulting distance matrix was subjected to a principal coordinate analysis to visualise relationships among populations.

Second, we used STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) to find the most likely cluster membership of the British samples. STRUCTURE uses a Bayesian inference method to assign individuals to a predefined set of clusters (denoted as K) with the objective of minimising deviations from Hardy–Weinberg equilibrium. We used the admixture-model implemented in STRUCTURE with 1,000,000 repeats and a burn-in period of 100,000. We compared different models (K-values from 2 to 5) with the software STRUCTURE HARVESTER (Earl & von Holdt, 2012) to find the most likely number of clusters. This pre-selected limited

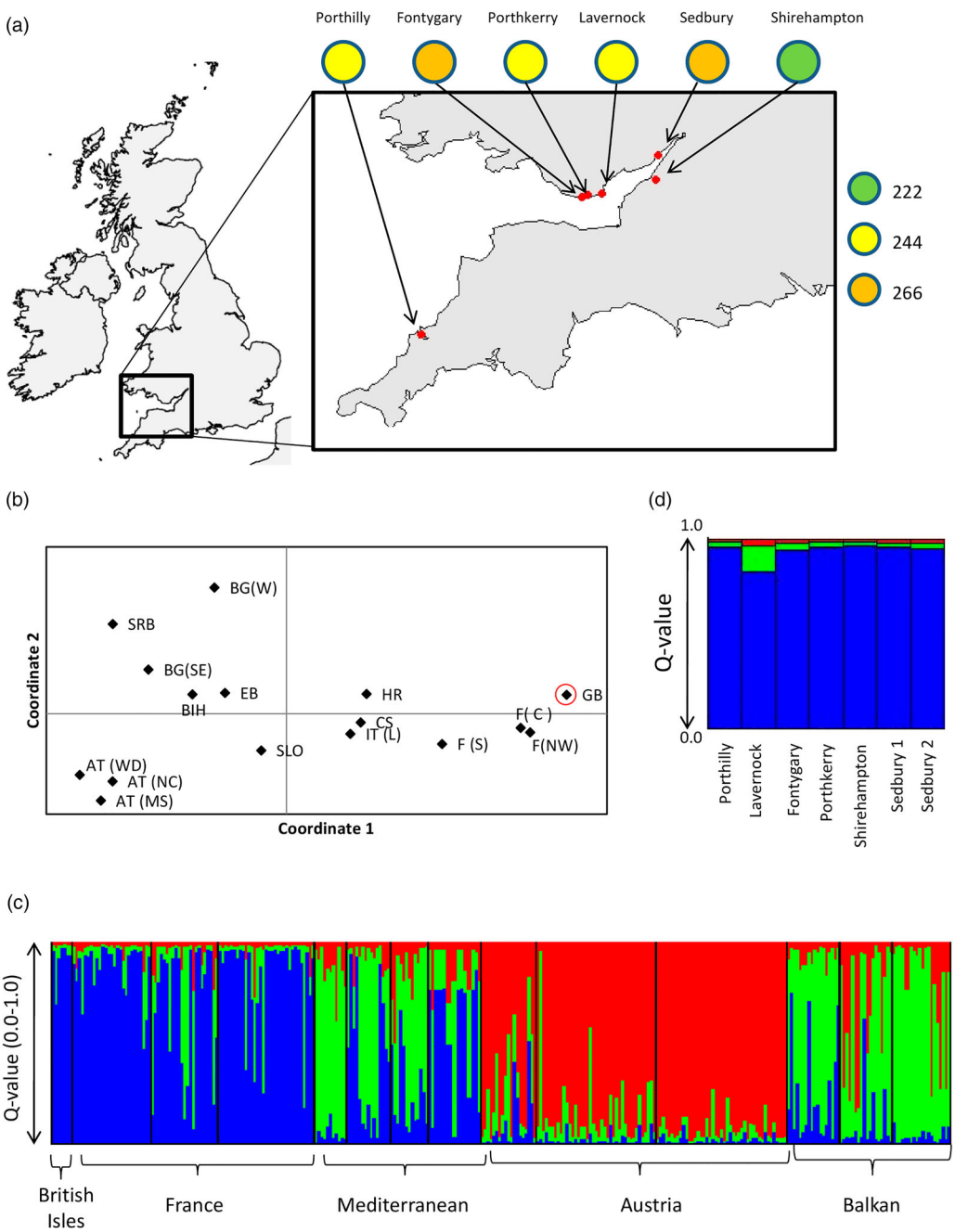

Figure 1 **(a) Distribution of cpDNA alleles across the British sample sites; (b) principal co-ordinate analysis of pairwise allelic distances according to Gregorius (1984); (c) structure barplot including all seven nuclear microsatellites, British *S. domestica* samples are shown at the left margin (denoted as 'GB'); (d) detailed structure barplot for single individuals from GB, assignment coefficient for each of the three cluster (blue, red and green; 0–100%) is given on the y-axis; population codes are: AT(xx) = Austria, F(C) = Central France, F(NW) = North-West France, F(S) = South France, IT(L) = Italy, CS = Corsica, HR = Croatia, SLO = Slovenia, EB = Elba, BIH = Bosnia-Herzegovina, BG(SE) = South-East Bulgaria, BG(W) = West Bulgaria, SRB = Serbia.**

number of K-values was chosen since earlier studies had already suggested weak population genetic structure in *S. domestica* across Europe (George *et al.*, 2015).

Third, to examine whether the scarce occurrence of *S. domestica* in the British Isles is likely to be the result of a recent introduction, we tested for the existence of first-generation migrants using GENECLASS2 (Piry *et al.*, 2004). Here, we used the Bayesian criterion according to Baudouin & Lebrun (2000) and calculated the likelihood of occurrence of the genotype within the population from which the individual has been sampled, a parameter denoted as *L_home* in Piry *et al.* (2004). This procedure was chosen in order to avoid a potential bias caused by non-sampled source populations. For probability computation, we chose the Monte Carlo re-sampling method suggested by Paetkau *et al.* (2004), using 100,00 simulated individuals and a *P*-value threshold of <0.001. All above mentioned analysis steps were carried out for the pooled sample set of all six sites, given that sample sizes per site were low in most cases and considering the fact that long-distance geneflow and weak population

genetic structure have been reported for *S. domestica* (George *et al.*, 2015; Kamm *et al.*, 2009).

Finally, observations made earlier at some of the sites (especially at Fontygary, Porthkerry and Sedbury) suggested that individual trees could simply be ramets (clones) of a few genets (genetically distinct trees). This idea was based on the strongly developed longitudinal roots of some trees and their steep-sided habitats with only limited space around, which could make establishment of offspring nearby difficult (Fig. 2). These observations prompted us to calculate the probability that two potentially identical multilocus genotypes that were found distantly spaced could have arisen from sexual reproduction, i.e. that they are really distinct genets. For this purpose we used the so called 'round-robin' method described in Parks & Werth (1993), which is implemented in the software GENCLONE 2.0 (Arnaud-Haond & Belkhir, 2007) by taking into account deviations from HW-equilibrium. However, this last analysis step was restricted to samples from Porthkerry, since only here did we have a sufficient sample size ($N = 14$), a promising high number of heterozygous loci (six out of seven, see results below) as well as a detailed map with relative positions of trees plotted from survey data by Mark Hampton (Fig. 3).

Results

Genetic diversity within and among sites

Primers for both nuclear DNA and cpDNA amplified for all seven loci and allowed for unambiguous genotyping without missing data. All trees sampled from Porthilly, Shirehampton, Lavernock, Fontygary and Porthkerry (including the tree from Fonmon Castle), respectively, had identical multilocus genotypes (MLGs). In contrast, samples from Sedbury could clearly be identified as two different genets, i.e. genetic individuals. Their genotypes were different at five of the seven analysed loci. As a consequence, one representative genotype of each identical MLG was used for further analysis of among-population-diversity as well as for genetic clustering and assignment, which led to an effective sample size of $N = 7$ (one individual from each of the five identical MLGs, and the two distinct MLGs from Sedbury). Diversity statistics are shown in Table 2, where it can be seen that the effective number of alleles, Ne, was 2.912 (standard error: 0.302) and there were no signs of an excess of homozygotes or heterozygotes ($F = -0.044$, not significantly different from 0); the information index, I, was comparatively high with a value of 1.156. Private alleles were not found within the British population. Diversity statistics

Figure 2 (a) General habitat of trees at Fontygary. Individuals of *S. domestica* are located in the centre. (b) The same as in (a), but larger scale, trees can be recognised by their brownish-coloured leaves. (c) Typical root sucker at Sedbury, only few metres distant from the sampled 'mother tree'. (d) Massive longitudinal root, connecting the shoot in (c) with the 'mother tree'.

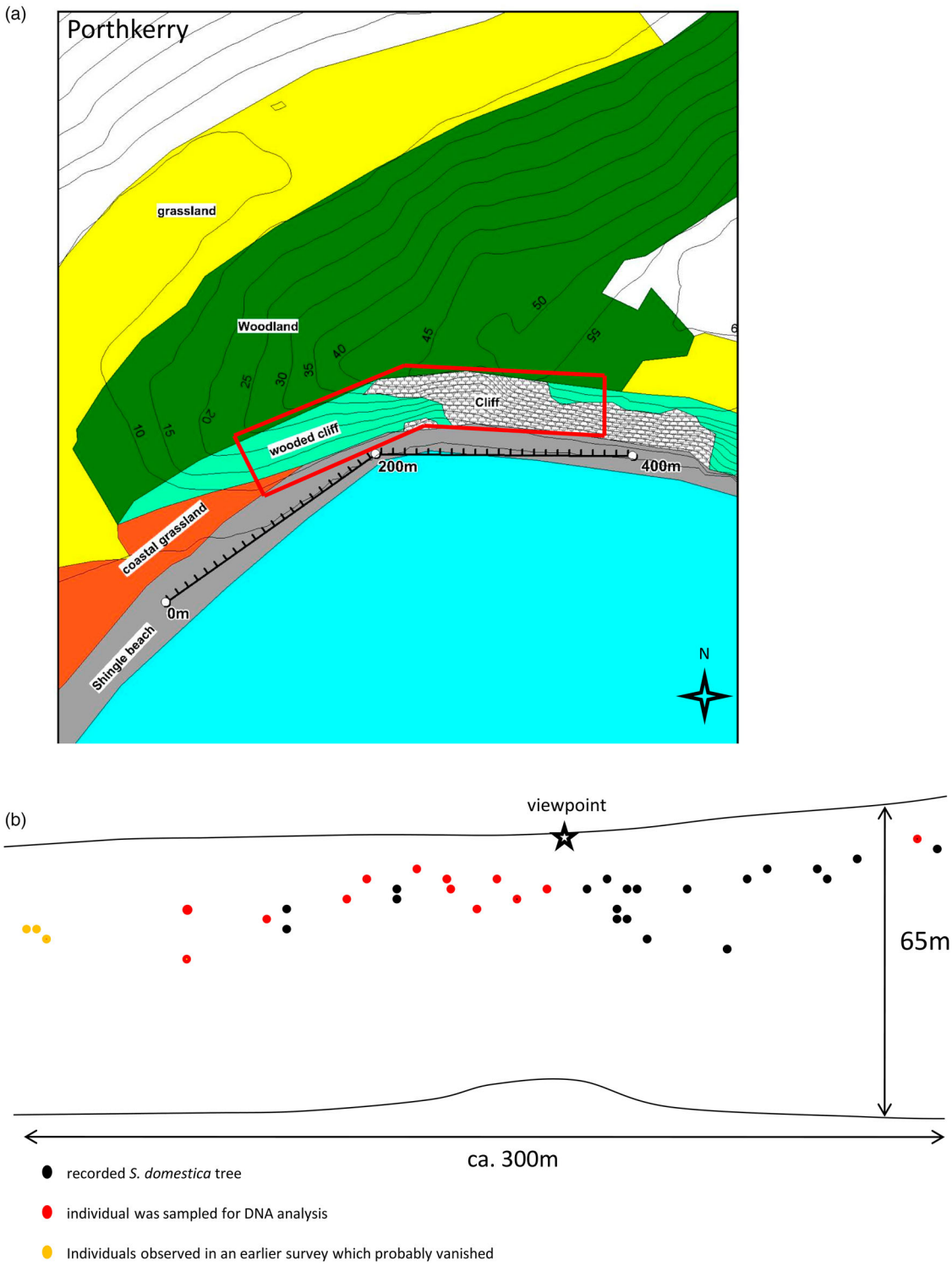

Figure 3 Map of the site at Porthkerry showing the spatial extent of clonal reproduction.

for European reference populations are summarised in Tables 3 and 4.

Chloroplast–DNA comprised three different alleles with number 244 (yellow in Fig. 1a) being the most frequent, occurring at Porthilly, Lavernock and Porthkerry. While individuals at Sedbury were different in their nuclear DNA, they had the same cpDNA allele (266, orange in Fig. 1a). This allele occurred also at Fontygary. Allele 222 (green in Fig. 1a) occurred only at Shirehampton. Unbiased haploid diversity for the whole British sample set was high at 0.714 and N_e was 2.579.

Genetic clustering and detection of first-generation migrants

The principal co-ordinate analysis revealed strong affiliation of the British population to populations from north-western and central France (Fig. 1b). Pairwise distances between these populations were 0.322 (Britain to north-west France) and 0.318 (Britain to

Table 2 Genetic diversity per locus for nuclear microsatellites calculated across all six sites.

Locus	N	N_a	N_e	I	H_o	H_e	uH_e	F
MSS5	7	5.000	3.920	1.451	0.857	0.745	0.802	–0.151
MSS16	7	3.000	1.782	0.759	0.571	0.439	0.473	–0.302
CH02C09	7	4.000	2.882	1.195	0.429	0.653	0.703	0.344
BGT23b	7	3.000	2.279	0.898	0.714	0.561	0.604	–0.273
CH01H10	7	4.000	2.390	1.055	0.429	0.582	0.626	0.263
CH01H01	7	4.000	3.630	1.334	0.714	0.724	0.780	0.014
MS14H03	7	5.000	3.500	1.400	0.857	0.714	0.769	–0.200
Mean		4.000	2.912	1.156	0.653	0.631	0.680	–0.044
SE		0.309	0.302	0.099	0.069	0.042	0.045	0.098

N, number of samples; N_a, number of alleles; N_e, effective number of alleles; I, information index; H_o, observed heterozygosity; H_e, expected heterozygosity; uH_e, unbiased heterozygosity; F, inbreeding coefficient; SE, standard error.

central France) and distances generally increased progressively with geographical distance (e.g. 0.376 between Britain and southern France, and 0.604 between Britain and Serbia, data not shown). The percentage of variation explained by the first two axes was 28.69% and 17.72%, respectively. These results were supported by genetic cluster analysis performed with STRUCTURE, which showed high assignment probability for all seven individuals to the French cluster for $K = 3$ (Fig. 1c and d). This model was also chosen as the most likely one, since Delta K values (i.e. the second order rate of change of the likelihood) decreased roughly from $K = 3$ to $K = 4$, but showed no further improvement of the model towards higher K-values (data not shown). There was no evidence for the occurrence of first-generation migrants within the British population sample (i.e. no genotype with a probability <0.001).

Probability that identical MLGs are a product of sexual propagation

The probability that two identical MLGs from Porthkerry could have also been arisen from sexual propagation just by chance ($p_{sex}(f)$) was 0.01 (1%) for the

Table 3 Genetic diversity (nSSR) for European reference populations.

Pop		N	N_a	N_e	I	H_o	H_e	uH_e	F
France (north-west)	Mean	27	5.000	2.740	1.163	0.603	0.602	0.615	–0.008
	SE		0.617	0.313	0.112	0.047	0.050	0.051	0.034
France (central)	Mean	33	5.429	2.794	1.181	0.595	0.588	0.597	–0.013
	SE		0.719	0.396	0.152	0.069	0.065	0.066	0.037
France (south)	Mean	23	5.286	3.419	1.323	0.693	0.675	0.690	–0.035
	SE		0.714	0.472	0.130	0.035	0.040	0.041	0.036
Elba	Mean	11	3.571	2.163	0.848	0.469	0.454	0.480	–0.071
	SE		0.719	0.367	0.191	0.089	0.091	0.097	0.095
Corsica	Mean	15	5.143	3.229	1.215	0.575	0.610	0.632	0.035
	SE		0.937	0.612	0.202	0.077	0.087	0.090	0.041
Italy (north)	Mean	13	5.286	3.674	1.269	0.648	0.607	0.632	–0.048
	SE		1.169	0.744	0.267	0.139	0.113	0.117	0.079
Italy (central)	Mean	4	3.286	2.497	0.970	0.571	0.545	0.622	–0.079
	SE		0.421	0.376	0.142	0.071	0.062	0.071	0.113
Sicily	Mean	3	2.857	2.506	0.904	0.571	0.532	0.638	–0.003
	SE		0.404	0.326	0.172	0.174	0.095	0.113	0.231
Slovenia	Mean	18	3.429	2.251	0.867	0.642	0.480	0.494	–0.274
	SE		0.369	0.358	0.151	0.134	0.085	0.087	0.086
Austria (National Collection)	Mean	19	4.857	2.696	1.067	0.575	0.544	0.559	0.003
	SE		0.705	0.480	0.178	0.122	0.086	0.088	0.112
Austria (Wolkersdorf)	Mean	41	5.857	2.687	1.184	0.604	0.587	0.594	–0.034
	SE		0.595	0.359	0.131	0.061	0.052	0.053	0.076
Austria (Merkenstein)	Mean	44	5.429	2.553	1.095	0.541	0.555	0.562	0.030
	SE		0.429	0.397	0.124	0.064	0.059	0.060	0.022
Croatia	Mean	18	4.571	2.622	1.043	0.612	0.533	0.548	–0.147
	SE		0.571	0.440	0.180	0.101	0.091	0.093	0.033
Bosnia-Herzegovina	Mean	18	5.714	3.320	1.282	0.643	0.614	0.632	–0.047
	SE		1.063	0.719	0.193	0.095	0.076	0.078	0.070
Serbia	Mean	7	4.286	3.252	1.120	0.626	0.557	0.603	–0.120
	SE		0.714	0.716	0.245	0.130	0.114	0.123	0.057
Bulgaria (west)	Mean	30	7.429	4.422	1.500	0.684	0.662	0.675	–0.027
	SE		1.360	0.925	0.264	0.119	0.107	0.109	0.035
Bulgaria (south-east)	Mean	20	6.143	3.218	1.294	0.609	0.611	0.628	–0.010
	SE		1.056	0.512	0.199	0.091	0.085	0.087	0.061

N, number of samples; N_a, number of alleles; N_e, effective number of alleles; I, information index; H_o, observed heterozygosity; H_e, expected heterozygosity; uH_e, unbiased heterozygosity; F, fixation index.

Table 4 Genetic diversity (cpDNA) for European reference populations.

Pop	N	N_a	N_e	I	h	uh
France (north-west)	33	3	2.889	1.078	0.654	0.674
France (central)	38	3	2.971	1.094	0.663	0.681
France (south)	24	3	2.642	1.024	0.622	0.649
Switzerland	18	2	1.385	0.451	0.278	0.294
Elba	14	4	3.161	1.240	0.684	0.736
Corsica	14	3	2.085	0.892	0.520	0.560
Italy (north)	4	2	1.600	0.562	0.375	0.500
Italy (central)	13	3	2.195	0.925	0.544	0.590
Sicily	4	2	1.600	0.562	0.375	0.500
Slovenia	31	2	1.538	0.534	0.350	0.361
Austria (national collection)	29	3	2.046	0.797	0.511	0.530
Austria (Wolkersdorf)	37	4	1.557	0.681	0.358	0.368
Austria (Merkenstein)	45	3	2.363	0.931	0.577	0.590
Croatia	34	4	3.158	1.196	0.683	0.704
Bosnia-Herzegovina	49	3	2.498	0.980	0.600	0.612
Serbia	32	2	1.679	0.594	0.404	0.417
Bulgaria (south-east)	21	2	1.800	0.637	0.444	0.467

N, number of samples; N_a, number of alleles; N_e, effective number of alleles; I, information index; uh, unbiased haploid diversity.

first re-encounter and decreased further the more identical MLGs were included in the calculation. For the last encounter, however, $p_{sex}(f)$ was only 2.08×10^{-37} (Table 5).

Discussion

Genetic diversity within and among sites

Our results show a contrasting pattern of diversity within and among sites in south-western Britain: on the one hand, the majority of sites encompassed only one and the same multilocus genotype, resulting in low within-population diversity. At least for the site at Porthkerry, we were able to confirm statistically that the 14 ramets that were sampled are very likely to belong to only one genet. The lateral extent of this genet can be estimated to be *c.*250 m (see Supplementary Material 1) and are in agreement with our earlier observations of extensive lateral root sprouting and underground growth (Fig. 2). Even though sprouting from roots or other organs is a common feature in many temperate forest trees and shrubs (e.g. Bond & Midgley, 2001; Del Tredici, 2001), estimates of clone size have rarely been reported. For instance, Hoebee *et al.* (2006) found that clonal patches in *Sorbus torminalis* in Switzerland occur over distances of 15–30 m and Kullman (1986) reported only maximum distances of 5 m for *Sorbus aucuparia*. We assume that this strategy of investing resources in vegetative reproduction could be the consequence of a limited number of potential mating partners, because self-fertilisation in many rosaceous species often is prevented by self-incompatibility mechanisms (De Nettancourt, 2001; Hiscock, 2002; Kao & Tsukamoto, 2004). A similar pattern at the northern distribution limit of *Sorbus torminalis* was found by Rasmussen & Kollmann (2008), who reported between 94 and 100% clonal reproduction across study sites in Denmark. More evidence comes from a study of the endangered rainforest tree *Elaeocarpus williamsianus* which grows in a very similar habitat in Australia: steep and actively eroding slopes with a high degree of fragmentation between populations (Rossetto *et al.*, 2004). The authors found that 170 potentially distinct trees of *E. williamsianus* can be traced back to only eight genets. Their evolutionary explanation is that movement of resources between ramets could be a competitive advantage when colonising disturbed habitats with limited resources. Without having more individual genetic data from the remaining five sites in our study, however, proving such hypotheses requires further study. However, individuals in Sedbury definitely originated from sexual reproduction, showing that sexual propagation in *S. domestica* does occur in the British Isles.

When we focus on genetic diversity among sites in the British Isles, samples harboured a notable overall amount both in nuclear microsatellites and in the plastid minisatellite. Usually, genetic diversity is expressed

Table 5 Summary statistics for the calculation of clonality probability at the Porthkerry site.

Ramet	pgen	pgen (fis)	No. ramets	Re-encounter	psex 1 reencounter	psex (fis) 1 reencounter
Porthkerry1	7.49×10^{-5}	3.32×10^{-4}				
Porthkerry2	7.49×10^{-5}	3.32×10^{-4}	2	1	2.39×10^{-3}	1.06×10^{-2}
Porthkerry3	7.49×10^{-5}	3.32×10^{-4}	3	2	2.78×10^{-6}	5.44×10^{-5}
Porthkerry4	7.49×10^{-5}	3.32×10^{-4}	4	3	2.08×10^{-9}	1.81×10^{-7}
Porthkerry5	7.49×10^{-5}	3.32×10^{-4}	5	4	1.13×10^{-12}	4.35×10^{-10}
Porthkerry6	7.49×10^{-5}	3.32×10^{-4}	6	5	4.73×10^{-16}	8.10×10^{-13}
Porthkerry7	7.49×10^{-5}	3.32×10^{-4}	7	6	1.59×10^{-19}	1.21×10^{-15}
Porthkerry8	7.49×10^{-5}	3.32×10^{-4}	8	7	4.43×10^{-23}	1.50×10^{-18}
Porthkerry9	7.49×10^{-5}	3.32×10^{-4}	9	8	1.04×10^{-26}	1.55×10^{-21}
Porthkerry10	7.49×10^{-5}	3.32×10^{-4}	10	9	2.07×10^{-30}	1.38×10^{-24}
Porthkerry11	7.49×10^{-5}	3.32×10^{-4}	11	10	3.56×10^{-34}	1.05×10^{-27}
Porthkerry12	7.49×10^{-5}	3.32×10^{-4}	12	11	5.33×10^{-38}	7.00×10^{-31}
Porthkerry13	7.49×10^{-5}	3.32×10^{-4}	13	12	6.98×10^{-42}	4.07×10^{-34}
Porthkerry14	7.49×10^{-5}	3.32×10^{-4}	14	13	8.04×10^{-46}	2.08×10^{-37}

pgen, genotype probability; psex, probability that genotype is the result of sexual reproductive event; fis in brackets accounts for deviations from random mating.

in terms of allelic richness with rarefaction to a fixed number of individuals to account for different sample sizes (e.g. Petit *et al*., 1998). However, from the microsatellite data, the effective number of alleles observed was higher for the British population compared with north-western France and central France (2.912 versus 2.740 and 2.794, respectively) despite its notably smaller sample size (7 versus 33 and 38, respectively). Thus, it can be concluded that the amount of neutral genetic diversity in the small British population is comparable to that observed in other, larger, European populations. This is also mirrored in chloroplast minisatellite DNA, which encompassed three different alleles (i.e. maternal lines) found in a sample of only seven individuals. The same three alleles were found across France by George *et al*. (2015) and it seems that no significant bottleneck in genetic diversity has occurred along this relatively long south to north distance. An introduction of *S. domestica* to the British Isles involving a single founder event thus seems unlikely. Generally, our results seem to confirm earlier findings that have shown only a very slight decrease in genetic diversity in both nuclear and chloroplast genomes from potential southern refugia to the current northern distribution limits (George *et al*., 2015). This might be the consequence of historic long-distance gene flow combined with self-incompatibility, which can be helpful in avoiding inbreeding and genetic drift even when the effective population size is small (Barrett & Kohn, 1991). Moreover, our results may give insights into the dispersal mechanism of the species. Considering the locations where *S. domestica* is mainly found today, e.g. the steep cliffs of Glamorganshire in south Wales, it seems unlikely that it was/is dispersed by large mammals such as wild boar, roe deer or red deer. The role of birds, either migratory or resident, as effective dispersers over long distances may be more likely.

Genetic clustering and first-generation migrants

Many tree species reached the British Isles after the last glacial maximum via France, either from south-western Europe (as was shown for *Quercus* spp. (Cottrell *et al*., 2002; Petit *et al*., 2002)) or from refugia located in south-eastern Europe (as was shown for *Alnus glutinosa* (L.) Gaertn. (King & Ferris, 1998)). However, principal co-ordinate analysis, as well as assignment of individuals to populations by STRUCTURE, revealed that the British population of *S. domestica* shares genetic similarities with populations from France, especially from the north-west and central regions. There is no evidence that individuals originated from further away, e.g. the Mediterranean or Balkan Peninsula, since assignment coefficients were consistently high (Fig. 1d). Moreover, a genetic cluster based on nuclear microsatellites that contained both British and French populations was also reported for the European crab apple (*Malus sylvestris* Mill.) by Cornille *et al*. (2012). Its scattered distribution across Europe is similar to that of *S. domestica* and we presume that the existence of such widespread genetic clusters, i.e. metapopulations with only little subpopulation structure, are the result of long-distance dispersal, which is a common feature in many rosaceous tree species (e.g. Bacles *et al*., 2004; Cornille *et al*., 2013; Oddou-Muratorio *et al*., 2005).

As expected, it seems unlikely that any of the analysed trees are the product of a migration event that took place only one generation ago. These results are in accordance with our finding of a probable clonal patch at Porthkerry, a patch which is unlikely to have developed during only one generation, and with the findings of Hampton & Kay (1995) who estimated that some ramets in Glamorganshire must be older than 300 years, based on counts of annual rings in some larger trunks. Hence, a very recent introduction of *S. domestica* into the British Isles seems to be improbable.

S. domestica *in the British Isles: native or an alien?*

Although a definitive clarification of the status of *S. domestica* in the British Isles was not the main objective of our study, our results do contribute to the debate. First of all, whether an organism is native or non-native in a certain region depends on the definition of 'native'. Very often, the word 'native' is used to mean 'long-established' and some authors have suggested that the beginning of the neolithic period (6000 BP) should be used as the dividing line to distinguish between native and non-native species (Manchester & Bullock, 2000; Webb, 1985). Because it still seems to be impossible to answer this question precisely for most of the species that are occurring today, Webb (1985) developed eight criteria, which serve as guidelines for a decision. These criteria are fossil evidence, historical evidence, habitat, geographical distribution, frequency of known naturalisation, genetic diversity, reproductive pattern and possible means of introduction. We have already addressed some of these criteria above (genetic diversity, reproductive pattern) and they seem to be congruent with the range-wide pattern of other *Sorbus* species at their northern distribution limit (Bacles *et al*., 2004; Rasmussen & Kollmann, 2004, 2008). While it is difficult to make statements about fossil evidence, because pollen within the genus is not identifiable to species, there are some interesting observations pertaining to the other criteria. For example, the habitat in which *S. domestica* grows in Britain seems to be anything but man-made. It is the same type of habitat that is

preferred by *S. domestica* across its entire distribution, namely open, temperate sites with a southerly exposure. This may explain why it also sometimes occurs together with *S. torminalis* (e.g. at Shirehampton and Sedbury). Are there possible means of introduction? Even though fruits had historic importance for cider production, there is no evidence that they were ever imported into Britain (e.g. in historic literature, trade documents, etc.). Of course, most of the botanical literature does not mention Britain as part of the geographical distribution of *S. domestica*, and it may well be that *S. domestica* has simply been overlooked because of its hard-to-reach and hidden habitat.

Nevertheless, there is some historic evidence regarding the occurrence of *S. domestica* in the British Isles. Kay *et al.* (1999) referenced what is probably one of the first records of the species in Britain. It appears in *Historia Brittonum*, a work attributed to the Welsh monk Nennius and dating from about 829 AD, and which includes the passage "… by the river called Guoy (Wye), apples are found on an ash tree in the wood near the mouth of the river." According to Kay *et al.* (1999), the tree referred to is undoubtedly *S. domestica*. Even though there is some uncertainty about the exact date, it is very likely that this early source refers to *S. domestica*, given the fact that there is still one extant individual in the Wye Valley today. It is growing on a vertical rock above a scree slope commonly known as 'Wintour's Leap'. The tree was identified as *S. domestica* by Mark Hampton in 2014, but it has not been possible to get any leaf samples from there due to its inaccessible location.

There is also a Welsh word that is given as 'Ceri, cerien, Pren Ceri, servis, sorbum' (Gairiadur Prifysgol Cymru, 1950–2002: 468) dated to 1592, and which probably refers to *S. domestica*. It occurs in the place names Porthkerry (1254) and Fontygary (1587) (Pierce, 1968), the latter syllables of both being pronounced -ceri (plus soft mutation $c > g$ in the latter). The word is listed in a Welsh-English-Latin dictionary in manuscript form (Evans, 1910). The name also occurs in the 14th century *Book of Taliesyn*, in the first comprehensive tree list in Welsh in the 'Cad Goddau' or Battle of Shrubs (Haycock, 1990). It is clear from the context that a tree name is meant, and the academic translation uses 'Service Tree', 'perhaps *S. torminalis*'. But *S. torminalis*, in Glamorganshire, does not grow on Lias Limestone (Wade *et al.*, 1994: 127), and historically was known under a different name, 'Sorff' (Gairiadur Prifysgol Cymru, 1950–2002: 468). In contrast, *S. domestica* occurs at both mentioned sites in sufficient number that it may have attracted attention, and several authors noted the particular status awarded to trees by the Celts (e.g. Green, 2004). If, as seems likely, 'Ceri' refers to *S. domestica*, the early date of 1254 would add support to the view that the species is long-established. Furthermore, the Irish equivalent, 'Caora', which translates as 'a berry or cluster of fruit, e.g. grapes' (Scott, 1981), and the derivative names for 'Rowan' in Welsh and Irish ('Cerddyn' and 'Caorthann') (Kelly, 1976) would suggest an even older origin. That the tree name is entirely a Celtic one does not support an introduction by the Romans, when, with over 300 years of Romano-British culture, one would expect a name of Latin origin, particularly when the first element of Porthkerry is the Latinate 'portus' (Pierce, 1968).

Conclusions

Our study showed surprisingly high levels of genetic variation in the scattered and highly fragmented population of *S. domestica* in the British Isles. Nevertheless, its status in Britain remains critically endangered, because population census size is very low. Since the observed phenotypes, viz. small, wind-stunted and with a pronounced ability to propagate vegetatively, could represent local adaptations to the harsh environmental conditions at the northern distribution limit, we recommend that conservation efforts should include the creation of ex situ seed orchards and the planting of offspring individuals as 'stepping stones' to connect sites genetically.

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Supplemental data

Supplemental data for this article can be accessed 10.1080/20423489.2016.1168092

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