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Research

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Population Genetics of *Dermacentor variabilis* Say 1821 (Ixodida: Ixodidae) in the United States Inferred From ddRAD-seq SNP Markers

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Abstract

Dermacentor variabilis Say is a tick species widely distributed in North America, where it is a common pest, and acts as vector for many tick-borne pathogens that affect both humans and livestock. In the United States, *D. variabilis* has a discontinuous geographic distribution. It is present eastwards of a line drawn from Montana to southern Texas, and a few disjoint populations western of the Rockies and in the intermountain region. It has been hypothesized that both groups may correspond to different species. The aim of this study was to investigate the population genetic structure of, and potential speciation within, *D. variabilis*. To do this, we generated a new data set based on nuclear markers (SNPs) discovered through next-generation sequencing. The results showed moderate population structure and supported the occurrence of gene flow between some genetic clusters. Maximum parsimony phylogenetic reconstruction showed a divergent monophyletic western clade and a generally eastern clade. Overall, the nuclear data set analyzed herein is congruent with previous findings based on mitochondrial markers, although it led to a higher level of resolution within the eastern clade. Additional lines of evidence are needed to determine whether eastern and western populations correspond to different species.

Key words: population structure, next generation sequencing, phylogenetic relationships, speciation

Knowledge of population genetics of parasites (vectors) may provide insight into ecological characteristics, population size and structure, and evolutionary processes (Nadler 1995, Tabachnick and Black 1995, Norris et al. 1996, de Meeûs et al. 2002, Barrett et al. 2008). Moreover, at larger geographic scales, genetic variability of vectors may relate to transmission patterns of pathogenic microorganisms (Gooding 1996). For example, in a study of genetic structure among different populations of *Ixodes scapularis* Say 1821, the authors suggested a genetic component associated with differences in the natural history of northern and southern *I. scapularis* populations, as well as a correlation with the prevalence of human Lyme disease cases (Gulia-Nuss et al. 2016).

Dermacentor variabilis Say 1821 (Ixodida: Ixodidae), known as the American dog tick, is a native three-host tick species that is widely distributed in North America, from Canada, through the United States to Mexico (Bishopp 1938, Gregson 1956, Wilkinson 1967, Dodds et al. 1969, Guzmán-Cornejo et al. 2016). Within the United States, it is present east of a line drawn from Montana to southern Texas, and in a few disjoint populations west of the Rockies and in the intermountain region (Bishopp 1938, Bishopp and Trembley 1945, Stout et al. 1971, Araya-Anchetta et al. 2013). This tick is a common pest and transmits a number of pathogens that affect humans and/or cattle, such as *Rickettsia rickettsii*, *Rickettsia montanensis*, *Francisella tularensis*, *Coxiella burnetii*, and *Anaplasma marginale* (Bishopp 1938, Burgdorfer and Brinton 1975, Goethert et al. 2004, Lankester et al. 2007, McQuiston et al. 2012).

Up to this point, only a few investigations have focused on genetic variability in this tick. Araya-Anchetta et al. (2013) found moderate population structure in three U.S. populations (two in MT and one in WA), with a weak signature of isolation by distance, based on amplified fragment length polymorphisms (AFLPs). Based on mitochondrial sequences, Canadian samples cluster in a monophyletic clade with central-eastern US samples, whereas *D. variabilis* from western populations form a separate monophyletic clade

(Krakowetz et al. 2010, Kaufman et al. 2018). No further structure could be distinguished within either of these two groups (Kaufman et al. 2018). Reports of incongruence between mitochondrial and nuclear inferences are frequent in tick literature, mainly due to the nature of the markers, but also to potential mitochondrial introgression and hybridization (Hwang and Kim 1999, Cruickshank 2002, Rubinoff and Holland 2005).

The consistent separation of western versus eastern populations in previous studies raises the question whether *D. variabilis* as currently defined is indeed a single species or could in fact correspond to two species. Species delimitation is of course a controversial topic, and the importance of considering different lines of evidence and methods when delimiting species has been underlined in the literature (Leo et al. 2010, Carstens et al. 2013, Nava et al. 2016). This is particularly relevant in tick studies. Successful cross-breeding experiments are commonly interpreted as supportive evidence of conspecificity, but several examples of successful interspecific hybridization have been reported in ticks, including between *Dermacentor* species (Pappas and Oliver 1972, Dergousoff and Chilton 2007, Araya-Anchetta et al. 2013). Therefore, using different approaches and integrating their outcomes is paramount.

The objective of this study was to investigate the population genetic structure of, and potential speciation within, *D. variabilis*. To do this, we generated a new data set based on nuclear markers (SNPs) discovered through next-generation sequencing.

Methods

Sampling

The study is based on 66 *D. variabilis* adults collected from the following US states (number of ticks in parenthesis): California (3), Georgia (2), Indiana (3), Maryland (4), Maine (3), Minnesota (5), North Dakota (1), Ohio (33), Oklahoma (5), Tennessee (5), Virginia (1), and Washington (1) (Fig. 1). Our sample thus included specimens from 26 localities, representing 12 states across the geographic distribution of *D. variabilis* in the United States (Table 1 and Fig. 1). Initially more samples were included (especially from the west coast), but they were removed from all downstream analyses due to the low quality of the reads obtained and the amount of missing data. All specimens were wild caught by dragging a 1- × 0.8-m cloth thorugh the vegetation. Ticks were killed and preserved in 95% ethanol. We aimed at using unfed ticks in an effort to limit working with DNA from the host. All collection data are accessible online through the OSAL (Ohio State Acarology Collection) database (https://acarology.osu.edu/database), and OSAL numbers are presented in Table 1.

DNA Samples Preparation and Processing

Genomic DNA from ticks was extracted using QIAGEN Blood & Tissue kit. Exoskeletons were preserved as vouchers following previously published protocols (Beati and Keirans 2001, Beati et al. 2012): a portion of the posterolateral idiosoma of individual ticks was cut off using a sterile scalpel and both parts of the specimen were incubated overnight in 180-µl Qiagen ATL lysis buffer (Qiagen, Valencia, CA) and 20 µl of proteinase K (Roche Applied Sciences, Indianapolis, IN). After complete lysis of the tick tissues, the exoskeleton was stored in 70% ethanol and kept as voucher specimen. Vouchers were barcoded and are available at the OSAL upon request.

To discover SNPs (single nucleotide polymorphisms), we used dd-RADseq (double digest restriction site associated DNA sequencing), following a modified protocol based on Peterson et al. (2012). Extracted DNA samples were quantified using a Qubit 2.0 fluorometer and normalized to 10 ng/µl. Twenty microliters of each sample were employed for the dd-RAD library preparation. Genomic DNA samples were digested with SbfI and MspI restriction enzymes. After DNA digestion and ligation of barcodes, the libraries were amplified using conventional PCR. Once the libraries were amplified, fragment size selection was performed employing a Blue Pippin (Sage Sciences) and selecting fragments between 300 and 450 base pairs. Samples were quantified using a bioanalyzer and sequenced using an Illumina HiSeq with single end 100-base pair reads.

Raw sequence reads were demultiplexed and processed using the ipyrad pipeline (Eaton and Overcast 2016). This pipeline inputs raw sequence reads and outputs loci, alleles, and SNPs. Base calls with Phred score below 33 were replaced by Ns, and a clustering

Fig. 1. Map showing the localities where the specimens used in this work were collected.

Table 1. General information of *D. variabilis* samples used in this study

Sample ID	Sample ID	U.S. state	Locality	Coordinates
OSAL00119592	119592–6	IN	Hoosier National Forest	38.52, –86.44
	119592–7	IN	Hoosier National Forest	38.52, –86.44
OSAL00119273	119273-2	IN	Splinter Ridge Wildlife area	38.75, –85.20
	119273-3	IN	Splinter Ridge Wildlife area	38.75, –85.20
	119273–4	IN	Splinter Ridge Wildlife area	38.75, –85.20
OSAL00119241	119241-1	OH	Battelle Darby Creek MP	39.9, –83.21
	119241–2	OH	Battelle Darby Creek MP	39.9, –83.21
	119241–3	OH	Battelle Darby Creek MP	39.9, –83.21
	119241–4	OH	Battelle Darby Creek MP	39.9, –83.21
OSAL00119243	119243-1	OH	Glacier Ridge MP	40.13, –83.18
	119243-2	OH	Glacier Ridge MP	40.13, –83.18
	119243-3	OH	Glacier Ridge MP	40.13, –83.18
	119243–4	OH	Glacier Ridge MP	40.13, –83.18
OSAL00119244	119244-1	OH	High banks MP	40.15, –83.03
	119244-4	OH	High banks MP	40.15, –83.03
OSAL00119247	119247-1	OH	Pickerington Ponds MP	39.88, –82.79
	119247-4	OH	Pickerington Ponds MP	39.88, –82.79
OSAL00119248	119248-1	OH	Pickerington Ponds MP	39.88, –82.80
	119248-4	OH	Pickerington Ponds MP	39.88, –82.80
OSAL00119250	119250–2	OH	Sharon Woods MP	40.11, –82.95
	119250–4	OH	Sharon Woods MP	40.11, –82.95
OSAL00110559	110559-1	OH	Fernald Preserve	39.29, –84.69
	110559-2	OH	Fernald Preserve	39.29, –84.69
OSAL00119600	119600B	OH	Roads intersection	39.13, –84.79
OSAL00115093	115093A	OH	Cuyahoga Valley	41.289, –81.573
	115093B	OH	Cuyahoga Valley	41.289, –81.573
	115093C	OH	Cuyahoga Valley	41.289, –81.573
	115093D	OH	Cuyahoga Valley	41.289, –81.573
	115093E	OH	Cuyahoga Valley	41.289, –81.573
OSAL00119928	119928A	OH	Strouds Run SP	39.369, –82.042
	119928B	OH	Strouds Run SP	39.369, –82.042
	119928C	OH	Strouds Run SP	39.369, –82.042
	119928D	OH	Strouds Run SP	39.369, –82.042
OSAL00119572	119572A	OH	Oak Openings MP	41.549, –83.854
	119572B	OH	Oak Openings MP	41.549, –83.854
	119572C	OH	Oak Openings MP	41.549, –83.854
	119572D	OH	Oak Openings MP	41.549, –83.854
OSAL00119567	119567A	TN	Knoxville	
	119567B	TN	Knoxville	
	119567C	TN	Knoxville	
	119567D	TN	Knoxville	
	119567E	TN	Knoxville	
N01358805	N8805A	OK	Washita Co.	
	N8805B	OK	Washita Co.	
	N8805C	OK	Washita Co.	
	N8806A	OK	Washita Co.	
	N8806B	OK	Washita Co.	
N01358464	N8464B	VA	Warren Co.	
OSAL00119951	119951	GA	Statesboro	32.42, –81.77
OSAL00119952	119952A	GA	Statesboro	32.42, –81.77
	119952B	GA	Statesboro	32.42, –81.77
OSAL00119955	119955	GA	Bryan Co.	32.12, –81.486
OSAL00115086	115086	MN	Carlos Avery	45.287, –93.122
OSAL00115087	115087A	MN	Camp Ripley	46.076, –94.349
	115087B	MN	Camp Ripley	46.076, –94.349
OSAL00119918	119918A	MN	Columbus	45.31, –93.02
	119918B	MN	Stutsman Co.	47.23, –98.87
N128168	N128168B	ND	Stutsman Co.	47.23, –98.87
OSAL00115139	115139A	ME	Crescent Beach	43.56, –70.23
	115139B	ME	Crescent Beach	43.56, –70.23
OSAL00115140	115140	ME	Unknown	Unknown
OSAL00119276	119276-2	MD	Aberdeen Providing Ground	39.46, –76.12
	119276-3	MD	Aberdeen Providing Ground	39.46, –76.12
	119276-5	MD	Aberdeen Providing Ground	39.46, –76.12

Table 1. *Continued*

Sample ID	Sample ID	U.S. state	Locality	Coordinates
	119276-6	MD	Aberdeen Providing Ground	39.46, –76.12
OSAL00115101	115101	CA	Napa Co	38.215, –122.33
OSAL00115102	115102A	CA	Lake Co.	39.139, –122.886
	115102C	CA	Lake Co.	39.139, –122.886
OSAL00115105	115105A	WA	Whitman Co.	46.623, –117.228
	115105B	WA	Whitman Co.	46.623, –117.228

All individuals are from the United States. Each row corresponds to an individual tick specimen and the columns to the collection information.

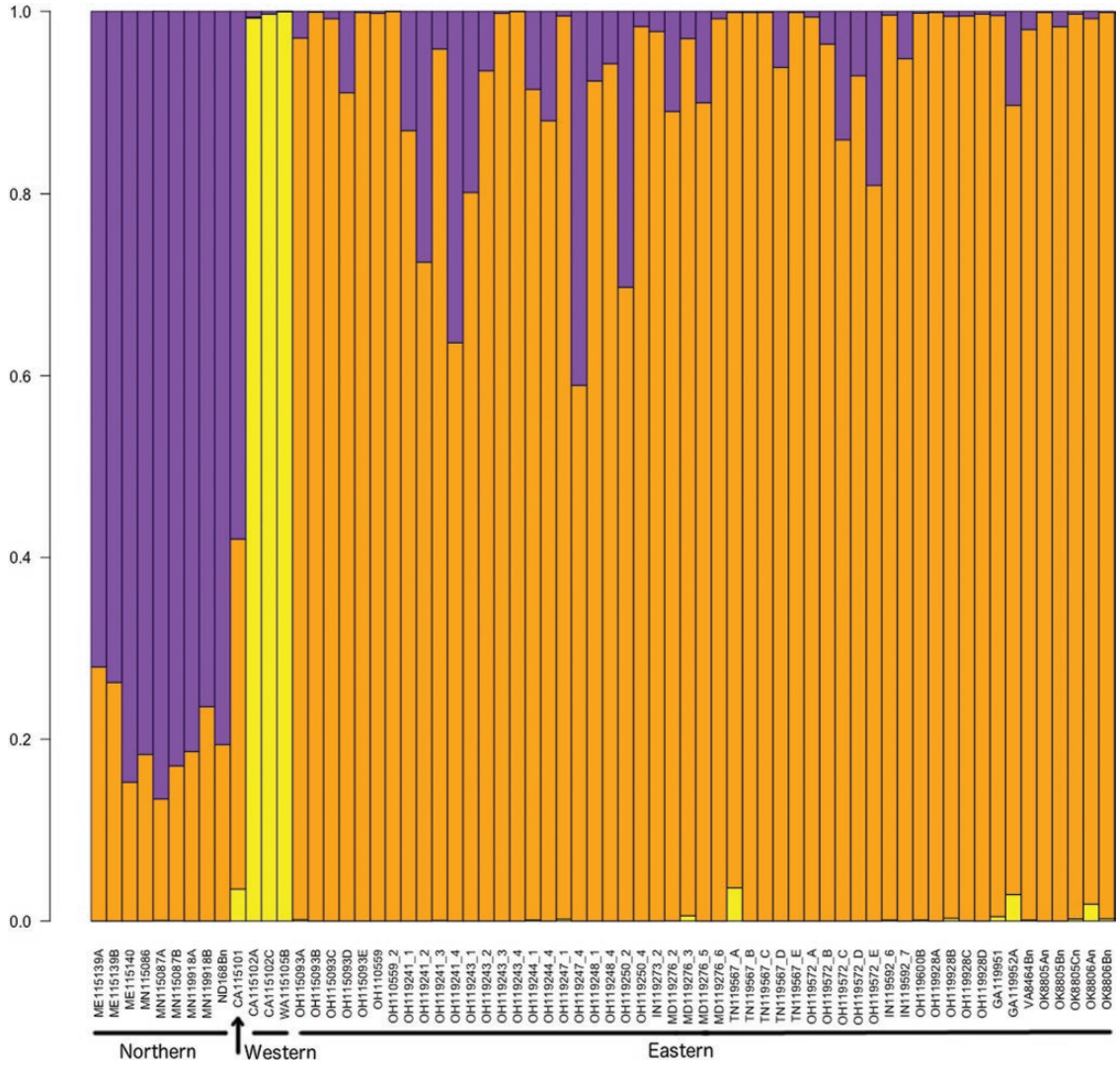

Fig. 2. Structure plot showing assignment probabilities for each sample at $K = 3$. Individual samples are in the x-axis, and membership probabilities in the y-axis.

threshold of 85% was employed to assemble reads into loci (which means the reads needed to be at least 85% identical to pass the filter). Both the minimum depth at which statistical base calls are made during consensus base calling and the minimum depth at which majority rule base calls are made was set to 6. Maxdepth was set to 10,000, attempting to minimize skewing results towards over amplified fragments during PCRs, for example. For a locus to be retained, it needed to be present in at least 25 samples. This was accomplished by setting the min_samples_locus parameter to 25.

Genetic Diversity, Heterozygosity, and *F* statistics

The R package hierfstat was used to calculate and summarize genetic diversity, as well as to test for homogeneity of variances (Null hypothesis: Hexp = Hobs) using the Bartlett test (Jombart 2008, Jombart and Ahmed 2011). F_{IS} values were also calculated in R, employing the basic.stats function of the hierfstat package (Goudet 2004). Hardy–Weinberg equilibrium was tested using the function hw.test from the pegas package, in R (Paradis 2010).

Population Genetic Structure

To infer genetic population structure within *D. variabilis*, two different approaches were used: a model based approach (STRUCTURE v2.3.4 software [Pritchard et al. 2000]) and a nonmodel based approach (discriminant analysis of principal components [DAPC] [Jombart et al. 2010]). STRUCTURE is a Bayesian clustering method that assigns individuals in the sample to populations, or to two or more populations if their genotypes indicate that they are admixed (Pritchard et al. 2010). The program assigns individuals to clusters assuming linkage equilibrium within populations (Pritchard et al. 2010). On the other hand, DAPC is a multivariate method that uses sequential K-means to identify clusters and does not make any assumptions about Hardy–Weinberg or linkage equilibrium. We decided to run both the model-based and the nonmodel based analyses. For both approaches, unlinked loci were analyzed, and no a priori information on the populations was used as input. Only loci present in at least 25 specimens were retained for this portion of the study. We assessed different values of k (k indicates the number of clusters), ranging from 1 to 10. For the STRUCTURE analysis, three replicated were performed for each k value, and the MCMC was set to 100,000 iterations. Independent STRUCTURE runs were combined using CLUMPP (Jakobsson and Rosenberg 2007), and the k value chosen using the Evanno method (Evanno et al. 2005) in STRUCTURE Harvester (Earl and vonHoldt 2012). For the DAPC analysis, 100 iterations were run for each k value assessed, and the optimal k was determined using the Bayesian information criterion (BIC) as recommended by the developers (Jombart et al. 2010). All plots were done in R (R Core team 2013). DAPC was also employed to determine the relationship between the clusters determined by the *find.clusters* function.

Phylogenetic Analyses

For maximum parsimony (MP) phylogenetic analyses, we used the same individual data set and ran the analyses with different amounts of missing data (3, 26, and 62%; see Results). Heuristic searches were performed by branch-swapping using the tree bisection-reconnection (TBR) algorithm, and branch support was assessed by bootstrap analysis (100 replicates) in PAUP (Swofford 2002). Additionally, a species tree was inferred using SDVquartets (Chifman and Kubatko 2014) also in PAUP. This is a method to infer relationships among quartets of taxa under the coalescent model. The resulting tree was built using a quartet-based phylogenetic tree reconstruction method (Reaz et al. 2014).

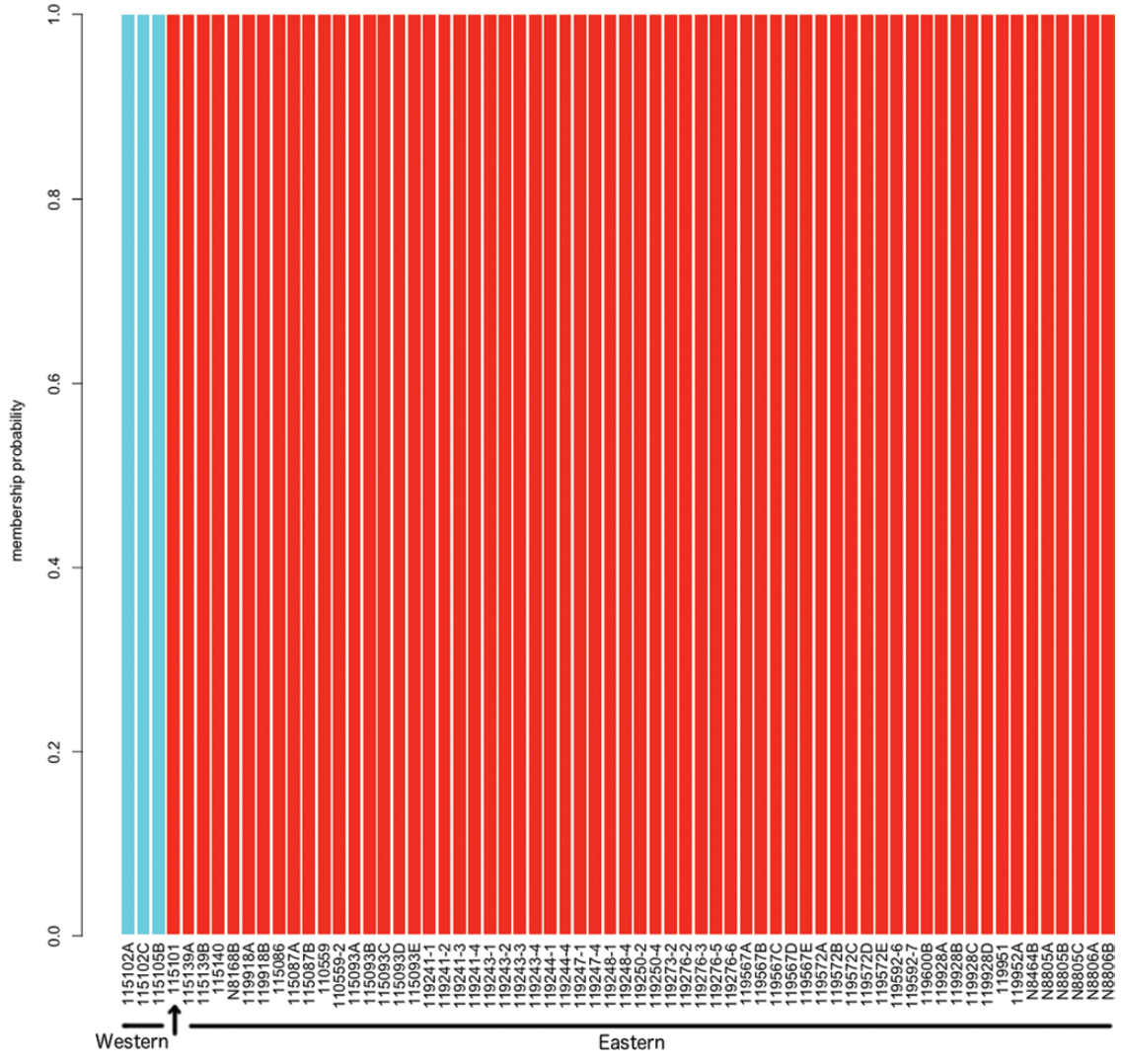

Fig. 3. Barplot showing the posterior probabilities of group membership from the DAPC for $k = 2$.

Results

DNA Sequencing

Sequencing of the libraries for the 66 *D. variabilis* analyzed resulted in ~80 million reads. The great majority of those reads passed the quality filters and were retained for de novo assembly, using the 85% threshold. A minimum of 6 reads was required to call a cluster, and the number of clusters varied among individuals between 23,657 and 74,669, for a total of 2,746,791. For all downstream analyses, only unlinked (independent) markers were used (*.ustr* output file from iPyRad). The final number of unlinked SNPs was 10,994 (from a total of ~60,000).

Genetic Diversity, Heterozygosity, and *F* statistics

The total number of individuals was 66, and the group sizes were 55 (Eastern), 7 (Northern), and 4 (Western). The number of alleles per group was 21,123, 13,859, and 10,888, respectively. Bartlett test showed that the average difference between expected and observed heterozygosity differs from zero (*P*-value 2.2e-16), and the same result was obtained using a paired *t*-test. Test results for H–W equilibrium per locus are presented in Supp Material S1 (online only). The level of heterozygosity per individual tick sample is generally low (Supp Material S2 [online only]).

F_{IS}, F_{ST}, and F_{IT} multilocus estimates for diploid data are as follows: 0.368, 0.172, and 0.477, respectively. Paired *F*st values between populations showed some differentiation, especially between western and both northern (0.388) and eastern (0.319) samples. However, the level of differentiation between eastern and northern samples is lower (0.09).

Population Genetic Structure

Structure

The optimal *k* value in STRUCTURE was *k* = 6, and the second-best *k* = 3. However, when analyzing the resulting bar plots for both *k* values (Supp Material S3 [online only] and Fig. 1), *k* = 3 showed the same overall groupings, and therefore was chosen as the best k. STRUCTURE results showed four distinct groups within the data set: 1) a "western" group, 2) individual OSAL 0115101, 3) a "northern" group (including all samples from ME, ND, and MN), and 4) an "east of the Rockies" group (Fig. 2). Tick OSAL 0115101 was collected in California, but it did not cluster within the "western" group. Instead, it appeared as a mixed individual, showing more similarities with the northern and eastern groupings than to the western cluster. Overall, the group of samples east of the Rockies showed little genetic structure, with only the northern group differentiated from the rest. It is worth noting that in the plot for *k* = 6, all samples from Oklahoma formed another group, different from the remaining eastern samples.

Because of the results obtained through the DAPC analysis (see below), we also decided to explore the STRUCTURE runs for *k* = 2. For *k* = 2 (Supp Material S4 [online only]), only two groups were present: 1) a western group and 2) all the remaining samples (including OSAL 0115101). The western cluster included the same samples as for *k* = 3.

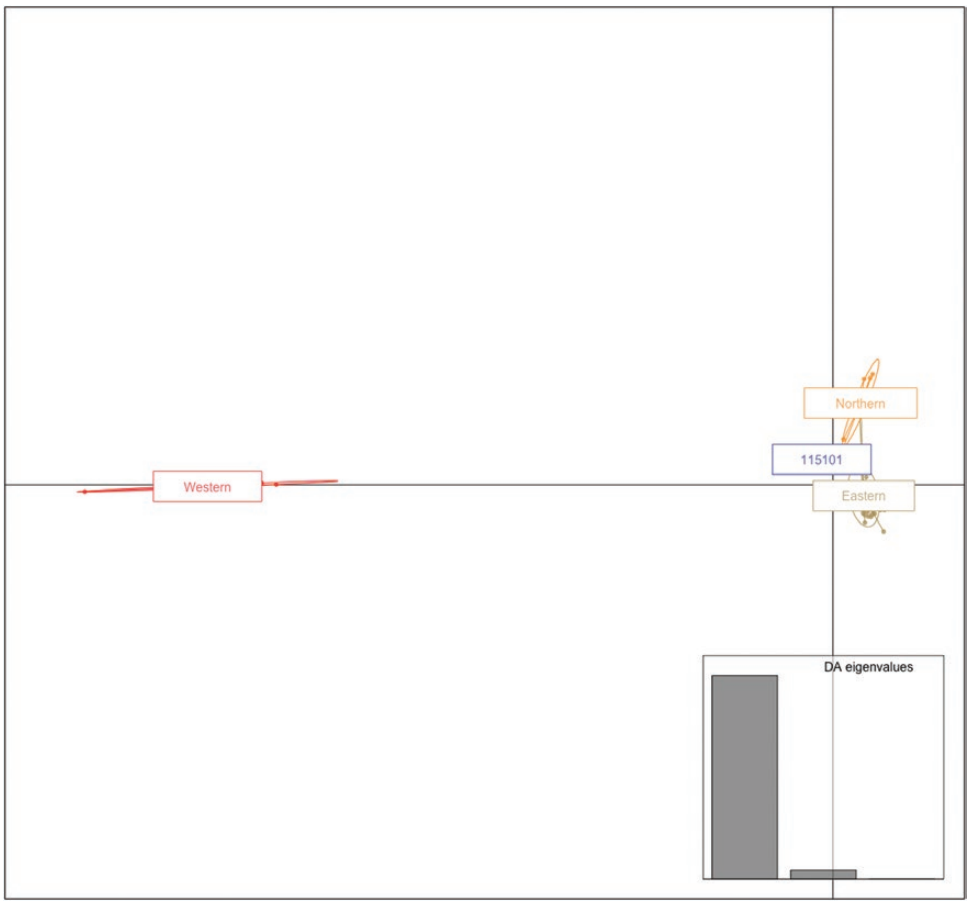

Fig. 4. Scatterplot of the results of the DAPC analysis. The scatterplot summarizes the genetic variation. Groups are shown by different colors and ellipses, whereas dots represent individual samples.

DAPC

For the DAPC data set, the optimal k value was $k = 2$. The individual membership probability plot (Fig. 3) once again showed a western group (including western samples except for OSAL 0115101), and another group to which the remaining samples belonged to. This result was similar to the STRUCTURE results for $k = 2$ (Supp Material S5 [online only]).

For comparison purposes, and to explore the relationship between clusters (which is not possible in the STRUCTURE analysis), we also did a DAPC analysis for $k = 3$. When three was chosen as the number of clusters, the individual membership probability plot (Supp Material S6 [online only]) showed three distinct groups: 1) western; 2) northern (including all samples from ME, ND and MN, plus two ticks from OH and individual OSAL 0115101; and 3) all remaining samples.

As for the relationships between clusters, the eastern and northern (inc. OSAL 0115101) clusters are distinctly more similar to each other than to the western cluster (Fig. 4).

Phylogenetic Analyses

Maximum Parsimony

Initially, the phylogenetic reconstructions were rooted at the midpoint. Because all phylograms showed a monophyletic western clade (except for OSAL 0115101), with a very long branch length, we decided to use that lineage as outgroup for the analysis. The strict consensus trees topologies were very similar regardless of the amount of missing data present. We describe here the tree inferred from the data set with up to 62% missing data as this was the data set used for the population structure analysis. The MP strict consensus tree showed moderate resolution, although overall bootstrap support was low (Fig. 5). Most groupings did not correspond to geography, with ticks from different regions clustering together. However, all specimens from OK constituted a monophyletic well-supported lineage, as did the "northern" samples (ME, MN, and ND, plus OSAL 0115101 from CA). The tick samples from ME formed a monophyletic clade within the "northern" lineage.

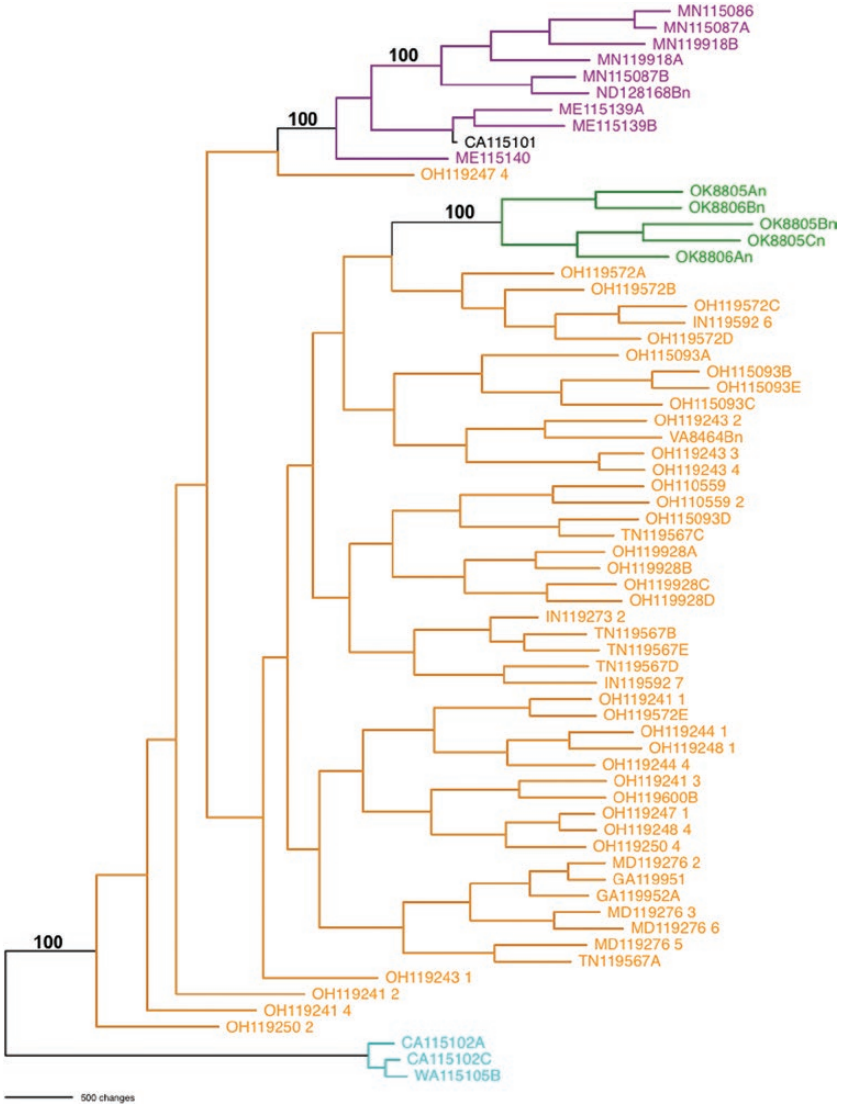

Fig. 5. Maximum Parsimony strict consensus tree (Bootstrap, 100 replicates) obtained from the analysis of concatenated sequence of dd-RAD loci. Bootstrap supports above 70% are shown.

SDVQuartets

The "species" tree inferred through SDVQuartets was rooted using the same approach as for the MP tree, with the western clade as outgroup to facilitate comparison between topologies. The SDVQ tree showed three distinct groups within the "ingroup": 1) the northern clade (ME, MN, and ND plus OSAL 0115101 from CA); 2) a well-supported clade including all samples from OK; and 3) all samples from the remaining states (Fig. 6).

Discussion

The results obtained through the different analyses, both population genetics and phylogenetics, among *D. variabilis* populations, showed a consistent east/west separation of the populations. Therefore, the nuclear data are largely congruent with the findings based on mitochondrial markers with a distinct western clade, and a group corresponding to all populations located east of the Rockies. However, this result is not absolute as tick OSAL 0115101 from California consistently grouped with the eastern, rather than the western lineage. Individual OSAL 0115101 may correspond to a recent migrant from the east to the west populations, or it may represent a significant part of western *D. variabilis* populations. A more extensive sampling of the western populations (we could use only four specimens) is needed to better understand the genetic population structure of this species in western locations, and to decide whether *D. variabilis* indeed corresponds to two different species.

With respect to the population genetics, it is noteworthy that even though the optimal number of clusters determined by STRUCTURE and DAPC was somehow different, the groupings were similar. The western group included all samples from CA and WA with exception of the one individual from CA (OSAL 0115101). Genetically, the east/west division was clearly the primary subdivision, with the eastern subgroups (STRUCTURE analysis) more similar to each other than to the western cluster. These results were further supported by the phylogenetic analyses, both the MP and the SDVQuartets analysis. STRUCTURE plots indicate some level of gene flow between populations, likely due to the movement of ticks on their hosts as previously suggested (Farlow et al. 2005, Araya-Anchetta et al. 2013,

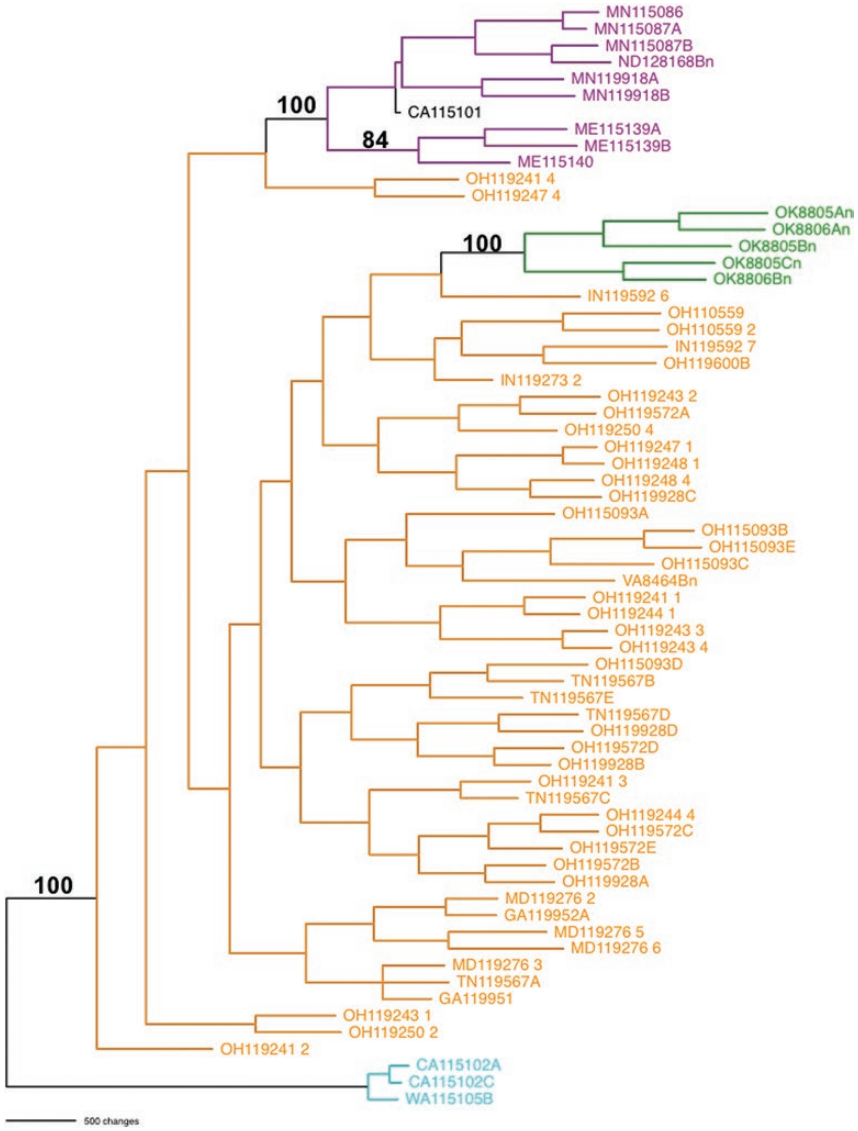

Fig. 6. SDVQuartets Tree obtained from the analysis of concatenated sequence of dd-RAD loci. Different colors were used for the different clades. The number on the nodes represent bootstrap support (support >70 are shown).

Kaufman et al. 2018). However, the gene flow between east and west (with exception of OSAL 0115101) appears to be very limited when compared with the other populations.

As mentioned above, there is broad agreement between mitochondrial and nuclear markers. However, mitochondrial markers have failed so far to further resolve genetic structure within the eastern clade. In contrast to that lack of resolution, the results presented here show moderate resolution within the "eastern" clade, consistently differentiating a "northern" clade (ND, MN, ME), and an "Oklahoma" clade from the "other eastern" ticks. The increased level of resolution may be due to the nature of the markers themselves and/or to the number of markers analyzed (over 11 thousand unlinked markers).

Congruence between mitochondrial and nuclear markers (especially among same individuals, data not shown) suggests that mitochondrial introgression is not frequent in this taxon, or that crosses between western and eastern clade ticks are rare. Crosses between eastern and western tick population are probably infrequent due simply to geographic isolation, and to, for example, genetic, morphological, or physiological incompatibility. The fact that colony ticks originally collected in WA and maintained through periodic introduction of local wild ticks have an eastern genetic signature suggests that interbreeding of ticks from eastern and western populations can be successful, and (perhaps) that there is movement of ticks towards the west as previously reported (Farlow et al. 2005, Araya-Anchetta et al. 2013). Such movement of ticks on hosts is likely bidirectional (East-West-East), even though there are no reports (yet) of western clade ticks in the east. This may be because the abundance is lower and therefore those individuals with a western genetic signature present east of the Rockies have not been sampled and sequenced as frequently. Finally, it is important to note that even if *D. variabilis* constitutes two species, interspecific hybridization in ticks and especially between *Dermacentor* species has been previously reported, and it appears to be common (Oliver et al. 1972, Ernst and Gladney 1975, Dergousoff and Chilton 2007). On the other hand, the existence of gene flow is not enough evidence to reject the hypothesis of two species, as speciation with gene flow is a phenomenon that is more frequent than previously thought (Morales et al. 2017).

In conclusion, across the United States, there are two distinct *D. variabili*s monophyletic clades: a western clade, and a generally "eastern" clade, both at the mitochondrial (Krakowetz et al. 2010, Kaufman et al. 2018) and nuclear level. Nuclear markers used in this study provided more resolution than that of the mitochondrial markers, showing some genetic structure within the eastern clade. To better understand the population genetic structure of this tick species in western locations, both more locations and more samples per location should be analyzed, and the authors hope this is accomplished in future studies. To determine whether the western and eastern clades represent two different species, more evidence is needed, especially considering that interspecific hybridization and speciation in the presence of gene flow are not rare processes.

Supplementary Data

Supplementary data are available at *Annals of the Entomological Society of America* online.

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