Population genomics of the invasive Argentine ant (*Linepithema humile*) – adaptive evolution in the introduced supercolonies despite low genetic diversity

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Abstract

The Argentine ant (*Linepithema humile*), native to South America, has spread globally over the past 150 years, forming extremely large supercolonies in its introduced range. For example, the European Main supercolony spans over 6,000 km along the Mediterranean coastline. Despite the recent demographic history, including founder effects, Argentine ants thrive in the introduced range and displace native ant species. We took a comprehensive approach to investigate this highly invasive species' recent demographic history and genome evolution, primarily focusing on introduced supercolonies. We used pooled whole genome sequence data of 100 workers from the species native range and each of the three invasive supercolonies – European Main, Catalonia, and Chile. Invasive supercolonies were highly differentiated from each other and harboured low genetic diversity. Despite this, we could detect signs of positive selection in their genomes – both supercolony-specific and shared patterns. Positively selected genes overlapped the most across invasive supercolonies, indicating altered and shared selective pressure in new habitats. These included, e.g., genes implicated in neurobiological functions. Moreover, we found balancing selection, both supercolony-specific and shared patterns, including many chemical signalling genes. We conclude that introduced Argentine ant supercolonies evolve adaptively, indicating that founder effects and low genetic diversity do not always hamper adaptability.

Keywords: pool-seq, founder effect, selective pressure, positive selection, balancing selection

1. Introduction

Invasive species increasingly threaten biodiversity and ecosystems globally (Mollot et al., 2017; Seebens et al., 2017). In the most harmful situations, they cause local extinctions, permanently changing the nature of colonised areas (Holway et al., 2002; Mack et al., 2000). However, factors causing the success of invasive species are still poorly understood, as are their long-term evolutionary prospects in the introduced ranges, which makes the possibility of controlling these species and their detrimental effects nearly impossible (Estoup et al., 2016; Pérez et al., 2006; Sherpa & Després, 2021).

Ants exist almost everywhere in the world, and they are one of the most successful taxa (Hölldobler & Wilson, 1990; La Polla et al., 2013). Ants have an enormous species richness, inhabit diverse ecological niches and play a crucial role in many ecosystems (Hölldobler & Wilson, 1990; Moreau et al., 2006; Wilson & Hölldobler, 2005). However, many ant species are among the most harmful invasive species (Holway et al., 2002; Lester & Gruber, 2016; McGlynn, 1999). One of these, the highly invasive Argentine ant (*Linepithema humile*), has spread from its original habitat in South America to all continents except Antarctica during the past 150 years (Suarez et al., 2001; Wetterer et al., 2006, 2009; Wetterer & Wetterer, 2006). Typical for invasive ant species, the Argentine ant also forms large polygynous (multi-queen) and polydomous (multi-nest) societies called supercolonies (Pedersen et al., 2006) in which territorial borders between nests are absent over large areas, and individuals move freely between them. In both the native and introduced ranges, these supercolonies form closed breeding units with intranidal mating, and the spread of the colony occurs by budding (Helanterä, 2022; Pedersen et al., 2006; Vogel et al., 2009). In the introduced range, Argentine ants dominate in interspecies competition, destroy local ecosystems and cause species extinctions (Giraud et al., 2002; Holway et al., 2002; Sanders et al., 2001, 2003). The Argentine ants' supercolony size in the introduced range can be enormous, e.g., the European Main supercolony spans over 6,000 kilometres along the Mediterranean coastline from France and Spain to Portugal (Giraud et al., 2002).

69 Previous microsatellite marker-data studies indicated that introduced Argentine ant supercolonies are
70 genetically highly differentiated from each other and the native range supercolonies with low genetic
71 diversity (Blight et al., 2012; Brandt et al., 2009; Giraud et al., 2002; Jaquiéry et al., 2005; Tsutsui et al.,
72 2000; Vogel et al., 2010). These introduced supercolonies originate from a small number, in some cases
73 only a dozen, of reproducing individuals (Giraud et al., 2002; Vogel et al., 2010), indicating strong founder
74 effects, i.e., loss of genetic variation. Genetic drift (random loss and fixation of alleles) is often amplified in
75 small, newly established founder populations with reduced genetic diversity (Keller & Passera, 1993; Nei et
76 al., 1975). Thus, initially, low diversity combined with strong drift resulting from low effective population
77 sizes should impede the potential for adaptive evolution in the introduced Argentine ant supercolonies.
78 Moreover, the general inefficiency of selection on worker traits in supercolonies with low relatedness due
79 to multiple egg-laying queens increases the accumulation of deleterious mutations and decline of fitness
80 (genetic meltdown) (Helanterä et al., 2009).

81 However, the success and dominance of Argentine ants in the introduced range (Vogel et al., 2010) suggest
82 local adaptation to the new environments. This success in novel ranges despite small effective population
83 sizes and decreased efficacy of selection raises important questions. First, can signals of natural selection
84 be detected in the genome, and if so, in what kind of genes? Second, are genes undergoing adaptive
85 evolution shared among invasive supercolonies, and between the native range supercolonies and invasive
86 supercolonies? Our recent population genetic study focusing on the evolution of immune genes in 18
87 Argentine ant supercolonies across the world, also including the invasive supercolonies of this study,
88 indicated that despite the recent demographic history, positive selection has affected immune genes'
89 evolution in the introduced range (Holmberg et al., 2024).

90 In this study, we took a more comprehensive approach by investigating signatures of selection across the
91 whole genome of the Argentine ant. We studied native range supercolonies and three invasive
92 supercolonies from different parts of the world using pooled whole-genome sequence data of 100
93 individuals from the native range and each of the invasive supercolonies. We aimed 1) to investigate

94 selection patterns within supercolonies and whether the same genomic regions are under selection across
95 supercolonies and 2) to confirm with genome-wide data the earlier findings based on microsatellite marker
96 data about demographic changes during introductions, supercolonial diversity and divergence.

97 2. Materials and Methods

98 2.1. Sampling

99 One of the Argentine ant samples represents the native supercolonies, and three represent invasive
100 supercolonies, one originating from South America and two from Europe, all including one hundred diploid
101 workers (Table S1) (Wild, 2004). All three invasive supercolonies arose from different primary introductions
102 from the native range (Vogel et al., 2010). The sample representing the native supercolonies (Native) was
103 collected in Argentina, while the samples representing the three invasive populations were collected in
104 France, Spain and Chile. The two European samples originated from the two known separate large
105 supercolonies, the European Main supercolony (European Main) and the Catalonian supercolony
106 (Catalonia), living partly next to each other along the Mediterranean coastline (Giraud et al., 2002). The
107 third invasive sample from the Chilean supercolony (Chile) originated from the only recognised supercolony
108 in the country (Vogel et al., 2010). Ten individuals were collected from ten different nests within each
109 supercolony (i.e., 100 individuals per supercolony) in the introduced range. In the native range, ten
110 individuals were collected from ten different supercolonies' nests from four separate localities (i.e., a total
111 of 100 individuals per ten supercolonies) to capture the total genetic diversity in the species' natural range
112 (Table S1).

113 2.2. DNA extraction and sequencing

114 Genomic DNA was extracted individually from each ant using DNeasy Blood & Tissue Kit (QIAGEN). Equal
115 amounts of DNA from ten individuals per nest were pooled and genome-amplified using REPLI-g Mini Kit
116 (QIAGEN). Finally, all the genome-amplified sub-samples were pooled so that each final sample represented
117 100 individuals. The samples were sent to BGI Genomics for library preparation and paired-end sequencing
118 using Illumina Hiseq™ 2000 sequencer, with a read length of 90 bp and an insert size of 200 bp. The clean

119 reads provided by BGI Genomics contained sequences where adapters, contaminations, and low-quality
120 sequences were removed.

121 2.3. Data processing

122 First, the quality-encoding of the sequenced raw reads was converted from Phred 64 to Phred 33 (known as
123 Sanger) as it is more widely used and required in many software programs. The converting was done using
124 the Seqtk tool (version 1.3) (*Seqtk*, 2018). After this, raw reads were filtered using trim-fastq.pl script from
125 the basic pipeline of PoPoolation (version 1.2.2) designed to analyse pooled next-generation sequencing
126 data (Kofler, Orozco-terWengel, et al., 2011). The reads were filtered using an average minimum base
127 quality of 20 and a minimum read length of 70 bp. The quality of the reads was controlled using FastQC
128 (version 0.11.8) analyses before and after filtering (Andrews, 2010).

129 Filtered reads were aligned to the reference genome (NCBI Assembly Lhum_UMD_V04) using the BWA-
130 MEM algorithm from the BWA software package (version 0.7.17) with default settings (Li & Durbin, 2009).
131 The resulting SAM files were sorted, quality-filtered and converted to more compressed binary versions
132 (BAM files) using the view command of SAMtools (version 1.10) (Li et al., 2009). Only aligned reads with a
133 mapping quality of at least 20 and a SAM flag "properly paired" were saved. Duplicates were removed using
134 Picard's MarkDuplicates tool (version 2.21.4) (*Picard Toolkit*, 2019).

135 The processed BAM files were converted to text-based pileup formats using the mpileup command of
136 SAMtools (Li et al., 2009). A minimum count of 2 was required for identification of indel, and 5 bp were
137 removed around each identified indel using identify-genomic-indel-regions.pl and filter-mpileup-by-gtf.pl
138 scripts from the basic pipeline of PoPoolation (Kofler, Orozco-terWengel, et al., 2011). Sample-specific
139 pileup files for Native, European Main, Catalonia, and Chile were filtered to keep only positions covered
140 with a minimum sequencing depth of 20 (a requirement in later sample-specific downstream analyses).
141 Finally, only the positions that met the sequencing depth criteria in all sample files were kept in analyses to
142 ensure the comparability of the results. As Catalonia had an uneven read alignment and lower data quality,
143 its inclusion in the analyses significantly reduced the number of shared positions. Thus, we decided to do

144 analyses that included and excluded Catalonia. The main text focuses on analyses with higher reference
145 genome coverage and better quality, excluding Catalonia, and analyses including Catalonia are presented in
146 Supplemental Information (see Text S2).

147 A multi-sample joint mpileup file was created using the mpileup command of SAMtools (Li et al., 2009). The
148 mpileup file was indel-filtered as above using corresponding scripts, identify-indel-regions.pl and filter-sync-
149 by-gtf.pl, from the pipeline of PoPoolation2 (version 1201) (Kofler, Pandey, et al., 2011). Before indel-
150 filtering, the mpileup file was synchronised using the script mpileup2sync.jar from PoPoolation2. The indel-
151 filtered mpileup file was further filtered to keep only positions with a sequencing depth of at least 4 (a
152 requirement in later multi-sample downstream analyses) in all considered samples (Kofler, Orozco-
153 terWengel, et al., 2011).

154 These sample-specific filtered pileup files and the multi-sample joint mpileup files, including and excluding
155 Catalonia, were used as input files for the downstream analyses detailed below, mainly according to the
156 pipelines of PoPoolation and PoPoolation2, respectively.

157 2.4. Population genetic analyses

158 2.4.1. Genomic diversity

159 The genomic diversity of supercolonies was evaluated with two genetic diversity estimators, Tajima's π (π
160 from now on) and Watterson's Θ (Θ_w from now on). These were calculated separately for Native and each
161 of the invasive supercolony samples using non-overlapping windows of 20 kbp with Variance-sliding.pl
162 script from PoPoolation (Kofler, Orozco-terWengel, et al., 2011). The window size was slightly larger than
163 the mean gene length (13 988 bp based on the NCBI Linepithema humile Annotation Release 100). The
164 minimum and maximum sequencing depth requirements of the sites used for SNP identifications were 20
165 and 250, respectively. A minimum count of 4 was required for a minor allele, and the minimum base quality
166 requirement was 20. Finally, at least 60% of each window had to meet the sequencing depth criteria for
167 acceptance when considering sample-specific pileup files containing shared positions. These parameter
168 values were used in all analyses unless otherwise stated. Furthermore, the effect of different sliding

169 window lengths and the inclusion of deletion sites were tested for the results. We calculated the genome-
170 wide mean and standard deviation for the estimated genetic diversity values and the number of single
171 nucleotide polymorphisms (SNPs) used in these analyses. We also estimated genetic marker diversity,
172 expected heterozygosity (H_{exp}) and allelic richness (*k'*) for five variable microsatellite loci obtained from
173 Vogel et al. (2010) applying a similar sample size and design as the genomic samples (see Text S3).

174 2.4.2. Test of neutrality and inference of adaptive evolution

175 We used Tajima's D to identify genomic windows evolving non-neutrally (Tajima, 1989). Tajima's D values
176 were calculated separately for Native and each of the invasive supercolony samples using Variance-
177 sliding.pl script from PoPoolation with the same parameter values as above (Kofler, Orozco-terWengel, et
178 al., 2011). Exceptionally, a minimum count of 2 instead of 4 was used for the minor allele as required for
179 the correcting calculation method (Achaz, 2008). In addition to the genome-wide sliding window analyses,
180 Tajima's D values were separately calculated for annotated genes (NCBI Linepithema humile Annotation
181 Release 100) in the genome. These calculations were done using the same sample-specific input pileup files
182 as above and the script Variance-at-position.pl from PoPoolation (Kofler, Orozco-terWengel, et al., 2011).
183 The script used genes as windows for calculations. Thus, the sliding window and step size parameters were
184 excluded from the analysis. We calculated the genome-wide statistics (minimum, maximum, mean, median,
185 and standard deviation) for Tajima's D values and the number of SNPs used in these analyses.

186 As demography and selection both affect the neutrality test statistic Tajima's D, it was crucial to disentangle
187 the effects of these two forces. It is widely accepted that demography affects the whole genome, whereas
188 natural selection acts more locally. We used an empirical outlier approach with strict cut-off values to
189 identify putative selection signals. The 1%, 5%, 95%, and 99% fractiles of observed Tajima's D value
190 distributions were used to recognise the 20 kbp genomic windows and annotated genes with the lowest
191 and highest, i.e., the extreme Tajima's D values, likely driven by selection rather than demography (Fabian
192 et al., 2012; Kolaczkowski et al., 2011; Nielsen, 2005).

193 Between-sample comparisons reveal the overlaps of these extremes, which might unveil genes encoding
194 crucial functions for invasion success (putative parallel adaptive evolution or pre-adaptation in the native
195 range). Comparisons were made separately for the lowest and highest extremes, and, in addition, a cross-
196 comparison was carried out between the highest extremes in Native and the lowest extremes in invasive
197 supercolony samples to determine particular patterns, such as if the invasions reversed the direction of the
198 selection of some genes. Pairwise and multi-set overlaps were evaluated using the R-package
199 SuperExactTest (*R Core Team*, 2022; Wang et al., 2015). The obtained p-values were adjusted for multiple
200 comparisons with Benjamini-Hochberg's method using a function p.adjust from R-package stats (Benjamini
201 & Hochberg, 1995; *R Core Team*, 2022). A p-value less than 0.05 is considered significant in all statistical
202 tests. Moreover, by using prop.test function in R, we evaluated if the proportions of overlaps were the
203 same with two-sample tests of equality of proportions (*R Core Team*, 2022), especially when comparing
204 Native and invasive supercolony samples' overlaps to the overlaps of invasive supercolony samples. In
205 addition, Jaccard indexes measuring the similarity of the two lists were calculated with a vegdist function
206 from R-package vegan (Oksanen et al., 2022; *R Core Team*, 2022).

207 Finally, the Blast2GO tool's enrichment analysis of Gene Ontology (GO) (Conesa et al., 2005) terms was
208 made separately for Native, and each of the invasive supercolony sample-specific gene sets with the
209 extreme values of Tajima's D and multi-set overlaps as test sets and a 9702 annotated genes described in
210 Viljakainen et al. (2018) as a reference set.

211 2.4.3. Genomic differentiation

212 A sliding window approach was used to measure genomic differentiation between samples as pairwise F_{ST}
213 values. The calculations were made using the multi-sample joint mpileup file as input for fst-sliding.pl script
214 from PoPoolation2 (Kofler, Pandey, et al., 2011). The same parameter values were used as above with the
215 PoPoolation analyses. SNPs used in the analysis were identified considering all samples simultaneously.
216 Again, at least 60% of each window had to meet the sequencing depth criteria in all samples included for

217 acceptance for the analysis. We calculated the genome-wide statistics (minimum, maximum, mean,
218 median, and standard deviation) for pairwise genetic differentiation values.

219 The 20 kbp genomic windows and annotated genes in these regions (identified based on the gene
220 annotation file: NCBI Linepithema humile Annotation Release 100) with the highest F_{ST} values were
221 recognised with 95% and 99% fractiles of the observed F_{ST} value distributions. Local adaptations might lead
222 to population differentiation over time as different alleles fixate in each population. The Blast2GO tool's
223 enrichment analysis of GO terms was carried out for these highly differentiated genes as above (Conesa et
224 al., 2005). We also estimated genetic marker differentiation as pairwise F_{ST} for five variable microsatellite
225 loci obtained from Vogel et al. (2010) by applying a sample size and design similar to the genomic samples
226 (see Text S3).

227 The Cochran-Mantel-Haenszel test (cmh-test) was used to assess if there were consistent allele frequency
228 changes in the genomes of the invasive supercolonies compared to the native supercolonies. This analysis
229 was done for every segregating site in the multi-sample joint mpileup files to detect statistically significant
230 (p-value<0.05) and consistent allele frequency changes between invasive supercolony samples and Native
231 using cmh-test.pl script from PoPoolation2 (Kofler, Pandey, et al., 2011). Again, the parameter values and
232 criteria requirements used were the same as detailed above. In addition, a quasibinomial generalised linear
233 model (qbGLM) for each segregating site in the genome was used to detect significant allele frequency
234 differences between the invasive supercolony samples and Native (see details in Supplemental Information,
235 Table S4).

236 3. Results

237 3.1. Properties of the data

238 Catalonia had an uneven read alignment and lower data quality, and its inclusion in the analyses
239 significantly reduced the number of shared positions. Thus, analyses including Catalonia are presented in
240 the Supplemental Information (see Text S2), and the main text focuses on the analyses with higher
241 reference genome coverage and better quality, excluding Catalonia. After filtering, the sample-specific

242 pileup files with shared positions of Native, European Main, and Chile with a minimum sequencing depth of
243 at least 20 covered 85% of the reference genome (Table S5 A-C). The corresponding multi-sample joint
244 mpileup file covered 93% of the reference genome (Table S5 D).

245 3.2. Genomic diversity

246 The number of SNPs found in Native was 1 121 307, twice the amount found in the two invasive
247 supercolonies, European Main and Chile (Table 1). Both genetic diversity estimators, π and Θ_w, indicated
248 that the native supercolonies are genetically more diverse than the invasive supercolonies (p-values<0.05 in
249 all pairwise comparisons, see details in Table S6; Table 1). We also tested the effect of different window
250 sizes and the inclusion of the deletion sites in calculating the genetic diversity estimators (Table S7 A-B).
251 The consequences for the results were minor; thus, we used only one window size, 20 kbp. Both H_{exp} and *k'*
252 estimates of genetic marker diversity gave us similar results as genomic diversity – the native supercolonies
253 harbour higher diversity than the invasive supercolonies (see Text S3).

254 3.3. Test of neutrality and inference of adaptive evolution

255 Tajima's D values for whole genomes (estimated with 20 kbp genomic windows) and separately for
256 annotated genes were estimated to detect deviations from neutrality (Tajima, 1989). Locations and shapes,
257 especially width and kurtosis, differed between Tajima's D density curves of the native and invasive
258 supercolonies, the European Main and the Chilean supercolonies (Figure 1). The density curves of the
259 native supercolonies are narrow with high kurtosis, while the density curves of the invasive supercolonies
260 are broad, with lower kurtosis and more extreme values. Moreover, the density curves of the native
261 supercolonies are located on the negative side. In contrast, the invasive supercolonies' density curves are
262 located on the positive side, but the density curves of whole genomes are especially skewed toward the
263 negative side (Figure 1). Similar observations could be made from genome-wide summary statistics (Tables
264 S8 & S9). These differences indicate varying selection pressure and evolutionary trajectories between the
265 native and invasive supercolonies.

266 Gene evolution differs from the evolution of the whole genome, as we observed differences in the
267 locations of corresponding density curves of each supercolony (p-values<0.05 in all pairwise comparisons,
268 see details in Table S6; Figure 1). Moreover, the invasive supercolonies' density curves of genes are
269 bimodal. Bimodality differentiates these gene curves of the invasive supercolonies from their whole
270 genome curves and the curves of the native supercolonies. The locations of peaks in the two invasive
271 supercolonies are the same, with one peak around zero and one on the positive side (Figure 1) – indicating
272 similar changes in selection pressures and evolutionary trajectories.

273 To identify putative targets of positive and balancing selection, we used an empirical outlier approach with
274 different thresholds to determine the genomic regions with the most extreme values of Tajima's D from
275 both ends of the distributions, i.e. genomic windows and genes with the lowest (positive selection) and
276 highest (balancing selection) Tajima's D values (File S10) (Fabian et al., 2012; Kolaczkowski et al., 2011). The
277 extreme regions might have been targets of recent selection events or linked to selected genes or other
278 genomic regions. Overlaps of genomic windows (Figure S11 A-B, File S10) and genes (Figures 2 & 3, File S10)
279 in the extreme ends of Tajima's D distributions, indicating similar selective regimes, were also found
280 between supercolonies.

281 The observed overlaps among the genomic windows were significantly larger than expected by chance
282 alone (BH-adj. p-values<0.05, see details in File S10) when considering the overlaps of the lowest and
283 highest extremes separately (Figure S11 A). All but one (overlap of 3 in the lowest 1% extremes of Native
284 and Chile) of the observed overlaps among the genes were significantly higher than expected by chance
285 alone (BH-adj. p-values<0.05; File S10) when considering the overlaps of the lowest and highest extremes
286 separately (Figure 2).

287 Invasive supercolonies' overlaps of genes and genomic windows in the lowest extremes of Tajima's D, i.e.,
288 in putative genes evolving under positive selection, were higher than overlaps between the native and
289 invasive supercolonies (p-values<0.05 in all pairwise comparisons, see details in Table S6; Figures 2 and S8
290 A).

291 Jaccard Indexes, which measure the similarity between the two lists, indicated the same pattern as
292 European Main's and Chile's indexes were higher than indexes between Native and the two invasive
293 supercolonies when comparing the lowest extremes of Tajima's D (Tables 2 & S12). The overlaps between
294 the highest extremes did not vary considerably in size (Figures 2 & S11 A), as detected in Jaccard indexes as
295 well (Tables 2 & S12**)**.

296 As putative regions where selective regimes may have changed between supercolonies, the highest
297 extremes of Native and lowest extremes of the invasive supercolonies overlapped more than expected by
298 chance for the 5% extremes for genomic windows (BH-adj. p-values<0.05, File S10; Figure S11 B), but not
299 for other comparisons of high and low extremes (Figures 3 & S11 B). The cross-comparison overlaps did not
300 vary considerably in size (Figures 3 & S11 B).

301 Scanning the gene sets having high Tajima's D values indicating balancing selection, the Enrichment
302 Analysis of GO terms found an overrepresentation of a biological process DNA integration GO:0015074
303 among the genes in the highest 1% and 5% of Native (BH-adjusted p-values<0.05, Table S13). Among the
304 genes in the highest 5% of Chile, a biological process term, DNA metabolic process GO:0006259 (BH-
305 adjusted p-value<0.05, Table S13), was found. Finally, one biological process, sensory perception of smell
306 GO:0007608, and two molecular function terms, olfactory receptor activity GO:0004984 and odorant
307 binding GO:0005549, were enriched among the genes in the highest 5% of European Main (BH-adjusted p-
308 values<0.05, Table S13).

309 Overlaps of the gene sets with the highest Tajima's D values revealed shared genes under balancing
310 selection among several supercolonies. The biological process term DNA integration GO:0015074 was
311 enriched among the overlapped genes in the highest 1% and 5% D values of Native and European Main, as
312 well as in the highest 5% D values of Native and Chile, and highest 5% D values of Native and both invasive
313 supercolony samples (BH-adjusted p-values<0.05, Table S13). No GO term enrichment was found in the
314 genes among the lowest extremes of Tajima's D values, i.e., genes evolving under positive selection.

315 Gene sets with the extreme Tajima's D values contain a broad set of genes with various functions. For
316 example, gene sets with the lowest Tajima's D values, i.e., those evolving under positive selection, contain
317 many genes implicated in neurological functions, and genes with the highest Tajima's D values, i.e., those
318 evolving under balancing selection, include genes encoding putative endogenous virus proteins (File S10).
319 Interestingly, genes functioning in chemical signalling, such as odorant receptors and fatty acid synthases,
320 are among the gene sets with both the lowest and the highest Tajima's D values, thus some of them
321 evolving under positive selection while others under balancing selection (File S10).

322 3.4. Genomic differentiation

323 We used pairwise F_{ST} values to evaluate genomic differentiation between supercolonies. These analyses
324 revealed that the invasive supercolonies were more differentiated from each other than from the native
325 supercolonies (p-values<0.05 in all pairwise comparisons, see details in Table S6; Figure 4, Table S14).
326 Similar observations were made from the genetic marker data, with the invasive supercolonies being the
327 most differentiated from each other (Text S3).

328 We searched for annotated genes among the 20 kbp genomic windows with the highest F_{ST} values (File S15)
329 to identify highly differentiated genes indicating local adaptation. The Enrichment Analysis of GO terms
330 showed an overrepresentation of two biological processes (calcium ion transport GO:0006816 and
331 inorganic cation transmembrane transport GO:0098662), three molecular functions (protein binding
332 GO:0005515, calcium channel activity GO:0005262, and transporter activity GO:0005215), and one cellular
333 component (cation channel complex GO:0034703) GO terms among the highest 5% of European Main and
334 Chile (BH-adjusted p-values<0.05, see details in Table S16).

335 Using a Cochran-Mantel-Haenszel test, we detected similar allele frequency changes between the invasive
336 and native supercolonies. 63% of the SNPs showed allele frequency changes significantly more similar than
337 expected by chance alone (BH-adjusted p-values<0.05, Table S4).

338 4. Discussion

339 This study aimed to shed light on the highly invasive Argentine ant's recent evolutionary history and
340 genome dynamics, specifically focusing on the introduced supercolonies. We searched for answers to the
341 following questions: Can selection be detected in the genomes of these highly invasive ants, and if so, in
342 which genes, and are selected genes shared between supercolonies? Moreover, we wanted to verify the
343 earlier population genetic findings based on marker data. We found similar diversity and divergence
344 patterns in the invasive supercolonies compared to the native supercolonies as in the earlier marker-based
345 studies. Moreover, despite the recent demographic history, including strong founder effects and low
346 genetic diversity of the invasive supercolonies, we could detect both shared and supercolony-specific signs
347 of selection, indicating adaptability.

348 The observed low diversity and high divergence in the invasive supercolonies confirm earlier Argentine ant
349 studies based on microsatellite loci and mitochondrial DNA (Blight et al., 2012; Brandt et al., 2009; Giraud
350 et al., 2002; Jaquiéry et al., 2005; Tsutsui et al., 2000; Vogel et al., 2010). Similarly, in another invasive ant
351 species, *Cardiocondyla obscurior,* genome-wide evidence shows reduced genetic diversity in invasive
352 populations (Errbii et al., 2021). Moreover, marker data has demonstrated reduced genetic diversity in
353 several other invasive ant populations of *Nylanderia fulva, Pheidole megacephala, Solenopsis invicta*,
354 *Solenopsis geminata*, and *Wasmannia auropunctata* (Fournier et al., 2005, 2009, 2012; Ross et al., 1993,
355 1996; Wauters et al., 2018). Together, these results indicate that introduced populations of invasive ant
356 species typically harbour less genetic diversity than native populations. This is in line with the typical
357 supercolonial lifestyle of the invasive ants, i.e., the formation of closed breeding units and the fact that
358 populations in the introduced ranges usually comprise single supercolonies. If the native range
359 supercolonies also form closed breeding units (Pedersen et al., 2006; Vogel et al., 2009), evolution could be
360 quite similar in both ranges as effective population sizes are always small in these types of supercolonies.

361 The invasive Argentine ant supercolonies included in this study have originated from different primary
362 introductions from the native range without secondary reinforcements (Giraud et al., 2002; Vogel et al.,
363 2010). Argentine ant workers are highly aggressive toward non-colony mates, efficiently preventing gene

364 flow between supercolonies (Krieger & Keller, 2000; Sunamura et al., 2011). In addition, gene flow is
365 prevented by the considerable geographic distance between the studied supercolonies, except the
366 European Main and Catalonian supercolonies (the latter considered in Supplemental Information – see Text
367 S2) living partly next to each other (Blight et al., 2012; Giraud et al., 2002; Jaquiéry et al., 2005; Vogel et al.,
368 2010). Although the European Main and Catalonia supercolonies live close to each other, there is no gene
369 flow between them (Blight et al., 2012; Giraud et al., 2002; Jaquiéry et al., 2005). The effective queen
370 number establishing the European Main as well as the Catalonian supercolonies has been estimated to be
371 between 6 to 13 individuals (Giraud et al., 2002; Vogel et al., 2010), while in native range supercolonies, it
372 has been estimated to be extremely high, reaching infinity (Pedersen et al., 2006). Thus, the founder effect
373 probably created the first shift toward genetic differentiation of the introduced supercolonies. In addition,
374 amplified genetic drift in the small founder populations might have caused further fluctuations in allele
375 frequencies, contributing to diversification in expanding supercolonies (Nei et al., 1975).

376 Other social insects, such as the yellow-legged hornet *Vespa velutina* and German wasp *Vespula germanica,*
377 have also successfully invaded regions outside their native range despite low genetic diversity (Arca et al.,
378 2015; Brenton-Rule et al., 2018; Eloff et al., 2020; Takeuchi et al., 2017). In the yellow-legged hornet,
379 successful invasion possibly originated from as few individuals as just one (one queen mated with multiple
380 males) (Arca et al., 2015; Takeuchi et al., 2017). In addition, other non-social insects, such as the African fig
381 fly *Zaprionus indianus* (Comeault et al., 2020), and other species, such as the invasive wart comb jelly
382 *Mnemiopsis leidyi* (Jaspers et al., 2021) have also proven that invasion success does not require high
383 genomic diversity. Thus, the general assumption of invasive populations' lower adaptive potential is
384 incorrect, as the founder effect and low diversity do not always prevent invaders' success.

385 Whilst the Mediterranean-like climate is shared across the native and invasive ranges of the Argentine ant
386 (Roura-Pascual et al., 2011), other biotic and abiotic factors, such as pathogens and competitors, likely
387 differ between regions, leading to distinct local selective pressures (Holmberg et al., 2024; Lester & Gruber,
388 2016). In the Argentine ant, pervasive selection in both the native and invasive supercolonies is suggested

389 by the different distributions of Tajima's D of the whole genomes (estimated with 20 kbp windows) and
390 genes.

391 The positively selected genes found in the lowest extremes of Tajima's D encoded different regulatory and
392 signalling proteins participating in several cellular processes and pathways despite no enrichment of
393 descriptive GO terms (File S10). For example, genes putatively involved in sociobiological traits (Privman et
394 al., 2018) through their roles in neurological functions, pheromonal signalling and caste determination were
395 positively selected in each supercolony. In line with our observation, genes associated with sociobiological
396 traits have also been targets of positive selection in another invasive ant species, the fire ant *S. invicta*
397 (Privman et al., 2018). Thus, evolutionary changes in genes responsible for sociobiological traits might be
398 crucial for the continued and long-term success of invasive ant species.

399 In addition to supercolony-specific adaptive evolution, we could detect some shared patterns (File S10). We
400 detected overlaps among the positively selected genes in different supercolonies. The overlaps between
401 the native and invasive supercolonies might indicate that some selection pressures have not changed after
402 introductions. Instead, the overlaps between the invasive supercolonies might indicate genes that
403 repeatedly experienced positive selection in the introduced range due to altered and shared selective
404 pressures. Overlaps of positively selected genes between the invasive supercolonies were higher than
405 overlaps between the native and invasive supercolonies. These overlaps between the invasive
406 supercolonies contained the same genes as mentioned above, including genes implicated in neurological
407 functions (File S10), again highlighting the importance of genes likely involved in sociobiological traits in the
408 evolution of the invasive supercolonies.

409 Supercolony-specific and shared patterns of evolution under positive selection were also evident in the
410 immune genes of Argentine ants (Holmberg et al., 2024). Despite the significant evidence of positive
411 selection acting on this species' immune genes, we could not detect immune genes among the genes under
412 the strongest positive selection when considering the whole gene repertoire of the species.

413 Positive Tajima's D values at the whole genome level in the case of the European Main and Chilean
414 supercolonies could indicate decreasing supercolony size. However, we can exclude the colony size
415 contraction since all three studied invasive supercolonies have tremendously expanded after introduction
416 events (Vogel et al., 2010).

417 The highest extremes of Tajima's D contain genes evolving under balancing selection, an important factor
418 for maintaining genetic variation in heterogeneous environments and allowing rapid response in the face of
419 environmental fluctuations and changing selection pressures (Davidson et al., 2011; Estoup et al., 2016;
420 Manfredini et al., 2019; Nielsen, 2005; Richards et al., 2006). One or a few GO terms were enriched in the
421 gene set under balancing selection in each supercolony. Three of the GO terms enriched in the European
422 Main supercolony were related to olfaction, including several genes encoding odorant receptors as well as
423 several fatty acid synthase genes. Such genes function in chemical signalling and could thus be relevant for
424 recognition and communication behaviours. Odorant receptors are necessary for perceiving chemical
425 signals (Anton & Rössler, 2021; Gadenne et al., 2016). In addition to within-colony communication,
426 perception of signals outside the colony, such as recognition of enemies, food, and other resources, are
427 crucial for colony survival. In turn, fatty acid synthases are implicated in cuticular hydrocarbon biosynthesis.
428 Insect cuticular hydrocarbons have two fundamental and vital functions – they protect the individual from
429 desiccation and mediate chemical signalling (Holze et al., 2020; Leonhardt et al., 2016; Menzel et al., 2017;
430 Sprenger & Menzel F., 2020; Walsh et al., 2020). In *C. obscurior,* chemical signalling genes are highly
431 variable (Errbii et al., 2021) and balancing selection has been observed among chemical signalling genes of
432 *Drosophila melanogaster* (Comeron, 2014; Croze et al., 2017).

433 Interestingly, overlaps were also observed among the gene sets under balancing selection in different
434 supercolonies. These overlaps contained the same chemical signalling genes discussed above. It has been
435 suggested that chemical signalling is the main evolutionary driver of cuticular hydrocarbons (Hefetz, 2007).

436 The overlaps of genes under balancing selection between the native and invasive supercolonies might
437 unveil pre-adaptations in the species' native range, a phenomenon that has been suggested to facilitate

438 invasion success in various species by providing a selection regime that allows rapid adaptation in response
439 to changing selection pressures in fluctuating environmental conditions (Gloag et al., 2016; Kaňuch et al.,
440 2021; Stern & Lee, 2020; Tepolt et al., 2022). It seems plausible that many species became successful
441 invaders due to the traits already present in their native range. An unstable environment due to frequent
442 floodings along the Paraná River in the native range (Wild, 2004) could maintain balancing selection in key
443 genes, preparing this species to become thriving dispersers.

444 We also found genes encoding putative endogenous virus proteins among the genes that have been targets
445 of balancing selection in all supercolonies and their overlaps. In general, endogenous virus elements seem
446 not to be an uncommon discovery in the genomes of insects or other animals (Blair et al., 2020; Flynn &
447 Moreau, 2019). In future studies, it could be interesting to compare the overall amount of these
448 endogenous virus proteins encoding genes in the genomes of native and invasive supercolonies and clarify
449 their functions. For example, in other insect and non-insect species, they have been found to function in
450 virus defence (Blair et al., 2020; Flynn & Moreau, 2019; Frank & Feschotte, 2017; Yap et al., 2014), which
451 could also be relevant in the Argentine ants since they are known to carry a high number of viruses
452 (Viljakainen et al., 2023; Viljakainen, Holmberg, et al., 2018).

453 The success of a wide range of invasive species populations with low adaptive potential due to low genetic
454 diversity has been explained by factors such as gene flow, stress-induced accelerated mutation rate or TE-
455 activation, epigenetic changes and the release from natural pathogens and enemies (Errbii et al., 2021;
456 Manfredini et al., 2019; Marin et al., 2019; North et al., 2021; Schrader et al., 2014). Despite the low genetic
457 diversity, genomes of the invasive Argentine ants continue to evolve adaptively. Pre-adaptation in the
458 native range has recently received more attention across species (Davidson et al., 2011; Elst et al., 2016;
459 Estoup et al., 2016; Guo et al., 2014; Hufbauer et al., 2012), and we suggest that this might also play a role
460 in the initial invasive success of the Argentine ant. The unstable environment in the native range of the
461 Argentine ant, characterised by frequent flooding, may enhance the species' adaptive potential. Balancing
462 selection, which maintains alternative alleles in fluctuating conditions, could contribute to their continued

463 and long-term success by adapting to new habitats following introductions. Moreover, the overlap of
464 positively selected genes is higher among the invasive supercolonies than between the native and invasive
465 supercolonies, indicating shared selective pressures in the new ranges. The exact roles of all selected genes
466 must be carefully resolved among the complex biological processes and pathways in upcoming genomic
467 surveys, such as comparative population genomic studies between native and introduced supercolonies
468 using both genomic and transcriptomic data at the individual level. Our study provides an excellent
469 groundwork and frame for future studies of the Argentine ant in the field of invasion genomics.

470 Tables and Figures

471 Table 1. The genome-wide means and standard deviations (sd) of π and Θ_W, and the number of SNPs used
472 for estimations.

Sample	mean π	sd π	Mean Θ_W	sd Θ_W	SNPs
Native	0.00189	0.00124	0.00203	0.00121	1 121 307
European Main	0.00103	0.00103	0.00095	0.00094	561 180
Chile	0.00115	0.00112	0.00105	0.00100	595 635

473 Table 2. Pairwise Jaccard Indexes for genes in the lowest 1% and 5% and highest 1% and 5% extremes of
474 Tajima's D values.

The extreme end of D values	Sample pair	Jaccard Index
Low 1%	Native & European Main	0.0330
Low 1%	Native & Chile	0.0138
Low 1%	European Main & Chile	0.1231
Low 5%	Native & European Main	0.0541
Low 5%	Native & Chile	0.0610
Low 5%	European Main & Chile	0.1457
High 1%	Native & European Main	0.1231
High 1%	Native & Chile	0.1458
High 1%	European Main & Chile	0.1711

High 5%	Native & European Main	0.1053
High 5%	Native & Chile	0.1128
High 5%	European Main & Chile	0.1130

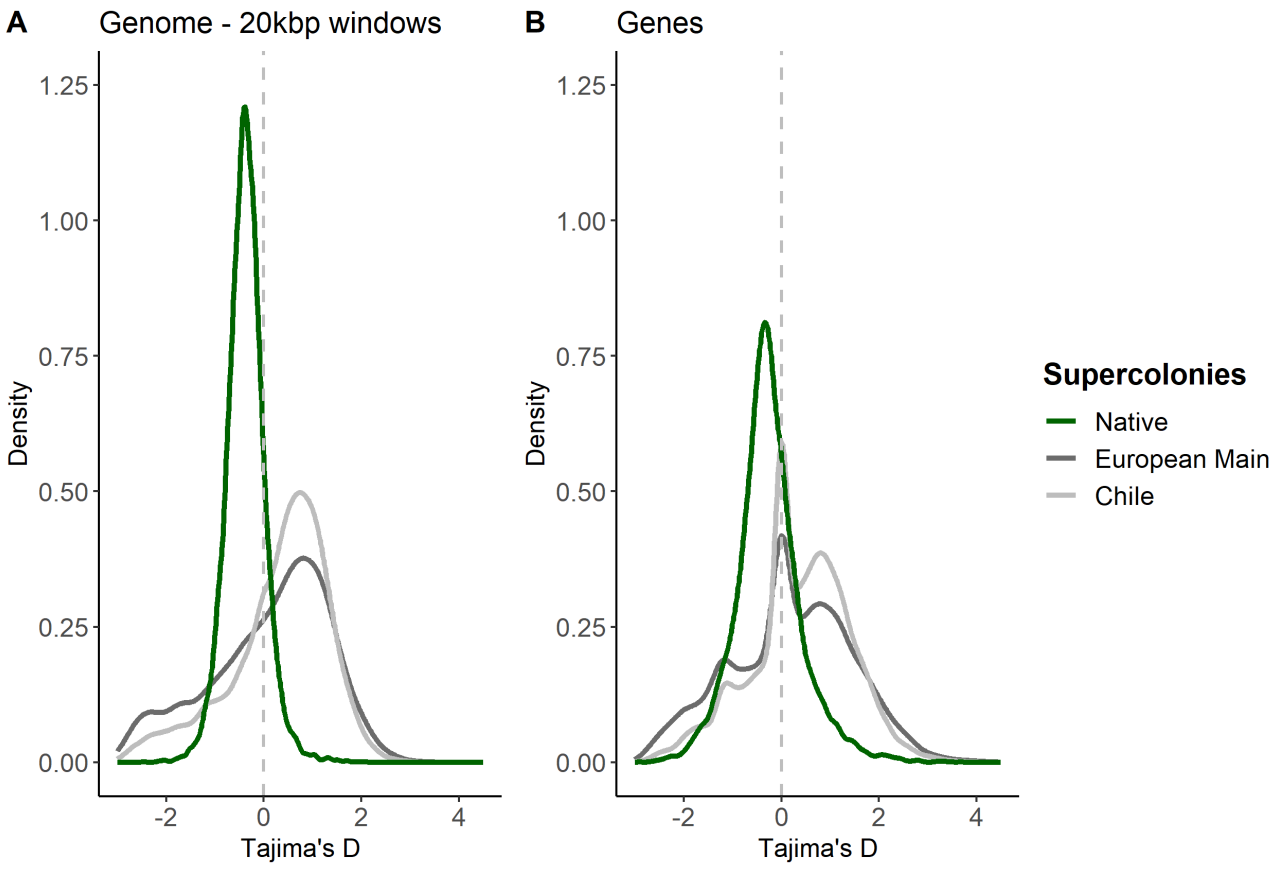


Figure 1. The density curves of Tajima's D values were estimated from (A) whole genomes with 20 kbp windows and (B) genes.

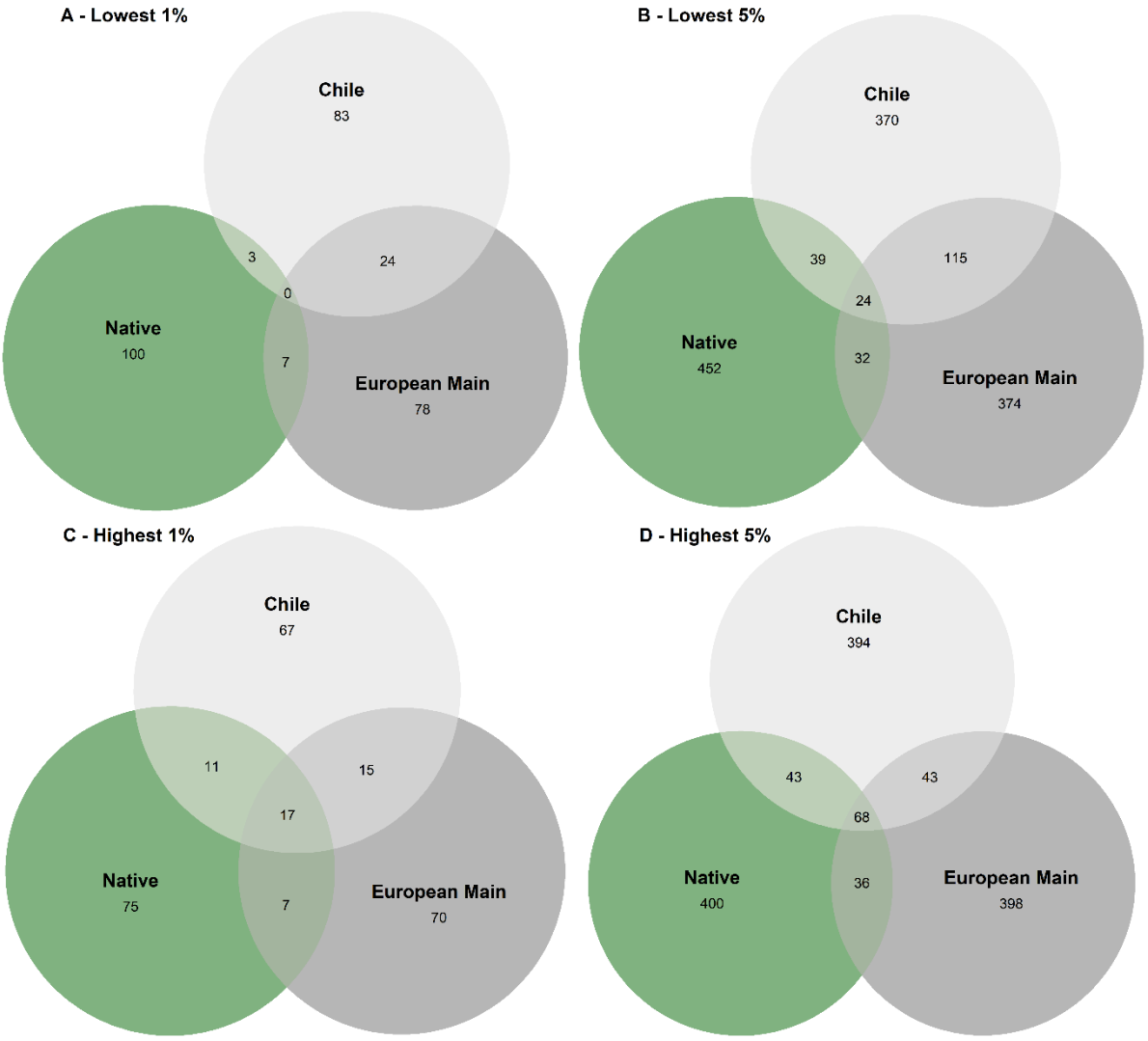
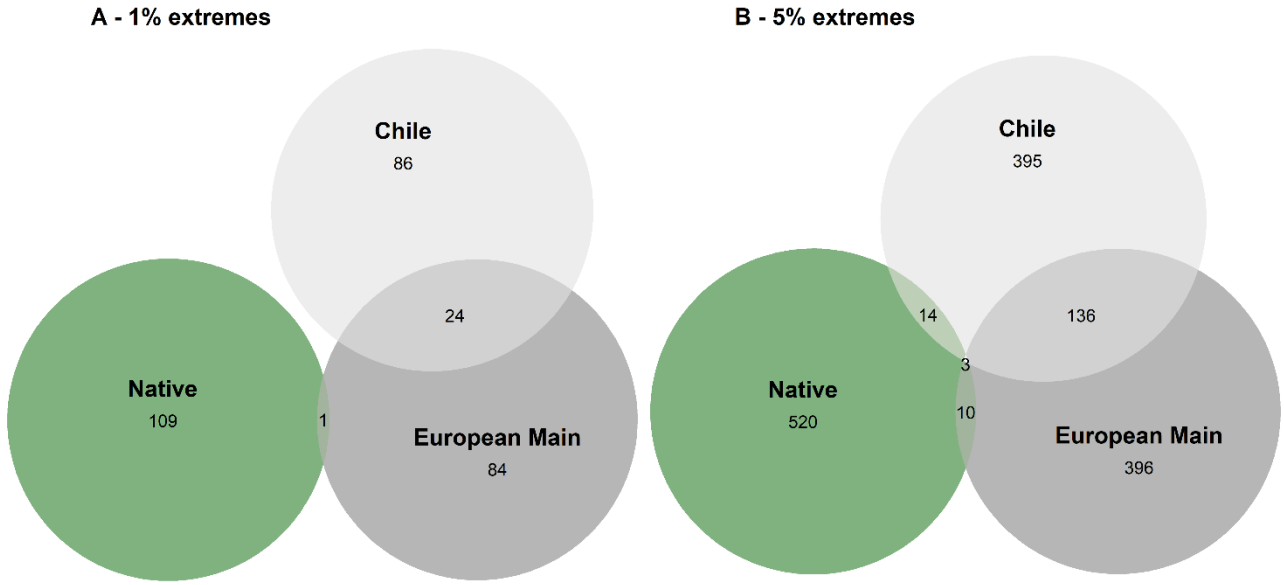


Figure 2. Overlapping genes in the (A) lowest 1%, (B) lowest 5%, (C) highest 1%, and (D) highest 5% extremes of Tajima's D values.



483

484 *Figure 3. Overlapping genes between the highest Tajima's D values of Native and the lowest Tajima's D values of the*
485 *invasive supercolonies with (A) 1% extremes and (B) 5% extremes.*

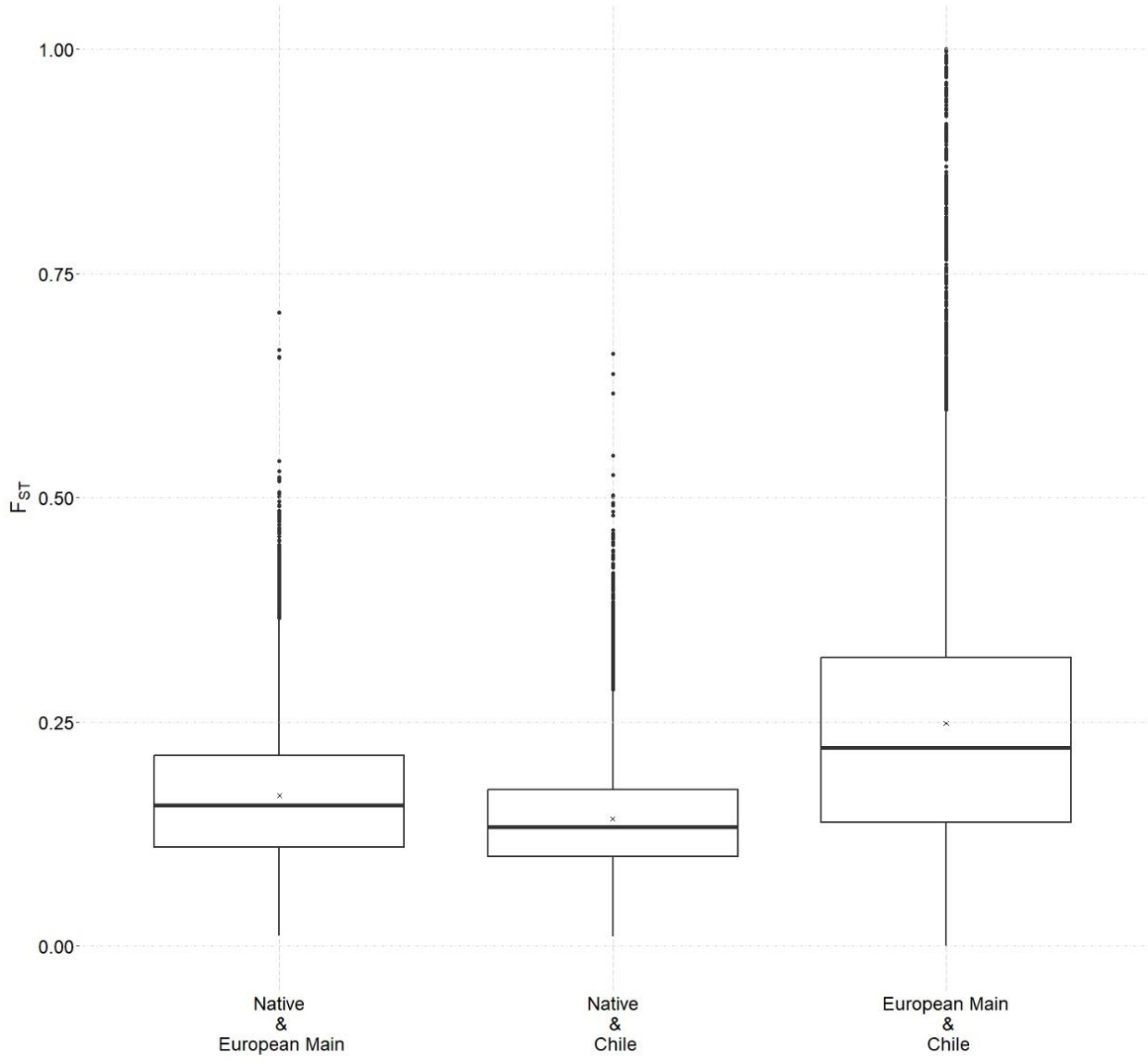


Figure 4. Boxplots of pairwise F_{ST} values. The mean values are indicated with "x".

Author contributions

JSP, HH, and LV designed the research. JP did the lab work. IH analysed the data and wrote the manuscript, but all authors contributed to editing and revising.

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Data Accessibility Statement

Raw sequence reads are deposited in the SRA (BioProject PRJNA846165).

Benefit-Sharing Statement

Benefits from this research accrue from sharing our data and results on public databases as described above.