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ORIGINAL PAPER

Distinct genotypes and phenotypes in European and American strains of *Drosophila suzukii*: implications for biology and management of an invasive organism

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Abstract

A clearer understanding of the structure of pest populations in newly invaded areas is a key step towards their effective management. Here, we use *Drosophila suzukii* as a model to highlight how populations from separate geographical regions differ in their genetic and phenotypic traits, including those associated with their invasiveness. New X-linked data indicate the presence of at most three *D. suzukii* genetic clusters in Europe, while North American populations are characterised by a larger genetic diversity. We found a likely new colonisation event from America to Italy and demonstrate that reference genomes from Italian and Californian populations lay in highly distant clusters. Comparative genomics indicate that these two genomes bear the traces of distinct evolutionary forces and are genetically distant, having diversified long ago in their native Asian range. Phenotypic studies further indicate that European and North American populations have differences in hatch rate, generation time, and parasitoid susceptibility. The observed genotypic and phenotypic differences likely represent a small fraction of the features unique to each of the two populations. The results provide some new insights towards both fundamental and management studies on invasive pests, particularly when findings are transferred across populations found in different geographical regions.

Keywords Invasive insect · Comparative genomics · Population genetics · Parasitoid · Life table · Intraspecific variations · *Drosophila suzukii*

Key message

- Understanding intraspecific variability in pest species is critical to identify management practices that are efficient across populations.
- We show that *Drosophila suzukii* populations from distinct invaded areas are fairly diverged, have undergone

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different selective forces, and are characterised by different reproduction capability and resistance to parasitoids.
- These differences may underlie a much wider, common, and underappreciated heterogeneity in populations of invasive pest species that should be taken into consideration when interpreting results from both fundamental and applied pest research.

Introduction

A crucial aspect in invasive biology is to characterise the genetic and phenotypic variability of a species occupying newly invaded territories. This knowledge can aid in the characterisation of key patterns such as invasion routes and success (Lee 2002). The genetic variability of any invasive species is, however, a complex issue depending on multiple factors. Bottleneck events often occur during such invasion, resulting in a relatively small number of founding individuals, and a consequent reduction in genetic diversity of colonising populations within geographically distinct regions (Estoup et al. 2016). It is also possible that the newly invaded region has been colonised by multiple independent events, whereby genetically diverged individuals are contributing to the present invading population. Indeed, it has been suggested that the genetic diversity created by the admixture of different genotypes may play a central role in invasive success (Rius and Darling 2014). On the other hand, invasions of separate geographical regions may result from temporal and genetically independent colonisation events, such that invasive populations may display both distinct genotypic and phenotypic traits (Dlugosch and Parker 2008). In order to develop effective management programs of a pest, it is therefore crucial not only to study the invasion dynamics (using, for example, population genetics), but also to evaluate the possibility that different populations are characterised by different phenotypes and/or genotypes associated with their invasiveness potential as exemplary shown in an invasive mussels (Reichard et al. 2015).

Understanding intraspecific variability in invasive agricultural pests is therefore highly relevant (Cesari et al. 2014; Van Damme et al. 2014), particularly because management practices defined on one population are generally applied to others from distant regions. *Drosophila suzukii* is a model pest species that offers the possibility of studying similarities and differences between separate invasions, especially since its invasion into both America and Europe was recorded in 2008 (Cini et al. 2012; Rota-Stabelli et al. 2013; Asplen et al. 2015). *Drosophila suzukii* genomes have been sequenced for both an Italian (Trentino, Ometto et al. 2013) strain and an American (California, Chiu et al. 2013) strain, providing valuable information on their genetic make-up. Although the two genomes revealed similar evolutionary patterns, such as reduced evolutionary rate and an expansion of odorant receptors compared to other Drosophila species (Ometto et al. 2013; Chiu et al. 2013), no direct comparison has been undertaken to identify differences that may underpin population-specific phenotypic features. Hints about their divergence first emerged in our study of odorant receptors (Ramasamy et al. 2016), in which we found that three key (pseudo)genes—OR85a, OR22a, and OR74—were extremely different in the two reference genomes, both in terms of gene sequence and intron structure. Studies involving the potential biocontrol agent *Wolbachia* (Cattel et al. 2016b) harboured by *D. suzukii* further pointed towards contrasting levels of infection frequencies and genetic variants present between European and North American populations (Cattel et al. 2016a; Kaur et al. 2017). A clear indication of intraspecific variation was confirmed by population genetic studies: both X-linked gene fragments (Adrion et al. 2014) and microsatellite markers (Fraimout et al. 2017) revealed different genetic backgrounds for the invading *D. suzukii* populations, suggesting distinct sources for the European (from northeast China) and the American populations (from southeast China and Hawaii, the latter colonised by Japanese flies). Approximate Bayesian computation analyses (Fraimout et al. 2017) also revealed ongoing admixture in the introduced populations between strains of different origins, both in America and in Europe. The extent of such differences and their phenotypic effects need, however, to be determined.

The common practice of extending the findings (and the consequent applications) obtained in the *D. suzukii* American populations to the European ones, and vice versa, may strongly bias the interpretation of otherwise population-specific evolutionary and ecological trends. For instance, nothing is known yet about possible morphological or physiological differences among *D. suzukii* populations. For example, the strong phenotypic response to environmental factors (e.g. temperature and host fruit; Clemente et al. 2018; Fraimout et al. 2018; Pajač Živković et al. 2018), and basic behavioural and population ecology parameters, including egg-laying behaviour, substrate choice, and parasitoid resistance, can have significant implications for the success of *D suzukii*. As a consequence, phenotypic differences that may exist between the American and the European populations can affect the local management strategies.

The goal of this study was therefore to characterise the intraspecific diversity of *D. suzukii* across the American and the European populations. To this aim, we sequenced X-linked data from new samples from Oregon and Italy to increase the accuracy of population genetics inferences; we compared European and North American reference genomes using phylogenomics and genetics; finally, we trialled three key insect phenotypic traits in flies coming from European and North American populations. Our results reveal

widespread genetic and phenotypic differences, which are important for the future interpretation of experiments and the transfer of knowledge from laboratories that use strains from different continents. The approach taken in this study is relevant not only for *D. suzukii*, but potentially for any type of invasive insect pest.

Materials and methods

Population genetics and specimen sampling

Drosophila suzukii males were collected between 2014 and 2016 from five orchards located, respectively, in Oregon (USA), Sardinia, Sicily, and two different localities in Trentino, Italy (TN, San Michele and VAL, Valsugana) using Droskidrink®-baited traps (Tait et al. 2018). Live individuals were identified according to (Hauser 2011), preserved in 96% ethanol (16,378, Sigma-Aldrich), and kept at 4 °C until DNA extraction. We also used *D. suzukii* from two laboratory-maintained stock populations, SMA13 and ORE14, established from natural population sampled, respectively, in 2013 in San Michele (Trentino, Italy) and in 2014 in the Willamette Valley (Oregon, USA). Japanese individuals were obtained from the Ehime-fly Stock Center (Kyoto, Japan). Genomic DNA was extracted from ten males from each of the eight populations using NucleoSpin Tissue (Macherey–Nagel) extraction kits and subsequently used to amplify five X-linked markers with the primers listed in (Adrion et al. 2014). Each pair of primers was used for PCR amplification in 25 µL final volume, containing 1X GoTaq G2 Master Mix, 0.5 µL of each prime, 10.5 µL of distilled deionised water, and 1 µL of genomic DNA. The PCR program was set with an initial period of denaturation at 94 °C (3 min), followed by 35 cycles of additional denaturation at 94 °C (30 s), 56 °C (1 min), 72 °C (1 min) and a final extension of 72 °C for 7 min. PCR products were then processed and sequenced on an Applied Biosystem DNA Analyser.

To explore how the genetic variation is structured in the dataset, we combined the five newly produced datasets (from Oregon and Italy) with those published by (Adrion et al. 2014) from Japan, Hawaii, Spain, North and South California, Michigan, Massachusetts, West Virginia, North Carolina, South Carolina, Georgia, and Florida. We applied a discriminant principal component analysis (DAPC) to analyse the genetic structure of our populations. This analysis defines a model in which genetic variation (summarised by a certain number of principal components) is partitioned into a between-group and a within-group component and yields synthetic variables which maximise the first while minimising the second (Jombart et al. 2010). We used K-means clustering to identify the most supported number of groups, assessed by Bayesian information criterion (BIC) and choosing the minimum K number after which the BIC increased or decreased by a negligible amount [as previously reported in Kanno et al. (2011) or Zalewski et al. (2016)]. Both analyses were run in the R environment, using the *adegenet* R package (Jombart et al. 2008). Because markers are X-linked, we performed a phylogenetic analysis on the concatenated five markers using *BEAST 1.8* and employing a *HKY* + *G* model of replacement, strict uncalibrated clock (normal distribution centred at 0 with SD 1), and coalescent constant demographic prior, and *MCMC* setting as described below.

Comparative genomics and phylogenomics

For comparative analyses, we integrated the orthologous datasets described in Ometto et al. (2013), which included sequences from *D. melanogaster*, *D. ananassae*, *D. takahashi*, *D. biarmipes*, and the Italian (ITA) reference strain of *D. suzukii*, with orthologues from the North American (USA) reference strain (Chiu et al. 2013) and from an unpublished draft of *D. subpulchrella* genome. In particular, we identified, and added to the aforementioned dataset, the best hits of a reciprocal BLAST between the ITA sequences and either the US transcriptome dataset and the draft transcriptome of *Drosophila subpulchrella* (which is the closest relative of *D. suzukii* for which genomic data are available; unpublished data). The final dataset included 2132 sets of 1:1 orthologues. Rates of molecular evolution were analysed using PAML 4.4 (Yang 2007). Because this approach assumes each species to be represented by a single branch, the European (ITA) and the North American (USA) sequences were analysed separately, namely by producing two datasets that could be described by unrooted trees in the form of (*D. melanogaster*, *D. ananassae*, (*D. takahashi*, (*D. biarmipes*, (*D. subpulchrella*, *D. suzukii*^{ITA} or *D. suzukii*-^{USA})))). We estimated the rate of non-synonymous substitution, d_N, and synonymous substitution, d_S, over all branches of the phylogenetic tree using the "free-ratio" model (M0', (Yang 1998); model = 1 and NSsites = 0). We then tested different models of substitution rates across coding sites (Yang et al. 2000; Yang and Nielsen 2000), with the aim of detecting genes that evolved at a different rate or that underwent positive selection along the *D. suzukii*^{ITA} and the *D. suzukii*^{USA} lineages (each consisting of a shared, ancestral *D. suzukii* branch, and a branch that would eventually correspond to a possible evolutionary history private to either of the two populations). In particular, we used the branch and branch-site models to detect genes with a branch-specific selective pressure (i.e. along the *D. suzukii* branch) and to detect positive selection affecting a few sites along the *D. suzukii* branch (Ometto et al. 2013). We tested for Gene Ontology (GO) term enrichment analysis in the candidate genes for positive selection using *topGO v.2.34.0* (Alexa

et al. 2006). This tool allows the identification of specific ontologies (i.e. the biological process, the molecular function, or the cellular component) preferentially associated with the putative targets of adaptive evolution along the Italian or the American *D. suzukii* lineages. We also tested ontology enrichment on the Drosophila anatomical ontology using the tool *TopAnat* (Komljenovic et al. 2018), which is based on the *Bgee* R package (Bastian et al. 2008). We used *BLASTn* on genome data to search matches to the insertion found in the OR22a pseudogene of the American genome strain (Ramasamy et al. 2016) and counted all the occurrences with a threshold of e^{-50}. We identify the family to which this insertion belongs using *RepeatMasker* at default settings.

We updated the phylogenomics dataset of Ometto et al. (2013), which included 91 orthologous datasets from 21 Drosophila species, by adding orthologues from the American reference transcriptome and the draft *D. subpulchrella* transcriptome using a reciprocal blast approach against the Italian *D. suzukii* sequences. The updated dataset spans 200,756 nucleotides. We conducted Bayesian analysis of this dataset in *BEAST* 1.8 using a *GTR* + *G* replacement model, a birth–death tree prior, a log normal relaxed clock calibrated using a root prior 80 MYA with a permissive SD of 20 for the Sophophora–Drosophila split (Ometto et al. 2013), and the instantaneous mutation rate of 0.028 (SD 0.01) taken from Keightley (2013) assuming either ten or five generations per year. Because we calibrated with an instantaneous mutation rate, we retained only the fourfold degenerate sites, consisting of an alignment of 22,156 nucleotides. We run the *MCMC* for 100 million generations, checked for actual convergence using *Tracer*, and generated a posterior consensus tree using 30% burn-in with *TreeAnnotator* from the *BEAST* package (http://tree.bio.ed.ac.uk/software/beast/). The concatenated alignment of the 91 markers for all individuals is available as Suppl. Materials.

Hatching and crossing experiment, life table parameters

Lines of SMA13 (ITA) and ORE14 (USA) were maintained under laboratory conditions on standard cornmeal agar media, on a 14:10 light–dark cycle, at 25 °C, and approximately 70% relative humidity (RH). We performed inter- and intra-strain crossing experiments to determine whether American and European *D. suzukii* populations differ in their fecundity levels. Males and females were separated upon eclosion (to ensure virginity), aged 5–7 days, and then placed in single mating pairs for each combination (ITA × USA, USA × ITA, ITA × ITA, USA × USA; where the first mate of the crossing represents the male) within a vial with 10 ml of medium. Flies were allowed to mate for 48 h. Males were then aspirated to avoid the use of anaesthesia, and females were left to lay eggs for another 24 h. Each combination was replicated ten times. Replicates containing females that did not produce larvae were removed from the final data set. The number of offspring eclosing from each vial was scored daily, until 12–15 days. This methodology allowed enough time for all larvae to emerge, ensuring the scoring of all offspring. The hatch rate was then assessed by counting the total adult progeny emerging from a vial. Hatched individuals counts for inter- and intra-strain crosses were compared with nonparametric Kruskal–Wallis tests (comparisons of more than two groups).

We conducted experiments for determining demographical parameters on SMA13 (ITA) individuals in order to compare the life table parameters with that of individuals from Oregon (Tochen et al. 2014); this population was the source of the ORE14 line, used for other physiological analyses presented in our study. We reared flies in a growth chamber at 22 ± 1 °C, 65% RH, 16:8 L:D, following the protocol described in (Tochen et al. 2014). The net reproductive rate (R_o), defined as the average lifetime production of eggs for a newborn female, was calculated using the equation:

$$R_o = \Sigma l_x * M_x$$

where l_x is the age-specific survival rate, and M_x is the age-specific fecundity (Birch 1948). The mean generation time (T), defined as the time required for a population to increase by a factor equal to the net reproductive rate, was calculated using the equation:

$$T = \Sigma l_x * M_x * \chi / R_o$$

where χ is the age in days. The intrinsic rate of population increase (r_m), defined as the rate of natural increase in a closed population that has been subject to constant age-specific schedules of fertility and mortality, was calculated using the equation:

$$r_m = lnR_o/T.$$

The doubling time (DT), defined as the period of time required for a population to double in size, was calculated using the equation $DT = ln(2)/r_m$ (Birch 1948).

Parasitoid susceptibility

We exposed *D. suzukii* individuals (from both SMA13 and ORE14) to three species of generalist Drosophila parasitoids (Rossi Stacconi et al. 2017). We used two stocks of the larval parasitoid *Leptopilina heterotoma* (Thomson), one stock of the pupal parasitoid *Trichopria* cf *drosophilae* (Perkins), and one stock of the pupal parasitoid *Pachycrepoideus vindemiae* (Rondani). Stock of *L. heterotoma* was established in 2017 from natural populations sampled in Trento (Italy) and Zurich (Switzerland). Pupal parasitoids stocks were established from natural populations sampled

in 2015 in Milan (Italy) and in 2017 in Trento (Italy) (*T. drosophilae* and *P. Vindemiae*, respectively). Each combination of *D. suzukii* population and parasitoid stock was replicated 30 times. Each replication consisted of ten *D. suzukii* second-instar larvae (for *L. heterotoma*) or pupae (for *T. drosophilae* or *P. vindemiae*) exposed to one adult female parasitoid for 2 h. After the exposure, we removed the parasitoids and incubated the parasitoid-exposed *D. suzukii* under controlled conditions (22 ± 1 °C, 70% RH, 16:8 L: D). After eclosion, we counted the number of parasitoids and the number of *D. suzukii* adults carrying melanized parasitoid eggs (Kacsoh and Schlenke 2012). Since assumption of normality was violated, we analysed the data using nonparametric Mann–Whitney *U* test.

Results

Distinct European and American clusters

We updated the population genetic dataset of Adrion et al. (2014) by sequencing ten individuals each from four Italian (two Trentino, Sicily, and Sardinia) and one American (Oregon) population. Bayesian information criteria (BIC, Fig. 1a) set the optimal number of clusters to 11. According to the discriminant analysis of principal component of Fig. 1b using $K = 11$, most of these clusters are well separated. Exceptions are the closely related clusters K1–*K*10, which include only European individuals, and clusters K3–K5–K6–K7, mainly formed by American individuals. Overall, we can identify three European and eight American clusters. This population structure is confirmed by the

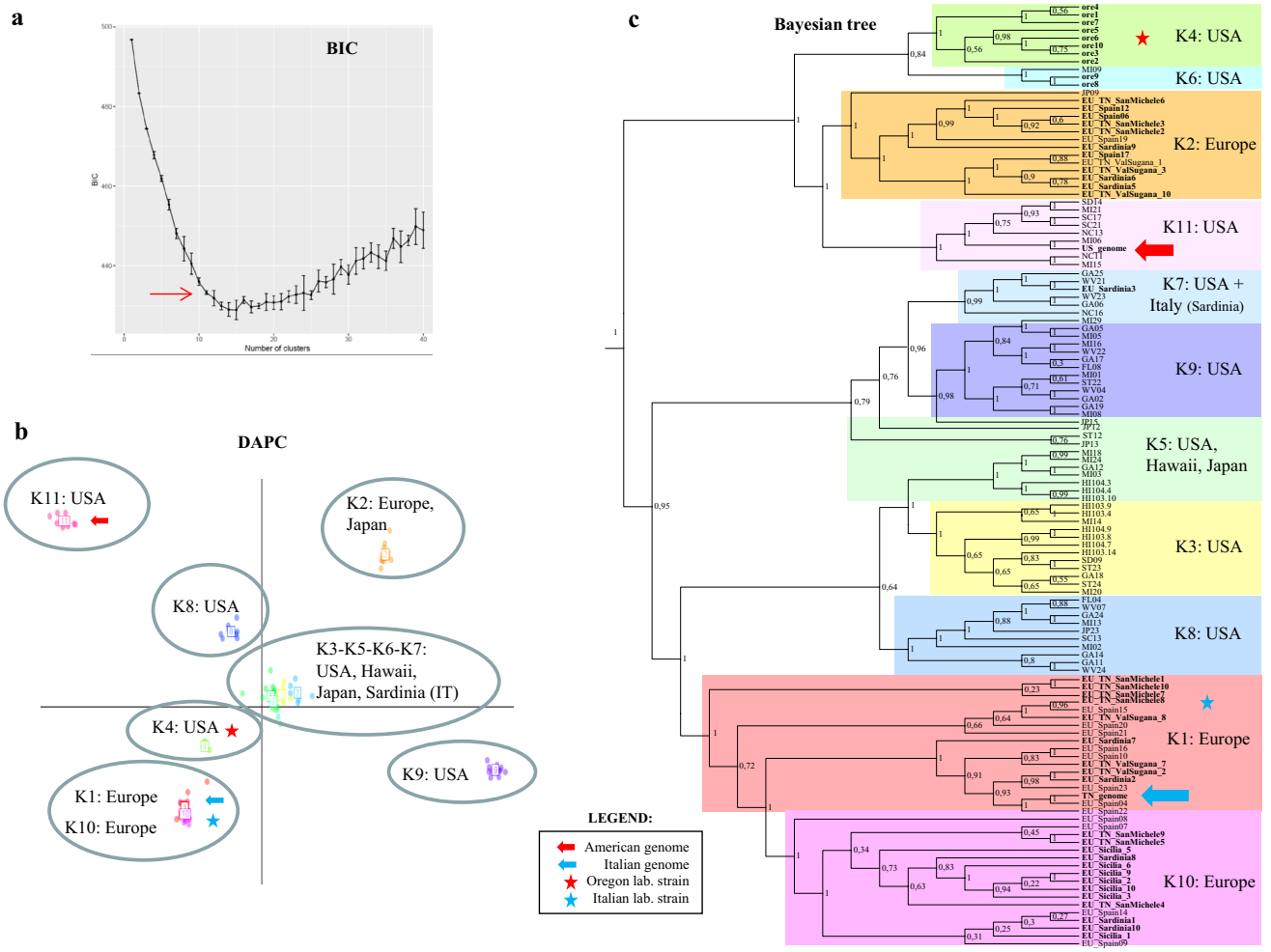

Fig. 1 Five X-linked markers support distinct European and American clusters. **a** Bayesian information criterion indicates $K = 11$ as the best fitting clustering. **b** Discriminant analysis of principal component of the five markers using $K = 11$; **c** Bayesian consensus tree of five concatenated markers. The two reference genomes available and the two laboratory strains used in this worked are indicated by arrows and stars, respectively

Bayesian phylogenetic analysis (Fig. 1c). All clusters except one contain either European or North American individuals only, compatible with a strong geographical structure and a genotypic distinction between the newly invaded continents. One of the American clusters (K7) contains one European individual sampled in Italy (Sardinia).

Distantly related reference genomes

The two reference *D. suzukii* genomes fall in distantly related clusters: the Italian genome in cluster K1 and the North American genome in K11 (blue and red arrows in Fig. 1b, c). Our Bayesian divergence estimates based on genome-scaled data set the diversification of the two genomes at 0.98 millions of years ago (mya), with 95% high posterior densities (HPD) between 0.44 and 1.96 mÅ (Fig. 2a and suppl. Figure 1). Since *D. suzukii* is likely characterised by fewer generations per year compared to other Drosophila species (Ometto et al. 2013; Rossi Stacconi et al. 2016), we repeated the analysis assuming five generations per year and obtained a mean of 1.5 mya (HPD 0.67–3.7; see suppl. Figure 2).

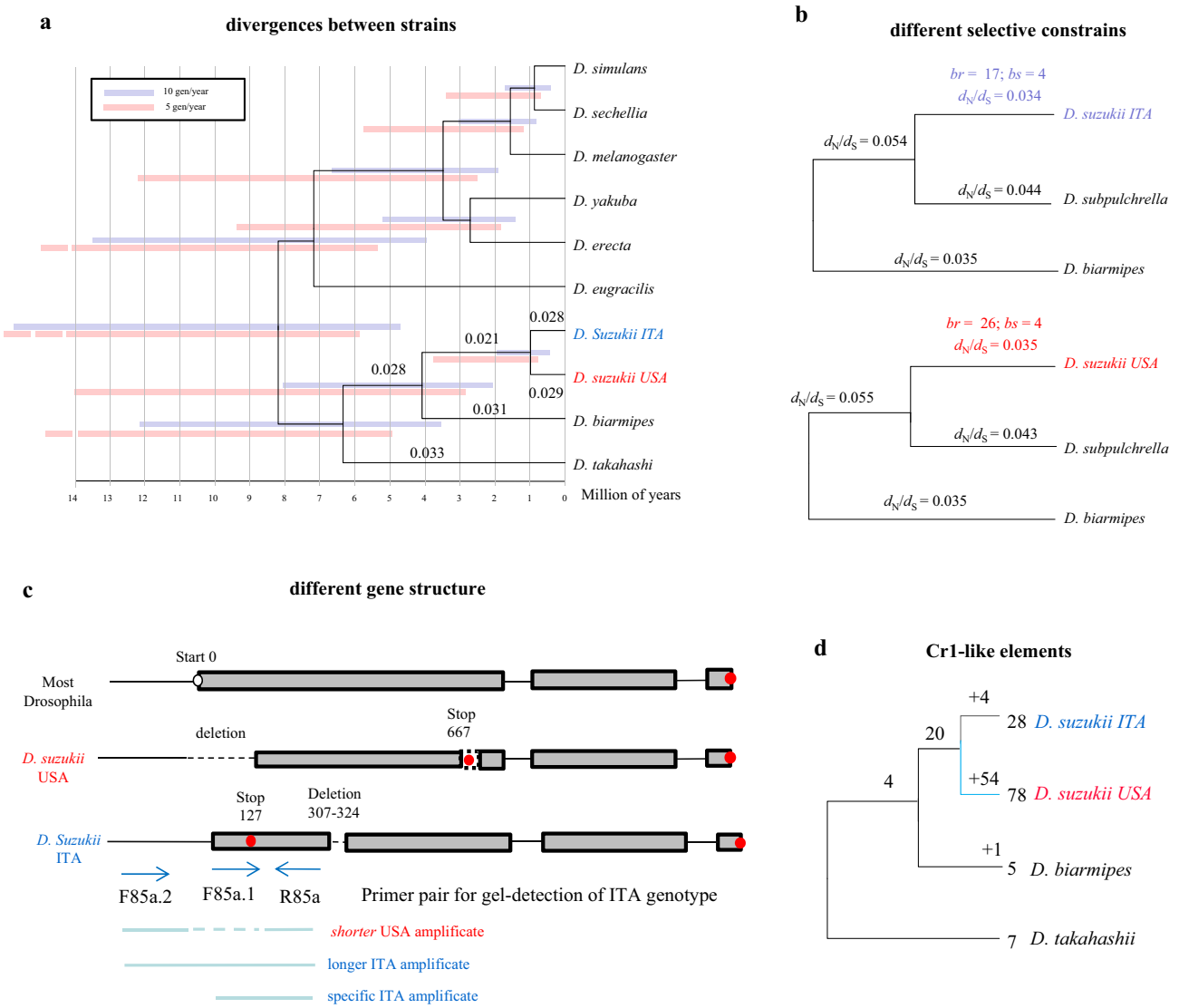

Fig. 2 Divergent reference Italian and California genomes. **a** Beast calibrated analyses based on the fourfold degenerate sites of 91 gene orthologues. Bars are the height of 95% high posterior densities. The tree depicts a zoom of a larger analysis: the full tree is available in supplementary Fig. 1. Number at selected nodes is mutation per site per millions of years. **b** The American genome is characterised by more genes with lineage-specific selective pressure (*br*) compared to the Italian genome, while the number of genes with sites under positive selection (*bs*) is similar in the two genomes (at $P < 0.001$; see suppl. Table 1). **c** Extensive genome variation in the odorant receptor OR85a pseudogene. The presence of one inserted element in the US strain allows defining a primer pair for the identification of the Italian genotype that lacks this insertion. **d** Uneven distribution of the *Cr1-like* element on the two genomes and in the genome of a sister species

Differently evolving reference genomes

We analysed the possible occurrence of positive selection in 2132 orthologous gene sets and found different patterns of evolution in the two reference genomes. There are nearly twice as many genes that evolved under a distinct selective pressure regime (*branch* test) in the North American genome compared to the Italian one, whereas the number of genes that contained sites that evolved under the action of positive selection (*branch-site* test) is identical (suppl. Table 1; Fig. 2b). The candidate genes for positive selection (*branch* and *branch-site* tests) were enriched in numerous GO terms according to at least one of the *topGO* algorithms (suppl. Tables 2–5). The biological process, molecular function, and cellular component identified distinct enriched terms for the Italian and for the American genomes, suggesting that the ancestral populations likely experienced different selective pressures. The same holds for the anatomical enrichment analysis, which revealed several anatomical regions where the candidate genes are expressed in Drosophila (suppl. Tables 2–5). As confirmed by our *BEAST* analysis (see numbers at selected nodes in Fig. 2a), the North American genome is significantly faster evolving than the Italian one (Fig. 2b; average $d_S = 0.069$ vs. $d_S = 0.065$, Wilcoxon test $P = 0.008$). We have re-annotated the gene structure of OR85a (Fig. 2c; see also Ramasamy et al. 2016) to highlight that a set of three primers (F85a.2, F85a.1 and R85a) are capable of discriminating among specific alleles of this ORs pseudogene. We further checked for the presence of a newly discovered repetitive element in the two genomes. We compared the fine-scaled structure of receptor OR22a (Ramasamy et al. 2016) and found that the American allele differs from the European: it contains a 217-nucleotide-long insertion which contributes disrupting the coding frame in the American allele only, while the European allele has frame disruptions elsewhere. We investigated the nature of this insertion and found that it is a fragment of a longer repetitive element of the *CR1* family within the *LINE* class of retroelements. Blast searches revealed that this fragment is highly abundant in *D. suzukii*, but rare in the closely related species *D. biarmipes* and *D. takahashi*, and absent from other Drosophila. This insertion is notably more abundant in the American genome (78 copies) than in the Italian one (28 copies, Fig. 2d, and suppl. Figure 3).

Higher reproductive capability in an Italian *Drosophila suzukii* strain

We measured eclosion rates in inter- and intra-strain crosses (Fig. 3a) (the Italian strain SMA13, belonging to K1, blue star in Fig. 1, and the American strain ORE14, cluster K4, red star in Fig. 1c). No differences were recorded for the inter-strain hatch rates of *D. suzukii*,

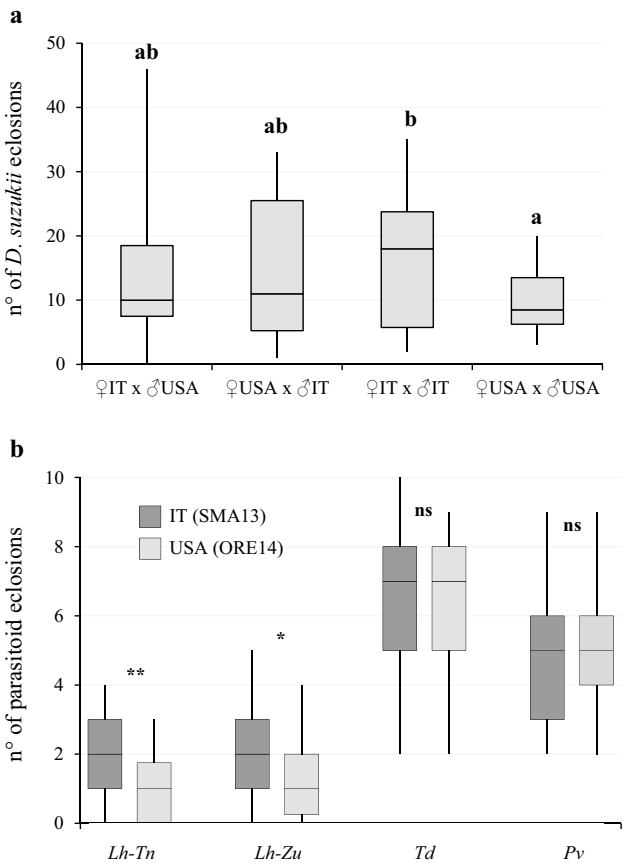

Fig. 3 Phenotypic differences. **a** Total number of eclosed *D. suzukii* individuals for inter- and intra-strain crosses between European (IT, SMA13) and American (USA, OR14) *D. suzukii* isofemale lines. Fertility was measured as the total progeny produced by single pairs of flies (sample size, $n = 10$). b) Susceptibility of the European (IT, SMA13) and American (USA, ORE14) *D. suzukii* to the most common European drosophila parasitoids. Data are presented as number of successful parasitisation events (sample size, $n = 30$). Letters and asterisks indicate statistical significance ($P < 0.05$) after nonparametric Kruskal–Wallis test on Mann–Whitney U test, followed by post hoc *Dunn's* multiple comparison test. Lh-Tn: *L. heterotoma,* Trento population; Lh-Zu: *L. heterotoma,* Zurich population; Td: *T. drosophilae*; Pv: *P. vindemiae*

indicating no signs of inter-strain incompatibility. The Italian strain of *D. Suzukii*, however, displayed a significantly higher hatching rate than the North American one (Kruskal–Wallis test, $H = 8.723$, $P = 0.033$). These results are partially confirmed by life table studies (see below). The values of the demographic growth parameters obtained from the life table studies conducted for SMA13 were: net reproductive rate (R_0) 181.61; mean generation time (T) 21.82 d; and intrinsic rate of increase (r_m) 0.238. For the North American *D. suzukii*, the demographic parameters reported in the literature were: net reproductive rate (R_0) 195.1; mean generation time (T) 24.2 d; and intrinsic rate of increase (r_m) 0.218. When comparing the

population doubling times, Italian *D. suzukii* (DT: 2.91 d) would double its population 8.6% faster than North American *D. suzukii* (DT: 3.18 d). Since we did not conduct side-by-side life table studies [SMA13 data were generated in the present study, while the Oregon data are taken from (Tochen et al. 2014)], we warn on a cautious interpretation of this comparison.

Higher *Leptopilina heterotoma* resistance in an American strain

We observed significantly higher eclosion rate of *L. heterotoma* in SMA13 (ITA) than in ORE14 (USA) ($U = 275.5$; $P < 0.01$ and $U = 311.5$; $P < 0.05$ for Trento and Zurich *L. heterotoma* populations, respectively; Fig. 3b). Similarly, we found significantly more *D. suzukii* adults carrying abdominal melanized parasitoid eggs in SMA13 (ITA) than in ORE14 (USA) ($U = 277$; $P < 0.01$ and $U = 298.5$; $P < 0.01$ for Trento and Zurich populations, respectively). We did not find any significant difference in the eclosion rate of the pupal parasitoids *T. drosophilae* and *P. vindemiae* among the two *D. suzukii* populations. Finally, we did not observe any fly carrying melanized egg in the pupal parasitoid treatments.

Discussion

In this article, we have gathered various genomic, evolutionary, and physiological evidences that helped us to characterise and quantify the divergence between *D. suzukii* populations/strains from Europe and North America. We compared the genomes of the Italian (Trentino) and California reference genomes (Ometto et al. 2013; Chiu et al. 2013) and compared the physiology of an Italian (SMA13 from Trentino) strain and an Oregon (ORE14) strain. We summarised the various differences found in this study with those from previous studies in Fig. 4. In particular, we found significant genetic divergence between the two strains with a sequenced genome, compatible with a long history of separation of the founder populations in Asia. Accordingly, we identified different genes under selection in the two strains, different copies of an evolutionary important repeat element, and a slightly different mutation rate. Even the number and sequence of pest related genes, such as odorant receptors, differ between strains, as well as the biology of host-Wolbachia interactions. These differences point towards population-specific biological and ecological features, as exemplified by differences in key phenotypic traits as fecundity and susceptibility to larval parasitoids in Trentino and Oregon strains.

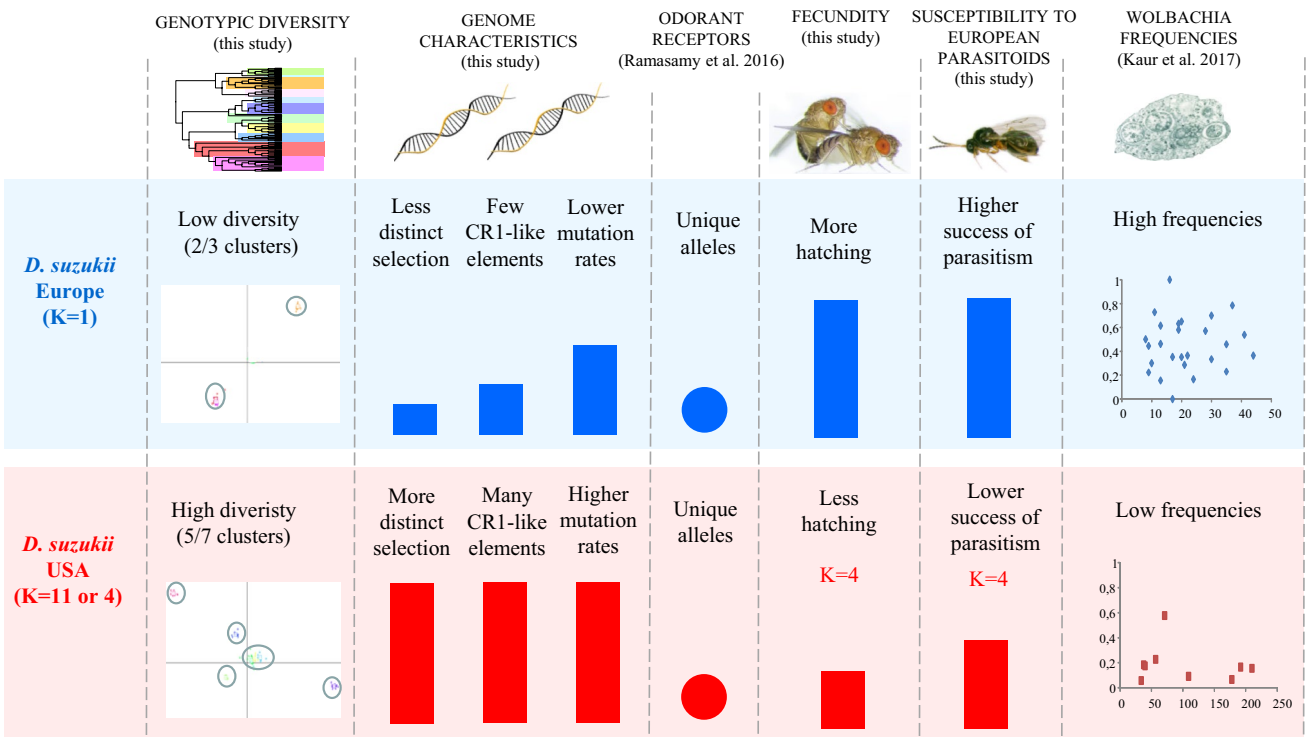

Fig. 4 Summary of the differences identified between American and European populations

Distinct continental populations

Our population genetic analyses identified a high divergence between strains originating from North America and Europe. Even by using only five markers, our analysis allowed us to get well-separated (Fig. 1b) and highly supported (Fig. 1c) genetic groups. Because of no continental overlap (excluding K7, see below) within each of the clusters, our results are compatible with at least two independent events of colonisation in the two continents (Fraimout et al. 2017). Results indicate the presence of two or three main genetic groups in Europe, the number depending on whether we consider K1 and K10 as the same cluster (Fig. 1). This scenario may reflect either two distinct invasion events or the concomitance entrance of two distinct genotypes, K2 and K1/K10. These two genotypes appear indeed well differentiated both in the DAPC plot and in the phylogenetic tree (Fig. 1b, c). One of the North American clusters (K7) contains, however, one European individual sampled in Italy (Sardinia). This can be interpreted either as an independent confirmation of the European–Eastern USA admixture that has been recently identified using microsatellite data (Fraimout et al. 2017), or a new independent, likely very recent, colonisation from the USA. More X-linked data from Europe (or alternatively more microsatellite data from Sardinia) are needed to clarify this issue.

Genetic and phenotypic disparities between Italian and American strains

Our results highlight a previously undetected diversity in the *D. suzukii* populations that have invaded Europe and North America. These differences do not stem simply from different genetic backgrounds in the corresponding populations/strains (Fig. 1) but are further confirmed by a genome-wide analysis of the two (North American and Italian) currently available sequenced genomes (Fig. 2). Both reference genomes are characterised by a reduced rate of nucleotide substitution compared to closely related species. This confirms the hypothesis that *D. suzukii* is characterised by a reduced number of generations per year compared with most other Drosophila species, which is compatible with a winter diapause (Ometto et al. 2013; Shearer et al. 2016). The North American reference genome accumulated, however, more substitutions than the Italian reference genome: assuming similar mutation rates in the two source strains, this difference may be explained by different number of generations in their native Asian ranges, possibly associated with slightly different climates (i.e. duration of the reproductive season and/or temperature-related developmental time). Therefore, we can hypothesise that different *D. suzukii* populations may be characterised by different levels of cold tolerance, something that should be tested in controlled conditions in the future. The possibility of different selective regimes acting in the two strains in their native areas is supported by the widespread presence of *D. suzukii* at a wide range of altitudes and climates in Asia (Mitsui et al. 2010), and the observation that the North American reference genome is characterised by a higher number of genes that evolved significantly faster (*branch* test) compared to the Italian reference genome.

In our phylogenomics analyses, divergence between the two strains (or more correctly, accounting for coalescence, the average divergence between alleles of the two strains) is around 1 million years: this is an unexpectedly high divergence, which in Drosophila is compatible with different species status within the same species complex (compared with the *D. simulans–D. sechellia* split, which dates at around the same age in Fig. 2a). We have shown, however, that such large genetic distance does not cause reduced crossing rate in comparison with intra-strain crossings (Fig. 3a): this excludes the possibility that the North American and the European strains belong to different cryptic (sensu not yet described) subspecies. Genetic differences can be extended to key genes that directly regulate the behaviour of *D. suzukii*, such as olfactory receptor genes. We suggest that the set of primers we have designed for one of them—OR85a—(Fig. 2c) can be routinely used to quickly distinguish European (K1) variants in uncharacterised populations. As soon as additional *D. suzukii* genomes will be available, it will be possible to refine primers for fast PCR discrimination of specific strains and populations (including other unsampled European populations). We have also explored the nature of an insertion responsible for OR22a frame disruption in the American genome and found that this insertion is part of a repetitive element, which is unevenly distributed in the two genomes. This is a first hint that differences in copy number and location of transposable elements in different *D. suzukii* strains may play key roles in strain differentiation. Further studies should focus on characterising the frequency and the position of repetitive elements in genomes characterised by longer scaffolds and better assembly.

To understand whether European and American populations are also characterised by different phenotypic traits, we compared their life table parameters and performed eclosion (Fig. 3a) and parasitisation (Fig. 3b) experiments. Results suggest differences between the two strains and pointed towards population-specific phenotypes that could ultimately affect their fitness. In particular, we observed a higher reproductive capability of the Italian strain compared to the North American strain: this is indicated by our eclosion experiments and with less significance (because of no side-by-side experiment) by our life table experiments. These differences are largely due to the shorter mean generation time and higher hatching rate in the Italian strain. This result apparently contrasts with the hypothesis that the Italian strain

derives from populations with lower number of generations per year than the ancestors of the American strain. In fact, one may hypothesise that the Italian (Trentino) strain is indeed (pre)adapted to colder climates: since low temperatures can both reduce metabolism, and hence reproduction, and shorten the reproductive season, there might have been local adaptation to increase reproduction capability during the warmer periods (see below). It is clear from the preliminary results that additional experiments need to be performed to verify these observations and test our hypotheses, particularly because our data refer to a single environmental condition (that of the experimental set-up).

Ecological impact and relevance for management strategies

Differences in the gene repertoire or in gene expression are likely to affect the way two distinct populations respond to the environment, including diapause, type of diet, and mortality factors such as susceptibility to parasitoids. Here, we have shown that different geographical strains (north Italy and Oregon, USA) are characterised by different reproductive capabilities (Fig. 3a). These differences are crucial as they may impact the worldwide applicability of *D. suzukii* demographic models and resistance forecasts (Tochen et al. 2014; Wiman et al. 2016) currently used for its management. Our results suggest that other management-relevant genes (particularly their expression level) should be tested in the future, for example, those involved with insecticide resistance (Li et al. 2007; Rivero et al. 2010; Silva et al. 2012). Currently, little is known about pesticide resistance in *D. suzukii* (Haye et al. 2016). However, if the genes involved in the production of detoxification enzymes that degrade or sequester insecticides are different, or differently expressed, we should expect a different probability of developing insecticide resistances between the two populations. Our data indicate that a similar scenario can be hypothesised for the use of specific attractive or aversive volatile compounds against *D. suzukii*. In this case, differences in the ORs repertoire/expression (Fig. 2c) may change the way such odours are perceived by the pest (Hickner et al. 2016; Ramasamy et al. 2016), resulting in different trapping/repellence efficacies among populations.

The adoption of cytoplasmic incompatibility (CI)-based control strategies using *Wolbachia* (Cattel et al. 2016a, 2018; Kaur et al. 2017) should also be carefully evaluated. This is because *D. suzukii* populations are characterised by the presence of different *Wolbachia* variants that may result in slightly different CI patterns due to specific host-*Wolbachia* associations. Indeed, we have previously identified a distinct Wolbachia infection frequency in wild populations *of* European and American *D. suzukii* (Cattel et al. 2016a) which has been linked to slightly dissimilar *wSuz* variants harboured by *D. suzukii* in these two continents (Kaur et al. 2017; see also suppl. Fig. 4 for an updated review of these differences). The data we have presented here do not exclude that *wSuz* frequency differences between continents are due to the genetic background of the *D. suzukii* host or to a combination of both the genetic background and the *wSuz* variants. For the same reason, we cannot exclude that the eclosion and parasitisation differences we have identified are at least partially due to different *wSuz* variants. This points to an urge of extending the comparison between American and European *D. suzukii* populations to various other life parameters using symbiont-free populations.

Parasitoids have the potential to regulate the populations of many Drosophila species. *Drosophila suzukii* is commonly attacked by specialised parasitoids in Eastern Asia (Daane et al. 2016), whereas in the newly invaded areas, a restricted community of generalist species attack *D. suzukii* (Rossi Stacconi et al. 2017; Chabert et al. 2012; Miller et al. 2015). We trialled the most commonly found European drosophila parasitoid species and found that the larval parasitoid *L. heterotoma* performs better on the European than on the American *D. suzukii* population. This is likely related to a lineage-specific immune response of *D. suzukii* in parasitoid's egg suppression through melanin encapsulation (Kacsoh and Schlenke 2012; Chabert et al. 2012), as supported by the higher number of flies carrying abdominal melanized parasitoid eggs in SMA13 (ITA) than in ORE14 (USA). Such mechanism has been shown to be highly dependent on the parasitoid recognition by the host (Colinet et al. 2013), which in turn depends on genes involved in the response to parasitoid's venom components by the *Drosophila* immunity (Carton et al. 2005; Asgari and Rivers 2011; Gatti et al. 2012; Strand 2012). Our results suggest that different *D. suzukii* lineages may be characterised by slightly different expressions of one or more of these genes. Such difference could be due either to pre-existing characteristics of the host strains or to a co-adaptation process between host and parasitoid in the same area. However, this falls out of the scope of this work and should be carefully characterised in future. *Drosophila suzukii* pupae have no obvious immunological defence reactions against parasitoids (Rossi-Stacconi et al. 2017; Chabert et al. 2012). However, a correlation between the thickness of the puparial wall, which the parasitoids have to drill through, and resistance has been observed in other *Drosophila* species (Kraaijeveld and Godfray 2003). Because the pupal parasitoids we have tested, *P. vindemiae* and *T. drosophilae*, maintain a similar level of efficacy on both the European and the American hosts, it is likely that there is no difference in the mechanical defence between the two fly populations. Over the last few years, many studies focused on the biological control of *D. suzukii* (Daane et al. 2016; Rossi Stacconi et al. 2018), and it is likely that a biological control agent (BCA) will be soon available on

the market. Our results on *L. heterotoma* advocate that BCA should be tested for their efficacy and specificity on different *D. suzukii* geographical variants before reaching the market.

Conclusions

We have used genetics, genomics, and physiology to characterise and quantify various differences between representative European and North American populations of the invasive pest *D. suzukii.* These differences may be the tip of the iceberg of a larger general disparity existing among wild populations, in particular between Europe and America. The differences we have highlighted in this article should be explored in depth, and comparisons among populations should be extended to other key genes and their level of expression, to more physiological characters, and to more populations. In particular, other relevant fitness-related traits such as cold tolerance or insecticide resistance should be tested in different populations.

More generally, we advocate that a comparative approach among different populations, as the one we have presented here for *D. suzukii*, should be applied to other invasive pests. In this context, we embraced the need for shifting "from a species-centred to a population-centred perspective" (Reichard et al. 2015) when studying invasive species, in particular those of agricultural relevance. As we have shown for *D. suzukii*, ecologically relevant intraspecific differences should be taken into account when interpreting results from both fundamental and applied research, particularly when management practices defined on one population/strain are transferred to others from a different continent.

Author contributions

OR-S conceived and coordinated the study. LO and OR-S performed genomic analyses. SG, OR-S, and GT performed population studies. RK performed crossing studies. OR-S and FD performed phylogenetic studies. JG and OR-S performed repeated element studies. GA and MVR-S performed parasitoid studies. VMW and MVR-S performed life table experiments. OR-S drafted the manuscript with major inputs from LO and MVR-S and inputs and edits from all authors.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals (Drosophila fruit flies) were followed. This article does not contain any studies with human participants performed by any of the authors.

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