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Dissecting the invasion history of Spotted-Wing *Drosophila* (*Drosophila suzukii*) in Portugal using genomic data

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Abstract

Background The invasive pest Spotted-Wing *Drosophila*, *Drosophila suzukii* (Matsumura), causes extensive damage and production losses of soft-skinned fruits. Native to Asia, the species has now spread worldwide, with first reports in Portugal in 2012. In this study, we focus on the genomic signatures of the recent Portuguese invasion, in the context of worldwide patterns established in previous works. We analyzed whole genome pool sequencing data from three Portuguese populations ($N = 240$) sampled in 2019 and 2021.

Results The correlation of allele frequencies suggested that Portuguese populations are related to South European ones, indicating a Mediterranean invasion route. While two populations exhibited levels of genetic variation comparable to others in the invasive range, a third showed low levels of genetic diversity, which may result from a recent colonization of the region. Genome-wide analyses of natural selection identified ten genes previously associated with *D. suzukii's* invasive capacity, which may have contributed to the species' success in Portugal. Additionally, we pinpointed six genes evolving under positive selection across Portuguese populations but not in European ones, which is indicative of local adaptation. One of these genes, *nAChRalpha7*, encodes a nicotinic acetylcholine receptor, which are known targets for insecticides widely used for *D. suzukii* control, such as neonicotinoids and spinosyns. Although spinosyn resistance has been associated with mutations in the *nAChRalpha6* in other *Drosophila* species, the putative role of *nAChRalpha7* in insecticide resistance and local adaptation in Portuguese *D. suzukii* populations encourages future investigation.

Conclusions Our results highlight the complex nature of rapid species invasions and the role of rapid local adaptation in determining the invasive capacity of these species.

Keywords Spotted-wing *Drosophila*, Natural selection, Invasive species, Invasive capacity

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Background

The increase in international trade has facilitated the introduction of new species in areas outside of their native range [1]. Although not all species can survive and thrive in a new environment, some biological and physiological traits, such as flexible metabolisms, polyphagy, or high dispersal ability by transportation in immature stages or assisted adult flight, can confer higher intrinsic invasive potential [2, 3].

The Spotted-Wing *Drosophila* (SWD), *Drosophila suzukii* (Matsumura), is an invasive polyphagous pest species of small fruits and berries, considered to be native to eastern and south-east Asia, even though this origin is still debated [4]. The species was described in 1931 in Japan, and was first collected outside its native area range in 1980 in Hawaii [5]. Over the last decade, *D. suzukii* has spread to several countries on almost all continents, causing high fruit losses and severe economic damages in the invaded areas [6–8]. The biggest and most relevant invasions occurred in 2008 in North America and Europe, with *D. suzukii* being detected near port areas (California, USA, and Rasquera, Spain). This suggested that the invasion originated from individuals at immature stages present in sea-traded fruits coming from the native range [9, 10]. The species rapidly spread and is currently present in almost all US states and European countries [9, 11]. A Mexico-South American invasion occurred in 2011, with the first *D. suzukii* detections in Mexico [12]. By 2017 it had already been established in Brazil, Chile, or Argentina. The presence of *D. suzukii* in Africa is yet unclear, although the report of its presence in Morocco in 2013 suggests it may be spreading across the continent as well [13–16].

The North American and European invasions are contemporaneous but independent demographic events. In a study based on X-linked gene fragments from *D. suzukii* flies collected across the invaded and native range, Adrion et al. (2014) detected signals of differentiation between the European, Asian, and North American populations, but no evidence of differentiation within the USA populations [17]. Then, Fraimout et al. (2017), using microsatellites, found evidence of multiple invasions from Asia into Europe and the USA, and differentiation of the eastern and western populations of the USA [18]. In contrast, studies of European populations suggest high genetic homogeneity, such as between the Italian and German *D. suzukii* populations [19, 20]. Important differences have been reported between the American and European populations. For example, European populations have lower genetic diversity, less divergent selection, lower mutation rates, higher fecundity rates, higher susceptibility to European parasitoids, and higher frequencies of *Wolbachia* when compared to the American populations [21]. Recent studies based on whole genome sequencing (WGS) have provided robust confirmation of these invasion routes [22–24]. In addition, signals of admixture originating from Eastern USA populations have been detected in Ireland, suggesting cross-migrations among invaded areas [23].

The rapid spread and invasion success of *D. suzukii* has been explained by its innate preference for temperate climates, coupled with high capacity to adapt to food availability and environmental stressors, and lack of specialized enemies in the invaded ranges [25–27]. Olazcuaga et al. (2020) identified single nucleotide polymorphisms (SNPs) associated with *D. suzukii* populations in invaded areas that may underlie its adaptive capacity.

The first records of *D. suzukii* in Portugal occurred in 2012 when adults were identified in a raspberry greenhouse on the coast of Alentejo [28]. In the last 10 years, it has spread across the Portuguese continental territory but also to its islands [29–31]. Here, we conduct the first genomic study of the Portuguese *D. suzukii*. Using new whole genome pool sequence data from three Portuguese populations of the species, and reanalyzing previously published data from the worldwide range, we (i) characterize the genetic diversity *D. suzukii* in Portugal, (ii) identify the invasion route originating these populations, and (iii) investigate whether local adaptation may underlie the invasive capacity of the species in the region.

Methods

Sample collection and genomic DNA extraction

Adults of *D. suzukii* (N=240) were sampled from three locations in the North of Portugal, Vieira do Minho (PT-VM19), Oliveira de Azeméis (PT-OAZ21), and Castelo de Paiva (PT-CP21), either by aspiration in the field or after emerging from infested fruits in the laboratory. Access to infested orchards was permitted by the owners, who also accompanied sampling and provided the infested fruits. As other *Drosophila* species could be present in the samples, adults were morphologically identified following the European Plant Protection Organization (EPPO) dichotomous key (OEPP/EPPO, 2013). Detailed information on sample location, year and collection method is indicated in Table S1. After identification, flies were surface sterilized in a 1.5% bleach solution (v/v, in Milli-Q H_2O) and stored at -80ºC until DNA extraction.

For genomic DNA extraction, the thoraxes of 8 males and 8 females were dissected under a magnifier and randomly pooled in a total of 5 pools per population (80 individuals per population), to decrease the possibility of unequal individual contribution to the final pools. DNA of each pool was isolated with the QIAamp DNA Micro Kit (Qiagen) according to the manufacturer's instructions for the isolation of Genomic DNA from Tissues, with some modifications: the thoraxes were disrupted in a

homogenizer with a 2.3 mm ceramic bead in Buffer ATL before adding proteinase K; samples were incubated for 4 h at 56°C; after incubation, carrier RNA was added to each sample following the protocol's instructions.

Library preparation, pool-sequencing, and data processing

DNA libraries were prepared for the fifteen pools of the three Portuguese populations with TruSeq DNA PCR-Free kit (Illumina) and IDT for Illumina TruSeq DNA UD Indexes. All libraries were quantified by quantitative polymerase chain reaction (qPCR) and run in a 2200 Tapesation (Agilent). Paired-end 150 bp sequencing was performed at Novogene Co. Ltd. (Cambridge, UK) on an Illumina NovaSeq, for a total of 90.97 Gb of raw sequencing data.

Raw population sequences from 22 worldwide populations of *D. suzukii* were retrieved from Olazcuaga et al. (2020) (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA576997) and processed with the same parameters as the raw sequences obtained for samples from Portugal (Fig. 1a, b). Adapter sequences were removed and reads trimmed for quality with Trimmomatic v0.39 [32] using the following parameters: TRAILING:15, SLIDINGWINDOW:5:15, MINLEN:25. Resulting single and paired-end reads were mapped to the *D. suzukii* LBDM_Dsuz_2.1.pri reference genome (https://www.ncbi.nlm.nih.gov/data-hub/genome/GCF_013340165.1/) [33] using BWA-MEM v.0.0.7.17 with default parameters [34]. Duplicates were removed with Picard v12.18.17 (http://broadinstitute.github.io/picard/) and reads with mapping quality below 20 (-q 20) were removed with SAMtools v1.3.1 [35]. Mapping stats were assessed with bamtools [36] and coverage per genomic position was retrieved with SAMtools v1.3.1. A mpileup file with all populations and reference genomes was created with SAMtools v1.3.1 and a variant call to VCF was performed using VarScan v.2.3.9 [37] with the following parameters: --min-coverage 20 --min-avg-qual 20 --min-var-freq 0.001. Autosome (A) and X chromosome (X) scaffolds were identified from Paris et al. (2020), and extracted from the vcf file with vcftools [38].

Population genetic diversity

Watterson's θ and genome-wide nucleotide diversity (π) were estimated with PoPoolation v1.2.2 [39] for all 25 populations. Bam files were indexed and separate bam files for Autosome (A) and X chromosome (X) were produced with SAMtools v1.3.1. Mpileup files for each population were created with SAMtools v1.3.1 with filters -q 20 and -Q 20. Diversity indices (π and θ) were estimated in non-overlapping 20 kb sliding windows, with the following filters adapted to each population and chromosome type (A or X): --pool-size haploid sample size for A or X, --min-count 3, --min-coverage ½ median coverage for A or X, --max-coverage 3x median coverage for A or X, --min-covered-fraction 0.50. ANOVA and Tukey's Honestly Significant Difference tests were conducted among populations. Values of pool-size, min-coverage, and max-coverage for each population are detailed in Table S1.

Population structure and relationships

BAYPASS v2.3 [24, 40] core model with default options was used to generate a scaled covariance matrix of population allele frequencies (Ω). VCF files of autosomes and X chromosome were converted to a pooldata object with the R package *poolfstat* v2.1.1 [22]. with the following parameters: min.cov.per.pool=4, min.rc=0, max.cov.per.pool=3x median coverage of each pool for A or X, min.maf=0.01, remove.indels=TRUE, nlines.per.read-block=1e+06 and verbose=TRUE. The pooldata object was converted to a BAYPASS input file (*pooldata2genobaypass*) using the R package *poolfstat* v2.1.1 with a subsample size of 75,000 using the thinning method [22]. The heatmap with clustering analysis and a singular value-decomposition (SVD) of the matrix Ω were plotted using the *baypass_utils* R file following the BAYPASS v2.3 manual instructions [24]. Additionally, population pairwise F_{ST} was estimated from the pooldata object with the *compute.pairwiseFST* of the *poolfstat* v.2.1.1 R package with default settings [22].

Population genetic relationships and migration were in addition inferred using TreeMix v.1.13 [41, 42], and the allelic frequencies subsampled with *poolfstat*. Standard errors (-SE) and bootstrap replicates (-bootstrap) were used to evaluate the confidence in the inferred tree topology without assigning a root. After constructing a maximum-likelihood tree, migration events were added (−m) and iterated 5 times for each value of m (1–9), to check for convergence of the model likelihood as well as the explained variance following the addition of each migration event. The best number of m migrations was determined using OptM [43]. The inferred maximum-likelihood population trees were visualized using the *popcorn* R package (https://github.com/andrewparkermorgan/popcorn).

Analyses of selection

Selective sweeps along the whole genome of Portuguese *D. suzukii* populations and three populations from the European range (DE-Dos, ES-Bar, and FR-Cor) were inferred separately for Autosome and X chromosome scaffolds with Pool-hmm v.1.4.4 using the hidden Markov model approach [44]. The settings -q 20, -e sanger, --pred, -k 1e-10 were common to all analyses. The threshold -k 1e-10 was set based on the study by Kapun et al. (2020) [45]. The number of chromosomes in the pool (-n), minimum coverage (-c), and maximum coverage

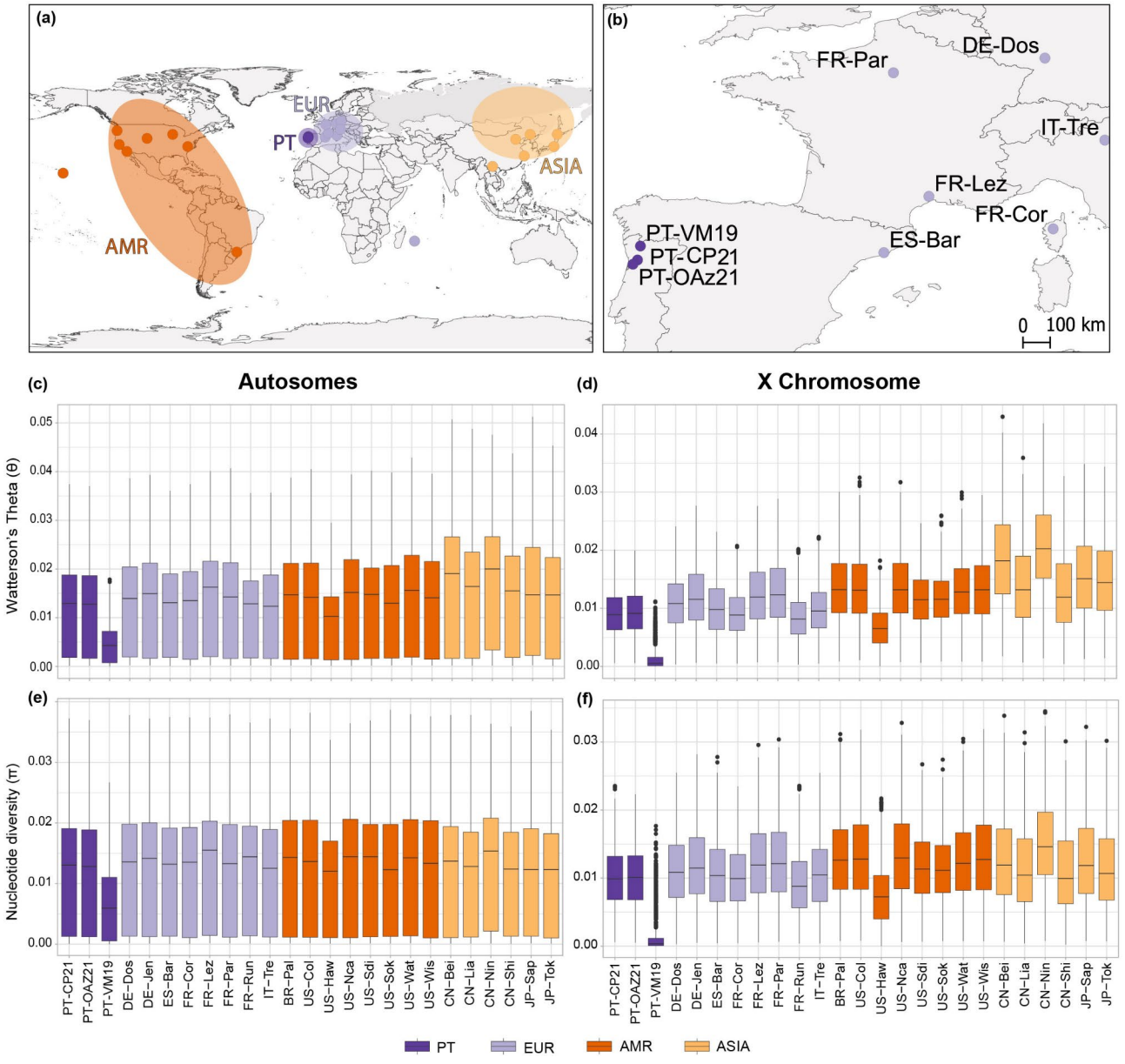

Fig. 1 Geographic location of the 25 populations (this study and Olazcuaga et al. 2020). Dots are colored according to their origin [Asia, America (AMR), and Europe (EUR), with the Portuguese (PT) populations highlighted with a darker color within the European populations]; (**b**) Zoom-in to sampling localities in Europe, from this study (three populations from Portugal) and Olazcuaga et al. (2020) [24] (scale bar = 100 km). (**c-d**) Watterson's theta (θ) for autosomes (**c**) and X chromosome (d) for all populations. (**e-f**) Nucleotide diversity (π) for autosomes (**e**) and X chromosome (**f**) for all populations. Original population codes from Olazcuaga et al. (2020) [24] were used (DE – Germany, ES – Spain, FR – France, IT – Italy, BR – Brazil, US – United States of America, CN – China, JP – Japan). Map downloaded from Natural Earth (naturalearthdata.com) and edited in QGIS v3.16.0 (qgis.org)

(-C) were the same as for the estimation of the diversity indices previously described. Genome-wide theta (-t) was determined based on the PoPoolation estimations of each population (mean value of θ estimated for each population, Autosomes and the X chromosome separately). Pool-hmm predicts three possible hidden states: 'neutral', 'intermediate', and 'selection'. We have primarily considered genomic fragments classified with hidden state 'selection' as our candidates to have evolved under selection. Secondarily, we inspected genomic fragments classified as 'selection' with probability of 1, to identify and highlight those with the strongest selection signals that are exclusive of Portuguese populations.

Tajima's D estimates were computed for each Portuguese population with PoPoolation v1.2.2 [39], adjusting the –min-count filter to 2 and –min-coverage to 1/3 of the median coverage of the population for Autosomes or the X chromosome. Selective sweeps along the genome

were visualized with the *ChromoMap* v0.4.1 R package [46].

Annotation of SNPs and gene enrichment analyses

For gene annotation, we used the strategy adopted by Olazcuaga et al. (2020), taking advantage of genomic resources available for *D. melanogaster*, a model species closely related to *D. suzukii*. We used bedtools getfasta [47] to extract 5-kb-long genomic sequences surrounding each SNP analyzed with Pool-hmm and aligned them onto the dmel6 *D. melanogaster* reference genome [48] using the BLAT algorithm, implemented in pblat [49] (Wang and Kong 2019). The gene annotation from the UCSC genome browser (https://genome.ucsc.edu/, last accessed January 2024) in combination with FlyBase converting tools vFB2023_06, released December 12, 2023 [50] allowed mapping and annotating the analyzed SNPs. While RefSeq IDs represent transcripts, FlyBase IDs correspond to genes, and consequently a given FlyBase ID (gene) may encompass multiple RefSeq IDs (transcripts). For consistency, we kept the number of genes retrieved from the FlyBase database.

Functional enrichment analysis was conducted using g: Profiler web server [51] to test the genes inferred as under selection. Only genes within retained segment windows were considered as the background list of genes. Categories with less than five genes were excluded and the g: SCS algorithm [51] was applied for computing multiple testing correction for *p*-values. We used the *D. melanogaster* GO term annotation.

Results

Whole genome population data and population diversity

Pool-sequencing of the three Portuguese populations had average median coverages of 91x and 72x for the autosomes (A) and the X chromosome (X), respectively, after the removal of reads with mapping quality below 20. Population PT-VM19 had the highest median coverage (103x and 81x for A and X, respectively), and population PT-OAZ21 had the lowest (77x and 60x for A and X, respectively) (Table S1). Data processing of the 22 world populations retrieved from Olazcuaga et al. (2020) resulted in median coverages for Autosomes and the X chromosome similar to the original study (Table S1).

Population diversity estimates for the 25 populations, for autosomes and X chromosome, revealed that PT-VM19 had the lowest diversity among all populations from the invaded and native range, including the other two Portuguese populations (PT-CP21 and PT-OAZ21; $p<0.05$, Tukey's HSD test) (Fig. 1c-f and Table S2, S3). This pattern was observed both for autosomes (Fig. 1c, e) and the X chromosome (Fig. 1d, f). Populations PT-CP21 and PT-OAZ21 had genetic diversity (θ and π) similar to the other populations in the native and invaded range, not only European but also American and Asian. Overall, Portuguese populations have significantly lower diversity than the other European populations ($p=0$, Tukey's HSD test), and these were also significantly less diverse than the Chinese populations, from the native range (θ, $p=0$, Tukey's HSD test) (Table S2 and S3).

Population structure and relationships

The scaled covariance matrix of population allele frequencies (Ω) separated the 25 populations into two clusters, one containing the Asian and American populations, and the other containing the European populations, including the Portuguese ones. In the analysis of the autosomes, the Portuguese populations clustered with those from France (FR-Cor), Spain (ES-Bar), and Italy (IT-Tre) (Fig. 2a). Population PT-VM19 appeared as a single branch in the European cluster. The singular-value decomposition of the matrix Ω (SVD of Ω) separated population PT-VM19 from all other populations in PC1, which explains 54.54% of the variance, while PC2 separated the European populations from the American and Asian ones (explaining 19.81% of the variance) (Fig. 2b). The other two Portuguese populations (PT-CP21 and PT-OAZ21) were grouped with the rest of the European populations, as seen in the matrix Ω heatmap (Fig. 2a). Similar results were obtained for the X chromosome (Figure S1a, b).

In the Treemix analysis, m=3 was identified as the best number of migrations, but no migration edges involved Portuguese populations, affecting e.g. US-Wat and FR-Par (Fig. 2c, S2 and S3). Treemix also placed the Portuguese populations in the European clade, but with some differences in the specific relationships within this clade (Fig. 2c and S3). Here, all Portuguese populations were grouped with the other South European populations (ES-Bar, FR-Cor, and IT-Tre), within the European clade. PT-VM19 showed a long drift branch, in keeping with its low level of genetic diversity. Pairwise F_{ST} estimates confirmed this large differentiation between PT-VM19 and the other analyzed populations for both autosomes and X chromosome (Figure S4a, b), with F_{ST} values of approximately 0.5 in all pairwise comparisons. In Treemix, allowing for migration events did not improve the estimated relationships involving the Portuguese populations, and produced broad heterogeneous topologies across replicates (Figure S3).

Selection analyses

Pool-hmm inferences along the genome of the Portuguese populations and European populations DE-Dos, ES-Bar and FR-Cor classified several regions under the hidden state 'selection' (Fig. 3a). PT-VM19 had the most widespread inferences of genomic windows under selection, with a total of 411 detected sweep windows (of

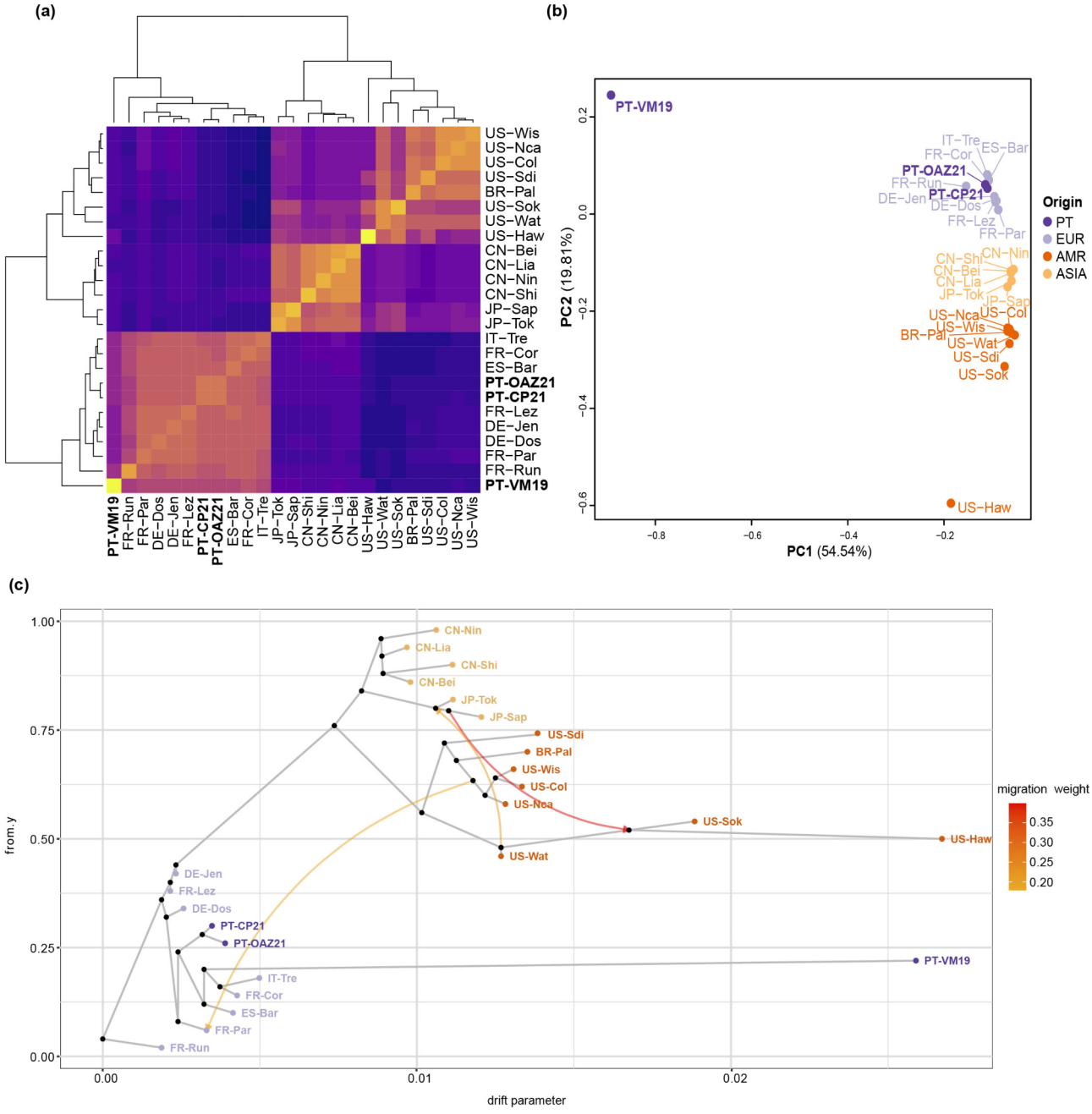

Fig. 2 Relationships of the Portuguese (PT) *D. suzukii* populations with other populations worldwide [European (EUR), American (AMR) and Asian (ASIA)]. (**a**) Heatmap of Ω for the autosomes; (**b**) SVD of Ω for autosomes with PC1 explaining 54.54% of the variance and PC2 explaining 19.81%. The analysis for the X chromosome shows qualitative similar results and are shown in Figure S1; (**c**) Treemix tree of relationships across analyzed populations (m = 3)

which 377 were in chromosome-placed scaffolds), whilst PT-CP21 and PT-OAZ21 had a lower number of inferences of genomic windows (a total of 75 and 54, respectively). Yet, the markedly low levels of diversity found in PT-VM19 may indicate an important deviation of demographic equilibrium and lead to an increase in the number of false positive inferences of selection targets. Interpretations should thus focus on consistent signals across populations. The three closely related European populations showed 92 (DE-Dos), 77 (ES-Bar) and 54 (FR-Cor) regions under selection. These results were compatible with Tajima's D estimates, with a broad coincidence of lower negative values in regions detected under selection by Pool-hmm both in Portuguese (Figure S6) and European (Figure S7) populations.

We then focused on genomic windows with consistent evidences of selection across the analyzed populations (three Portuguese and three European) (Fig. 3b). We

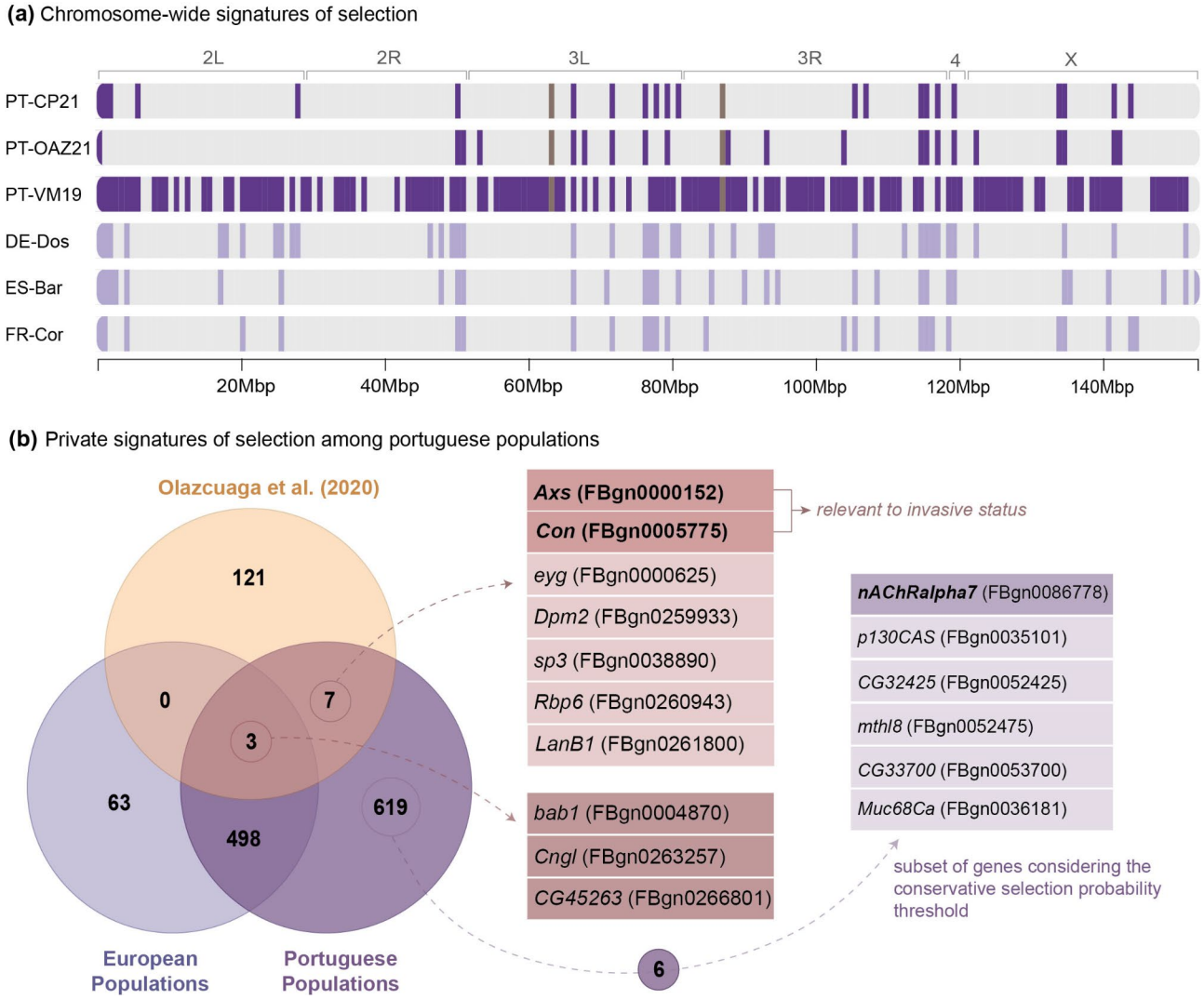

Fig. 3 Analyses of selection along the genome of the three Portuguese populations and three European populations (DE-Dos, ES-Bar, and FR-Cor). (**a**) Chromosome-wide signatures of selection for the six analyzed populations, with an indication of windows that are common and exclusive to the Portuguese populations (highlighted with different color). For visualization purposes and simplification, only the windows detected in chromosome-placed scaffolds are shown. (**b**) Intersection of the list of genes inferred to be under selection in the Portuguese and the other European populations, and those reported by Olazcuaga et al. (2020) [24], with the indication of concerned gene symbols and FlyBase IDs

identified and annotated 1,127 unique genes in common selection targets across Portuguese populations, and 564 unique genes in the three other European populations. Of these, 501 genes were common to all six analyzed populations. Intersecting these genes with those suggested by Olazcuaga et al. (2020) [24] as relevant for the invasive capacity of *D. suzukii* populations revealed seven common genes with the Portuguese populations (*Axs*, *Con*, *LanB1*, *eyg*, *Dpm2*, *sp3* and *Rbp6*), and three common to both Portuguese and the analyzed European populations (*bab1*, *Cngl*, *CG45263*) (Fig. 3b).

The analysis of gene ontology (GO) term enrichment in the set of genes inferred under selection across Portuguese populations (1,127 genes) identified terms associated with cell cycle molecular functions such as "structural constituent of chromatin" or biological processes such as "nucleosome assembly" or "nucleosome organization". Finally, a total of 626 genes were identified within selection targets exclusive and common across the Portuguese populations. Using a conservative criterion of selection inference with probability of 1, we identified six distinct genes: *p130CAS*, *CG32425*, *mthl8*, *CG33700*, *Muc68Ca* and *nAChRalpha7* (Fig. 3b and S5).

Discussion

The Portuguese invasion route

Native to Asia, *D. suzukii* is an invasive species on most of the continents outside its native area, especially in Europe and North America, where it has invaded almost all of its countries. In Portugal, it rapidly spread to the

entire country since its identification in 2012, but until now, no information was known on the genetic diversity of the Portuguese *D. suzukii* populations or about the route that led to the invasion of Portugal. Across the worldwide range, our results show that genetic diversity slightly decreases from the native to the invaded range, but levels are comparable (Fig. 2c, d), which is consistent with the maintenance of large effective population sizes along the invasion [18]. Yet, we did find a significant reduction of theta from the native Chinese populations to the European ones, consistent with an invasion bottleneck. The low levels of diversity in PT-VM19 strongly differ from the other analyzed Portuguese populations (Fig. 2c-f). These results could suggest some level of heterogeneity and complexity in the Portuguese invasion of the species. Previous studies based on molecular markers characterized the diversity of other European populations and while Petermann et al. (2021) found no differences among German *D. suzukii* populations, a result congruent with the estimated homogeneity in Italian populations [20], Ukrainian populations showed high genetic heterogeneity [52].

To further understand the heterogeneity in genetic diversity among the Portuguese populations, we estimated relationships among *D. suzukii* populations both following the methodology presented by Olazcuaga et al. (2020) [24] and using Treemix. This also aimed at inferring the route leading to the invasion of Portugal. The results of the two analyses were consistent in showing high similarity of populations PT-CP21 and PT-OAZ21, and placing them close to populations from Italy, Southern France, and Spain (Fig. 2a). This result suggests that these populations are part of the South European invasion route, likely through the Mediterranean Coast. This finding was not surprising, as the first individuals of *D. suzukii* in Portugal were identified near the country's Southern region [28, 30]. The low differentiation between these populations suggests they may form a large panmictic population. The relationship of PT-VM19 was again more heterogeneous across methods, either being placed as part of the European group but basal to it (BAYPASS; Fig. 2a), or close to the same south European populations reported above (Treemix; Fig. 2c). The marked low levels of genetic diversity found in PT-VM19 exacerbate differentiation levels (consistent with the long drift branch in Treemix), making the identification of relationships more difficult based solely on allele frequency differences. Furthermore, sampling of this population occurred two years prior to the sampling of PT-CP21 and PT-OAZ21, and homogenization of genetic diversity across populations may have occurred with time, and explain the differences. This hypothesis can be tested by resampling these populations to assess putative temporal changes. The detected heterogeneity can thus be not only spatial but also temporal. In addition, our Treemix analysis identified migration routes consistent with previous works [23, 25], but given the heterogeneity of the results across replicates, they should be interpreted with caution. Overall, our results identify a south European route originating the invasion of Portugal, but further analyses are required to understand the causes of the low diversity of PT-VM19 and clarify the relationship of this and the other populations.

Rapid local adaptation may underlie the Portuguese invasion

Biological invasions are often associated with a wide variety of evolutionary adaptive processes not only associated with the species itself but also the invasion process [53, 54]. Therefore, inferring selection targets along the genome of an invasive species such as *D. suzukii* may uncover local adaptation and the invasive potential of this species in Northern Portugal. A previous study had identified genetic loci that may have been targets of selection during the worldwide invasion of *D. suzukii*, and could have determined its invasive capacity [24]. The three Portuguese *D. suzukii* populations had a large set of common candidate selection target genes (1,127 genes), which may thus have played a role in the Portuguese invasion (Fig. 3b). Of these common candidate selection-target genes, ten had already been identified as candidate genes involved in adaptive processes associated with the invasive success of *D. suzukii* [24]. Yet, three were also included in genomic regions here inferred as putatively under selection in the analyzed European populations, which may result from common genomic features across all populations and not local adaptation [55]. Of the other seven genes, for which evidence of selection was found in the Portuguese but not the other European populations, only genes *Con* and *Axs* had significant SNPs in the contrast analysis of the C_2 statistics comparing native and invasive populations [24], the first in the European and the second in the American invasion routes. Whether these genes have also been targets of selection underlying the Portuguese invasion must be approached with caution and tested in future work.

To further understand the role of the candidate genes under selection across Portuguese populations of *D. suzukii*, a Gene Ontology (GO) enrichment analysis was performed. Such analysis aimed at understanding if particular functions were collectively recruited via natural selection during the invasion process. The most significant GO terms were associated with molecular processes and constituents, namely nucleosome, chromatin, and protein-DNA complexes, and these were common between all three Portuguese populations (Table S4). A few studies have previously explored the role of epigenetics on the invasion process and invasion success

of invasive species [56–60]. The fact that most of the candidate selection target genes were associated with molecular functions, biological processes, and cellular constituents involved in epigenetic mechanisms, raises the question if the *D. suzukii* Portuguese populations' invasive capacity and rapid local adaptation could be associated with epigenomic changes. Again, this hypothesis needs further investigation, as part of the evidence may result from common genomic features within the species and not marks of local adaptation.

Finally, we paid particular attention to genes present in regions found under selection in all Portuguese Populations but not in the closely related European ones. The specific signals in populations from Portugal and not elsewhere make them candidates for local adaptation in the Portuguese invasion (Fig. 3). Considering the fragments with strongest selection signal (conservative probability of selection of 1), we identified six genes, one of which is particularly interesting: *nAChRalpha7*. The nicotinic acetylcholine receptors (*nAChRs*) are the target for a wide variety of insecticides, such as neonicotinoids or spinosyns [61]. These are among the most used conventional insecticides for the control of *D. suzukii* [62, 63], particularly spinosyns (*spinosad*), as they are the only class of insecticides that are effective in controlling this pest in the context of organic farming [64]. Studies on *D. melanogaster* report an association between mutations on the α6 subunit of the *nAChRs* and spinosyn resistance [65, 66], but the same is yet to be explored in *D. suzukii*. Nevertheless, some studies are starting to report the emergence of a decrease in spinosyn sensitivity in some *D. suzukii* wild populations [64, 67]. To our knowledge, the only study in *D. suzukii* reporting changes in the *nAChRalpha7* gene is related to cold-acclimation, in which the gene was upregulated in cold-acclimated *D. suzukii* flies, with no correlation with insecticide resistance [68], although it appears that *D. suzukii* winter morphotypes are less susceptible to insecticides (spynosyns in particular) than summer morphotypes [69]. Further studies on insecticide resistance in the three Portuguese populations could give a better insight into the role of this gene in the local adaptation of the pest in Portugal.

Conclusions

Our first genomics assessment of the Portuguese populations of *D. suzukii* revealed that this invasion followed a Mediterranean route along Europe, but resulted in various levels of genetic diversity, which may have resulted from spatial but also temporal heterogeneity. Signatures of positive selection along the genome of the species in Portugal were found to overlap some genes previously suggested to underlie the invasive capacity of the species. Importantly, we detected signatures of positive selection that are private from Portuguese populations and may indicate local adaptation during the invasion. The identification of gene *nAChRalpha7* with such signature allows putting forth the hypothesis that local adaptation involved insecticide resistance, which should be tested in future research. Overall, this work highlights the complex nature of rapid species invasions and that natural selection may play an important role in determining invasive capacity. It is however relevant to broaden the temporal and geographic sampling range within Portugal to further clarify the complete context of the invasion of the species across the Portuguese territory.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions
S.S., C.S. and J.M.F. coordinated the study, designed research and acquired funding; S.S. contributed to sampling; S.S., L.F. and S.A. performed laboratory work; S.S., J.P.M. and J.M.F. analyzed data; S.S., J.P.M., C.S. and J.M.F. contributed to the interpretation of the results; S.S. wrote the paper; all authors revised the manuscript and approved the final version.

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Data availability
Whole genome sequencing data is available at NCBI under the BioProject PRJNA1081763 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1081763).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Westphal MI, Browne M, MacKinnon K, Noble I. The link between international trade and the global distribution of invasive alien species. Biol Invasions. 2008;10:391–8.
2. North HL, McGaughran A, Jiggins CD. Insights into invasive species from whole-genome resequencing. Mol Ecol. 2021;30:6289–308.
3. Nyamukondiwa C, Machekano H, Chidawanyika F, Mutamiswa R, Ma G, Ma C, Sen. Geographic dispersion of invasive crop pests: the role of basal, plastic climate stress tolerance and other complementary traits in the tropics. Curr Opin Insect Sci. 2022;50.
4. Walsh DB, Bolda MP, Goodhue RE, Dreves AJ, Lee J, Bruck DJ, et al. *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening Soft Fruit expanding its Geographic range and damage potential. J Integr Pest Manag. 2011;2:G1–7.
5. Hauser M. A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. Pest Manag Sci. 2011;67:1352–7.
6. De Ros G, Grassi A, Pantezzi T. Recent trends in the Economic Impact of *Drosophila suzukii*. *Drosophila Suzukii* Management. Cham: Springer; 2020. pp. 11–27.
7. Knapp L, Mazzi D, Finger R. The economic impact of *Drosophila suzukii*: perceived costs and revenue losses of Swiss cherry, plum and grape growers. Pest Manag Sci. 2021;77:978–1000.
8. Yeh DA, Drummond FA, Gómez MI, Fan X. The Economic Impacts and Management of Spotted Wing *Drosophila* (*Drosophila suzukii*): the case of wild blueberries in Maine. J Econ Entomol. 2020;113:1262–9.
9. Calabria G, Máca J, Bächli G, Serra L, Pascual M. First records of the potential pest species *Drosophila suzukii* (Diptera: Drosophilidae) in Europe. J Appl Entomol. 2012;136:139–47.
10. Rota-Stabelli O, Blaxter M, Anfora G. Drosophila suzukii. Curr Biol. 2013;23:R8–9.
11. Reyes JA, Lira-Noriega A. Current and future global potential distribution of the fruit fly *Drosophila suzukii* (Diptera: Drosophilidae). Can Entomol. 2020;152:587–99.
12. Garcia FRM, Lasa R, Funes CF, Buzzetti K. *Drosophila Suzukii* Management in Latin America: current status and perspectives. J Econ Entomol. 2022;115:1008–23.
13. Aouari I, Barech G, Khaldi M. First record of the agricultural pest *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) in Algeria. EPPO Bull. 2022;52:471–8.
14. Boughdad A, Haddi K, El Bouazzati A, Nassiri A, Tahiri A, El Anbri C, et al. First record of the invasive spotted wing Drosophila infesting berry crops in Africa. J Pest Sci (2004). 2021;94:261–71.
15. Kwadha CA, Okwaro LA, Kleman I, Rehermann G, Revadi S, Ndlela S, et al. Detection of the spotted wing Drosophila, Drosophila suzukii, in continental sub-saharan Africa. J Pest Sci (2004). 2021;94:251–9.
16. Ouantar M, Anfora G, Bouharoud R, Chebli B. First report of *Drosophila suzukii* (Diptera: *Drosophila*dae) in North Africa. Moroccan J Agricultural Sci. 2020;1:277–9.
17. Adrion JR, Kousathanas A, Pascual M, Burrack HJ, Haddad NM, Bergland AO, et al. *Drosophila suzukii*: the genetic footprint of a recent, worldwide invasion. Mol Biol Evol. 2014;31:3148–63.
18. Fraimout A, Debat V, Fellous S, Hufbauer RA, Foucaud J, Pudlo P, et al. Deciphering the routes of invasion of *Drosophila suzukii* by means of ABC random forest. Mol Biol Evol. 2017;34:msx050.
19. Petermann S, Otto S, Eichner G, Schetelig MF. Spatial and temporal genetic variation of *Drosophila suzukii* in Germany. J Pest Sci (2004). 2021;94:1291–305.
20. Tait G, Vezzulli S, Sassù F, Antonini G, Biondi A, Baser N, et al. Genetic variability in Italian populations of *Drosophila suzukii*. BMC Genet. 2017;18:87.
21. Rota-Stabelli O, Ometto L, Tait G, Ghirotto S, Kaur R, Drago F, et al. Distinct genotypes and phenotypes in European and American strains of Drosophila suzukii: implications for biology and management of an invasive organism. J Pest Sci (2004). 2020;93:77–89.
22. Gautier M, Vitalis R, Flori L, Estoup A. F -Statistics estimation and admixture graph construction with Pool-Seq or allele count data using the R package poolfstat. Mol Ecol Resour. 2022;22:1394–416.
23. Lewald KM, Abrieux A, Wilson DA, Lee Y, Conner WR, Andreazza F et al. Population genomics of *Drosophila suzukii* reveal longitudinal population structure and signals of migrations in and out of the continental United States. G3 Genes|Genomes|Genetics. 2021;11.
24. Olazcuaga L, Loiseau A, Parrinello H, Paris M, Fraimout A, Guedot C, et al. A whole-genome scan for Association with Invasion Success in the Fruit fly *Drosophila suzukii* using contrasts of Allele Frequencies Corrected for Population structure. Mol Biol Evol. 2020;37:2369–85.
25. Fraimout A, Monnet A-C. Accounting for intraspecific variation to quantify niche dynamics along the invasion routes of *Drosophila suzukii*. Biol Invasions. 2018;20:2963–79.
26. Little CM, Chapman TW, Hillier NK. Plasticity is key to success of *Drosophila suzukii* (Diptera: Drosophilidae) Invasion. J Insect Sci. 2020;20.
27. Ometto L, Cestaro A, Ramasamy S, Grassi A, Revadi S, Siozios S, et al. Linking Genomics and Ecology to investigate the Complex evolution of an Invasive *Drosophila* Pest. Genome Biol Evol. 2013;5:745–57.
28. OEPP/EPPO. EPPO Reporting Service no.10. 2012.
29. Melo M, Carvalho C, Pimentel R, João D, Lopes H, de Deteção. *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) na Ilha Terceira, Açores. Revista de Ciências Agrárias. 2019;41 spe:152–5.
30. Rego C, Aguiar AF, Cravo D, Boieiro M. Invasive fruit flies (Diptera: Drosophilidae) Meet in a Biodiversity Hotspot. J Entomol Res Soc. 2017;19:61–9.
31. Macedo FL, Ragonezi C, Reis F, de Freitas JGR, Lopes DH, Aguiar AMF, et al. Prediction of the potential distribution of *Drosophila suzukii* on Madeira Island using the Maximum Entropy modeling. Agriculture. 2023;13:1764.
32. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20.
33. Paris M, Boyer R, Jaenichen R, Wolf J, Karageorgi M, Green J, et al. Near-chromosome level genome assembly of the fruit pest *Drosophila suzukii* using long-read sequencing. Sci Rep. 2020;10:11227.
34. Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics. 2009;25:1754–60.
35. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25:2078–9.
36. Barnett DW, Garrison EK, Quinlan AR, Stŕmberg MP, Marth GT. BamTools: a C++API and toolkit for analyzing and managing BAM files. Bioinformatics. 2011;27:1691–2.
37. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L, et al. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res. 2012;22:568–76.
38. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, et al. The variant call format and VCFtools. Bioinformatics. 2011;27:2156–8.
39. Kofler R, Orozco-terWengel P, de Maio N, Pandey RV, Nolte V, Futschik A, et al. PoPoolation: a toolbox for Population genetic analysis of next generation sequencing data from pooled individuals. PLoS ONE. 2011;6:e15925.
40. Gautier M. Genome-wide scan for adaptive divergence and association with population-specific covariates. Genetics. 2015;201:1555–79.
41. Pickrell JK, Pritchard JK. Inference of Population splits and mixtures from genome-wide allele frequency data. PLoS Genet. 2012;8:e1002967.
42. Pickrell JK, Patterson N, Barbieri C, Berthold F, Gerlach L, Güldemann T, et al. The genetic prehistory of southern Africa. Nat Commun. 2012;3:1143.
43. Fitak RR. OptM: estimating the optimal number of migration edges on population trees using Treemix. Biol Methods Protoc. 2021;6.
44. Boitard S, Kofler R, Françoise P, Robelin D, Schlötterer C, Futschik A. Pool-hmm: a Python program for estimating the allele frequency spectrum and detecting selective sweeps from next generation sequencing of pooled samples. Mol Ecol Resour. 2013;13:337–40.
45. Kapun M, Barron MG, Staubach F, Obbard DJ, Axel R, Vieira J, et al. Genomic analysis of European *Drosophila* melanogaster populations reveals longitudinal structure, continent-wide selection, and previously unknown DNA viruses. Mol Biol Evol. 2020;37:2661–78.
46. Anand L, Rodriguez Lopez CM. ChromoMap: an R package for interactive visualization of multi-omics data and annotation of chromosomes. BMC Bioinformatics. 2022;23:33.
47. Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics. 2010;26:841–2.
48. Hoskins RA, Carlson JW, Wan KH, Park S, Mendez I, Galle SE, et al. The release 6 reference sequence of the *Drosophila* melanogaster genome. Genome Res. 2015;25:445–58.
49. Wang M, Kong L. Pblat: a multithread blat algorithm speeding up aligning sequences to genomes. BMC Bioinformatics. 2019;20:1–4.
50. Jenkins VK, Larkin A, Thurmond J. Using FlyBase: a database of *Drosophila* genes and Genetics. 2022. pp. 1–34.
51. Raudvere U, Kolberg L, Kuzmin I, Arak T, Adler P, Peterson H, et al. G:profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). Nucleic Acids Res. 2019;47:W191–8.

52. Lavrinienko A, Kesäniemi J, Watts P, Serga S, Pascual M, Mestres F, et al. First record of the invasive pest Drosophila suzukii in Ukraine indicates multiple sources of invasion. J Pest Sci (2004). 2017;90:421–9.
53. Fournier D, Aron S. Hybridization and invasiveness in social insects — the good, the bad and the hybrid. Curr Opin Insect Sci. 2021;46:1–9.
54. Sherpa S, Després L. The evolutionary dynamics of biological invasions: a multi-approach perspective. Evol Appl. 2021;14:1463–84.
55. Vijay N, Bossu CM, Poelstra JW, Weissensteiner MH, Suh A, Kryukov AP, et al. Evolution of heterogeneous genome differentiation across multiple contact zones in a crow species complex. Nat Commun. 2016;7:13195.
56. Carneiro VC, Lyko F. Rapid Epigenetic Adaptation in animals and its role in invasiveness. Integr Comp Biol. 2020;60:267–74.
57. Hawes NA, Fidler AE, Tremblay LA, Pochon X, Dunphy BJ, Smith KF. Understanding the role of DNA methylation in successful biological invasions: a review. Biol Invasions 2018. 2018;20:9.
58. Marin P, Genitoni J, Barloy D, Maury S, Gibert P, Ghalambor CK, et al. Biological invasion: the influence of the hidden side of the (epi)genome. Funct Ecol. 2020;34:385–400.
59. Neinavaie F, Ibrahim-Hashim A, Kramer AM, Brown JS, Richards CL. The genomic processes of Biological invasions: from Invasive species to Cancer metastases and back again. Front Ecol Evol. 2021;9:501.
60. Stajic D, Jansen LET. Empirical evidence for epigenetic inheritance driving evolutionary adaptation. Philosophical Trans Royal Soc B. 2021;376.
61. Casida JE. Neonicotinoids and other Insect Nicotinic receptor competitive modulators: progress and prospects. Annu Rev Entomol. 2018;63:125–44.
62. Shawer R. Chemical Control of *Drosophila suzukii*. Drosophila Suzukii Manage. 2020;:133–42.
63. Shawer R, Tonina L, Tirello P, Duso C, Mori N. Laboratory and field trials to identify effective chemical control strategies for integrated management of *Drosophila suzukii* in European cherry orchards. Crop Prot. 2018;103:73–80.
64. Ganjisaffar F, Gress BE, Demkovich MR, Nicola NL, Chiu JC, Zalom FG. Spatiotemporal variation of Spinosad susceptibility in *Drosophila suzukii* (Diptera: Drosophilidae), a three-year study in California's Monterey Bay Region. J Econ Entomol. 2022;115:972–80.
65. Homem RA, Buttery B, Richardson E, Tan Y, Field LM, Williamson MS, et al. Evolutionary trade-offs of insecticide resistance — the fitness costs associated with target-site mutations in the nAChR of *Drosophila* melanogaster. Mol Ecol. 2020;29:2661–75.
66. Lu W, Liu Z, Fan X, Zhang X, Qiao X, Huang J. Nicotinic acetylcholine receptor modulator insecticides act on diverse receptor subtypes with distinct subunit compositions. PLoS Genet. 2022;18:e1009920.
67. Deans C, Hutchison WD. Propensity for resistance development in the invasive berry pest, spotted-wing *Drosophila* (*Drosophila suzukii*), under laboratory selection. Pest Manag Sci. 2022. https://doi.org/10.1002/PS.7139
68. Enriquez T, Colinet H. Cold acclimation triggers major transcriptional changes in *Drosophila suzukii*. BMC Genomics. 2019;20.
69. Seong KM, Sun W, Huang J, Gut L, Kim YH, Pittendrigh BR. Comparative response of two seasonal spotted wing *Drosophila* (*Drosophila suzukii*) morphs to different classes of insecticides. Entomol Res. 2022. https://doi.org/10.1111/1748-5967.12624

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