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Complexity of the *Culex pipiens* complex in California

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Abstract

In California, the *Culex pipiens* complex consists of *Culex pipiens*, *Cx. quinquefasciatus*, their hybrids and *Culex pipiens* form *molestus*. Using 15 microsatellite markers and a variety of statistical analyses of within- and among-population variation, there is widespread introgression throughout the Central Valley with mostly *quinquefasciatus* genotypes in the south and *pipiens* in the north. Those specimens in the Sacramento County area consisted primarily of *pipiens-quinquefasciatus* and *pipiens-molestus* hybrids. Populations in Coachella Valley and Los Angeles, CA and Benton, WA were *Cx. quinquefasciatus* and *Cx. pipiens*, respectively. Studies are underway to relate these genotypes to phenotypes of autogeny, diapause and vector competence for West Nile Virus.

INTRODUCTION

In North America, the *Culex pipiens* complex consists of four entities: *Culex pipiens*, *Cx. quinquefasciatus*, their hybrids and *Culex pipiens* form *molestus* (Barr 1957, Barr 1967, Spielman 2001). Previous studies have demonstrated quantifiable genetic differentiation among these groups (Kothera et al. 2010). We are using microsatellite markers in this work. Microsatellites are located in non-coding stretches of DNA, and therefore are selectively neutral. Selectively neutral markers are preferred for estimating genetic diversity and differentiation, because their allele frequencies are influenced only by drift and admixture, not by natural selection. Therefore, they are good indicators of changes brought on by gene flow. Comparisons are made within and among populations, and several analyses assume that a population's allele frequencies conform to a state known as Hardy Weinberg Equilibrium (HWE). Departures from HWE can be caused by anything that changes allele frequencies in a population, including hybridization, where genetic admixture occurs among genetically distinct entities.

In 2009, a transect study that sampled *Cx. pipiens* complex mosquitoes from New Orleans, LA to Chicago, IL showed that the species composition at sample sites changed from *Cx. quinquefasciatus* in the south to *Cx. pipiens* in the north. Pure species were present at either end of the transect, and hybrids predominated at middle sites such as Memphis, TN (Kothera et al. 2009). In California, however, genetic relationships among members of the *Cx. pipiens* complex are not as clear (Cornel et al. 2003, McAbee et al. 2008). In the current study, we used microsatellite markers to elucidate the nature and extent of hybridization among members of the *Cx. pipiens* complex, an important group of disease vectors. Sixteen

sampling sites were chosen within the state, and several sites were chosen around Sacramento because there appears to be a high degree of genetic admixture in the area (Fig. 1).

METHODS AND MATERIALS

Individual specimens (N = 506) were homogenized with a copper BB and 500 ml of diluent BA-1 using a Mixer Mill (Qiagen, Valencia, CA). Genomic DNA was extracted on a Qiagen Universal Biorobot from a 220 ul aliquot of the homogenate. Individuals were assayed with two multiplex panels of microsatellite loci, with eight and nine markers, respectively, for a total of 17 markers. Amplification issues in two loci resulted in a final total of 15 polymorphic loci. The forward primer of each primer pair was fluorescently labeled and the PCR products were visualized on a Beckman Coulter (Brea, CA) CEQ8000 sequencer using its Fragment Analysis module. A multilocus genotype was generated for each individual, and the data were analyzed by the programs Arlequin (Excoffier and Lischer 2010) and FSTAT (Goudet 1995) to estimate within-population measures of genetic diversity. The program Structure (Falush et al. 2003) was used to describe among-population measures of genetic differentiation. The extent of hybridization was estimated using the program NewHybrids (Anderson 2002), and allele frequencies for one particularly informative locus were graphed for each population. Finally, sequence data were examined from a gene showing single nucleotide polymorphisms (SNPs) between *Cx. pipiens* and *Cx. quinquefasciatus*.

RESULTS AND DISCUSSION

Based on Observed (H_O) and Expected (H_E) Heterozygosity, genetic diversity levels are generally similar, although the *Cx. quinquefasciatus* populations (Coachella Valley Rural through Kern County Urban) show slightly lower values. H_O and H_E are statistically compared to determine instances of deviations from HWE. Such deviations can be present when genetically different individuals are mating in a population and can suggest the presence of genetic admixture, or hybridization. Populations with the most frequent of departures from HWE were Woodland, Manhole Sacramento, Zoo and Shasta.

Pairwise F_{ST} values represent the degree to which two populations are genetically divergent. Values between 0.05 – 0.15 indicate moderate levels of divergence. Most comparisons were statistically significant via permutation test, suggesting that significant levels of genetic differentiation exist among sampled populations. The highest pairwise F_{ST} values result from comparisons with the Manhole populations in Sacramento and Old Sacramento. Cluster analysis was performed using the program Structure, which determines the most likely number of genetic clusters (K) represented by the data. The first Structure analysis resulted in a most likely K value of six, meaning there were six genetic clusters of individuals. Linkage Disequilibrium (LD) occurs when allele frequencies within populations are significantly positively or negatively correlated. Linkage Disequilibrium can affect K values, so populations with the most instances of LD were removed and the Structure analysis repeated. This time the results indicated the most likely number of clusters was four (K = 4). Figure 2 shows the q values (i.e. proportion of membership in each cluster) for this

Structure run, and suggests the presence of *Cx. pipiens*, *Cx. quinquefasciatus* and two other genetic entities, possibly hybrid and autogenous individuals.

The same multilocus genotype data were used by the program NewHybrids to estimate the probability that an individual belonged to one of two parental species, or to several hybrid classes (F1 and F2 hybrids, and backcrosses to each parent). The results from the Sacramento area suggested the presence of both pure and hybrid individuals in several populations. In contrast, populations at the northern and southern ends of the sampled sites had few or no hybrids.

One genetic locus, Cxpq78, has been shown to be informative with regard to discriminating among *Cx. pipiens* and *Cx. quinquefasciatus* individuals due to the presence of species-diagnostic allele sizes (MERPDC et al. 2011). Populations from Kern County Rural southward showed allele frequencies and sizes consistent with the presence of *Cx. quinquefasciatus*. Populations north of Kern County Rural showed a typical *Cx. pipiens* allele distribution.

Finally, we examined a 500 bp portion of Vectorbase gene CPIJ000900 and noted single nucleotide polymorphisms (SNPs) among several individuals from CA as well as from other parts of the country. Specimens from outside the study area were from New York City, NY (*Cx. pipiens* and *Cx. pipiens* form *molestus*), Chicago, IL (*Cx. pipiens* and *Cx. pipiens* form *molestus*) and New Orleans, LA (*Cx. quinquefasciatus*). Preliminary results include the following: 1) There are *Cx. pipiens* - *Cx. quinquefasciatus* differences at several positions; 2) Both Manhole populations look like *Cx. pipiens* or *Cx. pipiens* form *molestus*, although one individual appears admixed; 3) Woodland has an insertion of one nucleotide; and 4) The Woodland and Heronry individuals show several unique nucleotides.

In summary, the amount genetic diversity among *Cx. pipiens* complex populations in California is comparable across populations. Several populations showed departures from HWE and LD (Woodland, Manhole Sacramento, Zoo) suggesting admixture among genetically distinct entities. The Structure results suggest a high degree of admixture, particularly around Sacramento. Also, when populations with a high frequency of LD were removed, Structure results are consistent with the presence of both hybrid and autogenous individuals. The addition of more populations could clarify genetic groupings among the populations in this area. Results from NewHybrids are consistent with Structure results in that the presence of hybrids is indicated in several populations. Sequence data from the CA individuals sampled also suggest hybridization when compared to known *Cx. pipiens* and *Cx. quinquefasciatus* individuals.

Future work will include sequencing more individuals to see if the observed patterns are maintained with additional individuals. Regions from another candidate gene may be of similar value to CPIJ000900 and will also be explored. The sequence data when complete will represent a SNP data set that may be useful in a phylogenetic analysis. Finally, we will compare genetic data with data on autogenous individuals to see if those individuals are genetically distinguishable.

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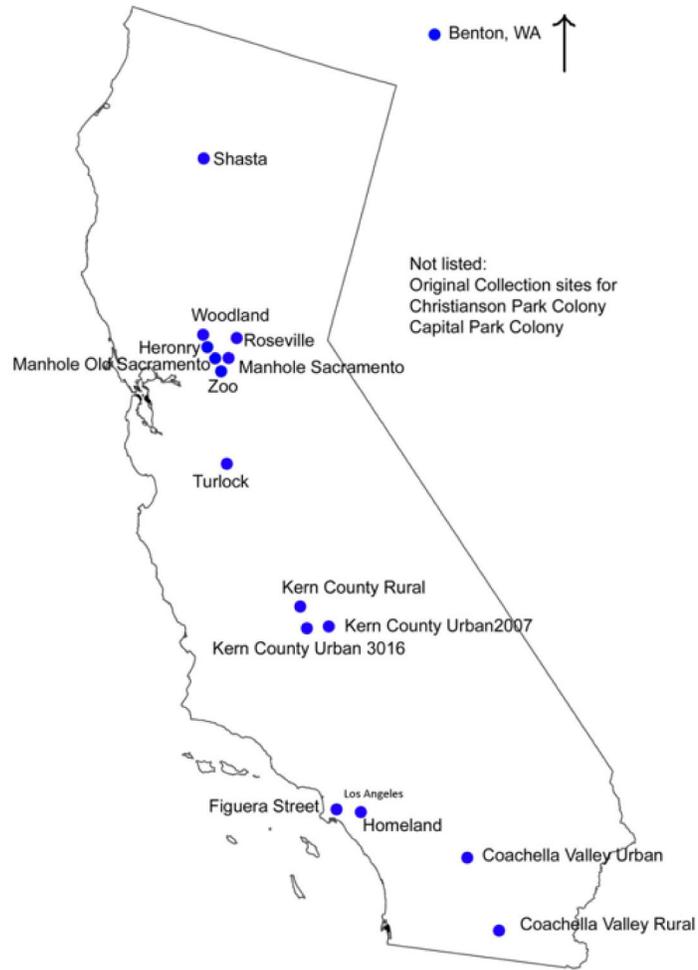


Figure 1. Map of 16 study areas where *Culex pipiens* complex mosquitoes were collected for genetic study.

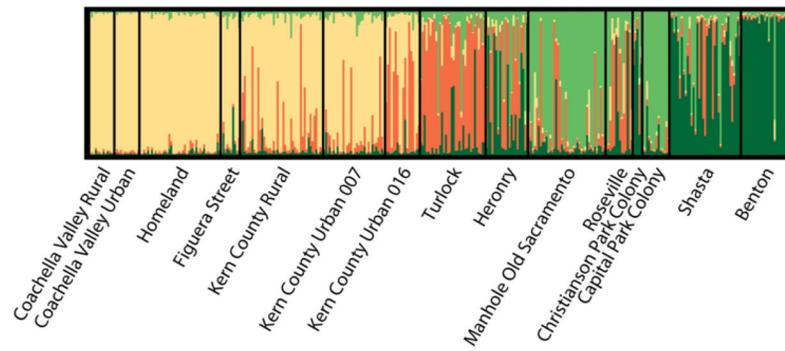


Figure 2. Individual mosquito assignments to each of four clusters ($K = 4$) in Structure. Each thin vertical line represents the proportion of membership in each cluster (q values) for one individual. Here, yellow are *Cx. quinquefasciatus* and dark green are *Cx. pipiens*; orange and light green show two forms of hybrids.