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THE DISTRIBUTION OF SPECIES OF THE *AEDES INCREPITUS* COMPLEX IN THE WESTERN UNITED STATES

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ABSTRACT. Maps of the California and Oregon distribution of members of the *Aedes increpitus* complex (*Aedes clivis* Lanzaro and Eldridge, *Aedes increptitus* Dyar, and *Aedes washinoi* Lanzaro and Eldridge) are presented that are based on collections reported by Lanzaro and Eldridge (1992) and new collections from various sites, many in the Central Valley of California. Analysis of individually reared specimens by polyacrylamide gel electrophoresis and conventional morphological methods confirmed the diagnostic value of isozymes for these species and of larval head hairs for distinguishing *Ae. clivis* from other members of the complex. Other larval characters and pupal hairs did not appear to have diagnostic value. An additional site was discovered where apparent hybrids between *Ae. washinoi* and *Ae. increpitus* occur, and a single possible hybrid between *Ae. washinoi* and *Ae. clivis* was found at a site where these species had previously been reported to occur sympatrically.

KEY WORDS Mosquitoes, systematics, genetics, *Aedes,* distribution, electrophoresis, isozymes

INTRODUCTION

Three species of the *Aedes increpitus* complex are known to occur in the western United States: *Aedes increpitus* Dyar, *Aedes clivis* Lanzaro and Eldridge, and *Aedes washinoi* Lanzaro and Eldridge. At the time of their original description, Lanzaro and Eldridge (1992) postulated the geographic distribution of the 3 species in the far western United States on the basis of collections made by them in 1988–89 and the examination of larval specimens present in the U.S. National Museum (USNM). Because 1988–89 were years of abnormally low rainfall in California, Lanzaro and Eldridge (1992) made no collections of mosquitoes of this complex anywhere in the Central Valley of California, and their presumption of the occurrence of *Ae. washinoi* in the Central Valley was on the basis of a few collections of that species made in the Sierra foothills. Darsie (1995), after an examination of the types of *Ae. washinoi* and *Ae. clivis* in the USNM, published a modification of the keys contained in the Darsie and Ward (1981) identification manual and suggested some additional morphological characters to distinguish the three species.

Precipitation in California returned to normal or above normal levels after 1994, and this afforded an opportunity to sample riverine larval breeding sites in the Central Valley that had been dry for several years. These collections yielded many new samples of the *Ae. increpitus* complex, enabling a clearer delineation of the geographic distribution of the 3 member species as well as validation of the additional taxonomic characters suggested by Darsie (1995). Lanzaro and Eldridge (1992) discovered 2 sites in the Sierra Nevada foothills where *Ae. clivis* and *Ae. washinoi* appeared to occur sympatrically, 2 sites on the eastern slope of the Sierra Nevada where *Ae. clivis* and *Ae. increpitus* appeared to occur sympatrically, and a single site in the southern Cascade range of California where specimens that apparently represented *Ae. washinoi-Ae. increpitus* hybrids occurred. A further analysis was made of specimens from several of these foothill sites.

METHODS

Collections were made between 1992 and 1997 in California, Nevada, and Oregon from habitats likely to yield *Ae. increpitus* complex mosquitoes. Nearly all collections were made as larvae. Collection procedures and processing of specimens were essentially identical to the methods described by Lanzaro and Eldridge (1992). A subsample of each collection of reared adults was analyzed for frequencies of allozymes for lactate dehydrogenase (Enzyme Commission [EC] number 1.1.1.27), aconitase (EC number 4.2.1.3), and diaphorase (EC number 1.6.4.3), the 3 enzyme systems previously found to be diagnostic (Lanzaro and Eldridge 1992). Procedures for electrophoresis differed from those used by those authors in that polyacrylamide gel, rather than starch gel, electrophoresis was used, but staining and other procedures were identical. The ROCK/DAVIS strain of *Aedes aegypti* was used as the standard on each gel, and in addition, 2 female specimens of each of the 3 species in the complex from localities where only that species is known to occur were also included for comparison. Allozyme designations were based on a comparison of migration distances with the *Ae. aegypti* standard. The same formula was used for calculating the relative migration distance as that used by Lanzaro and Eldridge (1992), and the allele designa-

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Table 1. Number of *Aedes* populations and specimens studied.

Species	Populations[1]	Specimens			
		Total	Gels	Larvae	Both
Ae. clivis	25	241	199	154	112
Ae. increpitus	9	119	118	88	87
Ae. washinoi	26	375	298	343	267
clivis/washinoi hybrids	1	1	1	1	1
increpitus/washinoi hybrids	4	24	24	6	6
Totals	65	760	640	592	473

[1] If two species were present at the same collection site, each species was counted as a population.

tions were presumed to be comparable. For a more detailed analysis of 1 of the pairs of sympatric populations discovered by Lanzaro and Eldridge (1992), a series of collections was made at Chicago Park, located in the Sierra Nevada foothills in Nevada County, California (elevation 750 m). Individually reared adults from this site were analyzed for allozyme patterns in parallel with morphological examination of the individual cast larval skins. The objectives of this particular study were to see if morphological evidence from larvae would furnish further support for lack of interbreeding between *Ae. washinoi* and *Ae. clivis* at those sites and also to determine if there were temporal differences in the relative abundances of the 2 species.

For the purpose of evaluating the morphological characters suggested by Darsie (1995) for separation of the 3 species, skins of individually reared larvae were examined from over 60 geographic populations. In most instances, the identification of the larval skins was confirmed by electrophoretic analysis of the emerged adults. Larval and pupal skins were examined from 592 individually reared mosquitoes. Of this total, 473 were also analyzed by electrophoresis (Table 1). The number of branches of setae 5-C and 6-C (upper and lower larval head hairs) for each larval skin and the number of branches of setae 10-C and 1-IV for each pupal skin were scored. A subset of the larval skins (205) was scored for configuration of the setal support plate (SSP) for setae 9, 10, and 12-M, T and configuration of the dentation of the terminal 5 pecten spines, following the character states suggested by Darsie (1995). The scoring of the larval skins was based on the following 3 conditions: 1) SSP with a single prominence bearing 3 or more spinules of about equal length, 2) SSP with a single prominence bearing spinules of unequal length, and 3) SSP with 2 or more prominences, each bearing 1 or more spinules.

RESULTS

Electrophoretic analyses: Altogether, 640 individual female mosquitoes belonging to the *Aedes increpitus* complex were analyzed on 38 separate gels (Table 1). Of these, 473 were individually reared specimens for which larval and pupal skins were available for analysis. On the basis of the analysis of allozyme patterns, 297 of these specimens were identified as *Ae. washinoi,* 199 as *Ae. clivis,* and 118 as *Ae. increpitus.* Twenty-four individuals showed evidence of hybridization between *Ae. increpitus* and *Ae. washinoi.* Of these, 17 were collected near Burney, Lassen County, California, approximately 50 km from the site in Lassen National Park where Lanzaro and Eldridge (1992) found evidence of hybridization between these 2 species (incorrectly listed by them as "Lassen State Park"). Twenty specimens were tested from Burney, and only 3 specimens appeared to be homozygous for all 3 loci tested.

Four additional apparent hybrids were from a collection made near Sandy, Clackamas County, Oregon. A single apparent hybrid individual was detected from each of 2 sites: Cottonwood, Tehama County, California, and Cedarville, Modoc County, California. At the Chicago Park site, 118 individual mosquitoes were analyzed by electrophoresis. Of these, 93 were diagnosed as *Ae. washinoi,* 23 as *Ae. clivis,* and 1 female was an apparent hybrid between these 2 species.

Morphological analyses: A summary of the resulting data is shown in Table 2. We found that the upper head hairs (5-C) of larvae could be used to distinguish most specimens of *Ae. clivis* from the other 2 members of the complex, confirming earlier

Table 2. Setal branching of upper and lower larval head hairs (5-C and 6-C) and pupal hairs 10-C and 1-IV for *Aedes increpitus* complex mosquitoes in California and Oregon.[1]

Species	Hair 5-C	Hair 6-C	Hair 10-C	Hair 1-IV
Ae. clivis (n = 154)				
Range	2–4.5	1–2.5	5–12	1–3.5
Mode	3	2	8	2
Mean ± SE	2.91 ± 0.03	1.59 ± 0.03	7.82 ± 0.12	2.20 ± 0.04
Ae. increpitus (n = 88)				
Range	1–3	1–2	6–12	2–4
Mode	2	1	10	3
Mean ± SE	2.18 ± 0.04	1.07 ± 0.02	8.31 ± 0.15	2.72 ± 0.05
Ae. washinoi (n = 342)				
Range	1–3	1–2	5–12.5	1–3.5
Mode	2	1	8	2
Mean ± SE	2.01 ± 0.02	1.13 ± 0.02	7.83 ± 0.07	1.98 ± 0.03

[1] Where branching of larval or pupal hairs is asymmetrical, the average number of hairs was recorded, e.g., 2 branches on 1 side and 3 on the other was scored as 2.5.

Table 3. Evaluation of electrophoresis vs. branching of seta 5-C for diagnosis of *Aedes clivis* and *Aedes washinoi* collected in California.

	Diagnosis by setal count	
	Ae. washinoi	*Ae. clivis*
Diagnosis by electrophoresis		
Ae. washinoi	230	37
Ae. clivis	9	103

results (Lanzaro and Eldridge 1992). To determine the reliability of 5-C to distinguish between specimens of *Ae. clivis* and *Ae. washinoi* at Chicago Park (a site where both exist sympatrically), an analysis used to evaluate tests in diagnostic medicine was employed (Armitage and Berry 1994). Identification by electrophoresis was arbitrarily considered to be the "true" diagnosis, and the identification by counts of branches of 5-C was the test to be evaluated (Table 3). Test specificity was highest when the 3-branched condition on either side of the larval head was considered to represent *Ae. clivis.* The results of this analysis showed that the proportion of "true" *Ae. washinoi* having 3-branched seta 5-C on either side was not significantly higher in specimens from Chicago Park than it was from specimens collected in areas where only that species occurs (0.18 Chicago Park vs. 0.14 all areas, $\chi^2 = 0.75$, $P = 0.39$, n.s.). Consequently, the data from all sites were combined to estimate the probability of misdiagnoses. On the basis of data from all sites, the probability of misdiagnosing a true *Ae. clivis* as *Ae. washinoi* on the basis of setal branching was about 1 in 12, of misdiagnosing a true *Ae. washinoi* as *Ae. clivis,* about 1 in 7.

On the basis of an analysis of all specimens, there is also a difference in the degree of branching for lower head hairs (6-C) of the larvae, with 2-branched being the condition occurring most frequently in *Ae. clivis* and unbranched for the other 2 species. However, overlapping in this character renders it unreliable.

Darsie (1995) pointed out that pupal hairs 10-C and 1-IV would distinguish most specimens of *Ae. increpitus* from the other members of the complex, but this was not confirmed by the results of this study (Table 2). Further, neither the structure of the SSP nor the dentation of the terminal 5 pecten spines appeared to have any diagnostic value for the populations studied. These characters were highly variable for all 3 species.

Seasonal distribution: Members of the *Ae. increpitus* complex are typical cold-adapted species, with larvae appearing in late winter and early spring at low elevations and in late spring at higher elevations. Because *Ae. washinoi* is adapted to lower elevations than is *Ae. clivis,* the possibility exists that *Ae. washinoi* and *Ae. clivis* are maintained in sympatry by temporal differences. However, no such pattern was evident from an analysis of collections made at various times at Chicago Park (Fig. 1).

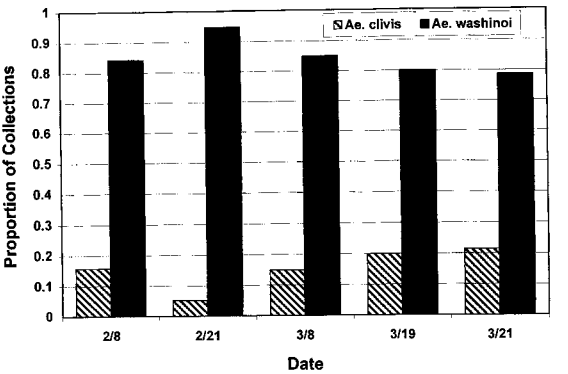

Fig. 1. Proportion of *Aedes clivis* and *Aedes washinoi* within samples made at Chicago Park at various times during a 3-year period.

Geographic distribution: Distribution maps of the species in the *Ae. increpitus* complex for California, Oregon, and Nevada are provided that are based on a composite of the records in the Lanzaro and Eldridge (1992) study and this study (Fig. 2). There are several sites at the interface between the ranges of *Ae. clivis* and *Ae. washinoi* where both species were sampled. These sites are not depicted on the maps, but possible areas of hybridization between *Ae. increpitus* and *Ae. washinoi* are shown.

Larval habitats: Aedes washinoi is mostly a riverine species. Collections from the Central Valley of California were often from borrow pits along highway and railroad rights-of-way but were nearly always within 1 km or so of rivers. Larvae were frequently collected in flooded willow thickets in river floodplains. Coastal habitats tended to be associated with estuaries and upper reaches of salt marshes, but larvae were never collected in brackish water. Larval habitats of *Ae. increpitus* were similar to those of *Ae. washinoi.* Collections of *Ae. increpitus* were made frequently in flooded thickets adjacent to rights-of-way, some of them formed from melted snow. *Aedes clivis* is a typical snowpool mosquito except that it may occur in habitats more typical of *Ae. washinoi* where the ranges of the 2 species overlap.

DISCUSSION

This study 1) established that *Ae. washinoi* is the only species of the complex in the Central Valley and coastal areas of California, 2) confirmed the area of apparent hybridization between *Ae. washinoi* and *Ae. increpitus* in Lassen County, California, 3) provided evidence for an additional such zone in the Cascade foothills of western Oregon, and 4) provided evidence that there is little hybridization occurring between *Ae. washinoi* and *Ae. clivis* in Nevada County, California.

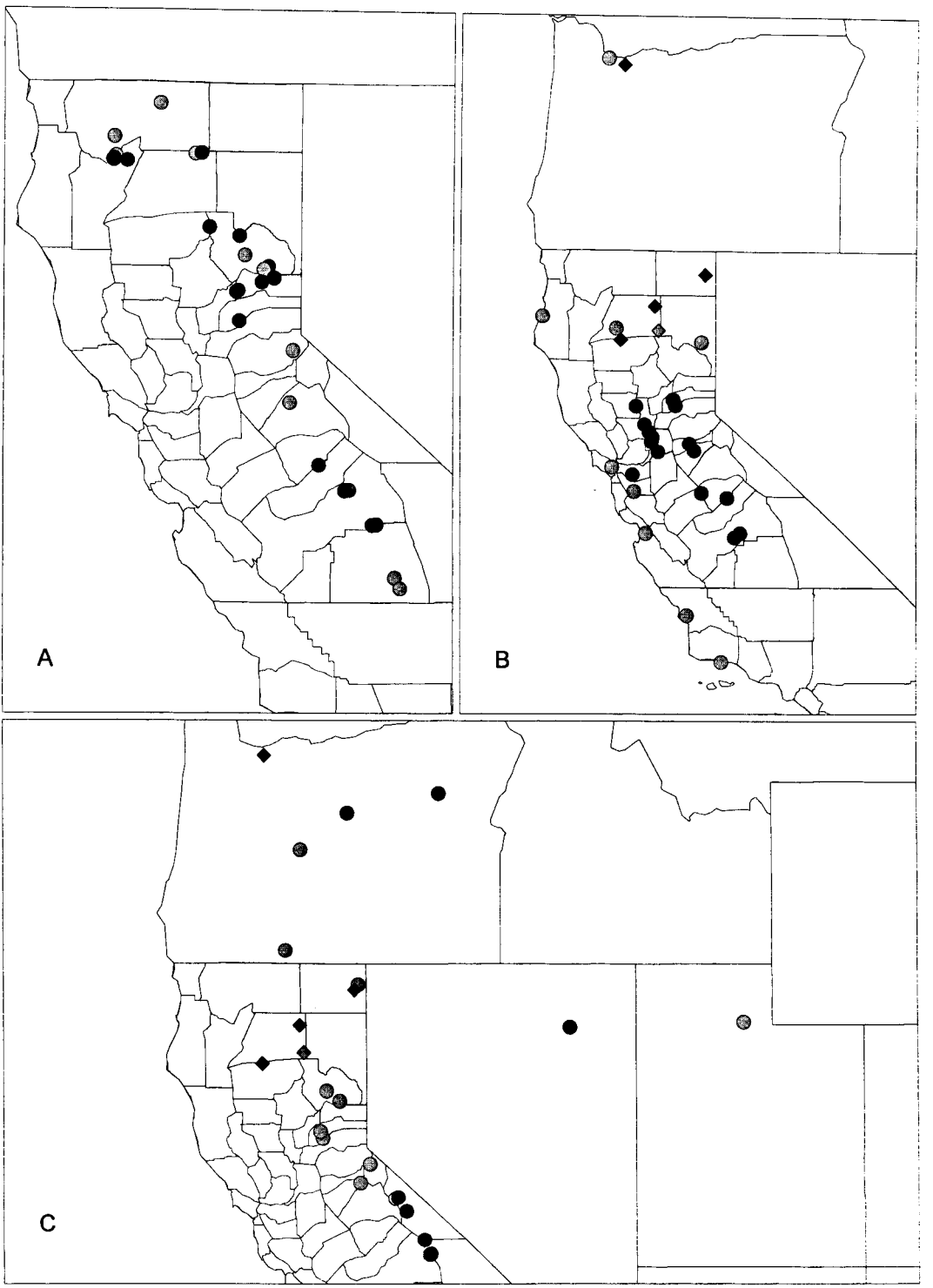

Fig. 2. Distributional maps for the *Aedes increpitus* complex in western North America (mainly California and Oregon). A. *Aedes clivis.* B. *Aedes washinoi.* C. *Aedes increpitus.* In all cases, black symbols refer to collections made in connection with this study; gray symbols refer to collections reported by Lanzaro and Eldridge (1992). Circles depict collections with no apparent hybrids. For B and C, diamonds depict the occurrence of apparent *increpitus-washinoi* hybrids.

These results appear to confirm the specific status of the 3 known species of the *Ae. increpitus* complex in the far western United States. There appears to be a narrow band of hybridization between *Ae. increpitus* and *Ae. washinoi* in Lassen County, California. The site reported here is in the Burney Basin, immediately to the north of Lassen National Park. A relatively low elevation gap in the Sierra Nevada–Cascade chain exists at this point, possibly permitting east–west movement of the 2 species. There is also evidence of some hybridization between *Ae. clivis* and *Ae. washinoi* at Chicago Park, but this is based on a single specimen from over 100 specimens tested. In all cases, the presence of alleles from both species at 1 or more loci among heterozygotes represents the only evidence for hybridization. Without additional evidence, such as that based on cross-mating experiments, the hybrids must be considered presumptive. If such specimens do represent true hybrids, they most likely represent examples of parapatric contact between species where occasional hybrids occur (Mayr and Ashlock 1991) and thus are indicative of specific status for these populations.

The above results, when combined with those of Lanzaro and Eldridge (1992), provide a clearer picture of the distribution of these species in California and Oregon. *Aedes washinoi* occurs in coastal and inland valley habitats in both states, extending into the foothills of the Sierra Nevada and Cascades. The only exception to this distribution is the collection of *Ae. washinoi* larvae near Honey Lake, Lassen County, well east of the Cascades. Again, this finding is consistent with the areas of apparent hybridization discussed above.

Aedes clivis apparently is a widespread species in California at high elevations in several mountain chains but has not been found outside of California. *Aedes increpitus* occurs throughout the Great Basin of the western United States.

Species of the *Ae. increpitus* complex do not appear to be important arbovirus vectors. No published record of a virus isolated from members of the complex in nature is known to the authors. Kramer et al. (1992) demonstrated differences in vector competence among mountain and coastal populations of members of the complex for California encephalitis and Morro Bay viruses, but vector competence was low. Among artificially infected females, few transmitted virus. Hardy et al. (1993) reported no isolation of California serogroup viruses from 5,719 *Ae. increpitus* and 1,096 *Ae. clivis* collected, and Fulhorst et al. (1996) failed to isolate any virus from 10,125 *Ae. washinoi.*

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