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).

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

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

In this study, we took a more comprehensive approach by investigating signatures of selection across the whole genome of the Argentine ant. We studied native range supercolonies and three invasive supercolonies from different parts of the world using pooled whole-genome sequence data of 100 individuals from the native range and each of the invasive supercolonies. We aimed 1) to investigate selection patterns within supercolonies and whether the same genomic regions are under selection across supercolonies and 2) to confirm with genome-wide data the earlier findings based on microsatellite marker data about demographic changes during introductions, supercolonial diversity and divergence.

2. Materials and Methods

2.1. Sampling

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

2.2. DNA extraction and sequencing

Genomic DNA was extracted individually from each ant using DNeasy Blood & Tissue Kit (QIAGEN). Equal amounts of DNA from ten individuals per nest were pooled and genome-amplified using REPLI-g Mini Kit (QIAGEN). Finally, all the genome-amplified sub-samples were pooled so that each final sample represented 100 individuals. The samples were sent to BGI Genomics for library preparation and paired-end sequencing using Illumina Hiseq^{TM} 2000 sequencer, with a read length of 90 bp and an insert size of 200 bp. The clean reads provided by BGI Genomics contained sequences where adapters, contaminations, and low-quality sequences were removed.

2.3. Data processing

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

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

The processed BAM files were converted to text-based pileup formats using the mpileup command of SAMtools (Li et al., 2009). A minimum count of 2 was required for identification of indel, and 5 bp were removed around each identified indel using identify-genomic-indel-regions.pl and filter-mpileup-by-gtf.pl scripts from the basic pipeline of PoPoolation (Kofler, Orozco-terWengel, et al., 2011). Sample-specific pileup files for Native, European Main, Catalonia, and Chile were filtered to keep only positions covered with a minimum sequencing depth of 20 (a requirement in later sample-specific downstream analyses). Finally, only the positions that met the sequencing depth criteria in all sample files were kept in analyses to ensure the comparability of the results. As Catalonia had an uneven read alignment and lower data quality, its inclusion in the analyses significantly reduced the number of shared positions. Thus, we decided to do analyses that included and excluded Catalonia. The main text focuses on analyses with higher reference genome coverage and better quality, excluding Catalonia, and analyses including Catalonia are presented in Supplemental Information (see Text S2).

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

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

2.4. Population genetic analyses

2.4.1. Genomic diversity

The genomic diversity of supercolonies was evaluated with two genetic diversity estimators, Tajima's π (π from now on) and Watterson's ϴ ($ϴ_w$ from now on). These were calculated separately for Native and each of the invasive supercolony samples using non-overlapping windows of 20 kbp with Variance-sliding.pl script from PoPoolation (Kofler, Orozco-terWengel, et al., 2011). The window size was slightly larger than the mean gene length (13 988 bp based on the NCBI Linepithema humile Annotation Release 100). The minimum and maximum sequencing depth requirements of the sites used for SNP identifications were 20 and 250, respectively. A minimum count of 4 was required for a minor allele, and the minimum base quality requirement was 20. Finally, at least 60% of each window had to meet the sequencing depth criteria for acceptance when considering sample-specific pileup files containing shared positions. These parameter values were used in all analyses unless otherwise stated. Furthermore, the effect of different sliding window lengths and the inclusion of deletion sites were tested for the results. We calculated the genome-wide mean and standard deviation for the estimated genetic diversity values and the number of single nucleotide polymorphisms (SNPs) used in these analyses. We also estimated genetic marker diversity, expected heterozygosity (H_{exp}) and allelic richness (*k'*) for five variable microsatellite loci obtained from Vogel et al. (2010) applying a similar sample size and design as the genomic samples (see Text S3).

2.4.2. Test of neutrality and inference of adaptive evolution

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

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

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

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

2.4.3. Genomic differentiation

A sliding window approach was used to measure genomic differentiation between samples as pairwise F_{ST} values. The calculations were made using the multi-sample joint mpileup file as input for fst-sliding.pl script from PoPoolation2 (Kofler, Pandey, et al., 2011). The same parameter values were used as above with the PoPoolation analyses. SNPs used in the analysis were identified considering all samples simultaneously. Again, at least 60% of each window had to meet the sequencing depth criteria in all samples included for acceptance for the analysis. We calculated the genome-wide statistics (minimum, maximum, mean, median, and standard deviation) for pairwise genetic differentiation values.

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

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

3. Results

3.1. Properties of the data

Catalonia had an uneven read alignment and lower data quality, and its inclusion in the analyses significantly reduced the number of shared positions. Thus, analyses including Catalonia are presented in the Supplemental Information (see Text S2), and the main text focuses on the analyses with higher reference genome coverage and better quality, excluding Catalonia. After filtering, the sample-specific pileup files with shared positions of Native, European Main, and Chile with a minimum sequencing depth of at least 20 covered 85% of the reference genome (Table S5 A-C). The corresponding multi-sample joint mpileup file covered 93% of the reference genome (Table S5 D).

3.2. Genomic diversity

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

3.3. Test of neutrality and inference of adaptive evolution

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

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

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

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

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

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

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

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

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

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

3.4. Genomic differentiation

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

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

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

4. Discussion

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

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

The invasive Argentine ant supercolonies included in this study have originated from different primary introductions from the native range without secondary reinforcements (Giraud et al., 2002; Vogel et al., 2010). Argentine ant workers are highly aggressive toward non-colony mates, efficiently preventing gene flow between supercolonies (Krieger & Keller, 2000; Sunamura et al., 2011). In addition, gene flow is prevented by the considerable geographic distance between the studied supercolonies, except the European Main and Catalonian supercolonies (the latter considered in Supplemental Information – see Text S2) living partly next to each other (Blight et al., 2012; Giraud et al., 2002; Jaquiéry et al., 2005; Vogel et al., 2010). Although the European Main and Catalonia supercolonies live close to each other, there is no gene flow between them (Blight et al., 2012; Giraud et al., 2002; Jaquiéry et al., 2005). The effective queen number establishing the European Main as well as the Catalonian supercolonies has been estimated to be between 6 to 13 individuals (Giraud et al., 2002; Vogel et al., 2010), while in native range supercolonies, it has been estimated to be extremely high, reaching infinity (Pedersen et al., 2006). Thus, the founder effect probably created the first shift toward genetic differentiation of the introduced supercolonies. In addition, amplified genetic drift in the small founder populations might have caused further fluctuations in allele frequencies, contributing to diversification in expanding supercolonies (Nei et al., 1975).

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

Whilst the Mediterranean-like climate is shared across the native and invasive ranges of the Argentine ant (Roura-Pascual et al., 2011), other biotic and abiotic factors, such as pathogens and competitors, likely differ between regions, leading to distinct local selective pressures (Holmberg et al., 2024; Lester & Gruber, 2016). In the Argentine ant, pervasive selection in both the native and invasive supercolonies is suggested by the different distributions of Tajima's D of the whole genomes (estimated with 20 kbp windows) and genes.

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

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

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

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

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

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

The overlaps of genes under balancing selection between the native and invasive supercolonies might unveil pre-adaptations in the species' native range, a phenomenon that has been suggested to facilitate invasion success in various species by providing a selection regime that allows rapid adaptation in response to changing selection pressures in fluctuating environmental conditions (Gloag et al., 2016; Kaňuch et al., 2021; Stern & Lee, 2020; Tepolt et al., 2022). It seems plausible that many species became successful invaders due to the traits already present in their native range. An unstable environment due to frequent floodings along the Paraná River in the native range (Wild, 2004) could maintain balancing selection in key genes, preparing this species to become thriving dispersers.

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

The success of a wide range of invasive species populations with low adaptive potential due to low genetic diversity has been explained by factors such as gene flow, stress-induced accelerated mutation rate or TE-activation, epigenetic changes and the release from natural pathogens and enemies (Errbii et al., 2021; Manfredini et al., 2019; Marin et al., 2019; North et al., 2021; Schrader et al., 2014). Despite the low genetic diversity, genomes of the invasive Argentine ants continue to evolve adaptively. Pre-adaptation in the native range has recently received more attention across species (Davidson et al., 2011; Elst et al., 2016; Estoup et al., 2016; Guo et al., 2014; Hufbauer et al., 2012), and we suggest that this might also play a role in the initial invasive success of the Argentine ant. The unstable environment in the native range of the Argentine ant, characterised by frequent flooding, may enhance the species' adaptive potential. Balancing selection, which maintains alternative alleles in fluctuating conditions, could contribute to their continued and long-term success by adapting to new habitats following introductions. Moreover, the overlap of positively selected genes is higher among the invasive supercolonies than between the native and invasive supercolonies, indicating shared selective pressures in the new ranges. The exact roles of all selected genes must be carefully resolved among the complex biological processes and pathways in upcoming genomic surveys, such as comparative population genomic studies between native and introduced supercolonies using both genomic and transcriptomic data at the individual level. Our study provides an excellent groundwork and frame for future studies of the Argentine ant in the field of invasion genomics.

Tables and Figures

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

Table 2. Pairwise Jaccard Indexes for genes in the lowest 1% and 5% and highest 1% and 5% extremes of 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

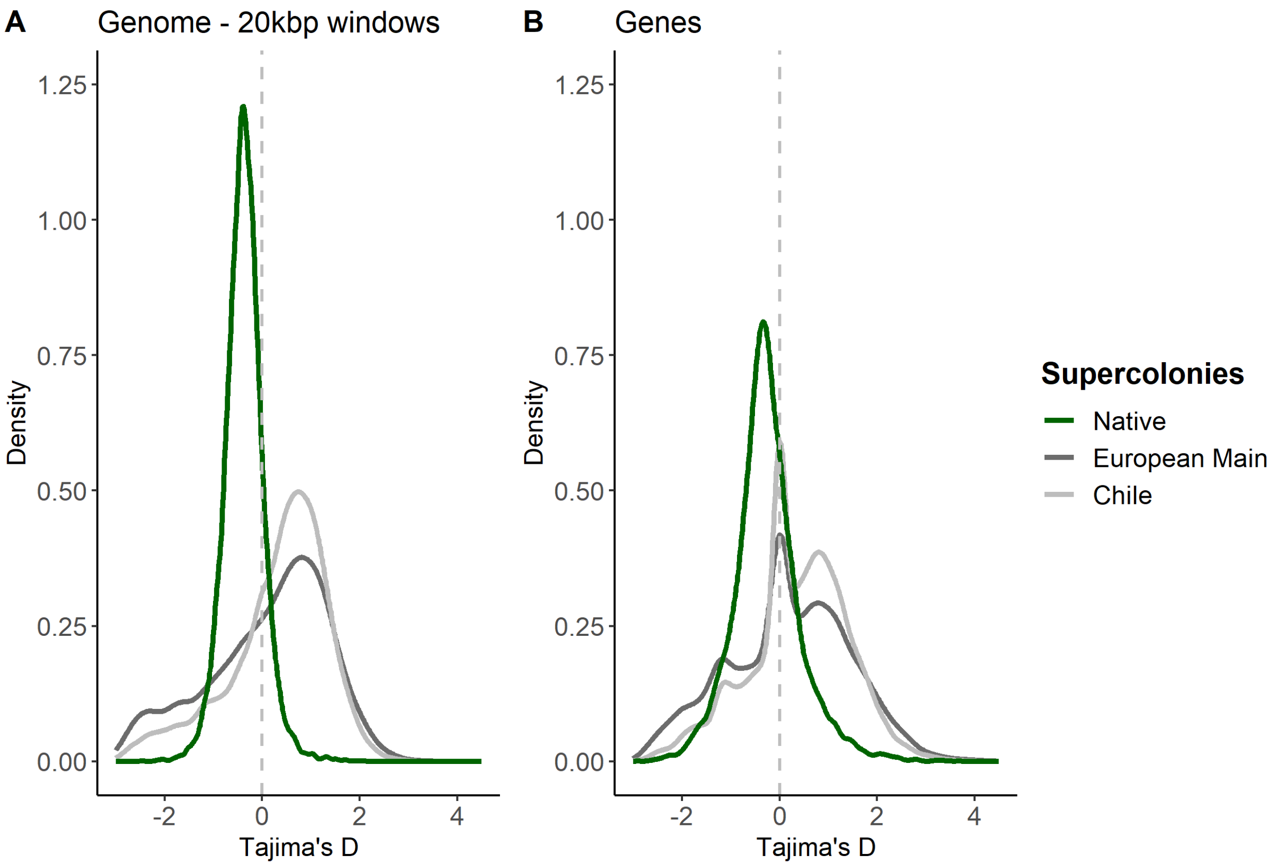


Figure 1. The density curves of Tajima's D values were estimated from (A) whole genomes with 20 kbp windows and (B) genes.

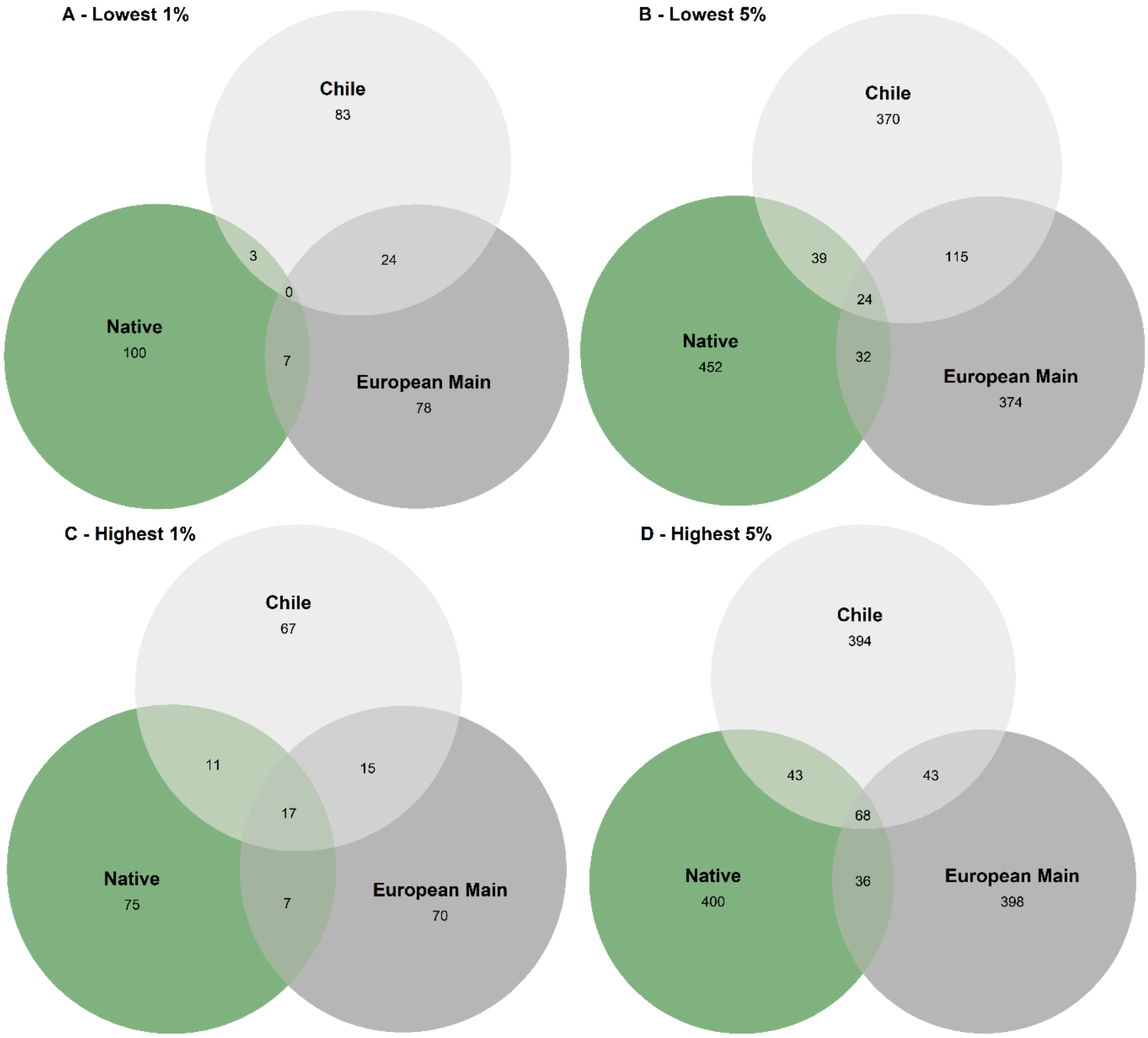
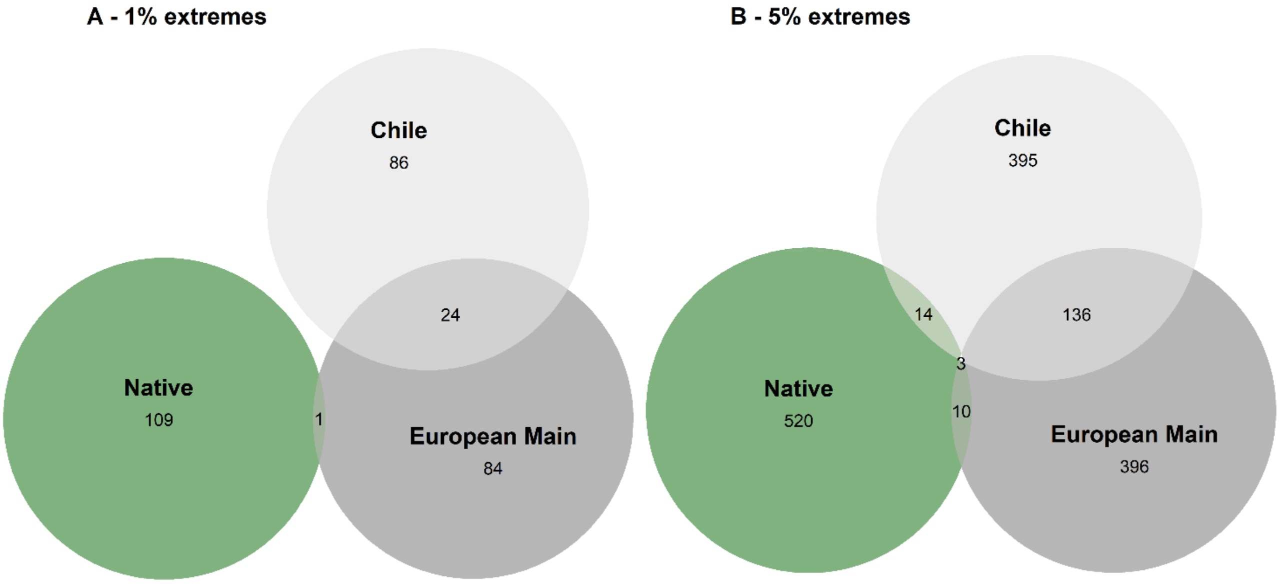


Figure 2. Overlapping genes in the (A) lowest 1%, (B) lowest 5%, (C) highest 1%, and (D) highest 5% extremes of Tajima's D values.



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.*

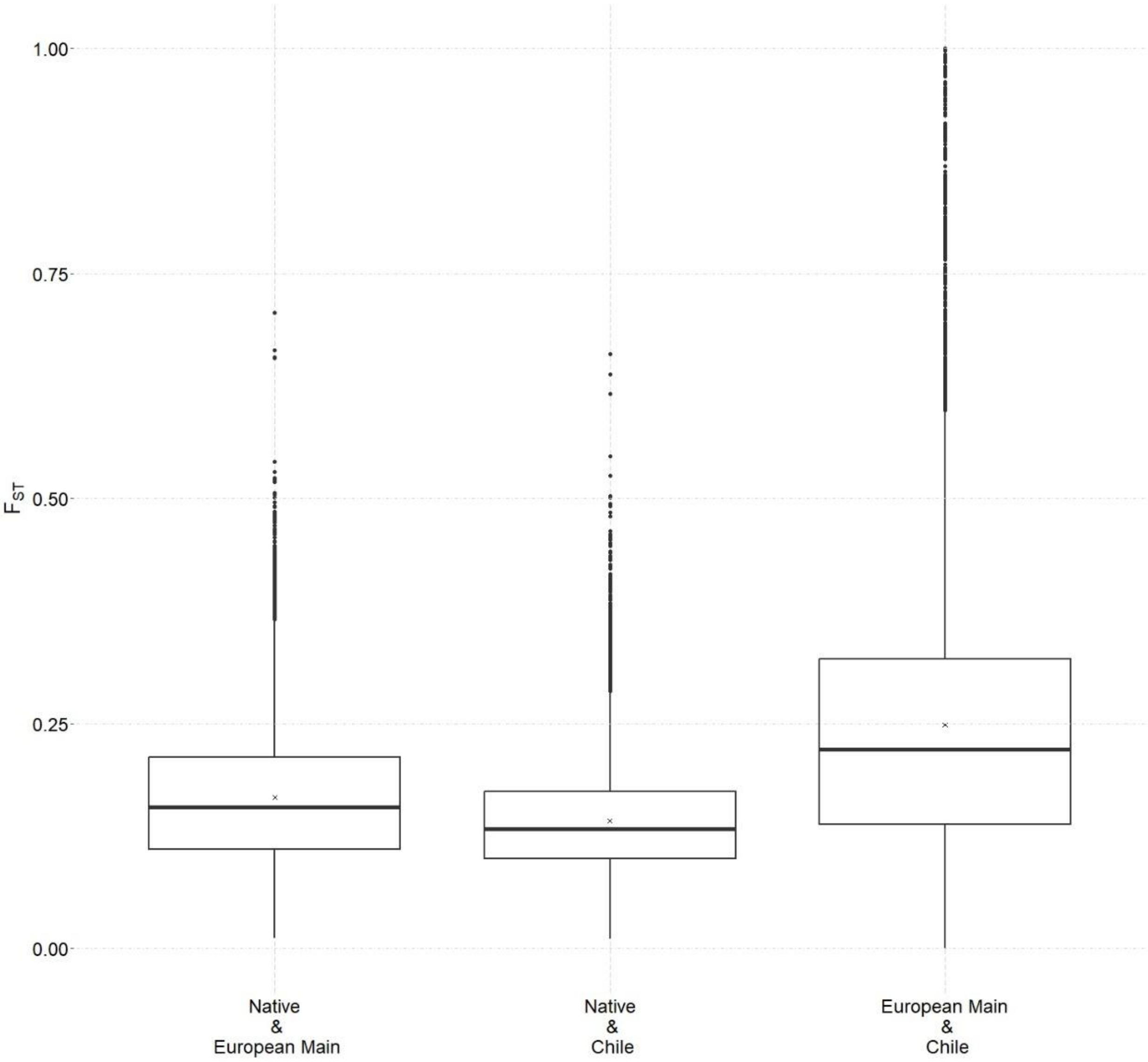


Figure 4. Boxplots of pairwise F_{ST} values. The mean values are indicated with "x".

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.