

SCIENTIFIC NOTE

# Spotted-wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae), exhibits large-scale spatial genetic structure across Canada but not fruit host-associated genetic structure

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## Abstract

Spotted-wing drosophila, *Drosophila suzukii*, is a global pest of soft fruits that is capable of reproducing on a wide range of cultivated and wild plant species. In Canada, *D. suzukii* was first reported in British Columbia in 2009 and is now widespread across the country. Understanding the genetic structure of *D. suzukii* populations could be important for pest management if there are phenotypic differences between genetically distinct populations. For example, insect pest populations could respond differently to directional selection imposed by insecticides, differ in their host plant preferences, and vary in their susceptibility to biological control agents. Here, we used double-digest restriction site-associated DNA sequencing to examine large- and fine-scale patterns of the genetic structure of *D. suzukii* reared from fruit hosts in Canada. We found that this species has a large-scale spatial genetic structure; the flies collected formed two distinct genetic clusters, one of which was distinct to western Canada and the other to eastern Canada. At the local scale, *D. suzukii* populations showed no evidence of host-associated structuring in British Columbia, suggesting that pest management tactics may be best applied at the landscape level. Our results highlight the need to investigate phenotypic differences between western and eastern *D. suzukii* populations in Canada.

The invasive spotted-wing drosophila, *Drosophila suzukii* (Matsumura, 1931), is native to southeast Asia and has become a global pest of fruits (Asplen *et al.* 2015; Tait *et al.* 2021). Over the past 15 years, it has spread rapidly across North America. In Canada, it was first detected in British Columbia in 2009 and in Quebec and Ontario by 2011 (Thistlewood *et al.* 2013) and is now widespread across the country. Current management of *D. suzukii* populations in Canada relies on cultural practices such as short harvest intervals and the removal of unmarketable fruit combined with chemical insecticide applications, applied at regular intervals of 7–14 days through fruit ripening (Ontario Ministry of Agriculture, Food, and Rural Affairs 2022; British Columbia Ministry of Agriculture and Food 2023). In addition, biological control agents *Ganaspis*

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*brasiliensis* Ihering, 1905 and *Leptopilina japonica* Novković and Kimura, 2011 (both Hymenoptera: Figitidae) have recently established in British Columbia (Abram *et al.* 2022a), and their role in suppression of *D. suzukii* remains an active area of research.

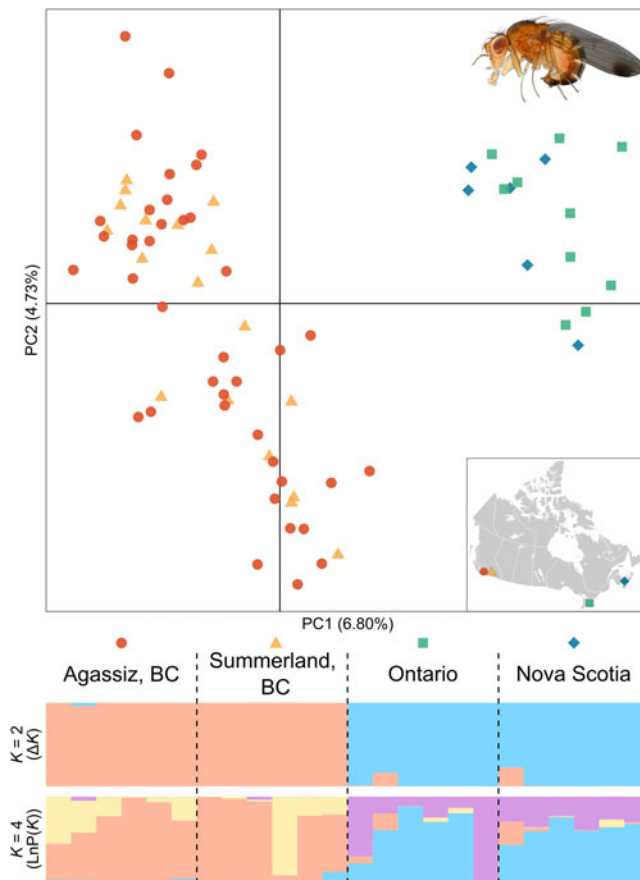
Understanding the population genetic structure of *D. suzukii* is potentially useful for refining existing management strategies because genetically distinct populations may differ in phenotypic traits (e.g., hatch rate, generation time, insecticide resistance, susceptibility to parasitoids, and host plant association) that could impact pest management practices (Rota-Stabelli *et al.* 2020; Olazcuaga *et al.* 2022). Given that current management strategies rely heavily on the use of insecticides for population suppression, selection for resistance is a serious threat (e.g., Gress and Zalom 2019; Ganjisaffar *et al.* 2022). Knowledge of population size, genetic diversity, and rates of gene flow can help build a more resilient management strategy (Green *et al.* 2020); for example, the use of untreated host plant refugia in areas where gene flow is known to occur can help to impede insecticide resistance development (Tabashnik *et al.* 2013). The genetic structure and invasion history of *D. suzukii* have been the focus of past investigations (Fraitout *et al.* 2017; Lewald *et al.* 2021) that each found evidence of multiple *D. suzukii* introduction events from Asia into the United States of America, with a geographic subdivision of eastern and western populations. However, patterns of population structure remain unknown for Canadian *D. suzukii* populations.

The success of *D. suzukii* is partially due to its ability to exploit a wide range of host plants that fruit throughout the growing season in different landscapes (e.g., cultivated cherry (*Prunus avium* Linnaeus), Himalayan blackberry (*Rubus armeniacus* Focke), and elderberry (*Sambucus racemosa* Linnaeus); Kenis *et al.* 2016; Abram *et al.* 2022a). Under laboratory conditions, adaptation of *D. suzukii* to different host fruits has been observed over multiple generations (Olazcuaga *et al.* 2022), but it is unclear whether similar adaptation could occur with the spatial and temporal patterns of fruit use by *D. suzukii* under natural conditions. If *D. suzukii* populations are able to associate with or specialise in specific host plants, host fruit-associated genetic structure may occur at a local scale.

Here, we examined the genetic structure of Canadian *D. suzukii* using double-digest restriction site-associated DNA sequencing (ddRADseq). First, using specimens from four regions, we asked whether there exists large-scale genetic structuring in *D. suzukii* populations across the country. Second, using *D. suzukii* that emerged from fruits collected from several host plant species in British Columbia, we assessed whether there is evidence of local population genetic structuring associated with host plant species.

In 2020, we collected fruit samples infested with *D. suzukii* eggs, larvae, and pupae from four fruit-growing regions across Canada (Fig. 1; Agassiz, British Columbia; Summerland, British Columbia; Vineland, Ontario; and Kentville, Nova Scotia). We collected ripe fruits from six cultivated or wild host plant species between the beginning of June and early October (Table 1). Following the rearing protocol of Abram *et al.* (2022b), we stored fruits in indoor locations in ventilated containers (12 × 12 × 8 cm, Ziploc® Medium Square Containers) at ambient or controlled (24 °C) temperatures without wire mesh lining the container bottom. We checked containers for adult *D. suzukii* emergence two to three times per week. If found, we mouth aspirated adult flies into vials containing 95% ethanol, which we labelled and stored at –20 °C for later molecular analysis. We randomly selected and sexed 10 flies from each host plant + site combination for genetic analysis. We retained representative voucher specimens from each host plant + site combination at –20 °C at the Agassiz Research and Development Centre (Agriculture and Agri-Food Canada, Agassiz, British Columbia) and deposited vouchers at the Royal British Columbia Museum, Victoria, British Columbia.

We extracted genomic DNA from 80 adult *D. suzukii* with DNeasy Blood & Tissue Kits (QIAGEN, Hilden, Germany) with the manufacturer's suggested addition of pancreatic ribonuclease A (RnaseA; 4 µL at 100 mg/mL; QIAGEN) to digest RNA. We eluted DNA into 2 × 50 µL of 56 °C Buffer AE to increase DNA yield and concentration, which we kept at –20 °C until library preparation. *PstI*-*MspI* restriction enzyme ddRADseq libraries were prepared by staff



**Figure 1.** Principal component analysis and structure barplots depicting spatial genetic clustering among *Drosophila suzukii*. STRUCTURE results from the first of 10 subsampled replicates of 24 individuals.  $K$  values with the greatest statistical support using  $\Delta K$  and  $\text{LnP}(K)$  are presented. Photo of male *D. suzukii* by Yoichiro Watanabe.

at the Molecular Biology Service Unit at the University of Alberta (Edmonton, Alberta, Canada) using the protocol of MacDonald *et al.* (2020). The two enzymes cut DNA at select restriction sites across the genome to reduce complexity before sequencing. Single-end, 75 base-pair (bp) reads were generated from 200 ng of DNA per individual on one high-output flow cell of a NextSeq 500 (Illumina, California, United States of America).

We demultiplexed DNA sequence data using the *process\_radtags* programme in Stacks 2, version 2.55 (Rochette *et al.* 2019), discarding reads that failed the Illumina purity filter, had uncalled bases, or had Phred scores below 20 over a 15% sliding window of the read length. Resulting reads were 67 bp long after the index sequences were removed. Due to sequencing error associated with the adaptor sequence in the *PstI* restriction enzyme site, we used Cutadapt, version 3.4 (Martin 2011), to trim an additional 5 bp from the 5' ends, yielding final read lengths of 62 bp. We aligned reads to the near-chromosome level assembly of the *D. suzukii* genome (Paris *et al.* 2020) with the Burrows–Wheeler Aligner, version 0.7.17 (Li and Durbin 2009), using the BWA–MEM algorithm. We converted SAM files to BAM format and sorted them with SAMtools, version 1.9 (Li *et al.* 2009), and then used the *ref\_map* programme in Stacks 2 to call single-nucleotide polymorphisms (SNPs), specifying one population containing all individuals. We retained loci that were present in at least 80% of individuals (Paris *et al.* 2017), with a minor allele frequency cut-off of 0.05. We completed further filtering in VCFtools, version 0.1.16 (Danecek

**Table 1.** Summary of collection locations, latitude and longitude coordinates, host plant species, collection dates, and sex ratios for *Drosophila suzukii* collected for molecular analysis.

Location	Coordinates	Host plant species	Emergence dates (2020)	Sex ratio (♀:♂)
Agassiz, BC	49.241985, -121.765744	Elderberry ( <i>Sambucus racemosa</i> )	13 June–8 July	6:4
		Raspberry ( <i>Rubus idaeus</i> )	1–8 July	5:5
		Cultivated blackberry ( <i>Rubus fruticosus</i> )	19–26 August	7:3
		Himalayan blackberry ( <i>Rubus armeniacus</i> )	19–26 August	6:4
Summerland, BC	49.566056, -119.638397	Cherry ( <i>Prunus avium</i> )	9–18 August	4:6
		Kousa dogwood ( <i>Cornus kousa</i> )	8–19 October	7:3
Vineland, ON	43.17366, -79.35599	Cultivated blackberry ( <i>R. fruticosus</i> )	21 August–25 September	7:3
Kentville, NS	45.162394, -64.5011944	Cultivated blackberry ( <i>R. fruticosus</i> )	8–11 September	5:5

BC, British Columbia; NS, Nova Scotia; ON, Ontario.

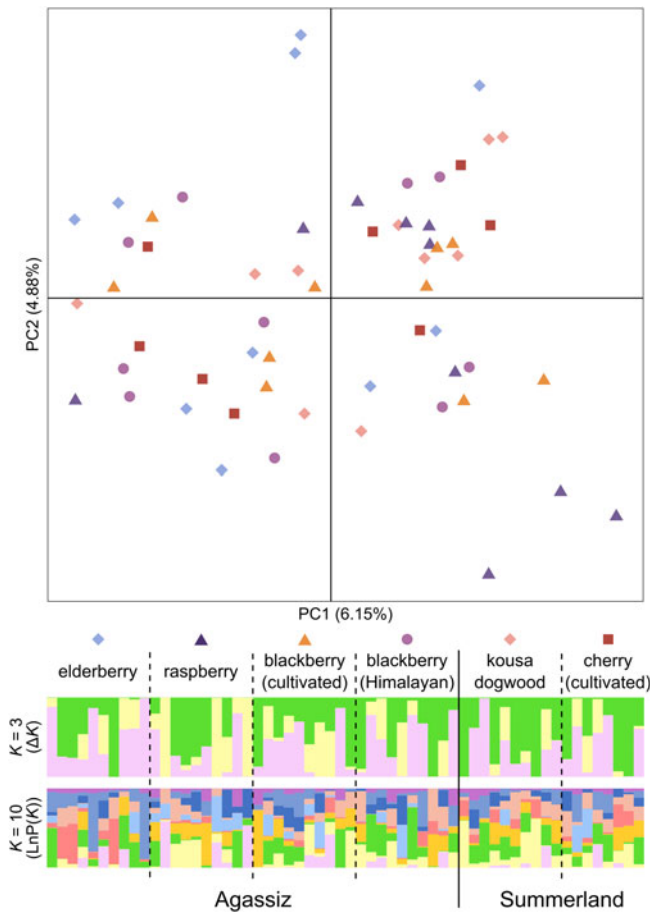
*et al.* 2011), removing individuals with more than 10% missing data and loci with more than 5% missing data. We thinned SNPs to ensure that no two were within 10 000 bp of one another, minimising physical linkage (MacDonald *et al.* 2020). Our demultiplexed ddRADseq data are published in fastq format in the National Center for Biotechnology Information Sequence Read Archive under accession number PRJNA995753.

We used three independent analyses to quantify population structure. First, we performed principal component analysis in *adegenet*, version 2.1.5 (Jombart and Ahmed 2011), and visualised output with *ggplot2*, version 3.3.5 (Wickham 2016). Then we used Bayesian model-based clustering analysis implemented in STRUCTURE, version 2.3.4 (Pritchard *et al.* 2000), to assess potential genetic clusters ( $K$ ). To ensure sample sizes were equal for the global STRUCTURE analysis (Puechmaille 2016), we used VCFtools to create 10 subsampled data sets from the global data set, each containing all six individuals from Nova Scotia and six randomly subsampled individuals from Agassiz, Summerland, and Ontario (24 of 74 potential individuals per subsampled data set). Sampling three to six individuals per population permits robust assessment of genetic structuring when using SNP data (Shi *et al.* 2010; Nazareno *et al.* 2017; Qu *et al.* 2020). For our third step, we calculated pairwise  $F_{ST}$  values (Weir and Cockerham 1984) using *weir-fst-pop* in VCFtools and calculated the number of sites with private alleles among groups using *private\_alleles* in poppr, version 2.9.4 (Kamvar *et al.* 2015).

For each STRUCTURE analysis, we ran 10 replicates for each  $K$  value 1–10, stipulating a burn-in of 100 000 followed by 1 000 000 Markov chain–Monte Carlo repetitions. Using the admixture model, we set the  $\alpha$  prior to 0.5 based on the greatest expected support for  $K = 2$  in principal component analysis (Wang 2017), and we used the *locpriors* option to better resolve spatial structure across the four collection locations (Porrás-Hurtado *et al.* 2013). We used StructureSelector (Li and Liu 2018) to assess the  $K$  values with the greatest statistical support, considering both  $\ln P(K)$  (Pritchard *et al.* 2000) and  $\Delta K$  (Evanno *et al.* 2005), and CLUMPAK, version 1.1, to average the 10 replications of each  $K$  value and generate Q-matrices (Kopelman *et al.* 2015). To assess hierarchical genetic structuring (Vähä *et al.* 2007), we re-ran the individuals that constituted separate genetic clusters in the global analyses through Stacks 2 using the above filtering options and then through principal component analysis and STRUCTURE. We set the  $\alpha$  value to 1 for each hierarchical data set, and we did not use *locpriors* for data sets that were derived from a single collection location.

After filtering in VCFtools, the global 74-individual data set consisted of 6766 biallelic SNPs with a mean read depth of  $31.38 \times$  across 15 502 225 filtered reads and 1.56% total missing data. We found two major genetic clusters among the 74 retained *D. sukukii* individuals in principal component analysis. The first of the 10 subselected, 24-individual STRUCTURE data sets consisted of 5058 biallelic SNPs with a mean read depth of  $32.43 \times$  across 3 883 504 filtered reads and 1.44% total missing data.  $K = 2$  was best supported using the Evanno *et al.* (2005) method, and  $K = 4$  was best supported using the Pritchard *et al.* (2000) method, although  $K = 2$  was also highly supported (Supplementary material, Figs. S2 and S3). We found similar STRUCTURE results in all subselected data sets (Supplementary material, Fig. S1). Overall, we found that *D. sukukii* from Agassiz and Summerland comprised one genetic cluster and that *D. sukukii* from Ontario and Nova Scotia comprised the other cluster (Fig. 1; Supplementary material, Table S1). Mean pairwise  $F_{ST}$  between the western (“BC”) and eastern (“ON + NS”) clusters was 0.071, and the clusters had 1493 and 217 sites with private alleles, respectively. The “BC” cluster had a mean observed heterozygosity of 0.22 (range 0.15–0.30) with a mean expected heterozygosity of 0.25 (range 0.24–0.25), and the “ON + NS” cluster had a mean observed heterozygosity of 0.24 (range 0.19–0.36) with a mean expected heterozygosity of 0.27 (range 0.27–0.28).

Our finding of two geographic genetic clusters is consistent with the strong population structure identified between western and eastern populations of *D. sukukii* in the United States of America by Lewald *et al.* (2021). Although the differences in population genetic methods used in the current study prevent direct comparison of our SNPs to the genotype likelihoods of Lewald



**Figure 2.** Principal component analysis and structure barplots of 58 *Drosophila suzukii* reared from fruit host species in Agassiz and Summerland, British Columbia.  $K$  values with the greatest statistical support using  $\Delta K$  and  $\text{LnP}(K)$  are presented.

*et al.* (2021), it is likely that the western and eastern Canadian populations we identified are congruent with those found in the United States of America. Neither our study nor Lewald *et al.* (2021) investigated the genetic structure of *D. suzukii* populations from central Canada and central United States of America. It is possible that “central” individuals are genetically intermediate to the eastern and western clusters on a hypothetical cline that spreads across North America. However, we hypothesise that they belong to the eastern or western genetic cluster. We did not detect significant genetic differences between *D. suzukii* from Ontario and those from Nova Scotia despite over 1000 km of overland separation, which is good evidence that individual clusters can remain genetically similar over large parts of their range (*e.g.*, along the coasts of North America; Lewald *et al.* 2021) without forming a genomic cline.

The final hierarchical data set of *D. suzukii* from British Columbia contained 7063 biallelic SNPs with a mean read depth of  $30.50\times$  across 12 361 221 filtered reads and 1.29% total missing data. We found little evidence of principal component analysis clustering within this data set (Fig. 2;  $n = 58$ ). In the STRUCTURE data set, three clusters ( $K = 3$ ) had the greatest statistical support using the Evanno *et al.* (2005) method, whereas 10 clusters ( $K = 10$ ) had the greatest support using the Pritchard *et al.* (2000) method (Supplementary material, Figs. S6 and S7). Such

incongruence between principal component analysis and each  $K$  method is expected when there is little genetic clustering in hierarchical analysis (e.g., Nelson *et al.* 2022). Mean overall  $F_{ST}$  between host plant groups was very low, varying from  $-0.010$  to  $+0.016$  (Supplementary material, Table S2). Females had 34 sites with private alleles, whereas males had three. Hierarchical principal component analysis, STRUCTURE, and  $F_{ST}$  analysis found little evidence of genetic clustering within *D. suzukii* collected in British Columbia, indicating that there is little host-associated, sex-associated, or phenological genetic clustering (Fig. 2; Supplementary material, Figs. S4 and S5), although several individuals that emerged from elderberry or raspberry in Agassiz formed two small outlier groups.

Hierarchical principal component analysis and STRUCTURE analysis found no evidence of genetic clustering among the individuals collected in Ontario and Nova Scotia (Supplementary material, Figs. S8–S10;  $n = 16$ ) or in Summerland and Agassiz when analysed separately (not shown due to similarity with Fig. 2). Under laboratory conditions, Olazcuaga *et al.* (2022) found that there is potential for host plant-associated local selection and adaptation in *D. suzukii*, but our results show no evidence of this occurring under natural conditions in coastal and interior regions of British Columbia. The likelihood of host plant-associated local selection may be relatively low under natural conditions, perhaps due to (1) low genetic diversity among the founding members of the British Columbia cluster of *D. suzukii* preventing rapid evolution, (2) frequent spatial co-occurrence of different host plants at small scales (Abram *et al.* 2022a), coupled with dispersal ability of *D. suzukii* between host plants (Tait *et al.* 2021), and (3) fruiting phenology of single host plants not extending long enough throughout the season to be the only host supporting *D. suzukii* during its reproductive period (Thistlewood *et al.* 2019). As such, *D. suzukii* would need to reproduce on at least two different host plants to complete its seasonal cycle. Despite our relatively limited sample size at a few sites during a “snapshot” in time, our results showing a lack of host plant-associated genetic structuring suggest that current pest management decisions are best implemented for British Columbia at the landscape level and provide a baseline against which future potential adaptation and selection of invasive *D. suzukii* populations in western Canada can be measured.

Our finding of a distinct subdivision of western and eastern Canadian *D. suzukii* populations is consistent with that of Lewald *et al.* (2021), who found similar subdivision of eastern and western *D. suzukii* populations in the United States of America. These findings could have implications for the management of *D. suzukii* if the two populations have different phenotypic parameters or different levels of susceptibility to integrated pest management tactics. For example, there may be geographic variation in the susceptibility of these *D. suzukii* populations to native, adventive, or intentionally introduced natural arthropod enemies (reviewed in Wang *et al.* 2020; Abram *et al.* 2022b), as has been found in other *Drosophila*–parasitoid associations (e.g., Kraaijeveld and Godfray 1999). It is also possible that these two *D. suzukii* populations respond differently to insecticide treatments or develop resistance (e.g., Gress and Zalom 2019; Ganjisaffar *et al.* 2022) at different rates. Whether there are phenotypic differences relevant to pest management in western versus eastern *D. suzukii* populations associated with the genetic structure identified by ourselves and others (Framout *et al.* 2017; Lewald *et al.* 2021) remains an open question that should be addressed in the coming years.

**Supplementary material.** To view supplementary material for this article, please visit <https://doi.org/10.4039/tce.2023.24>.

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**Competing interests.** The authors declare that they have no competing interests.

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