GUEST EDITORIAL

The ecological and evolutionary implications of microrefugia

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ABSTRACT

Pleistocene microrefugia (or cryptic refugia) may be distinguished from macrorefugia (or conventional refugia) on the basis of two characteristics. First, microrefugia were smaller than macrorefugia and consequently supported smaller refugial populations. Second, microrefugia harboured less diverse biotic communities than macrorefugia. We propose that these characteristics have important implications for the ecology and evolution of species and populations that have a history of isolation in microrefugia. We propose four hypotheses regarding the evolution of microrefugial populations: (1) small effective population sizes associated with survival in microrefugia lead to reduced genetic diversity and influence the evolution of mating systems; (2) differences in environmental conditions between macro- and microrefugia lead to local adaptation; (3) reduced diversity increases ecological opportunity and promotes ecological divergence in microrefugia; and (4) reduced species diversity in microrefugia allows more specific species interactions and promotes coevolution among species. We urge biogeographers to study the evolutionary implications of isolation in microrefugia.

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The consensus view of Pleistocene biogeography was, until recently, that most mid- to high latitude Northern Hemisphere species survived the last glaciation in refugia south of the major ice sheets (Bennett *et al.*, 1991; Hewitt, 1996, 2000; Bernatchez & Wilson, 1998; Taberlet *et al.*, 1998). Accumulating evidence of the importance of small, isolated refugia, however, has led to the replacement of this simple conclusion by a much more pluralistic understanding – not only of where species survived the last glaciation (Stewart & Lister, 2001; Provan & Bennett, 2008), but also of what constitutes a refugium (Stewart *et al.*, 2010; Dobrowski, 2011; Hampe & Jump, 2011).

Recently, the concept of *microrefugia* (a.k.a. *cryptic refugia* or *climate relicts*) has emerged to describe glacial refugia that are distinct from conventional refugia or *macrorefugia*. Stewart *et al.* (2010, p. 662) defined cryptic refugia as 'refugia situated at different latitudes or longitudes than would normally be expected, and [which] often resemble climatic islands in which conditions differ favourably from the surrounding areas'. Rull (2010) pointed out (1) that the notion of 'different than expected' is vague and subjective, and (2) that these refugia are 'cryptic' (i.e. hidden or

obscured) only from an anthropocentric point of view, and only until they are discovered. Rull (2009, pp. 482–483) suggested instead the term 'microrefugium', which he defined as 'a small area with local favourable environmental features, in which small populations can survive outside their main distribution area (the macrorefugium), protected from the unfavourable regional environmental conditions'. Hampe & Jump (2011, p. 314) described climate relicts as populations that remain outside of the retracting range limit, 'isolated in enclaves of benign environmental conditions within an inhospitable regional climate.' For clarity and consistency herein, we use the term microrefugia.

Recently, Hampe & Jump (2011) have urged researchers to adopt a more ecological and (micro-)evolutionary focus when studying refugia, and to emphasize refugial populations rather than whole species. An important first step in the examination of the evolutionary and ecological implications of survival in microrefugia is thus to 'identify what actually characterizes a refugium beyond the fact that its environmental conditions have presumably allowed species to persist' (Hampe & Jump, 2011, p. 317). In this paper we identify two characteristics that we believe are integral in

distinguishing microrefugia from macrorefugia, and we then propose four testable ecological and evolutionary hypotheses that are suggested by these characteristics of microrefugia.

TWO CHARACTERISTICS OF MICROREFUGIA

First, we agree with Rull (2009) that small geographical area is an important part of what constitutes microrefugia. Most recognized 'macrorefugia' (e.g. Beringian and Mississipian refugia for freshwater fishes) encompassed huge areas. Even peninsular refugia (Balkan, Iberian) were much larger than most refugia embedded in the ice sheets (e.g. nunataks) or situated north of the ice sheets. Small geographical area is generally associated with small effective population size, and the consequences of inbreeding and demographic stochasticity are therefore expected to be exacerbated in microrefugia (Lande, 1988; Magri et al., 2006; Stewart et al., 2010).

Second, microrefugia were likely to have been less diverse biotic environments than macrorefugia. This is probably because (1) smaller area implies lower species diversity (Arrhenius, 1921; MacArthur & Wilson, 1963, 1967), and (2) only a subset of species possesses traits (e.g. small home ranges, vegetative reproduction) that would have facilitated survival in microrefugia (Bhagwat & Willis, 2008; Mosblech et al., 2011). There is also evidence in the fossil record for non-analogue communities in glacial refugia; species that were found together during the glacial maxima that are not found together in the present (Stewart & Lister, 2001). Similarly, we expect microrefugial communities to have differed from macrorefugial communities. While it is absent from most discussions of microrefugia, we believe that this difference in biotic communities between macro- and microrefugia is a highly relevant aspect to consider in the evolution of divergence and local adaptation associated with isolation in glacial refugia.

While we mainly discuss examples related to northern glacial refugia (i.e. those that allowed temperate species to persist during glacial periods), the general message that there are important evolutionary implications of differences in the characteristics of macro- and microrefugia is relevant to different categories of refugia (sensu Stewart et al., 2010). Indeed, Stewart et al. (2010) argued that low-latitude macrorefugia and high-latitude microrefugia during glacial periods are analogous to high-latitude macrorefugia and low-latitude microrefugia during interglacial periods. Stewart et al. (2010) also point out, however, that Pleistocene glacial periods are an order of magnitude longer than interglacial periods, and the resulting evolutionary implications are probably more relevant for species isolated in glacial refugia than interglacial refugia.

ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS OF SURVIVAL IN MICROREFUGIA

1. Small effective population sizes associated with survival in microrefugia lead to reduced genetic diversity and the evolution of low levels of inbreeding depression and/or high rates of self-fertilization or self-compatibility

Reduced effective population size, or a population bottleneck, can affect genetic diversity in a variety ways. Expected heterozygosity is predicted to decrease for neutral loci during a demographic bottleneck (Nei et al., 1975). In addition, inbreeding in small populations is expected to lead to further decreases in heterozygosity over subsequent generations (Falconer & Mackay, 1996; Frankham, 1998). These decreases in heterozygosity, however, occur at a slower rate than decreases in allelic richness (Nei et al., 1975). Indeed, allelic richness is expected to decline relatively rapidly in small populations, with a biased loss of rare alleles (Luikart et al., 1998a,b).

The expected losses in genetic diversity in small microrefugial populations, and the associated increased probability of inbreeding, may have had important evolutionary implications. We hypothesize that selection favouring reduced inbreeding depression in small microrefugial populations may have influenced the evolution of mating systems. In particular, species or populations that have a history of extended periods with small effective population size are expected to have evolved low levels of inbreeding depression and/or high rates of self-fertilization or self-compatibility (Lande & Schemske, 1985). Among vascular plant species, there is a significant negative correlation between selfing rate and inbreeding depression, which is consistent with the theoretical prediction that selfing (or inbreeding) should allow deleterious alleles to be purged, thereby reducing inbreeding depression (Husband & Schemske, 1996). A test of our hypothesis might involve a comparison of populations that were isolated in macro- and microrefugia, with the prediction that microrefugial populations (which are expected to have a history of higher rates of inbreeding) have evolved lower inbreeding depression than macrorefugial populations.

2. Differences in environmental conditions between macro- and microrefugia lead to local adaptation

Macro- and microrefugia probably differed in their environmental conditions. For example, glacial microrefugia in Europe and North America, which were generally located north of the main macrorefugial areas, were likely to have been colder and experienced shorter growing seasons than macrorefugia south of the ice sheets. Analogously, interglacial microrefugia situated in alpine areas are generally found at much lower latitude than the main polar macrorefugia.

At the interspecific level, there is evidence that survival in northern microrefugia selected for species with specific traits (Bhagwat & Willis, 2008). For example, tree species (e.g. *Salix* spp.) that survived in high-latitude refugia in Europe during the last glaciation tended to be wind-dispersed habitat generalists with the ability to reproduce vegetatively (Bhagwat & Willis, 2008). Similarly, mammals that survived in high-latitude Northern Hemisphere glacial refugia (e.g. *Vulpes vulpes*) tended to be habitat generalists with present-day northerly distributions (Bhagwat & Willis, 2008). These interspecific examples provide indirect evidence that environmental conditions in microrefugia differed from those in

macrorefugia, and suggest that these differences could also lead to divergence at the intraspecific level.

We therefore hypothesize that, at the intraspecific level, populations that survived in both micro- and macrorefugia should be differentially adapted to environmental conditions that differed between micro- and macrorefugia. A test of this hypothesis would be to compare populations of the same species that survived in a micro- and macrorefugia, and assess differences in trait values that are associated with specific environmental conditions (e.g. cold tolerance, increased vegetative growth, etc.).

3. Reduced diversity increases ecological opportunity and promotes ecological divergence in microrefugia

Reduced species diversity in microrefugia may lead to reduced interspecific competition or to under-used niches. This in turn can increase ecological opportunity, which has long been hypothesized to be a catalyst for diversification (Simpson, 1953; Schluter, 2000a; Losos, 2010). Evidence for the role of ecological opportunity in priming diversification is reviewed in Schluter (2000b), and famous examples involving lizards (*Anolis* spp.) and fish (*Gasterosteus aculeatus*) are provided by adaptive radiations in species-poor archipelagos (e.g. Losos *et al.*, 1998) and in newly colonized post-glacial lakes (e.g. Schluter, 1996), respectively.

We hypothesize that microrefugial populations should exhibit more ecological divergence than macrorefugial populations due to increased ecological opportunity in species-poor microrefugia. At least one of three patterns is expected in cases where competition is released (Schluter, 2000b): (1) increased trait variance in the microrefugial population; (2) directional changes in trait value if the population switches to occupying a different niche left empty in the microrefugial environment; or (3) the novel evolution of distinct ecomorphs that utilize alternative niches within the microrefugia. Tests of this hypothesis would involve comparisons of mean (or variance in) trait values between populations of the same species that survived in micro- and macrorefugia. Note that at least in the case of the first two patterns, differences in trait value could occur via random processes and that competition for niche space with a species in the macrorefugium should be demonstrated to make a more convincing case. For example, a limnetic form of lake whitefish (*Coregonus clupeaformis*) has evolved from the benthic ancestral form several times independently in northern temperate lakes (Bernatchez & Dodson, 1990). There is strong evidence suggesting that isolation in refugial lakes without a limnetic competitor (*Coregonus artedi*) was a necessary condition for the evolution of the limnetic form of *C. clupeaformis* (Pigeon *et al.*, 1997; Lu & Bernatchez, 1999). In addition, there is evidence that isolation of lake whitefish in a microrefugium that existed in a corridor between the Mackenzie Mountains and the Alaskan slope contributed to the origin of the limnetic divergence in at least one population in the Lower Liard River, British Columbia (Prest, 1970; Ford, 1974; Foote *et al.*, 1992).

4. Reduced species diversity in microrefugia allows more specific species interactions and promotes coevolution among species

Isolation in microrefugia may lead to the evolution of locally adapted species interactions. The geographical mosaic theory of coevolution suggests that interactions between species vary across the landscape (Thompson, 1994, 1999a,b), such that coevolution is an important process in some locations (i.e. coevolutionary hotspots), but not in others (i.e. coevolutionary coldspots). Theory suggests that community diversity has a strong influence on the specificity of interactions among species (Brown & Vincent, 1992). Also, with decreased community diversity, we expect more specific antagonistic interactions to evolve (Thrall *et al.*, 2007; Poisot *et al.*, 2011). There is empirical evidence that coevolutionary hotspots occur in communities with reduced diversity. For example, the absence of red squirrels (*Tamiasciurus hudsonicus*) in isolated stands of Rocky Mountain lodgepole pine (*Pinus contorta* ssp. *latifolia*) has led to the coevolution of red crossbills (*Loxia curvirostra*) and Rocky Mountain lodgepole pine (Benkman *et al.*, 2001). We hypothesize that, if reduced species diversity (e.g. the removal of competitors) results in more specific species interactions, microrefugia may be associated with coevolutionary hotspots. A test of this hypothesis might compare populations of the same pairs of species that survived in a macro- and microrefugia (e.g. with and without competitors, respectively), and test for the presence of differential coevolution. The most likely candidates here may be species with strong interactions, such as pollinating insects and their host plants, or parasites and their hosts. Margraf *et al.* (2007) studied local adaptation among alpine leaf beetles (*Oreina elongata*) in interglacial refugia (i.e. isolated alpine habitats) with different host plant assemblages. Contrary to our hypothesized effect of community diversity on the evolution of specific species interactions, Margraf *et al.* (2007) found no evidence for more local adaptation or greater host plant specificity in leaf beetle populations with less diverse host plant communities. Nonetheless, the study is an example of how this hypothesis might be tested.

In summary, we contend that an important but largely ignored implication of microrefugia is that they harboured less diverse communities than macrorefugia. We further argue that the reduced diversity of communities in microrefugia and the smaller size of populations in microrefugia have important implications for the ecology and evolution of the populations that survived in such refugia. We have proposed a series of hypotheses that attempt to catalyse two conceptual shifts urged by Hampe & Jump (2011): to go beyond descriptive biogeography and adopt an ecological and evolutionary focus, and to emphasize refugial populations as opposed to whole species. Research that aligns with these conceptual shifts has already begun to evaluate the importance of microrefugia to the evolution of species and populations (e.g. Margraf *et al.*, 2007). We hope that the field will continue to move from documenting the presence of microrefugia towards testing specific hypotheses about their

ecological and evolutionary consequences. As Hampe & Jump (2011) point out, microrefugial populations are important components of local and regional biodiversity, and can tell us much about the ecological and microevolutionary processes that occur during the process of climate change.

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BIOSKETCHES

Jonathan Mee and **Jean-Sébastien Moore** are broadly interested in the evolution of ecological divergence. They were sharing an office and finishing their PhDs on the diversity and phylogeography of North American fishes (*Chrosomus* spp. and *Salvelinus alpinus*, respectively) in Rick Taylor's lab at the University of British Columbia when their ideas for this editorial first took shape.

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