Letters

Beyond skepticism: uncovering cryptic refugia using multiple lines of evidence

The increasingly popular opinion that temperate plant and animal species persisted in many small refugia in Europe north of the main southern peninsular refugia (Tzedakis *et al.*, 2013) directly challenges a classical paradigm (Huntley & Birks, 1983; Bennett *et al.*, 1991). As indirect evidence for the existence of such glacial microrefugia readily accumulates (reviewed in Tzedakis *et al.*, 2013 for plants; Schmitt & Varga, 2012 for animals), it is interesting to note that the few direct *in situ* occurrences of fossils testifying their local existence are almost always debated (Kullman, 2002; Birks *et al.*, 2005, 2012; Parducci *et al.*, 2012). Our recent study on European beech (*Fagus sylvatica*) refugia in south-western France was no exception to this trend (de Lafontaine *et al.*, 2014; Huntley, 2014, in this issue of *New Phytologist*, pp. 715–729 and 447–449, respectively). Yet, as mentioned by the philosopher Bertrand Russell (1959), it is not possible 'to get anywhere if we start from skepticism. We must start from a broad acceptance of whatever seems to be knowledge and is not rejected for some specific reason.' We therefore believe that no *a priori* preference should be given to one of the two scenarios of migration from a distant macrorefugium or of survival *in situ* in small refugia. Microrefugia are by definition spatially restricted and include few and possibly less fit individuals, so that a direct assessment for their existence at the stand-scale is extremely challenging (Gavin *et al.*, 2014). In fact, without prior information on the possible location of these refugia, finding fossils testifying to their existence is akin to looking for a needle in a haystack. We believe that to uncover glacial microrefugia, the fossil record needs not to be abundant, as long as the claims are reasonable and the dataset includes both indirect and robust direct field evidence for local presence during the most severe climatic conditions.

The criticisms of Huntley (2014) extend beyond our own study to other recently published studies on so-called cryptic glacial refugia, that is putative microrefugia inferred indirectly from genetic analyses of extant populations (Cruzan & Templeton, 2000), species distribution modeling (SDM) or regional-scale pollen analyses (Tzedakis *et al.*, 2013). As we will now show, considerable progress has been made recently in these fields that deserve to be more widely appreciated (Gavin *et al.*, 2014).

Is our claim that beech survived the last ice age in the Landes de Gascogne area plausible?

We infer beech survival in microrefugia located between 43°54′N and 44°24′N in south-western France, merely *c.* 100 km north of

an established beech refugium in the Pyrenees (Magri *et al.*, 2006). In the most recent review of the distribution of such refugia in Europe, Tzedakis *et al.* (2013) also suggest the possible survival of temperate trees close to this location (see their Fig. 1).

European beech can be extremely hardy, surviving at higher altitudes than most other temperate broadleaved tree species. In particular, it is known to endure extreme conditions above the alpine timberline thanks to clonal reproduction through layering despite a near total lack of generative reproduction (Vacek & Hejcman, 2012). Crucial for such survival under extreme conditions is the microclimate as modulated by local topography. In this respect, the two locations where we found the oldest beech charcoal particles in south-western France are moist ravines along permanent rivers (Fig. 1), a habitat shown to have acted as a microrefugium for the species (Papageorgiou *et al.*, 2014).

Climatic niche models also support the view that beech persisted in south-western France (Svenning *et al.*, 2008), although Huntley (2014) criticized the supposed propensity of the Maxent model to overestimate species distribution. In a recent study, Saltré *et al.* (2013) used another modeling approach relying on process-based SDM to assess whether beech postglacial colonization was in equilibrium with climate during the last 12 kyr. According to their model, beech colonization in western Europe lagged behind favorable climate, a finding consistent with the postglacial migration lag hypothesis proposed by Svenning *et al.* (2008). Our finding that beech was already present in south-western France 12 kyr ago thus contributes to solve the discrepancy between beech

Fig. 1 European beech (*Fagus sylvatica*) stand at site Ciron B (44°23′N, 0°18′W). A gorge along the Ciron River where beech charcoal particles were found dating back 40.0–43.3 and 31.3–32.9 kyr ago (2σ calibrated ranges). This population bears a genetic signature unique to the region and different from other nearby populations, as expected if this population had been isolated for extended periods of time.

presence and climate detected by both SDM studies in western Europe. Yet, as pointed out by Huntley (2014) and as discussed by Gavin *et al.* (2014), scale is still an issue when addressing glacial microrefugia using SDM approaches given the lack of high-resolution climate datasets. Well dated and precisely geolocalized fossils such as our soil charcoals will ultimately help progress on such issues (Maiorano *et al.*, 2013).

Finally, high-resolution pollen analysis of marine sediments core MD04-2845 from the Bay of Biscay suggest the persistence of broadleaved tree species (including beech) between 25 and 23.8 cal kyr before present (BP; i.e. during Heinrich stadial-2 (HS2)) in the region (M. F. Sánchez-Goñi, unpublished). Although a precise location of the corresponding Pleistocene populations is not possible with such an approach, the results imply

the existence of glacial temperate forest refugia somewhere in the Loire and Garonne Valleys.

Is our fossil evidence ambiguous in terms of species presence during the coldest episodes of the last ice age?

Huntley (2014) specifically questioned the relevance of the reference period that we used to determine the existence of a refugium. Following Tzedakis *et al.* (2013), we used the Late Pleniglacial (LPG) interval (24.0–14.6 cal kyr BP), defined as *the interval of most extreme glacial conditions devoid of major oscillations* (in the North Atlantic), as reference period for detecting glacial refugia, whereas the last glacial maximum (LGM; 23–19 cal kyr BP),

Fig. 2 The period post-dating last glacial maximum (LGM) was colder and/or drier than the LGM in Europe. (a) δ¹⁸O record from the NGRIP ice core (NGRIP members, 2004); (b–e) regional paleovegetation indicating percent semi-desert plants in marine pollen record from the south-western European margin (MD99-2331 + MD03-2697, Naughton *et al.*, 2007, 2009), percent arboreal taxa in marine pollen records from the Mediterranean Sea (ODP site 976, Combourieu Nebout *et al.*, 2002 and MD99-2043, Fletcher & Sánchez-Goñi, 2008), as well as percent arboreal pollen (AP) in a terrestrial record from northeast Iberia (Lake Banyoles, Pérez-Obiol & Julià, 1994); (f) periods of either cold or dry climate or of arboreal taxa minima/semi-arid taxa maxima identified from recently published records (see details and data sources in Supporting Information Table S1); (g) the 2σ calibrated age ranges for the nine Pleistocene macrofossil charcoal reported by de Lafontaine *et al.* (2014; this issue of *New Phytologist*, pp. 715–729). Late Pleniglacial (LPG) interval (24.0–14.6 cal kyr before present (BP)) according to Tzedakis *et al.* (2013); LGM interval (23–19 cal kyr BP) according to Mix *et al.* (2001); Heinrich stadial-2 (HS2) interval (26.5–24.3 cal kyr BP) according to Sánchez-Goñi & Harrison (2010); Heinrich stadial-1 (HS1) interval (18.1–14.6 cal kyr BP) corresponds to the mean period of either cold or dry climate or of arboreal taxa minima/ semi-arid taxa maxima identified by the cited studies; SST, sea surface temperature; EOF, empirical orthogonal function.
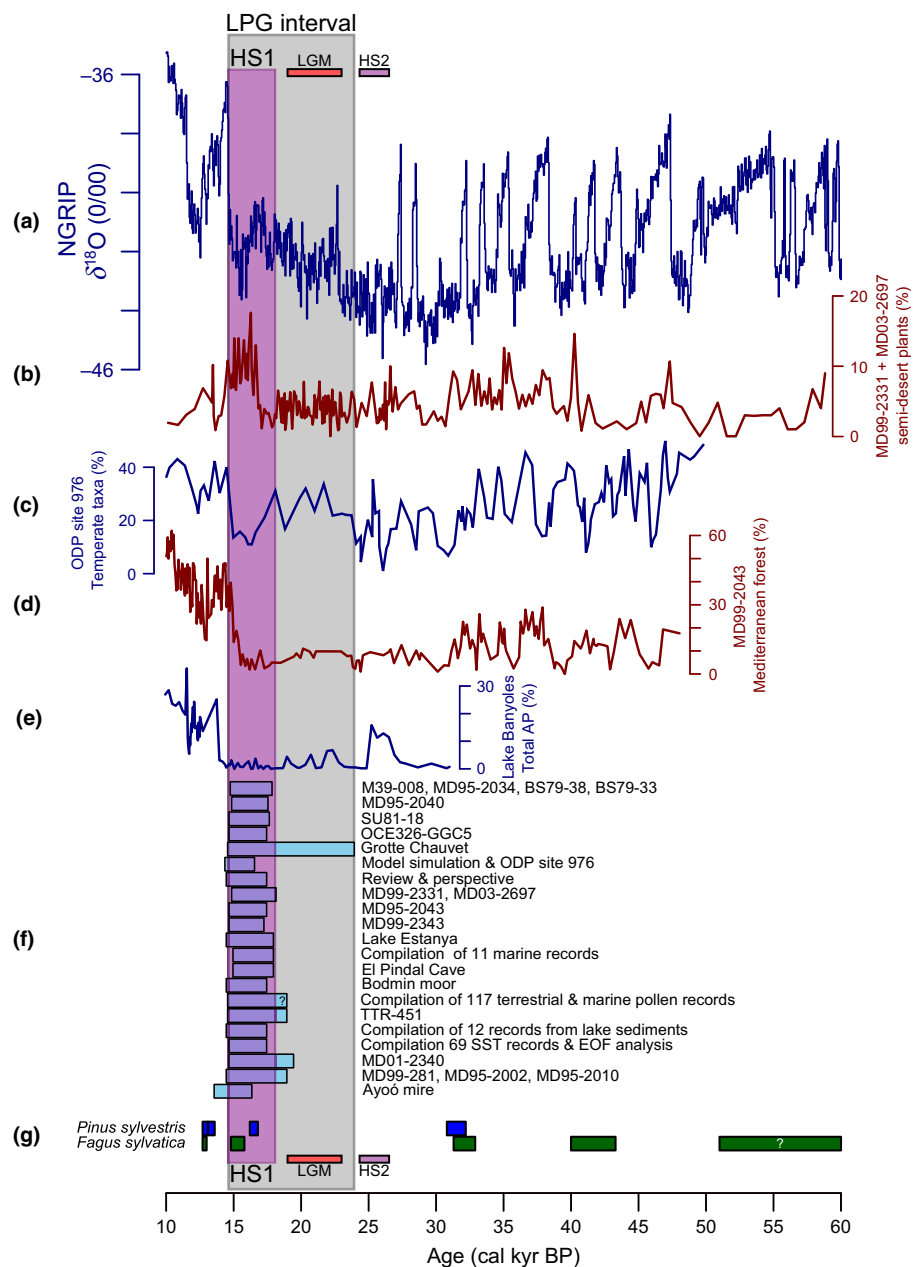
defined in terms of *the most recent interval when global ice sheets reached their maximum integrated volume* (Mix *et al.*, 2001), was considered less relevant. The difference is not only semantic: the LPG interval ends with the onset of the abrupt warming of the Lateglacial whereas the LGM reference period excludes *c.* 4400 yr with extremely cold and arid conditions, including Heinrich stadial-1 (HS1) that was colder and drier than the LGM itself in western Europe. Heinrich events *sensu stricto* (as invoked by Huntley, 2014) are included in a cycle of recurrent global climate degradations named Heinrich stadials that can be longer than Heinrich events (Sánchez-Goñi & Harrison, 2010). The termi-nology used by Huntley (2014) ambiguously confuses processes (i.e. Heinrich event *sensu stricto*) and their climatic impacts (i.e. Heinrich stadial). In our study, we rather focused on the climatically relevant stadial, HS1.

A large body of recent research shows that the period post-dating LGM was in fact colder or drier than the LGM in Europe. A thorough (quantitative-based) review on the topic would be highly desirable to formally disentangle this issue. Here we simply provide a preliminary summary of these studies, showing that from 18.1 to 14.6 cal kyr BP the climate was particularly cold and dry, arboreal taxa were at a minimum while semi-desert taxa were at a maximum (Supporting Information Table S1; Fig. 2). This period spans the calibrated age ranges of one beech and one Scots pine macrofossils that we reported in our study. So we conclude that we have indeed found (scattered) fossils during one of the coldest and driest interval in the region. Specifically, given that drought rather than temperature has been shown to limit beech (Jump *et al.*, 2006; Piovesan *et al.*, 2008), a beech charcoal macrofossil found during one of the driest phase of the LPG (HS1, see Fig. 2b) suggests local persistence of the species in a climatically unusual microrefugium. While Huntley (2014) argues that some records and a vegetation model suggest that HS2 was colder than HS1, it is still not clear whether HS1 or HS2 was colder. Indeed, either scenario can be inferred depending on the climatic tracer used, the precise geographic region or the period of the year considered. For example, the coldest interval recorded at the Galicia Margin was HS2 according to alkenone-based annual sea surface temperature and HS1 according to foraminifera-based winter sea surface temperature (Naughton *et al.*, 2009). What is clear however is that both intervals (HS1 and HS2) were climatically harsher (colder and dryer) than the LGM in Europe (e.g. Eynaud *et al.*, 2009). Given that the nearest marine pollen record suggests regional beech presence during HS2 (M. F. Sánchez-Goñi, unpublished) and that our charcoal record indicates local beech presence during HS1, we conclude that beech was present during the two coldest episodes of the last ice age (immediately before and after the LGM).

Could beech have colonized the region in response to millennial climatic fluctuations?

The scenarios of rapid response to millennial climatic fluctuation through colonization from a distant macrorefugium vs persistence in a microrefugium cannot be rigorously distinguished on the basis

of our discontinuous macrofossil charcoal record, as pointed out by Huntley (2014) and as acknowledged in our paper. Instead, we must consider the most parsimonious between these two hypotheses.

First, we note that quantitative reconstructions of postglacial beech spread in Europe suggest that colonization started in multiple regions from a mosaic of sparse stands, at densities too low to be detectable by pollen analysis (Magri, 2008), as expected if beech populations persisted in several microrefugia. Beech is also known for its particularly low dispersal ability (Bradshaw & Lindbladh, 2005; Feurdean *et al.*, 2013; Saltré *et al.*, 2013), so hypotheses involving multiple episodes of invasions within short periods at times when conditions were still very harsh for the species appear unlikely. In fact, while significant expansions of the Atlantic flora occurred during interstadials 12 and 14 (Sánchez-Goñi *et al.*, 2008; Fletcher *et al.*, 2010), our charcoal record in south-western France corresponds to completely distinct periods (interstadials 10–11, 5–6, and HS1), implying that its presence was not strictly a consequence of those warm episodes in response to millennial climatic fluctuations.

Another reason why we consider the microrefugium hypothesis more parsimonious comes from our previous genetic study of beech across 65 populations (2510 individuals typed at 16 highly polymorphic microsatellite loci) covering the species range in France and northern Spain, with a particular focus on south-western France (de Lafontaine *et al.*, 2013). In this region, we detected a complex genetic structure bearing the signature of glacial
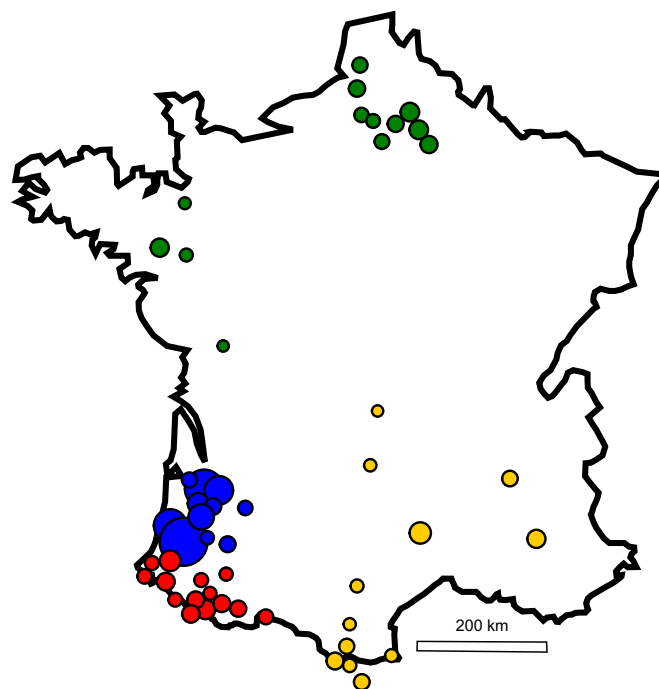

Fig. 3 The European beech (*Fagus sylvatica*) populations from Landes de Gascogne are genetically divergent from other nearby populations. Blue, red, yellow, and green circles indicate populations from the Landes de Gascogne, the western Pyrenees, south-eastern France, and northern France, respectively. Circle size proportional to mean differentiation (F_{ST}) with all other sampled populations.

refugia, that is a lack of isolation by distance, high genetic differentiation between populations and the presence of many outlier populations (Fig. 3). The presence of outlier populations in this region was interpreted as being caused by strong genetic drift caused by small population size and long isolation in unsuitable regional habitat matrix at the rear edge, suggesting the ancient origin of present-day range fragmentation. This interpretation was supported by the lack of evidence for recent bottlenecks in these populations. This pattern is characteristic of other well-established refugial areas, whereas newly colonized regions in Europe show a clear pattern of isolation by distance and little differentiation between populations, with no outlier populations. A review of the existing literature shows that these predictions are robust both in plants and in animals (de Lafontaine *et al.*, 2013).

Huntley (2014) only takes note of the fact that in the study of de Lafontaine *et al.* (2013) a few genetically divergent populations in south-western France characterized by reduced genetic diversity were spuriously assigned to other parts of the range, depending on the assignment method used. In fact, many populations are not only unique to this region but also differ from the other nearby populations, as expected if these populations had been isolated for extended periods of time, given the lack of signal of recent bottleneck (Fig. 3). This is especially true for the Ciron site, where beech charcoal dated from before and after the LPG was found (Fig. 1). This evidence supports the idea of glacial survival of beech *in situ* rather than of early postglacial colonization of the Landes de Gascogne from the Pyrenees or from south-eastern France.

Conclusion

Given that a microrefugial scenario for temperate trees in the Landes de Gascogne area is plausible and is supported by genetic data (de Lafontaine *et al.*, 2013), paleoclimatic models (e.g. Svenning *et al.*, 2008) and high-resolution pollen record from nearby marine sediments core (M. F. Sánchez-Goñi, unpublished), we were interested in assessing whether it was possible to detect the presence of such putative cryptic microrefugia from direct *in situ* fossil evidence. Our approach to target macrofossils isolated from mineral soil samples allowed us to specifically pinpoint those few unusual sites in the region that still harbor beech. The same reasons that explain beech survival today in these climate refugia within an inhospitable matrix (increased humidity caused by local edaphic and topographic conditions) could explain the species persistence at the exact same location during the last ice age. This might also explain why these microrefugia were not a major source of Holocene expansion (Edwards *et al.*, 2014). The 140 dated charcoal particles reported by de Lafontaine *et al.* (2014) represent hard-won macrofossils providing robust direct *in situ* field evidence for the presence of at least two forest tree species (beech and Scots pine) before, during, and after the LPG interval in multiple stands within the study region (for details on the numerous precautions taken to ensure reliable charcoal dating, see de Lafontaine *et al.*, 2014). The microrefugia scenario therefore stands out as the most parsimonious one to explain the existing data. While the questions raised by Huntley (2014) are legitimate, a balanced view should consider both alternatives on the basis of all available evidence.

Guillaume de Lafontaine[1,2,3]*, **Carlos Alberto Amasifuen Guerra**[1,2], **Alexis Ducousso**[1,2], **Maria-Fernanda Sánchez-Goñi**[4] **and Rémy J. Petit**[1,2]

[1]INRA, UMR 1202 BIOGECO, F-33610 Cestas, France;
[2]Univ. Bordeaux, UMR 1202 BIOGECO, F-33600 Pessac, France;
[3]Canada Research Chair in Forest and Environmental Genomics, Centre for Forest Research, Institute for Systems and Integrative Biology, Université Laval, 1030 avenue de la Médecine, Québec, QC, Canada G1V 0A6;
[4]Ecole Pratique des Hautes Etudes, UMR-CNRS 5805 EPOC, Univ. Bordeaux, F-33405 Talence, France
(*Author for correspondence: tel +1 418 656 2131 ext 12328; email guillaume.de-lafontaine.1@ulaval.ca)

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Some references explicitly suggesting that Heinrich stadial-1 was colder and drier than the global last glacial maximum in western Europe and 2σ ranges of the calibrated ages of two charcoal fragments reported by de Lafontaine *et al.* (2014; this issue of *New Phytologist*, pp. 715–729) completely included within Heinrich stadial-1 interval

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Key words: cryptic glacial refugia, *Fagus sylvatica* (European beech), genetic structure, Heinrich stadials 1 and 2, last glacial maximum, Late Pleniglacial interval, microrefugia, soil macrofossil charcoal.