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Microsatellite Variation of two Pacific Coast *Drosophila suzukii* (Diptera: Drosophilidae) Populations

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ABSTRACT The vinegar fly, *Drosophila suzukii* (Diptera: Drosophilidae), is a recent invader in North America that has become a serious threat to small fruit production. It was first detected in California in 2008 and in Washington state in 2009. In this study, *D. suzukii* populations from the area of the original detection on California's central coast and from eastern Washington, the United States, were sampled over a 3-year period to determine genetic variation in both using microsatellite markers. Six different loci were successfully amplified and included in the analysis. These loci included *nanos*, *elf1*, *antennapedia*, *mastermind*, *z600*, and *tenA*. The population from eastern Washington was highly monomorphic with one locus, *mastermind*, having multiple alleles. There was greater genetic variation in the coastal California population with all loci having multiple alleles, with the exception of *tenA*. Owing to the relatively low levels of genetic variation in the eastern Washington population compared with the coastal California population, it appears that the *D. suzukii* population in the eastern Washington region has undergone a significant bottleneck.

KEY WORDS microsatellite, invasion, bottleneck, *Drosophila suzukii*, selection

Invasive species are a significant problem, both globally and locally, that negatively impact humans by causing significant yield loss in agricultural systems or by creating new public health issues (Pimentel 2005). Invasive species can also negatively impact the environment by outcompeting, predating, or parasitizing local flora and fauna (Pimentel 2005). The successful establishment of an invasive species relies on genetic variation that allows it to adapt to the environment into which it was introduced. When invasions do occur, there is commonly a founder effect, causing a genetic bottleneck to occur (Williamson-Natesan 2005), often lowering the fitness of the founding population and making it less adaptable to new selection pressures. The use of microsatellite markers is a useful tool for studying founder effects (Williamson-Natesan 2005), the potential source of an invading population (Rollins et al. 2006, Rugman-Jones et al. 2007), and ability to withstand new selection pressures such as genes that confer resistance to some pesticides (Liu et al. 2002, Weng et al. 2005). Understanding the genetic variation and ultimately the evolutionary potential of an introduced species is an essential element to developing a successful management program.

The invasive vinegar fly, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is native to southeast Asia (Hauser et al. 2009). Following its almost simultaneous initial detections in North America (Bolda et al. 2010) and Europe (Cini et al. 2012), it spread rapidly, and owing to its propensity to attack ripening fruit as opposed to over-ripened fruit, which is the case with the endemic *Drosophila* species, it has become a key economic pest of berry and cherry crops across both continents (Lee et al. 2011). The full genome of *D. suzukii* has recently been published (Chiu et al. 2013, Ometto et al. 2013), and Adrion et al. (2014) examined population genetics of 12 *D. suzukii* populations (nine from the continental United States, one from Hawaii, one from Japan, and one from Spain) using six loci. Their analysis suggested that the recent colonization of Europe and the continental United States were separate demographic events and that bottlenecks occurred for each (Adrion et al. 2014). While these studies provide novel molecular data and information on the global status and movement of *D. suzukii,* between continents, they do not address the dispersal and radiation of the pest once it had become established in a new country or geographic region. Two California populations were included in the analysis, though neither was from the county of first detection, and no populations from other Pacific coast states were included. In the United States, environmental conditions and production practices in agricultural systems can vary significantly from state-to-state as well as within states. As a result, *D. suzukii* populations introduced into different geographic regions may adapt and evolve at different rates, ultimately resulting in different levels of genetic diversity. Evaluation of the genetics of these populations may inform the types of management practices appropriate to different regions.

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Although microsatellites have been widely applied to a variety of studies in other *Drosophila* species (Goldstein and Clark 1995, Noor et al. 2000, Barker et al. 2009, Haddrill et al. 2013), no work has been published to date using microsatellites in *D. suzukii*. In this study, we utilized microsatellite loci conserved between *Drosophila melanogaster* and *D. suzukii* to evaluate genetic variation in populations of *D. suzukii* from California and Washington collected across three years (2011–2013). Populations from Watsonville, CA (located on California's central coast), were chosen because this was the region of first record of *D. suzukii* establishment in the continental United States, and populations from eastern Washington state were chosen because this is a region where preliminary climate-based *D. suzukii* range-expansion models did not predict successful establishment, and therefore, climatic selection pressures may occur. Additionally, *D. suzukii* was detected in eastern Washington within a year of its original detection in CA.

Materials and Methods

Sample Collection. Specimens of *D. suzukii* from eastern Washington state were collected between 2011 and 2013 near Prosser and Paterson. A total of 132 individual flies representing 44 individuals for each of the 3 years of this study collected from organic fruit crops, including blueberries, apricots, cherries, Concord juice grapes, wine grapes, and peaches, from eastern Washington were selected for analysis. Flies were trapped using apple cider vinegar and were subsequently transferred to 95% ethanol and stored at 22°C until processed. Specimens of *D. suzukii* were collected between 2011 and 2013 near Watsonville, California, from coastal organic raspberries near Watsonville, California, nearby the site where *D. suzukii* was first reported in North America in fall 2008. A total of 42 individual flies representing 14 individuals for each of the 3 years of this study were collected using a D-VAC Vacuum Insect Net Model 122 (Rincon-Vitova Insectaries, Inc. Ventura, CA). Live *D. suzukii* from the vacuum samples were flash-frozen on dry ice and then stored frozen at −80°C until being transferred to 95% ethanol for shipping.

Microsatellite Loci Selection and DNA Extraction. Microsatellite markers have not been developed to date for *D. suzukii* because the genome sequence has only recently been published (Chiu et al. 2013). Owing to the lack of microsatellite information for *D. suzukii*, 10 loci of *D. melanogaster* were selected and tested to determine if loci were conserved between these two closely related species (Table 1). Total genomic DNA was extracted from individual flies using the DNeasy Blood & Tissue Kit (Qiagen, Frederick, MD).

PCR Amplification, Visualization, Cloning, and Sequencing. All polymerase chain reactions (PCRs) were set up as 20 µl reactions containing 2 µl of DNA extract, 0.5 U *Ex Taq* DNA polymerase (Takara, Mountain View, CA), 200 µM dNTPs, 1 µM forward primer, 1 µM reverse primer, and 1.5 mM 10× reaction buffer. Thermal cycling conditions were as follows: initial denaturing for 5 min at 95°C, followed by 30 cycles of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C. All reactions were run on a T100 Thermal Cycler (Bio-Rad, Hercules, CA). Following PCR amplification, product was visualized using gel electrophoresis on 4% MetaPhor agarose to determine allele numbers and sizes. MetaPhor agarose is a high-resolution agarose that allows for differentiation of products that differ in size from 1 to 2%. All samples that produced bands were subsequently cloned using TOPO® TA Cloning® Kit (Invitrogen, Carlsbad, CA) and sequenced by ELIM Biopharmaceuticals (Hayward, CA) to verify allele size and presence of the desired repeat motif. Allele sizes were scored based on the PCR product size that included flanking regions of DNA and the tandem repeat region.

Data Analysis. Plasmid sequence data were edited and contiguous sequence files were created using Vector NTI (Life Technologies, NY). Contiguous sequences were then scored for allele size and recorded as six-digit numbers to represent both copies of the allele. Genotype, allele frequencies, heterozygosity for each locus, and an Analysis of Molecular Variance (AMOVA) were completed using GenoDive v.2.0b25 (Miermans 2013). The detection of a bottleneck was calculated using the Wilcoxon test under the stepwise mutation model owing to the use of fewer than 20 polymorphic loci in the BOTTLENECK program (Cornuet and Leikart 1999).

Results

Amplification of Microsatellite Loci. Of the 10 loci that were tested for *D. suzukii*, six were reliably amplified across all 132 samples tested from Washington and across all 42 samples from California. The loci that successfully amplified were *nanos, mastermind, antennapedia, z600, elf1,* and *tenA*. The four loci that failed to amplify consistently were *Mlcl*, *expanded*, *zeste-white 3* (3′UTR), and *sevenless*.

Microsatellite Variation Within Populations. In the eastern Washington population, all loci were monomorphic, with the exception of *mastermind*, where nine alleles were detected (Table 2). At this locus, 51 heterozygotes were detected. The frequencies of *mastermind* deviated significantly ($P = 0.001$) from Hardy–Weinberg equilibrium. From the coastal California samples, only one locus, *tenA*, was monomorphic, while all other loci were polymorphic (Table 2). All polymorphic loci in the coastal California population deviated significantly ($P < 0.01$) from Hardy–Weinberg equilibrium, with the exception of *z600* ($P < 0.058$), and the population overall showed a significant deviation ($P = 0.001$) from equilibrium. Also, the same alleles were detected each year for each locus examined in the California population, and for the *mastermind* locus in the Washington population with comparable levels of frequencies between each year (Table 3). In all loci for the California population, the predominant (most frequent) allele represented in Table 2 had the highest frequency for each year of the study, with the exception

Table 1. Microsatellites selected from *Drosophila melanogaster* for use in analysis

Locus	Primer (5′ to 3′)*	Repeat Motif	Location
zeste-white 3	TCCAGCAATCACAGCAAACTTCTTTTGAAATTGCGGTTGA	CAG	3′ UTR
sevenless	AATTTGTTATGGCCACTTCCCCATGGCCAATAAGTCACGC	TTG	Intron
tenA	CTCTTAGTGCGCAGGGATTCGAGTCGCTCAATGGCAGG	TA(CC)AT	3′ UTR
expanded	GTGATCGATCCCGCTGTCGAGTCGCTCAATGGCAGG	CAG	Expressed
mastermind	CAGCAGCAGATCCAAGTTCAGTTTGCATTGTAGGGCGAGT	CAG(CAA)CAG	Expressed
elf1	ACAGCAACAACGGAGCAACTCTGCAACCTGGGAGTCTG	CAG	5′ UTR
z600	AAATCTGTTGCTCATACTGCCCAACCGGCGAAATGTTCAG	TTC	Intron
antennapedia	TGCATTGCTAATGATCGTGGGCTTTTTCTTACACAATTCGCA	CT(GT)CT(TT)CT	Unknown
nanos	CGCAAGTATTCATTTCAACACATGCTGGCGGTTGTTTCAT	TA	3′ UTR
Mlcl	GGGTAATCCCTTGCTAATATGGAATGGTTGTCGCTAAAAGTT	CAA(CAC)CAA	3′

*Goldstein and Clark 1995

Table 2. Microsatellites amplifying in *Drosophila suzukii* from eastern Washington and coastal California

Locus	No. of alleles$_{He*}$		Allele sizes (bps)$_{Frequency}$	
	Washington	California	Washington	California
nanos	1	$4_{0.92}$	$52_{1.0}$	$52_{0.46}$, $102_{0.13}$, $103_{0.01}$, $104_{0.40}$
elf1	1	$5_{0.06}$	$77_{1.0}$	$75_{0.16}$, $77_{0.74}$, $80_{0.01}$, $82_{0.07}$, $87_{0.02}$
antennapedia	1	$8_{0.43}$	$47_{1.0}$	$47_{0.79}$, $80_{0.006}$, $85_{0.16}$, $86_{0.01}$, $90_{0.02}$, $95_{0.006}$, $100_{0.006}$, $120_{0.006}$
tenA	1	1	$75_{1.0}$	$75_{1.0}$
mastermind	$8_{0.39}$	$15_{0.55}$	$50_{0.01}$, $60_{0.13}$, $63_{0.01}$, $67_{0.01}$, $77_{0.02}$, $80_{0.03}$, $83_{0.05}$, $85_{0.74}$, $90_{0.02}$.	$60_{0.02}$, $63_{0.01}$, $65_{0.04}$, $67_{0.006}$, $70_{0.006}$, $73_{0.02}$, $75_{0.08}$, $77_{0.10}$, $80_{0.19}$, $83_{0.04}$, $85_{0.28}$, $87_{0.10}$, $90_{0.07}$, $93_{0.006}$, $95_{0.02}$ $93_{0.17}$, $95_{0.83}$
z600	1	$2_{0.38}$	$90_{1.0}$	

*$_{He}$: Heterozygosity.

Table 3. Allele frequencies for the *mastermind* locus in the Washington population for each year of the study

Allele	2011	2012	2013
50	0.01	0.01	0.01
60	0.13	0.14	0.12
63	0.01	0.01	0.01
67	0.01	0.01	0.01
77	0.02	0.02	0.02
80	0.03	0.03	0.03
83	0.05	0.05	0.05
85	0.74	0.73	0.75
90	0.02	0.02	0.02

Table 4. *F*-statistics analysis with significance levels for population differentiation at all loci for eastern Washington and coastal California populations of *Drosophila suzukii*

Locus	F_{ST}	P
nanos	0.498	0.001
elf1	0.228	0.001
Antennapedia	0.204	0.001
tenA	0.000	1.000
mastermind	0.207	0.001
z600	0.887	0.001
Overall	0.511	0.001

of *tenA*, which was homozygous. The same trend was seen for the *mastermind* locus in Washington population where the 85-bp allele was predominant throughout the study (Table 3).

Microsatellite Variation Between Populations and Bottleneck Test. There was a highly significant population differentiation (F_{ST}) between the eastern Washington and coastal California populations (Table 4). This differentiation extended to each locus tested, with the exception of *tenA*. All alleles present in the eastern Washington population were present in the coastal California population, with the exception of the allele at the *z600* locus that was absent from the California samples. The overall deficiency in heterozygotes was significant for the eastern Washington population (0.25; $P < 0.0001$) with each loci expressing significant deficiency in heterozygotes, with the exception of the *mastermind* locus (0.075; $P = 0.69$). Overall, the coastal California population did not display a significant deficiency in heterozygotes (0.078; $P = 0.647$) (BOTTLENECK version 1.2.02) (Cornuet and Luikart 1999). The overall heterozygosity between populations was significantly different ($P < 0.001$) (BOTTLENECK version 1.2.02) (Cornuet and Luikart 1999).

Discussion

The conservation of loci between *D. melanogaster* and other closely related species of *Drosophila* was previously reported by Harr et al. (1998) and Adrion et al. (2014); however, our specific loci were not included in these comparisons. Our results confirm that multiple microsatellite loci (*nanos, mastermind, antennapedia, z600, elf1,* and *tenA*) are conserved between *D. suzukii* and *D. melanogaster*, as indicated by the successful

amplification of those loci using primers designed for *D. melanogaster* in *D. suzukii*.

Where the initial North American invasion took place, and from where the founding population originated remains unclear. If *D. suzukii* was first introduced into the coastal California area where it was initially found, its rapid and ultimately widespread distribution elsewhere along the length of the Pacific coast may be attributed to human movement because significant geological barriers including major mountain ranges would have impeded its natural spread. An alternate scenario is that *D. suzukii* was introduced simultaneously at multiple locations, resulting in independent detections throughout the Pacific coast states over a period of less than two years. Multiple North American introduction events cannot be dismissed, as no data currently exist to support or refute this possibility. Regardless of where *D. suzukii* was first introduced or how it came to be so widely distributed, our results indicate that there is much less genetic diversity in the population from eastern Washington state than in the coastal California population, suggesting a strong bottleneck has occurred in the former population. While different loci were analyzed, our findings are consistent with those of Aldrion et al. (2014), who determined that that there is a loss of genetic diversity in the regions where *D. suzukii* has been introduced relative to Japan (presumed to be part of the ancestral range).

The significantly lower genetic diversity in terms of both the number of alleles and heterozygotes also supports a founder effect that produced a genetic bottleneck in the establishment of the *D. suzukii* population in eastern Washington. That the most common alleles in the coastal California populations are also fixed in the eastern Washington population could support that the coastal California population was the source of the eastern Washington population. Also, it is plausible that the more extreme climate in eastern Washington could affect *D. suzukii* survival, both contributing to the bottleneck detected in the eastern Washington population and ultimately to maintaining a low level of genetic diversity at the observed loci. This also provides evidence that specimens of *D. suzukii* collected from eastern Washington are from an established population in the region as opposed to annual, re-introduction events. The presence of homozygous individuals for the same allele for each year of the study period indicates no detectable gene flow over time. Also, in the case of the *mastermind* locus, where variation does occur, the same alleles are detected each year in similar frequencies, with the predominant locus being the most frequent for each year. If *D. suzukii* was not surviving year-round and had to be re-introduced each year, some level of variation in alleles present in the population would be expected as well as an increase in the level of heterozygosity.

Evaluation of the genetic diversity among *D. suzukii* populations is crucial from a pest management perspective based on well-documented cases of reduced fitness in populations of organisms with low levels of genetic diversity (Hitchings and Beebee 2002, Reed and Frankham 2003, Spielman et al. 2004). The low levels of genetic diversity in eastern Washington *D. suzukii* populations could be an indicator of a lower level of overall evolutionary fitness that may translate to slower adaptation, which will ultimately determine what the most appropriate management practices should be given that genetic variation is a source for natural selection to occur. While mutation is the primary source of genetic variation, and can happen randomly, independent of population size, the invasion of a species into a new region will likely expose it to a variety of new environmental factors and in the case of a significant bottleneck, as was seen in the Washington population, lowering its overall fitness and requiring more time to adapt to all conditions, given that the likelihood of gaining simultaneous mutations that confer beneficial traits to deal with all the new environmental conditions is extremely low. This may also prove true for *D. suzukii* populations elsewhere in North America, especially where a founding population has undergone similar bottlenecks in its establishment due to extreme cold or hot temperatures or other environmental factors as in eastern Washington. Further studies of allele diversity and frequency elsewhere in the native and introduced range of *D. suzukii* may assist in determining the source(s) of this pest beyond the western United States, help to describe its spread and adaptation throughout North America, and identify if gene flow is occurring through new introductions via importation of small fruit from Asia or elsewhere in its current range.

The introduction of *D. suzukii* into North America represents a classic biological invasion. The initial introduction is presumed to have occurred in California, but it is not known if the population in California originated from the population in Hawaii (Kaneshiro 1983) or if it came directly from its ancestral range or yet another region. While a reduction in genetic diversity is likely to have occurred at the initial introduction, this study demonstrated a loss of genetic diversity as a likely result of a secondary introduction that possibly originated from the California population, although the possibility of a different source for the introduction cannot be dismissed. The use of genetic testing to monitor biological invasions was well-reviewed by Chown et al. (2015), and the data presented in this study highlight the principles presented therein. By providing baseline data on the invasion of *D. suzukii* into North America, its subsequent movement into other geographic areas of North America, as well as basic genetic tools to monitor this movement, future studies can build on these data and gain higher resolution on the local scale that can then be combined with similar work from other countries to establish the origins of various populations, how quickly they move, and, ultimately, the most likely routes and means of invasion on a global scale.

The results presented herein are consistent with those of Adrion et al. (2014), in that *D. suzukii* undergoes a bottleneck where it is newly introduced, albeit using different loci and examining populations on a smaller geographical scale. The consistency in results highlights the potential and need to examine more populations at finer resolution to get a more precise

understanding of the dispersal of this species. The evolutionary processes visible between populations of *D. suzukii* within North America using these microsatellite markers are fundamentally the same as those seen at the global scale and thus can be used by more researchers on a larger geographic scale to gain a more resolute understanding of the invasion biology of *D. suzukii*.

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