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BIOGEOGRAPHY OF THE *AEDES (OCHLEROTATUS) COMMUNIS* SPECIES COMPLEX (DIPTERA: CULICIDAE) IN THE WESTERN UNITED STATES

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ABSTRACT. It has been demonstrated recently that *Aedes communis* (De Geer) represents a complex of cryptic species, at least three of which are found in the western United States. Because members of this group have been implicated as vectors of Jamestown Canyon virus, it is important to delimit the distribution of individual species in order to clarify their viral associations. Specimens of *Ae. communis s.l.* collected in California and neighboring states were identified electrophoretically using allozyme markers. Populations from the Sierra Nevada, southern Cascades, and Trinity Alps in California were determined to be *Ae. tahoensis* Dyar. Specimens from Oregon, Washington, Nevada, and Utah were identified as *Ae. nevadensis* Chapman and Barr. Based on our collections and on previous literature, it appears these two species are allopatric, with *Ae. tahoensis* restricted to the high mountains of California.

INTRODUCTION

Aedes communis (De Geer) was first described in northern Europe (De Geer 1776) and has been reported to be holarctic in distribution, although at least in North America it represents a complex of cryptic or near-cryptic species (Ellis and Brust 1973), three of which occur in the western United States (Brust and Munstermann 1992). Dyar (1916) first described *Ae. tahoensis* from Gold Lake Camp, Plumas County, California, but later synonymized it with *Ae. communis* (Dyar 1928). Chapman and Barr (1964) described a subspecies, *Aedes communis nevadensis,* from Elko County, Nevada, on the basis of differences in larval comb scales. Subsequently, Ellis and Brust (1973) elevated *Ae. nevadensis* to specific status and described an additional sibling species, *Ae. churchillensis,* from western Canada. Gjullin et al. (1968) and Gjullin and Eddy (1972) reported several collections from the northwestern U.S. as *Ae. communis,* despite the fact that the larval comb scales, although variable, more closely matched Chapman and Barr's description of *Ae. communis nevadensis.* Apparently, Ellis and Brust (1973) misinterpreted the records of Gjullin et al. (1968) to suggest that *Ae. nevadensis* and *Ae. communis s.s.* occurred sympatrically in Washington, Oregon, and Idaho. An electrophoretic investigation of specimens from California and elsewhere recently led to the resurrection of *Ae. tahoensis* Dyar as a valid species (Brust and Munstermann 1992). Other than the pointed larval comb scales characteristic of *Ae. nevadensis,* there are no diagnostic morphological characters to separate species within this group.

Owing to the past taxonomic confusion within the Communis Complex, and the lack of diagnostic characters, most published collection records and distribution maps do not distinguish among the sibling species. Therefore, accurate information on the distributions of individual species is lacking. Such information is of considerable importance in light of the fact that *Ae. communis s.l.* has been implicated as a vector of Jamestown Canyon (JC) virus in California (Campbell et al. 1991) and elsewhere. The recent publication of an electrophoretic key to the Communis Complex (Brust and Munstermann 1992) provides the means to map the distributions of individual species. In the present study, we examine and identify specimens

from California, Oregon, Washington, Nevada, and Utah and map species distributions relative to major geographic features.

MATERIALS AND METHODS

Collection and mass rearing. Mosquitoes were collected as larvae and/or pupae in montane snow pool and flooded meadow habitats in California, Oregon, Washington, Nevada, and Utah (Table 1). Living specimens were returned to the laboratory at University of California, Davis where they were reared to adulthood, identified as *Ae. communis s.l.,* sexed, and pooled for arbovirus testing (Campbell et al. 1991) and electrophoretic studies. In addition, voucher specimens of larval and pupal exuviae and pinned adult specimens were retained from each collection whenever possible. These vouchers are deposited in the Bohart Entomology Museum, University of California, Davis.

Preparation of specimens. Pooled specimens were stored in cryovials at −80°C prior to analysis. Frozen specimens (we used adult females only) subsequently were macerated with a glass rod in individual 1.5. ml microfuge tubes containing 40 μl distilled water and then centrifuged for 10 min at 12,000 rpm.

Electrophoresis. Approximately 2 μl of supernatant solution from previously centrifuged specimens was pipetted into wells in 5% polyacrylamide slab gels (2 μl/well) and subjected to electrophoresis for *ca.* three hr (Matthews and Munstermann 1983, Eldridge et al. 1986). One buffer (Tris-citrate pH 7.0) was used. Several specimens of the Rock-Davis strain of *Ae. aegypti* Linnaeus (Eldridge et al. 1991) were run on each gel as a reference standard. After electrophoresis, individual gels were stained for the five isozyme loci used in Brust and Munstermann's (1992) key, following standard histochemical staining procedures (Steiner and Joslyn 1979, Murphy et al. 1990). These five loci, plus 10 additional loci, also were examined using 12.5% horizontal starch gels (Steiner and Joslyn 1979). Supernatant solution from centrifuged specimens was loaded into preformed wells (10 μl/well) and subjected to electrophoresis at 4°C for *ca.* four to five hr at 60 mA. Three buffers were used (Table 2). After electrophoresis, gels were cut into five horizontal slices, which were stained individually for a total of 15 loci. Allozyme frequency and genetic distance data were derived from the starch gel results and analyzed with the BIOSYS-1 program (Swofford and Selander 1981).

RESULTS

Initial species identifications based on polyacrylamide gel results were consistent with the electrophoretic key devised by Brust and Munstermann (1992). We found that starch gels produced equivalent resolution and were more efficient in terms of equipment required and number of loci that could be examined; therefore we used starch gels for the remainder of our study.

Specimens of the Communis Complex from all collection sites in California were identified as *Ae. tahoensis* (Fig. 1). As reported by Brust and Munstermann, this species exhibits relatively little genetic variability; mean population heterozygosities ranged from 1.3–3.3%, and genetic distances among populations were small (Table 3). Collections of this species were made at elevations ranging from 1,500–2,990 m (mean = 2,200 m). Specimens from the Ruby Mountains in Nevada, the Cascades in Oregon and Washington, and the Wasatch Range in Utah (Fig. 1) were all *Ae. nevadensis.* There was considerable variation in allozyme frequencies between collection sites (mean $F_{ST} = 0.336$), resulting in greater genetic distances among populations than those found in *Ae. tahoensis* (Table 3), and we confirm the diagnostic difference at the *Hadh* locus between Oregon and Utah *Ae. nevadensis* noted by Brust and Munstermann (1992). However, we found populations from Washington and the type locality in Nevada to possess both allozymes (Fig. 2); therefore *Ae. nevadensis* does appear to represent a single species, albeit with considerable geographic variation in allozyme frequencies. We found no evidence of the presence of *Ae. communis s.s.* in any of our collections.

Table 1. Collection sites.

State	County	Town	Locale	Longitude	Latitude	Elevation (m)	Date	Sample size	Species
CA	Alpine	Hope Valley	Blue Lakes Road	119° 55′ 14″	38° 42′ 20″	2,256	17 May 89	63	*Ae. tahoensis*
CA	Alpine	Caples Lake	Dam outflow	120° 03′ 49″	38° 42′ 16″	2,490	30 May 91	12	*Ae. tahoensis*
CA	Calaveras	Cabbage Patch	CalTrans Meadow	120° 08′ 15″	38° 24′ 47″	1,900	30 May 90	6	*Ae. tahoensis*
CA	Fresno	Shaver Lake	Huntington Lake	119° 14′ 18″	37° 13′ 59″	2,200	11 May 90	4	*Ae. tahoensis*
CA	Lassen	Silver Lake	9 mi east	121° 07′ 30″	40° 30′ 00″	1,900	23 May 89	29	*Ae. tahoensis*
CA	Sierra	Bassetts	Harney Lake	120° 38′ 10″	39° 40′ 29″	2,024	12 Jun 91	3	*Ae. tahoensis*
CA	Shasta	Lassen Nat. Pk.	South Summit Lake	121° 15′ 30″	40° 29′ 00″	1,900	7 Jul 88	24	*Ae. tahoensis*
CA	Trinity	Union Creek	Union Creek	122° 55′ 00″	41° 09′ 00″	1,500	24 May 89	30	*Ae. tahoensis*
CA	Tuolumne	Yosemite Nat. Pk.	Tuolumne Meadows	119° 21′ 56″	37° 52′ 31″	2,650	20 Jun 90	26	*Ae. tahoensis*
NV	Elko	Lamoille	Lamoille Canyon	115° 22′ 00″	40° 37′ 30″	2,560	13 May 92	36	*Ae. nevadensis*
OR	Lane	Waldo Lake	Shadow Bay	122° 02′ 18″	43° 43′ 34″	1,650	8 Jul 89	30	*Ae. nevadensis*
UT	Summit	Lofty Lake	Lofty Lake trailhead	111° 40′ 34″	41° 02′ 10″	1,544	2 Jul 89	24	*Ae. nevadensis*
WA	Chelan	Washington Pass	Overlook	120° 39′ 23″	48° 31′ 27″	1,725	16 Jun 92	27	*Ae. nevadensis*

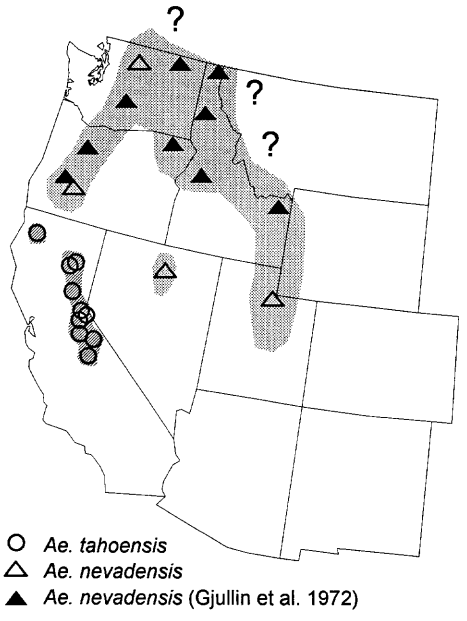

Fig. 1. Collection sites and hypothesized ranges (shaded areas) of *Ae. tahoensis* and *Ae. nevadensis.*

Examination of larval comb scale types in individual specimens from California, Oregon, and Washington (no voucher material was available from Utah) revealed considerable overlap between species. Because scale types vary within individual larvae, we scored larvae as possessing a particular scale type if 60% or more of their comb scales were that type. A total of 35% of specimens from California, where we have identified only *Ae. tahoensis* on the basis of isozyme data, fell within the range of variation described by Ellis and Brust (1973) for *Ae. nevadensis,* although no *Ae. tahoensis* possessed type I comb scales and no *Ae. nevadensis* possessed type IV (Fig. 3). Based on the collection records of Gjullin and Eddy (1972), larvae with comb scale types I, II, and III, consistent with *Ae. nevadensis,* but none with type IV scales, characteristic of *Ae. tahoensis* or *Ae. communis s.s.,* were found in Oregon, Washington, and the Bitterroot Mountains in Idaho (Fig. 1). Specimens from Yellowstone National Park in Wyoming, kindly provided by L. Nielsen, possessed a mixture of comb scale types ranging from I to IV and could not be conclusively identified.

Collections of *Ae. nevadensis* were made at elevations of 1,500–2,560 m (mean = 1,701 m); this was significantly lower than the mean elevation for *Ae. tahoensis* collections (*t*-test, $P < 0.001$). However, elevations of collection sites varied with latitude for both species, indicating that the observed species differences are probably attributable to the effect of latitude.

DISCUSSION

Based on our collection records, examination of material in the Bohart Museum collection, and previous literature, *Ae. nevadensis* and *Ae. tahoensis* appear to be allopatric. *Aedes tahoensis* is restricted to the Sierra Nevada, southern Cascades, and Klamath Mountains in California. Populations separated by as much as 500 km show little genetic differentiation. *Aedes tahoensis* may be restricted to California by lack of appropriate habitat between the Trinity Alps in northwestern California and the Crater Lake area in Oregon. We found no evidence for the presence of this species in adjoining states, although it is likely to be present on the Nevada side of Lake Tahoe in the Sierra Nevada. Therefore, all known isolates of JC virus from Communis Complex mosquitoes in California are referable to *Ae. tahoensis.*

Aedes nevadensis is more widespread than *Ae. tahoensis,* occurring in the northern Cascades, Ruby Mountains, and Bitterroot and Wasatch ranges. This species has not been found in California; the southernmost collection record along the Cascades is Crater Lake, Oregon. Ellis and Brust (1973) and Brust and Munstermann (1992) stated that the shape of the larval comb scales is consistently diagnostic of *Ae. nevadensis.* However, the presence of *Ae. nevadensis*-like comb scales in some California specimens of *Ae. tahoensis* and the presence of all four comb scale types in individual specimens from Wyoming suggest there may be sufficient overlap between species to question the reliability of this character. The electrophoretic key of Brust and Munstermann (1992) indicated diagnostic

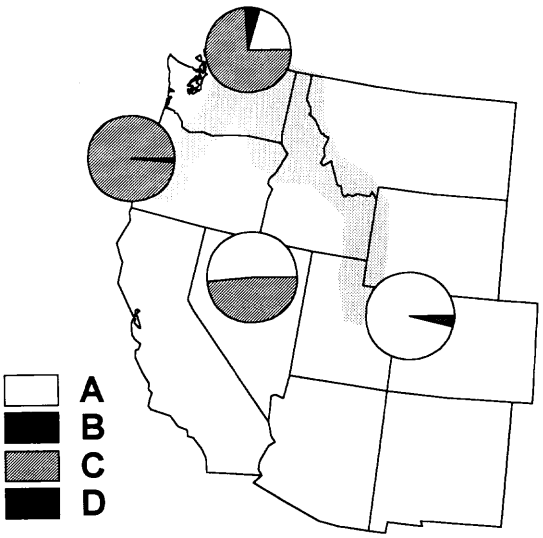

Fig. 2. Frequencies of 2-hydroxyacid dehydrogenase (*Hadh*) allozymes in *Ae. nevadensis* collections from Utah, Nevada, Oregon, and Washington.

Table 2. Isozyme loci assayed.

Locus	Enzyme	EC number	Buffer[1]
DDH	dihydrolipoamide dehydrogenase	1.8.1.4	C
G3PDH	glycerol-3-phosphate dehydrogenase	1.1.1.8	B
GPI	glucose-6-phosphate isomerase	5.3.1.9	A
HADH	2-hydroxyacid dehydrogenase	1.1.99.6	A
HK-1,2,3,C	hexokinase	2.7.1.1	A
IDH-1,2	isocitrate dehydrogenase	1.1.1.42	A
MDH-1,2	malate dehydrogenase	1.1.1.37	B, C
MDHP	malate dehydrogenase (NADP+)	1.1.1.40	B
PGDH-1	phosphogluconate dehydrogenase	1.1.1.44	A
PGM	phosphoglucomutase	5.4.2.2	A

[1] A = tris-citrate, pH 7.0 (Ayala and Powell 1972); B = tris-citrate, pH 8.0 (Murphy et al. 1990); C = aminopropylmorpholine-citrate, pH 6.9 (Clayton and Tretiak 1972).

Table 3. Coefficients of Nei's (1978) genetic distance (above diagonal) and Rogers' (1972) genetic similarity (below diagonal).

Species		Population	1	2	3	4	5	6	7	8	9
Ae. nevadensis	1	Lane Co., OR	****	0.045	0.166	0.029	0.221	0.236	0.242	0.234	0.225
Ae. nevadensis	2	Elko Co., NV	0.885	****	0.043	0.027	0.282	0.299	0.301	0.296	0.289
Ae. nevadensis	3	Summit Co., UT	0.820	0.892	****	0.110	0.434	0.456	0.461	0.453	0.444
Ae. nevadensis	4	Chelan Co., WA	0.908	0.902	0.820	****	0.201	0.213	0.220	0.211	0.205
Ae. tahoensis	5	Alpine Co., CA	0.782	0.697	0.628	0.770	****	0.001	0.004	0.000	0.000
Ae. tahoensis	6	Lassen Co., CA	0.774	0.687	0.618	0.758	0.983	****	0.003	0.000	0.000
Ae. tahoensis	7	Trinity Co., CA	0.761	0.691	0.620	0.745	0.970	0.984	****	0.002	0.003
Ae. tahoensis	8	Shasta Co., CA	0.774	0.690	0.620	0.758	0.984	0.994	0.986	****	0.000
Ae. tahoensis	9	Tuolumne Co., CA	0.781	0.692	0.623	0.764	0.986	0.992	0.979	0.989	****

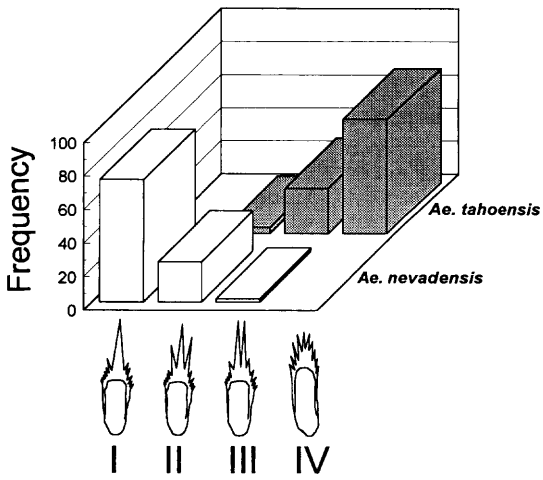

Fig. 3. Frequencies of comb scale types in larval collections, n = 183 larvae (*Ae. tahoensis,* California), 41 larvae (*Ae. nevadensis,* Oregon, Washington, Nevada).

allozyme differences between *Ae. nevadensis* populations in Utah and Oregon. Our data confirm these differences but show populations in Nevada (the type locality) and Washington to be genetically intermediate, suggesting clinal variation in allelic frequencies consistent with restricted gene flow among populations. We found no evidence for the sympatric occurrence of *Ae. nevadensis* and *Ae. communis s.s.* within our study area.

Based on the data of Brust and Munstermann (1992), *Ae. churchillensis,* although possessing *Ae. communis s.s.*-type comb scales, is separated from *Ae. nevadensis* by smaller genetic distances than some *Ae. nevadensis* populations are separated from each other and could represent a geographic and morphological variant of the latter species. Examination of additional material from western and central Canada still is necessary to clarify the status of *Ae. churchillensis.* In addition, *Ae. communis s.s.* in North America should be compared with European specimens to determine whether they are truly conspecific. Detailed examination of European material might also reveal the presence of additional cryptic species.

Electrophoretic keys are problematical because they require fairly elaborate equipment, precise replication of the original lab-

oratory conditions (often difficult, especially in cases where methods are not clearly detailed), and destruction of specimens. In cases where individual morphological characters are not diagnostic, multivariate analyses of morphological characters (Sofield et al. 1984, Schutz et al. 1989) have proven useful in the development of practical keys based on simple measurements. When Brust and Munstermann measured several morphological characters in the *Ae. communis* species group, they found some differences in mean values but considerable overlap in ranges for values between species. A multivariate approach using combinations of these characters might well prove diagnostic where individual characters were not and assist in the resolution of this difficult group.

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