Genomics of microgeographic adaptation in the Amazonian hyperdominant tree *Eperua falcata* Aubl. (Fabaceae)

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Abstract

Plant populations are able to undergo very localized adaptive processes, that allow continuous populations to adapt to divergent habitats in spite of recurrent gene flow. Here, we carried out a genome scan for selection through whole-genome sequencing of pools of populations, sampled according to a nested sampling design, to evaluate microgeographic adaptation in the hyperdominant Amazonian tree *Eperua falcata* Aubl. (Fabaceae). A high-coverage genomic resource of ~250 Mb was assembled *de novo* and annotated, leading to 32 789 predicted genes. 97 062 bi-allelic SNPs were detected over 25 803 contigs, and a custom Bayesian model was implemented to uncover candidate genomic targets of divergent selection. A set of 290 divergence outlier SNPs was detected at the regional scale (between study sites), while 185 SNPs located in the vicinity of 106 protein-coding genes were detected as replicated outliers between microhabitats within regions. These genes possibly underlie ecologically important phenotypes and tend to indicate that adaptation to microgeographic habitat patchiness would affect genomic regions involved in a variety of physiological processes, among which plant response to stress (for e.g., oxidative stress, hypoxia and metal toxicity) and biotic interactions. Identification of genomic targets of microgeographic adaptation in the Neotropics supports the hypothesis - frequently raised at the community level - that local adaptation would be a key driver of ecological diversification, probably operating across multiple spatial scales, from large- (i.e. regional) to microgeographic- (i.e. landscape) scales.

Key words: Amazonia, *Eperua falcata*, Pool-Seq, genome scan, SNPs, Bayesian modelling, genomic divergence, local adaptation.

Introduction

Plant populations are able to undergo very localized adaptive processes, that allow continuous populations to adapt to divergent habitats in spite of recurrent gene flow. This phenomenon has been repeatedly observed in annual plants growing in strongly contrasting yet geographically adjacent habitats, particularly in cases with large differences in soil composition (Bradshaw 1960; Jain and Bradshaw 1966; Antonovics and Bradshaw 1970; Antonovics 2006; Gould *et al.* 2014); in some cases, molecular evidence also supports adaptation to habitat mosaics (Turner *et al.* 2010; Fustier *et al.* 2017). In the case of *Howea* palm trees, Savolainen *et al.* (2006) have elegantly provided cogent evidence of a link between adaptation to habitat mosaics and sympatric speciation. The process underlying these instances of adaptation with gene flow has been dubbed 'microgeographic adaptation', whereby adaptive divergence occurs at geographical scales of the same order of magnitude as gene dispersal distance (Richardson *et al.* 2014). Adaptation to multiple optima within a continuous population has been identified as a major mechanism in the maintenance of genetic diversity (Delph and Kelly 2014); conditions under which adaptive divergence can occur with gene flow have been analyzed theoretically, mostly in models including two environmental patches connected by varying levels of gene flow (Bulmer 1972; Hendry *et al.* 2001; Yeaman and Guillaume 2009; Yeaman and Otto 2011; Schmid and Guillaume 2017). Quite counter-intuitively, these theoretical results show that a wide range of migration-selection equilibrium states can lead to adaptation to each patch with maintenance of polymorphism (i.e. maintenance of patch specialists, as opposed to emergence of generalist genotypes).

Data on perennial plants, and on trees in particular, suggest that this may be a general phenomenon, that can be readily observed at the phenotypic level (Yeaman and Jarvis 2006; Brousseau *et al.* 2013; Vizcaíno-Palomar *et al.* 2014; Lind *et al.* 2017) (but see Latreille & Pichot (2017) for a case of complete lack of microgeographical adaptation). At the molecular level, association between alleles (or genotypes) and habitats has been detected in a variety of cases (Cobb *et al.* 1994; Mitton *et al.* 1998; Kelly *et al.* 2003; Jump *et al.* 2006; Pluess and Weber 2012; Audigeos *et al.* 2013; Csilléry *et al.* 2014; Brousseau *et al.* 2015; Eckert *et al.* 2015; Lind *et al.* 2017), which led us to a reconsideration of the adaptive role of intra-specific and intra-populational variation (Scotti *et al.* 2016). The advent of genome-wide approaches has increased detection power, making it possible to more precisely evaluate the genetic bases of microgeographic adaptation (Turner *et al.* 2010; Eckert *et al.* 2015; Izuno

et al. 2017; Fustier *et al.* 2017; Lind *et al.* 2017). In general, those genome scan studies have found only a minority of loci (in the order of few percent) to be submitted to microgeographic disruptive selection. Yet the advantages of genome-wide, sequencing-based approaches go beyond a finer estimation of the number of loci involved in the process, because they permit formulating functional interpretations of the divergence process, when sequence annotation discloses the role of the target loci (Turner *et al.* 2010; Eckert *et al.* 2010, 2015; Fustier *et al.* 2017).

Microgeographic adaptation involves the filtering of phenotypes and genotypes that favor adaptation to habitat patches, and therefore can be viewed as a preliminary step towards diversification of populations, ecotypes and eventually species. In particular, environmental filtering has frequently been evoked as a driver of niche partitioning and species diversification in the Neotropics (Fine *et al.* 2004, 2005, 2013; Baraloto *et al.* 2007; Kraft *et al.* 2008; Fine 2015); intra-populational diversification processes may play a key role in the generation of functional diversity. Moreover, because it has the effect of maintaining adaptive genetic diversity, microgeographic adaptation can play a role in the determination of a species' short-term adaptive potential (Harrisson *et al.* 2014; De Kort and Honnay 2017), which largely depends on standing genetic variation. Measuring its extent in the wild is thus of major importance, as it could provide insights into the ability of populations to evolve towards new phenotypic and genetic optima. In this respect, retrospective analyses of past adaptation ('space-for-time' approach; Pickett (1989)) provide useful knowledge about the ability of local populations to cope with environmental variations and about the identity of the genomic regions responding to selection. In particular, local adaptation may improve species survival under global climate change (GCC). Theoretically, two evolutionary processes can allow populations to cope with GCC and avoid extinction (Aitken *et al.* 2008; Harrisson *et al.* 2014): populations can migrate to new areas to track ecological niches, causing shifts in the species' distribution, and/or adapt locally to the new conditions through the action of natural selection (Hoffmann and Sgro 2011). These processes are likely to occur simultaneously and populations' response to GCC can be viewed as a 'race where populations are tracking the moving optima both in space and time' (Kremer *et al.* 2012).

Trees are particularly good models to study microgeographic adaptation: many wild tree species have large, continuous stands, which span over a variety of microhabitats, harbor large amounts of genetic diversity, and display large dispersal distances (Hamrick and Godt 1990; Petit and Hampe 2006; Neale and Kremer 2011; Sork *et al.* 2013; Fetter *et al.* 2017). They therefore correspond quite closely to the conditions under which selection-migration

equilibrium theory has been developed. Moreover, methods to detect locus-specific divergence (and infer selection) should work quite well with microgeographic processes in forest tree stands, because their relatively shallow (spatial) population structures and large effective population sizes should minimize the introduction of biases (e.g., because of drift) and the generation of false positives.

Lowland Amazonian rainforests, whose landscapes show fine patchiness of contrasting topographic and edaphic conditions varying over microgeographic scales (**Fig. 1a,c,d**), are an ideal place to seek microgeographic adaptation processes. In these communities, a minority of hyperdominant tree species accounts for half of all stems (ter Steege *et al.* 2013). Some of these widespread species are also ecological generalists, whose preferences could be explained by individual phenotypic plasticity or by microgeographic adaptation to habitat patchiness (Fortunel *et al.* 2016). We focus here on dense, continuous stands of *Eperua falcata* Aubl. (Fabaceae) in French Guiana, with the goal of identifying genomic loci undergoing divergence at the microgeographic scale between habitats. To do this, a genome scan was carried out through a nested sampling design combining regional and microgeographic spatial scales (i.e. replicates of microhabitats, ~300 m apart, in two study sites, ~300 km apart); the data were submitted to analyses of divergence within a hierarchical Bayesian framework (Brousseau *et al.* 2016) designed to identify locus-specific departures from genome-wide divergence in nested population samples.

Materials and Methods

Species model

Eperua falcata Aubl. (**Fig. S1**) is a supposedly non-nodulating (Villadas *et al.* 2007) tree species of Fabaceae (Caesalpinioideae). It is widespread in North-East Amazonia, from North Brazil to Venezuela trough the Guiana shield (https://demo.gbif.org/species/2943522), and it is a 'hyperdominant' species, the 13th most abundant tree species of the Amazonian tree flora (ter Steege *et al.* 2013). It is an evergreen, late-successional canopy tree species which is often emergent. It is shade hemi-tolerant (Bonal, Barigah, *et al.* 2000) and well tolerant to both drought (Bonal, Atger, *et al.* 2000; Bonal *et al.* 2001) and water-logging (Baraloto *et al.* 2007). Its distribution is aggregative and populations are commonly found at high densities. It is a generalist with respect to microgeographic edaphic conditions, albeit it is more abundant

in water-logged forests of bottomlands (Baraloto *et al.* 2007). *E. falcata* flowers and fruits during the rainy season, and seeds reach maturity in April-May, synchronously among trees in all microhabitats. Its large pollen grains (>100µm) are dispersed by animals such as bats (Cowan 1975), while seed dispersal is autochorous: heavy seeds are dispersed around crowns of mother trees through explosive pod dehiscence.

Sampling design and study sites

We conducted a sampling design that replicated the environmental contrast 'bottomland *versus* terra-firme' (~300 m apart) at two regional locations (i.e. study sites) of the Guiana shield (~300 km apart), **Fig. 2a**. Laussat (western French Guiana) and Regina (eastern French Guiana) study sites are located on the coastal Guiana shield, about 300 km apart, and experience different rainfall regimes (~2 500 and 3 500 mm/years, respectively) (Brousseau *et al*. 2015). Each regional location harbors different microhabitats (hygromorphic sandy bottomlands and ferralitic *terra-firme*) separated by short distances (~300 m), and *E. falcata* trees are found through the study area, thus allowing gene flow between tree populations occupying the two different microhabitats (Brousseau *et al*. 2015), **Fig. 2b**. In Laussat, a plateau of low elevation is adjacent to a permanently water-logged bottomland, while Regina is composed of higher elevation hilltops bordered by abrupt slopes surrounding a seasonally flooded bottomland forest, **Fig. S2**. *E. falcata* stem (> 20 cm diameter at breast height) density varies between 30 and 50 trees / ha at these locations (Brousseau *et al.* 2015). Twenty adult trees for each of the four *regional location* × *microhabitat* combination were selected, totaling 80 individuals.

Molecular methods

Genomic DNA was extracted from fresh leaves according to a modified CTAB protocol (Doyle and Doyle 1987): CTAB buffer was supplemented in proteinase K in concentration 80 µg/ml, and the deproteinization step was realized twice (once with Dichloromethane:Chloroform:Isoamylalcool 24:24:1 and once with Chloroform:Isoamylalcool 24:1). Genomic DNA was eluted in Tris-EDTA 0.5X with RNAse A in concentration 10 µg/ml, and quantified twice using a Nanodrop 2000. Individuals belonging to the same regional location and microhabitat were pooled together in equimolarity, resulting in four libraries of pooled individuals with total DNA concentrations

ranging between 180 and 240 ng/µl. The four libraries were sequenced (paired-end) on three lanes of an Illumina HiSeq 2500. Each library was distributed over the three lanes, and each lane received the different libraries in equal quantity.

Bioinformatics pipeline

Reads pre-processing. Paired-end reads (100 nt) were pre-processed (cleaned and filtered) before assembly. Individual bases of low quality were masked using the 'Fastq_masker' tool of the suite fastx_toolkit (http://hannonlab.cshl.edu/fastx_toolkit) under a quality threshold of 25. Reads-ends were trimmed using Fastq_trimming' (fastx_toolkit) by trimming the first and last ten bases of each reads (resulting in reads of 80 nt). Reads were further sorted and orphan reads were excluded using Sickle (Joshi and Fas 2011) with the following parameters: minimum per base quality threshold = 25 and minimum read length = 70. The quality of the cleaned reads was assessed using 'fastqc'. Reads pre-processing resulted in 2 332 116 952 high-quality reads (**Table S1**). Cleaned reads have been deposited on the European Nucleotide Archive (ENA, EMBL), under project accession code PRJEB9879, sample accession codes ERS791957, ERS791958, ERS791959, ERS791960.

***De novo* assembly and mapping.** Because *E. falcata* is a non-model species with no reference genome, cleaned reads were assembled into a crude *de novo* genome assembly which constitutes a large new pan-genomic resource. Because classical assemblers do not easily support the numerous polymorphisms contained in libraries of pooled individuals (resulting in the segregation of reads from different populations pools into different assembled contigs and scaffolds), the sequenced libraries were *de novo* assembled using RayMeta (Boisvert *et al.* 2012), an assembler originally developed for meta-communities. The different libraries were assembled together in order to maximize the coverage of the *de novo* assembly, with a kmer size of 21. The assembled contigs were then aligned together in order to re-align redundant contigs using CD-HIT-EST (Li and Godzik 2006; Fu *et al.* 2012) with a sequence identity threshold of 95%. Non-redundant contigs were retained and used as reference to map *a posteriori* each library separately using bwa (Li and Durbin 2009): 'bwa aln' and 'bwa sampe' (only paired reads were mapped). The .sam files (one by library) were converted into .bam, sorted, indexed and merged into a unique .mpileup file using the samtools suite (Li *et al.* 2009): 'samtools view', 'samtools sort', 'samtools index' and 'samtools mpileup'.

Structural and functional annotation. The crude pangenomic reference was annotated with the suite implemented in Maker mpi v. 2.31.8 (Cantarel *et al.* 2008) and associated tools. In

summary, repeats elements were detected by repeatMasker v.4.0.3 (http://www.repeatmasker.org/) and *ab initio* gene prediction was realized by Augustus v.2.6.1 (Stanke *et al.* 2006) at the scaffold level using both the reference transcriptome of *E. falcata* (Brousseau *et al.* 2014) and a composite proteome reference composed of all published reference proteomes of Fabaceae (*Cajanus cajan*, *Cicer arietinum*, *Glycine max*, *Lupinus angustifolius*, *Medicago truncatula*, *Phaseolus angularis*, *Phaseolus vulgaris* and *Vigna radiata*) plus the reference proteomes of *Arabidopsis thaliana*. The predicted proteins were further blasted against the non-redundant protein database with NCBI blastp v.2.6.0. At last, gene ontology terms of each predicted gene were retrieved using Interproscan 5.21.60 (Jones *et al.* 2014).

SNP detection and Filtering. The absolute abundance of different nucleotides was counted for each assembled position through PoPoolation2 mpileup2sync.jar script (Kofler *et al.* 2011). The .java.sync output file containing nucleotide counts for each position of the assembly was post-processed through custom perl scripts. (1) Monomorphic sites were discarded and rare SNPs rare were masked (replaced by 'N') under a minimum allele frequency (maf) threshold of 0.05, all libraries confounded (meaning that a minimum number of 8 alternative variants out of 160 sequenced gametes in the whole dataset was required for a polymorphism to be retained). (2) Among the remaining SNPs, only bi-allelic polymorphisms with a total maximum coverage of 200X over all libraries to avoid SNPs caused by potential misassembly of paralogs into the same contigs and scaffolds. (3) Bi-allelic SNPs with library-level coverage < 20X were discarded, resulting in a SNP dataset composed of SNPs with coverage > 20X within each of the four libraries. (4) Contiguous SNPs were further filtered out to avoid polymorphisms caused by copy number variation (CNV). (4 and 5) SNPs located within repeat elements (detected by repeatMasker) as well as SNPs located in contigs with SNP density > 0.02 were filtered out, once again to avoid SNPs located in paralogs.

Computation of basic diversity statistics

Nei's molecular diversity π (Nei 1987) was computed for each of the four sub-populations at the contig level on unphased SNP data, under the hypothesis of random mating, as the sum of unbiased estimates of heterozygosity at the nucleotide (SNP) level (Nei 1987):

$$\pi = \sum_{i}^{S} H_{e,i}$$

with S = number of polymorphic loci in a contig, and

$$H_{e,i} = H'_{e,i}\frac{n}{n-1}$$

where n = sample size and $H'_{e,i}$ = expected heterozygosity at the ith polymorphism in the contig. Because different contigs have different lengths, to compare across contigs we present $\pi_L = \pi/L$, where L is contig length, that is, molecular diversity per base.

Bayesian modelling of genomic divergence and outlier detection

A Bayesian modelling approach was used to identify outlier SNPs under selection. Brousseau *et al.* (2016) has described this Bayesian framework in details and its power has been assessed through simulations, revealing low false discovery rate and low false non-discovery rate. Briefly, allele frequencies were inferred from read counts and pairwise locus-specific G_{ST} was estimated in the model, thus integrating coverage variability across the genome and libraries.

$$n_{i,m} \sim Bin(p_{i,m}, N_{i,m})$$

$$q_{i,m} = 1 - p_{i,m}$$

where $n_{i,m}$ is the read count of the reference allele and $N_{i,m}$ the total number of read counts at marker m in population i. $p_{i,m}$ and $q_{i,m}$ are allele frequencies of the reference and the alternative alleles respectively.

$$GST_{(i,j),m} = (Ht_{(i,j),m} - Hs_{(i,j),m})/Ht_{(i,j),m}$$

$G_{ST(i,j),m}$ is the Nei's fixation index (i.e. genetic differentiation) between populations i and j at marker m. $H_{T(i,j),m}$ and $H_{S(i,j),m}$ are the expected heterozygosities in the total metapopulation and within subpopulations respectively:

$$Hs_{(i,j),m} = (p_{i,m}q_{i,m}) + (p_{j,m}q_{j,m})$$

$$Ht_{(i,j),m} = 2\bar{p}_m\bar{q}_m = 2\left(\frac{p_{i,m} + p_{j,m}}{2}\right)\left(\frac{q_{i,m} + q_{j,m}}{2}\right)$$

Pairwise G_{ST} was further partitioned into genome-wide and locus-specific components according to a hierarchical framework: neutral (genome-wide) divergence both between study sites and between microhabitats within each study sites (**Fig. 2a**), and adaptive (locus-specific) divergence both between sites and between microhabitats common to both sites.

$$GST_{(i,j),m} \sim N(\mu_{(i,j),m}, \sigma_R^2)$$

$$\mu_{(i,j),m} = kr_{(i,j)}(\mu r + \theta r_m) + kl_laussat_{(i,j)}(\mu l_laussat + \theta l_m) + kl_regina_{(i,j)}(\mu l_regina + \theta l_m)$$

$$kr_{(i,j)} = \begin{cases} 0 \\ 1 \end{cases}; kl_laussat_{(i,j)} = \begin{cases} 0 \\ 1 \end{cases}; kl_regina_{(i,j)} = \begin{cases} 0 \\ 1 \end{cases};$$

μr and μl are the global mean of differentiation (over all markers) at regional and local scales respectively: μr is the global mean of differentiation between sites, $\mu l_laussat$ is the global mean of differentiation between microhabitats within the study site of Laussat, and μl_regina is the global mean of differentiation between microhabitats within the study site of Régina.

$kr_{i,j}$ and $kl_{i,j}$ are binary matrices describing whether the populations i and j belong to the same study site (regional scale) and to the same microhabitat within each study site (local scale): $kr_{i,j} = 1$ if the two populations i and j belong to the different study sites, $kr_{i,j} = 0$ otherwise; $kl_laussat_{i,j} = 1$ if the two populations i and j belong to different microhabitats within the study site of Laussat, $kl_laussat_{i,j} = 0$ otherwise; $kl_regina_{i,j} = 1$ if the two populations i and j belong to different microhabitats within the study site of Régina, $kl_regina_{i,j} = 0$ otherwise.

θr_m and θl_m are locus-specific parameters describing the departure from the global means of differentiation at regional (i.e. between sites) and local (i.e. between microhabitats) scales. Posteriors of θr_m and θl_m are normally distributed allowing thus to attribute an empirical Bayesian p-value (eBP-value) to each SNP. SNPs with an eBP < 1% were considered as outliers under divergent (upper tail) or homogenizing (lower tail) selection between sites (θr_m) or microhabitat within sites (θl_m).

The Bayesian model was encoded in stan (Carpenter *et al.* 2016) and compiled into C++ using Rstan. Because Bayesian inference is computationally expensive, the model was applied to subsets of 10 000 SNPs on a Dell Power Edge CG220 Intel Xeon (CPU E5-2670 v2 @2.50Ghz) cluster node with parallelization on 5 CPU (with parameters: virtual memory vmem = 25G, virtual memory limit h_vmem=30G and parallel_fill = 5) and explicit chains parallelization in R with the package 'parallel'. Both dataset hashing and explicit parallelization optimized computation times and the model ran in ~ 4 hours / subset. Outputs were post-processed with R, including chain convergence checking and eBP values inference from the posterior distribution of locus-specific parameters. Due to the large number of SNPs screened, a correction for multiple-testing was applied on eBP values with the package 'fdrtool' with a FDR threshold of 10%. The proportions of outliers under selection was compared between geographic scales with chi-squared tests ('prop.test' function). Enrichment tests were further conducted through X^2 tests in order to test (i) the enrichment of outliers located within exons, within a predicted gene and in the neighborhood (< 5 kb) of a predicted

gene compared to the whole SNP dataset, as well as (ii) the enrichment of GO terms between predicted genes neighboring outlier SNPs compared to all predicted genes of the entire reference.

Results

The crude reference genome produced here, although incomplete and fragmented, constitutes a valuable pan-genomic resource containing key information about population-level genetic diversity (i.e. tens of thousands of loci scattered throughout the genome and supposedly representative of it, a first for a non-model tree species of Amazonia). The four population pools were assembled *de novo* into a high-coverage pan-genomic reference: *de novo* assembly led to 325 249 contigs in 272 865 scaffolds for a total assembly length of about 250 Mb (252 364 908 bases assembled *de novo* of which 249 308 901 were successfully mapped *a posteriori* by the different libraries, **Table S1)** and a mapping coverage of 57.81X on average, **Fig. S3**. The structural annotation predicted 32 789 genes and 117 278 exons, and detected 124 287 repeated elements. The functional annotation of the predicted genes resulted in 32 075 successfully blasted genes, and only 732 predicted genes did not return any blast hit. Additionally, gene-ontology terms (GO terms) were retrieved for 18 577 predicted genes, while the remaining 14 212 genes were not associated to any GO term.

A total of 97 062 bi-allelic SNPs scattered across 25 803 contigs and 23 276 scaffolds were identified, according to a stringent procedure, with a final SNP density of 0.003/base (*sd*=0.004) with a Ti/Tv=1.80, **Table S2** and **Fig. S4**. Normalized molecular diversity π_L varied between 9×10^{-6} and 8×10^{-3} (**Table S3**), with little variation among populations. 41 050 SNPs (42.3%) were located in scaffolds with at least one predicted gene while 56 012 SNPs (57.7%) were located in scaffolds without any predicted gene. 26 435 SNPs (27.2%) were located within a predicted gene, of which 10 863 (11.2%) within exons, while 14 615 SNPs were in the neighborhood of a predicted gene with a mean distance to a predicted gene of 737 bp (with min = 1bp and max = 27.2 kb).

The Bayesian model revealed low genome-wide differentiation between populations and significant departures from neutral expectations for a small subset of SNPs. Genome-wide genetic differentiation (average G_{ST} across loci) ranged between 0.026 and 0.032 at both geographical scales, **Fig. S5**. Accordingly, genome-wide parameters of divergence estimated through G_{ST} partitioning were not higher between regions than between microhabitats within regions, as confirmed by overlapping credible intervals, **Table S4**. Locus-specific departures

from genome-wide divergence (i.e. outlier SNPs) were identified from the distribution of locus-specific parameters (θr_m and θl_m). Between 510 and 2 045 outlier SNPs were detected to be under selection with an eBP threshold of 1%: 375 SNPs under homogenizing selection at regional scale, 2 045 SNPs under divergent selection at regional scale, 510 SNPs under homogenizing selection at local scale and 1 770 SNPs under divergent selection at local scale. After multiple-testing correction, the number of significant SNPs fell to 475 outlier SNPs (0.49% of all SNPs screened): 290 SNPs (in 268 contigs) under divergent selection at the regional scale and 185 SNPs (in 168 contigs) under divergent selection at the local scale, with no outlier shared between the regional and the local scales. Outlier SNPs under divergent selection were characterized by clines in allele frequencies between regional locations and/or local microhabitats. Clines in allele frequency across sites and microhabitats are illustrated in **Fig. 4** for different kinds of neutral and outlier SNPs. A total of 140 outlier SNPs (29.5% out of 475 outlier SNPs) were located within predicted genes, among which 39 (8.2%) were within exons, and 86 are in the neighborhood (< 5kb) of one or several predicted genes (**Supplementary files S1 and S2**). However, outlier SNPs were not more likely to be located within exons (X^2 = 3.9368, df = 1, p-value = 0.05), within predicted genes (X^2 = 1.0846, df = 1, p-value = 0.30), or in the neighborhood of predicted genes (X^2 = 3.1964, df = 1, p-value = 0.07), than any SNP of the entire dataset. Nevertheless, 87 GO-terms were significantly enriched in the subset of genes surrounding outlier SNPs (84 genes) compared to all predicted genes of the entire reference at the regional scale, and 100 GO-terms were significantly enriched in the subset of genes surrounding outlier SNPs (106 genes) at the local scale, **Table S5**.

Discussion

This study analyzes patterns of genomic divergence associated to microgeographic (topographic and edaphic) habitat patchiness in *E. falcata* in French Guiana. In this species, former studies reported high heritability for quantitative growth and leaf traits in a common garden experiment (Brousseau et al., 2013), suggesting high levels of standing genetic variation for functional phenotypic traits. In addition, seedlings native to different microhabitats displayed inherent differences in phenotypic traits probably due to a combination of neutral processes (i.e. short-distance seed flow and local inbreeding) and local adaptation (Audigeos *et al.* 2013; Brousseau *et al.* 2015). However, preliminary investigations

about the evolutionary drivers behind microgeographic genetic divergence were based on datasets restricted to few (e.g. candidate gene approach, Audigeos *et al.* (2013)) or anonymous markers (AFLPs, Brousseau *et al.* (2015)), and the study of microgeographic adaptation lacked a comprehensive survey at the genomic scale.

Patterns of genome-wide divergence.

Genome-wide (supposedly neutral) differentiation was low whatever the geographic scale, ranging from 2 to 3% (**Fig. S5**), similarly to what previously found by Brousseau *et al.* (2015), who reported F_{ST} values between 2 and 3% between microhabitats within sites, and about 3% between these study sites (based on a screening of about 1 100 AFLPs). In two additional study sites in French Guiana (Paracou and Nouragues), another study (Audigeos *et al.* 2013) provided comparable estimates: $F_{ST} \cong 1\%$ between study sites and F_{ST} from 1 to 2% between microhabitats within sites. SNP density estimated here, a mean of 3 SNP every 1 000 bp (**Table S2**), was quite low: Neale and Kremer (2011) for example reported that SNP frequency in trees is generally in the range 1 SNP per 100 bp. However, in our study the stringent SNP filtering probably filtered out real polymorphisms, leading to an under-estimation of genome-wide SNP density.

Low genome-wide differentiation suggests that high levels of gene flow regularly homogenize genome-wide genetic diversity both between regions and between microhabitats within regions. The consequences of strong gene flow on population adaptability can be paradoxical. On one hand, extensive gene flow can allow the spread of advantageous alleles between populations, thus granting high adaptive potential for populations established in heterogeneous environments. On the other hand, gene flow may also counteract the effect of natural selection, with migration load halting adaptive divergence of populations into locally adapted subpopulations (Lenormand 2002; Le Corre and Kremer 2012; De Kort *et al.* 2015; Pujol *et al.* 2018). The antagonist effect of gene flow on adaptive divergence could explain the low proportion of outliers detected (see below). Nevertheless, high gene flow (and resulting low genome-wide differentiation) did not completely prevent adaptive divergence between populations.

Microgeographic adaptation.

Locus-specific (putatively adaptive) departures from genome-wide divergence were detected for 0.49% of the 97 062 SNPs analyzed. We detected significantly more outliers at the regional than at the microgeographic scale: 290 *versus* 185 outlier SNPs (significant X^2 = 22.826, df = 1, p = 1.77×10^{-6}). This can be viewed as the logical consequence of an order of magnitude difference in the geographical distances between populations: 300 meters between microhabitats within sites versus 300 km between study sites. Indeed, ecological conditions are probably more variable - and divergent selection stronger - at the regional scale than divergent selective pressures between microhabitats separated by hundreds of meters, and the effect of divergence may be reinforced by more limited gene flow. The proportion of outliers detected over microgeographic scales, albeit lower, remains nevertheless non negligible. Because the Bayesian model focuses on replicated footprints of adaptation only, it is extremely unlikely that the detected outliers could have been structured between microhabitats in two different regions only by chance (Savolainen *et al.* 2013). Moreover, no outlier was detected jointly at both spatial scales, as shown by the heart-shaped joint distribution of regional- and local-scale locus-specific parameters (**Fig. 3**). Rather, outliers were specific to one spatial scale, suggesting that different portions of the genome respond to different types of environmental constraints; in future studies, local adaptation in wild populations should be reconsidered by integrating different spatial scales, including microgeographic habitat patchiness. It is however important to stress that our Bayesian approach may miss footprints of polygenic adaptation, if adaptation acts in different ways in the two study sites (through different selection strengths and/or different loci affected), even more in case of weak selection strength at many SNPs (Brousseau *et al.* 2016), which is known to be common in nature (Berg and Coop 2014; Lind *et al.* 2017; Harrisson *et al.* 2017). Indeed, F_{ST}-based methods are more prone to detect adaptation caused by loci of large effects than by loci with small effects, especially since adaptation occurs with ongoing gene flow (Hedrick 2006; Yeaman and Whitlock 2011; Le Corre and Kremer 2012; Savolainen *et al.* 2013). In other words, the set of outliers detected is probably enriched in loci with large effects. Even if the Bayesian model employed here proved its robustness to detect footprints of polygenic adaptation caused by weak selection strengths (s=0.05) in simulations (Brousseau *et al.* 2016), it may still miss footprints of polygenic adaptation if the strength of selection is variable among loci (Sork *et al.* 2013).

By providing evidence of adaptation to microgeographic habitat patchiness, our results agree with previous studies showing soil variation as a driver of adaptation in trees: for example, adaptation to soil nutrient composition such as potassium deficiency in *Cornus*

florida (Cornaceae) (Pais *et al.* 2017), to aluminum and phosphate ion concentrations in *Pinus contorta* (Pinaceae) (Eckert *et al.* 2012), to substrate age in *Metrosideros polymorpha* (Myrtaceae) (Izuno *et al.* 2017), to soil types in *Abies religiosa* (Pinaceae) (Méndez-González *et al.* 2017), or to soil water availability in *Pinus albicaulis* (Pinaceae) (Lind *et al.* 2017). Preliminary evidence of microgeographic adaptation has been recently reported in Neotropical tree species, including *E. falcata* (Audigeos *et al.* 2013; Brousseau *et al.* 2013, 2015; Torroba-Balmori *et al.* 2017). At the community level, the study by Fortunel *et al.* (2016) on differential seedling mortality in different habitats suggests a mechanism through which microgeographic adaptation may take place. Topography-linked microgeographic patchiness is likely driven by a bundle of abiotic and biotic factors (including soil types (Misiewicz and Fine 2014), water-logging and flooding events, water availability (Clark *et al.* 1999), seasonal soil drought (Markewitz *et al.* 2010), soil fertility (John *et al.* 2007), light availability (Ferry *et al.* 2010), competition and predation (Fine *et al.* 2004)), hence a variety of classes of loci may respond to selection for microgeographic adaptation. It is, however, difficult to identify the exact genomic targets of natural selection, particularly in non-model species, because of potential hitchhiking between causal mutations and the analyzed SNPs, and because of the lack of chromosome-level genome assembly and of reliable genome-wide estimates of linkage disequilibrium in this species. Despite these limitations, the identity of the genes neighboring outlier SNPs may provide useful information, as many 'divergence' outlier SNPs detected here were located within or near (< 5 kb) protein-coding genes involved in a variety of biological functions (**Supplementary files S1 and S2**), with 106 genes neighboring a genomic footprint of microgeographic divergence and 100 Gene Ontology terms (corresponding to 49 genes) significantly enriched in the subset of genes neighboring outlier SNPs, **Table S5**. Some of them are particularly meaningful regarding their biological significance in terms of survival, growth, defense and reproduction (**Supplementary files S1 and S2**).

We notably detected several genes involved in plant response to stress near outlier SNPs. For example, we detected a glutaredoxin-C11 (predgene_010110) involved in managing the level of reactive oxygen species (ROS) in plant cells (Mittler *et al.* 2004), a H_2O_2-responsive gene encoding a metallopeptidase M24 family protein which is up-regulated under stress in *Arabidopsis thaliana* (Ordoñez *et al.* 2014), and a gene encoding a plant cysteine oxidase 4-like (predgene 003921) involved in response to flooding and soil hypoxia (Weits *et al.* 2014; White *et al.* 2017). Oxidative stress is very common in bottomlands, where water-logging and seasonal flooding events cause soil hypoxia and oxygen deprivation in root

cells, leading to the production of ROS (Blokhina *et al.* 2003). This observation is concordant with a previous candidate-gene approach in which a catalase (i.e. a gene involved in ROS detoxification) has been reported under selection in this species (Audigeos *et al.* 2013). We also detected five genes with possible implications in lipid biosynthesis (predgenes 006075, 019500, 002577, 001066, 000689), as for example a palmitoyl-acyl carrier protein thioesterase which is known to play an essential role for plant growth and seed development (Dörmann *et al.* 2000; Bonaventure *et al.* 2003). Lipid metabolism is of high importance for plant development, from early germination to reproduction. Indeed, lipids are the primary material for cutin, and they are largely involved in stress signaling and resistance (Okazaki and Saito 2014). We also detected two genes involved in proline amino acid biosynthesis (predgenes 007023 and 007024), which is accumulated in response to drought stress in plants (Fu *et al.* 2018). A calmodulin-like protein (probable CML48, predgene 022002) was also detected in the neighborhood of a microgeographic outlier. Calmodulin and calmodulin-like plant calcium sensors have evolved unique functions of high importance in the regulation of plant development and plant response to stress (Perochon *et al.* 2011). We also detected a probable cellulose synthase A (predgene 000745) whose mutations can alter morphology or induce resistance to cellulose biosynthesis inhibitors as reported in *A. thaliana* (Richmond 2000), a gene for plant metal tolerance (predgene 014347), and a mRNA-decapping enzyme-like (predgene 011170) which is of importance for early seedling development (Goeres *et al.* 2007).

We also detected two genes mediating biotic interactions - through plant defense against pathogens and recognition of symbiotic bacteria - as potential targets of microgeographic adaptation. In particular, plant chemical defense against herbivores and pathogens is supposed to play a key role in microgeographic adaptation; previous studies have experimentally demonstrated that a trade-off between growth and antiherbivore defenses strengthens habitat specialization between congeneric species within large Neotropical plant families (e.g. Annonaceae, Burseraceae, Euphorbiaceae, Fabaceae, Malvaceae) (Fine *et al.* 2004, 2013). Here, we detected a chitinase (predgene 000254), a family of enzymes known for their role in resistance to pathogens (Punja and Zhang 1993; Grover 2012). We also discovered a gene involved in recognition of symbiotic bacteria (LysM receptor-like kinase, predgene 002974) which notably enables the model legume *Lotus japonicus* to recognize its bacterial symbiont (Madsen *et al.* 2003; Radutoiu *et al.* 2003; Ryals *et al.* 2008). Symbiotic nitrogen fixation is a specific feature of legumes, and nutrient availability largely varies across microhabitats (Ferry *et al.* 2010). The presence of genes involved in the perception of

symbiotic bacteria near genomic footprints of microgeographic adaptation in this supposedly non-nodulating legume (Villadas *et al.* 2007) is particularly interesting. However, accurate functional characterizations of the proteins surrounding outlier SNPs are required to draw reliable conclusions about the physiological processes involved in microgeographic adaptation.

Microevolution in the (Neo)tropics.

Although largely exploratory, our results open the way to uncover the evolutionary processes underlying the generation of (Neo)tropical biological diversity, by supporting the hypothesis of niche as a driver of diversification (Leibold 2008). Microgeographic adaptation driven by a bundle of abiotic and biotic factors may be an initial step towards tree specialization and ecological (sympatric) speciation (Savolainen *et al.* 2006; Feder *et al.* 2012). In this study we provide support for this process at the intra-populational level in an Amazonian tree species. However, the efficacy of microgeographic adaptive divergence at a small but non negligible fraction of loci in driving ecological speciation (as opposed to the effect of genome-wide neutral divergence, caused by strong restrictions in gene flow, in assisting allopatric speciation) need to be carefully assessed. It is also possible that microgeographic adaptation contributes to the generalist and hyperdominant distribution of *E. falcata* (ter Steege *et al.* 2013). In this view, local adaptation to habitat patches would maintain multiple adaptation optima (i.e. multiple genetic combinations of alleles at different loci), thus pre-adapting the populations to a multitude of environmental situations. When observed from a higher standpoint (that is, when looking at a population as a whole, containing multiple optima), this is tantamount to population-level balancing selection, which is considered as a major component of adaptive potential (Barrett and Schluter 2008; Delph and Kelly 2014). Such a reservoir of adaptively useful genetic diversity may help the species deal with GCC and other environmental challenges, including potential habitat fragmentation.

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Author contribution

LB, ED and IS designed the experiment. LB sampled leaf material, extracted and conditioned DNA, analyzed the data including the development of the Bayesian model under the supervision of IS and GVV. All authors wrote the article.

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Author information, data availability

Sequencing data have been deposited on the European Nucleotide Archive (ENA, EMBL), under the project accession code PRJEB9879. It contains the raw reads (accessions ERS791957, ERS791958, ERS791959, ERS791960) and the draft *de novo* genome (accession ERS791961). SNPs data are available on Figshare under the DOI 10.6084/m9.figshare.4212126. Data are under embargo until formal publication of this manuscript. In the meanwhile, correspondence and requests should be addressed to Louise Brousseau (louise.brousseau@ird.fr).

Online supporting information

Supplementary figures S1 to S5.

Supplementary tables S1 to S5.

Supplementary file S1. Interactive gene network to easily navigate through the functional annotation of genes neighboring 'divergence' outlier SNPs (< 5 kb) at the microgeographic scale. Green bubbles indicate predicted genes ; blue and pink bubbles indicate Gene ontology terms, with size depending on the number of predicted genes neighboring an outlier SNP flagged with each GO term ; pink bubbles indicate GO terms significantly enriched between the subset of predicted genes neighboring outlier SNPs compared to the all predicted genes.

Supplementary file S2. Blast and functional annotation of genes neighboring (< 5 kb) 'divergence' outlier SNPs at the microgeographic scale.

Figures legend

Fig. 1. (a) Relief map of forest landscapes in the region of Régina, French Guiana. The map was realized using the French 'Géoportail' (https://www.geoportail.gouv.fr/): official geospatial data were provided by the French Institute of Geographic and Forest Information ©IGN 2017. (b) *Eperua falcata* sapling. (c) and (d) Forest communities associated with topography and related soil types: *terra-firme* forests on ferralitic soils at the top of hills (c), water-logged forests on hydromorphic soils in bottomlands (d).

Fig. 2. (a) Sampling design scheme and correspondence with genome-wide parameters of divergence estimated through the hierarchical Bayesian model at regional (μr) and local scales ($\mu l_{Laussat}$ and $\mu l_{Régina}$ respectively). (b) Distribution of geographic distances between trees in the study sites of Laussat (Western French Guiana) and Régina (Eastern French Guiana). Vertical dotted bars indicate the range of habitat turnover in each study site.

Fig. 3. (a) Locus-specific parameters (θr_m and θl_m) and normal distribution inferred by the Bayesian model. Vertical bars indicate the location of outliers under homogenizing (lower tail) and divergent (upper tail) selection. Left: regional scale (θr_m), right: local scale (θl_m). (b) Proportion of outlier SNPs under selection at regional (i.e. between study sites) and at local (i.e. between microhabitats within sites) scales. (c) Joint distribution of regional and local-scale locus-specific parameters inferred through the Bayesian model. Blue dots indicate outliers under divergent selection at the regional scale, green dots indicate outliers under divergent selection at the local scale.

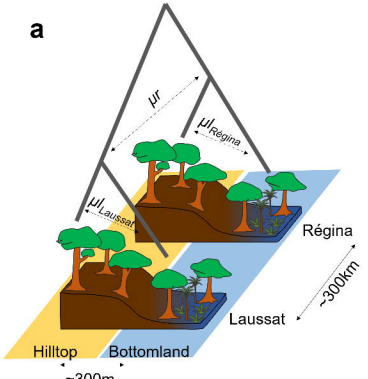
Fig. 4. Clines in allele frequency between regional locations (i.e. study sites) and microhabitats for the different kinds of (randomly sampled) neutral and outlier SNPs at different geographical scales.

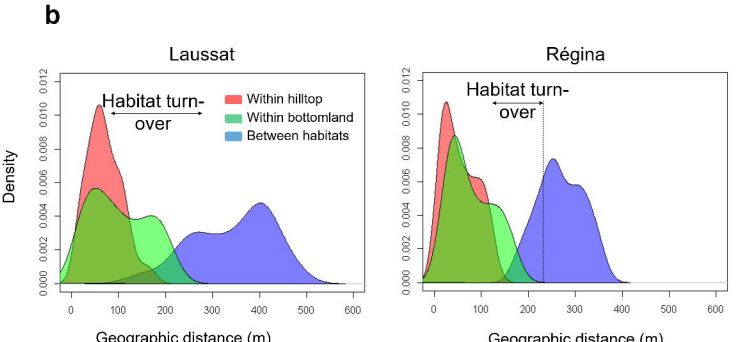
a

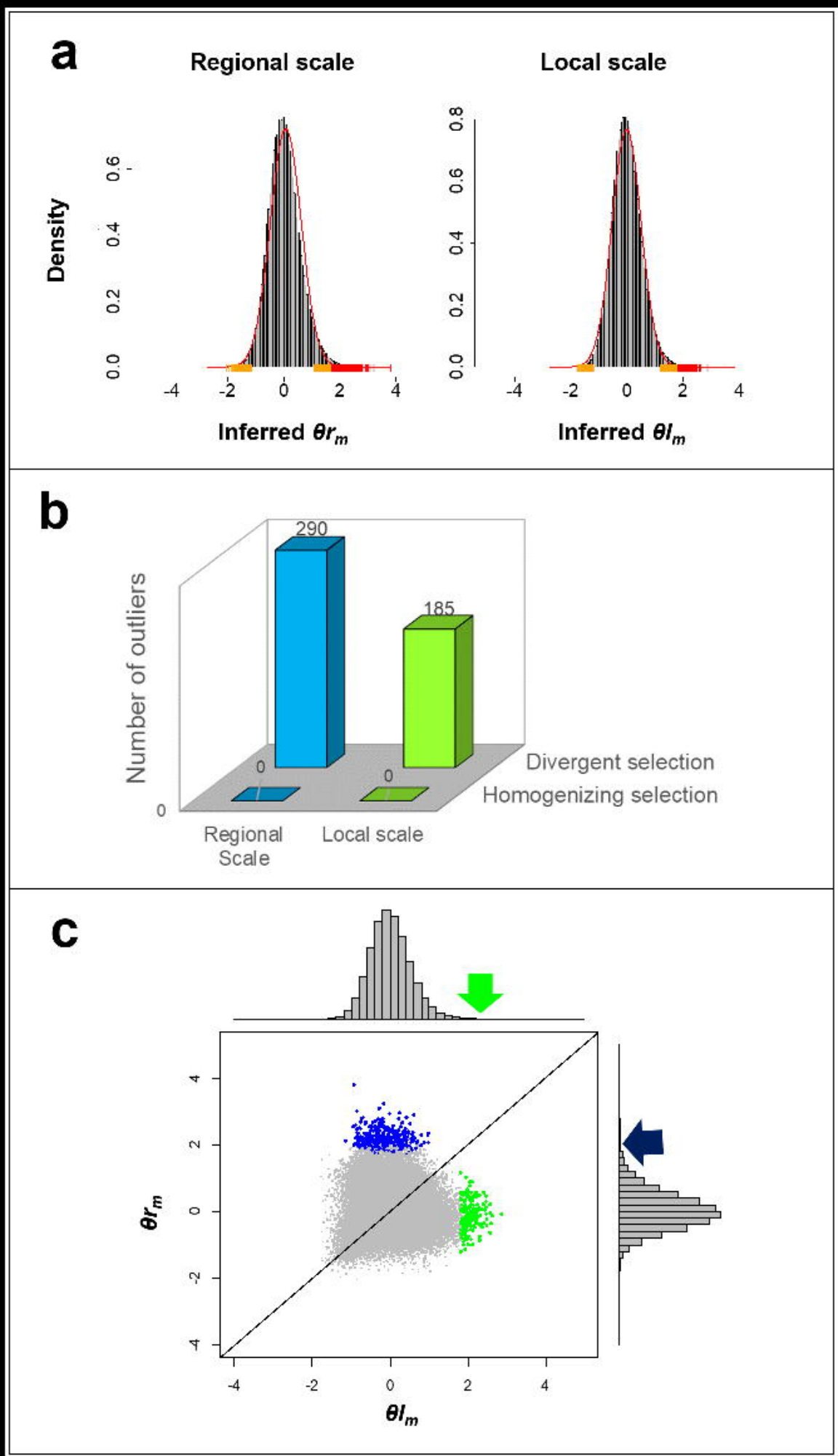
b

c

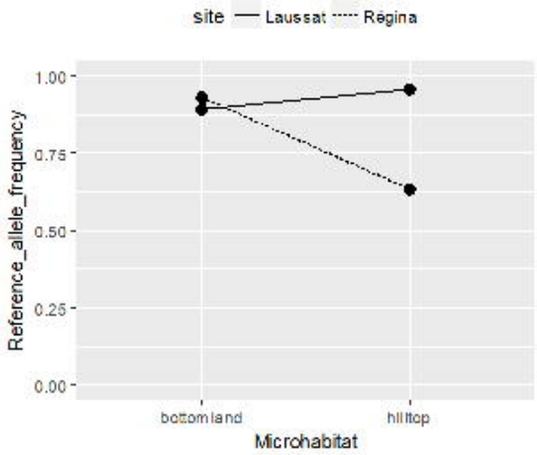
d


a
μr
μl Régina
μl Laussat
Régina
Laussat
~300km
Hilltop
Bottomland
~300m

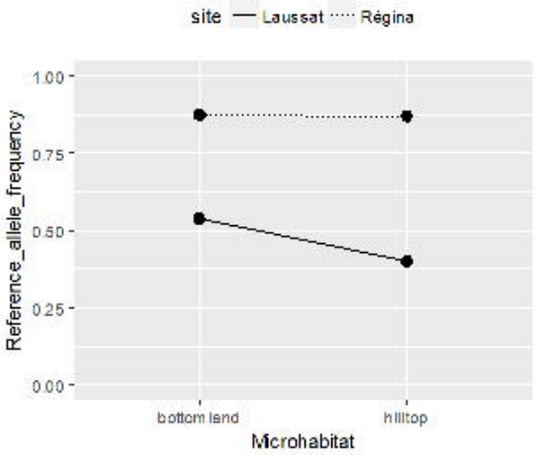

b
Laussat
Régina
Habitat turn-over
Within hilltop
Within bottomland
Between habitats
Density
0.000
0.002
0.004
0.006
0.008
0.010
0.012
0
100
200
300
400
500
600
Geographic distance (m)


a
Regional scale
Local scale
Density
0.0
0.2
0.4
0.6
0.8
-4
-2
0
2
4
Inferred θr_m
Inferred θl_m
b
Number of outliers
290
185
0
0
0
Divergent selection
Homogenizing selection
Regional Scale
Local scale
c
θr_m
θl_m
-4
-2
0
2
4

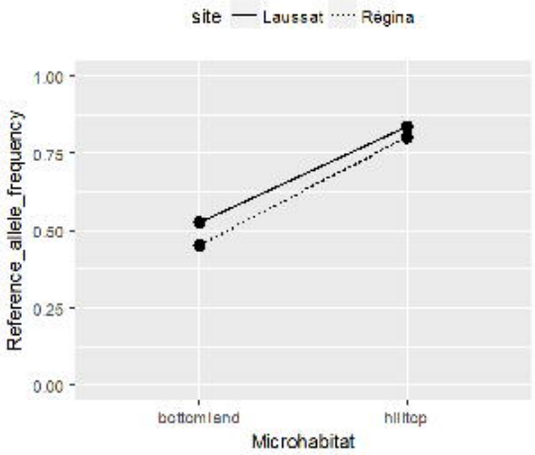
Regional scale	Neutral	Divergent selection	Neutral
Local scale	Neutral	Neutral	Divergent selection



contig-2712000008 pos.550



contig-6243000001 pos.1017



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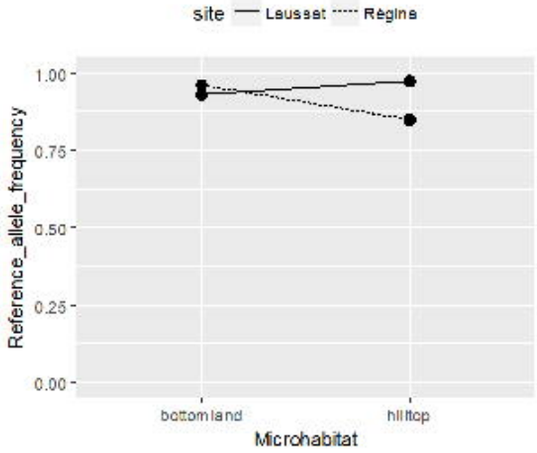

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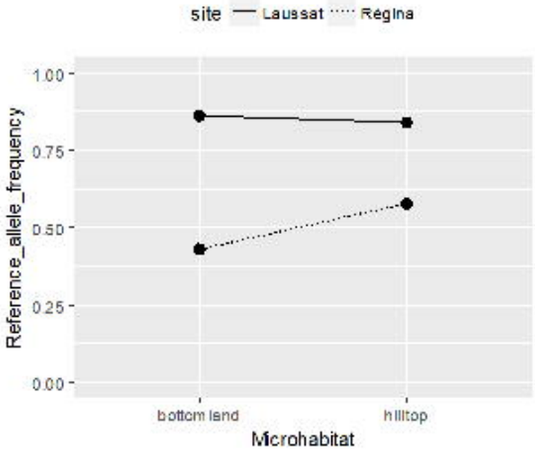

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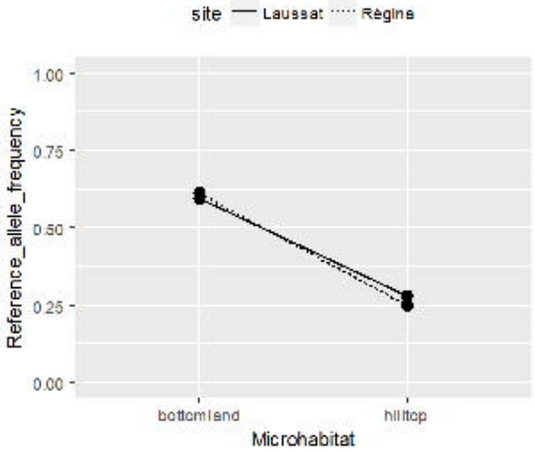

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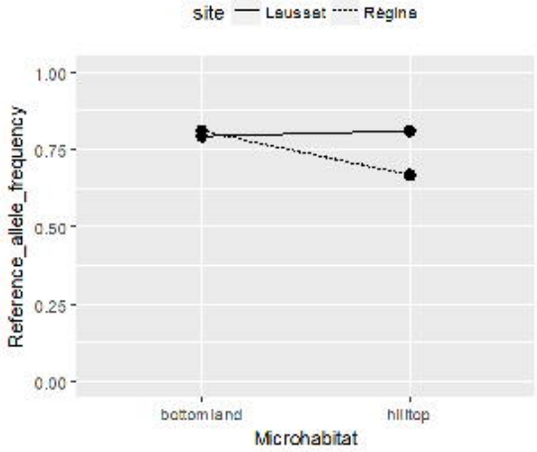

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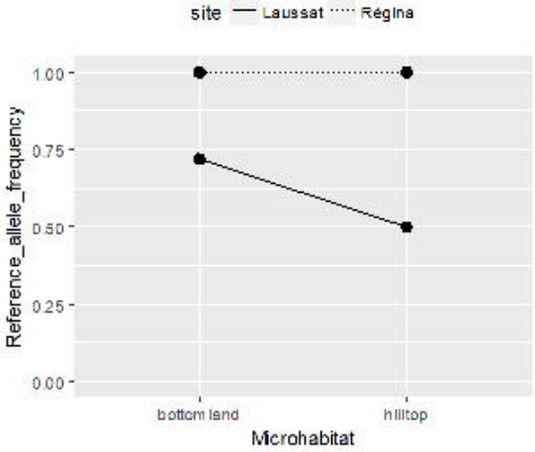

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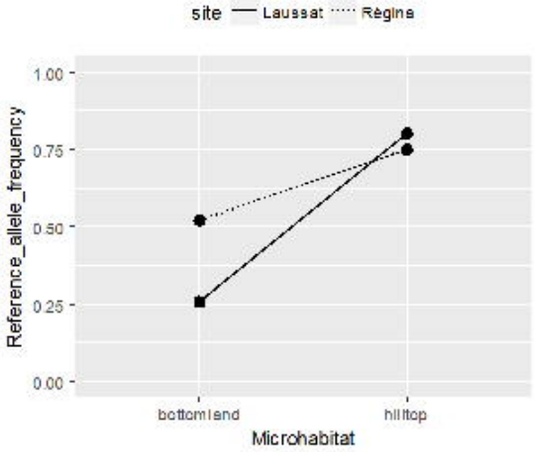

contig-17997000007 pos.579