Field Morphological Variation and Laboratory Hybridization of *Culicoides variipennis sonorensis* and *C. v. occidentalis* (Diptera: Ceratopogonidae) in Southern California

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ABSTRACT Two field populations of *Culicoides variipennis* (Coquillett) from southern California, *C. v. occidentalis* Wirth & Jones from the Salton Sea and *C. v. sonorensis* Wirth & Jones from a dairy wastewater pond in the Chino Basin, were sampled monthly from February to July (6–7 mo). Morphometric analyses of slide-mounted adults reared from field-collected larvae and pupae indicated that females of the 2 forms were indistinguishable. Two of the standard characters, wing length and mandibular teeth, were correlated with seasonal temperature changes. Males of *C. v. sonorensis* were distinguishable by the presence of spicules on the aedeagus, which were entirely lacking in *C. v. occidentalis*. Two populations of *C. v. occidentalis* (Salton Sea and Bolsa Chica Marsh) and a laboratory strain of *C. v. sonorensis* hybridized successfully in the laboratory and were maintained for 6 generations. Differential hybrid viability (F_1) was observed in reciprocal crosses. Males of *C. v. occidentalis* mated with females of *C. v. sonorensis* resulted in a lower egg hatch (7.4%) than did the reciprocal cross (75.6%). Hybrid males displayed spicules on the aedeagus (a character of *C. v. sonorensis*), but the number of spicules was sometimes reduced compared with parental *C. v. sonorensis* (AA strain). Spicules in a field population of *C. v. sonorensis* were similar in number to the laboratory *C. v. sonorensis–C. v. occidentalis* hybrids. Based on successful hybridization, the 2 forms should be considered closely related. The 2 forms are separated ecologically by the nature and distribution of their larval habitats.

KEY WORDS *Culicoides variipennis occidentalis*, *Culicoides variipennis sonorensis*, bluetongue, hybridization, character variation, Diptera

THE TAXONOMIC STATUS of biting midges in the *Culicoides variipennis* (Coquillett) complex is of considerable interest because they are important vectors of bluetongue viruses to ruminants in North America (Tabachnick 1996). Wirth and Jones (1957) divided the complex into 5 subspecies based primarily on morphology but supported by differences in distribution and habitat—*C. v. albertensis* Wirth & Jones, *C. v. australis* Wirth & Jones, *C. v. occidentalis* Wirth & Jones, *C. v. sonorensis* Wirth & Jones, and *C. v. variipennis* (Coquillett). Subsequent morphometric analyses (Hensleigh and Atchley 1977) showed that some of the salient morphological characters were influenced by environmental conditions. Electrophoretic analyses of isozyme loci have supported the existence of 3 genetically distinct entities (Tabachnick 1990, 1992a, 1996). *C. variipennis* sensu strictum is a fresh-water (often polluted by manure) form which is predominant in the northeastern and north-central United States and southern Canada. *C. v. sonorensis* also is a fresh-water form and is abundant in manure-polluted habitats in the southern United States; it is found from Florida to California and as far north as British Columbia, Maryland, and Ohio. *C. v. occidentalis* inhabits saline–alkaline habitats in western North America from southern California north to British Columbia but has been found as far east as western Texas (F. R. Holbrook and C. McKinnon, personal communication).

Although morphological studies have included a wide geographic and seasonal range of collections from North America, no published study has evaluated adequately seasonal variation in adult morphology within a single field population. Hensleigh and Atchley (1977) observed marked morphological variation in both spatially and temporally separated field populations of *C. variipennis* complex midges from New Mexico, but the temporal samples were limited to 2 sampling dates (7–13 mo apart) at 2 geographically isolated sites. Size-related features would be expected to vary substantially over time (e.g., wing length of *C. variipennis* varies inversely with temperature in the laboratory) (Hensleigh and Atchley 1977, Akey et al. 1978) and field (Mullens 1987, Linhares and Anderson 1989).

Two members of the *C. variipennis* complex are common in California. *C. v. sonorensis* is wide-

spread and abundant in manure-polluted fresh-water habitats, whereas *C. v. occidentalis* has been characterized using isozyme analyses from saline–alkaline habitats (Holbrook and Tabachnick 1995). The *C. v. occidentalis* habitats tended to be large, permanent, and somewhat isolated from likely *C. v. sonorensis* habitats. The identified *C. v. occidentalis* sites include 2 large inland lakes (Salton Sea in southern California and Borax Lake in northern California), a salt marsh adjacent to the Pacific Ocean in southern California (Bolsa Chica Marsh in Orange County), and a smaller, presumably saline–alkaline seep in Death Valley.

The current study was conducted to characterize seasonal morphological variability within a population of each subspecies and to determine whether and to what extent hybridization might occur in laboratory crossing studies.

Materials and Methods

Seasonal Variation in Morphology. Morphological variation was studied using specimens of *C. v. sonorensis* from the Aukeman dairy in the Chino Basin, western Riverside County, and *C. v. occidentalis* from the Salton Sea (north shore), Riverside County, California. The identity of the respective subspecies was confirmed earlier using isozyme characterization (Holbrook and Tabachnick 1995). Surface mud samples (upper 1–2 cm) containing pupae and larvae were collected from the edge of the water using a trowel. Collections were made from the same location within these field sites, monthly from February through July (6 mo spanning winter, spring, and summer). An additional September sample was collected from the Chino Basin dairy. Occasionally samples had to be collected from adjacent pools (<20 m away) when water in the regularly sampled pools dried or yielded no immatures. Samples were placed in plastic bags in an ice chest and transported to the laboratory. The mud and water from the habitat, with insects therein, were shaped into an artificial slope in a 1-liter plastic cup, creating an aquatic–terrestrial interface. The plastic cup was placed into a cardboard container (3.8 liters) with an organdy-mesh lid, and the samples were held in the laboratory at ambient photoperiod and 22°C for adult emergence. Containers were examined daily, and emerged adults were aspirated into vials of 70% ethanol. Adults eclosing from pupae or mature larvae within 4 d of collection were mounted on slides for further study. Using insects reared in natural habitat mud over a restricted time frame probably minimized possible laboratory influence on character states.

From 8 to 15 specimens of each sex were measured, per collection date, using a Leitz compound microscope with a camera lucida which integrated specimen and digitizing tablet images (Bioquant Hipad Digitizer, Houston Instruments, Austin, TX). Point-to-point lengths were captured on an IBM personal computer before transfer to a mainframe computer for analysis. Linear measurements were made using standard origin and end points; emphasis was placed on characters used in the original separation of subspecies in the *C. varipennis* complex (Wirth and Jones 1957). Female characters included number of mandibular teeth, length and width of 3rd maxillary palpal segment (length/width, palp ratio), length of 5th maxillary palpal segment, length of antennal flagellomeres i–viii and ix–xiii (ix–xiii length/i–viii length, ant ratio), and wing length (basal arculus to tip). The length of the 5th palpal segment was not a character used by Wirth and Jones (1957), but the ratio of 3rd palpal segment width/5th palpal segment length (palp ratio 2) was thought to be useful for separating *C. v. sonorensis* and *C. v. occidentalis* (W. L. Grogan, personal communication). The shape of the female spermatheca was observed but not quantified. Male wing length was measured, and the aedeagus was scored for presence or absence of spicules. Exact spicule counts could not be made from slide-mounted material, but anomalous counts were noted.

Hybridization Experiments. Hybridization experiments used field collections of immature *C. v. occidentalis* from 2 sites, the north shore of the Salton Sea and the coastal salt marsh habitat in Bolsa Chica (near Huntington Beach). The *C. v. sonorensis* specimens came from a laboratory colony designated the AA strain, which was colonized in Texas (Hunt 1994). Colony *C. v. sonorensis* males are aggressive and highly stenogamous in pursuit of mates, which assured numerous mating attempts (Hunt 1994). Insects were raised following standard protocols (Hunt 1994). Pupae of wild and laboratory insects were sorted by sex (Fig. 1) and held in groups for emergence. Sexed adults were inspected (confirming unisexuality) before crossing. Crosses were accomplished using pooled groups of adults (≈50–100 virgins of each sex) maintained for 3 d in 237-ml containers with 10% sucrose. Reciprocal crosses were attempted (i.e., field *C. v. occidentalis* females and AA strain *C. v. sonorensis* males and viceversa). Females were given the opportunity to feed on cow blood through an artificial membrane 4 d after emergence. Engorged females were removed and held with sucrose water for an additional 3 d before being offered an ovipositional substrate consisting of Whatman #1 filter paper wetted with culture-pan water.

Females from crosses involving Bolsa Chica *C. v. occidentalis* and AA strain *C. v. sonorensis* were removed from the pooled groups after they fed on blood and were held individually for egg collection on moist filter paper. If oviposition had not occurred by day 6 after the blood meal, specimens were decapitated to induce oviposition. Eggs of individual females were maintained under normal culture conditions until all hatching had ceased. Counts of total and hatched eggs were taken as an

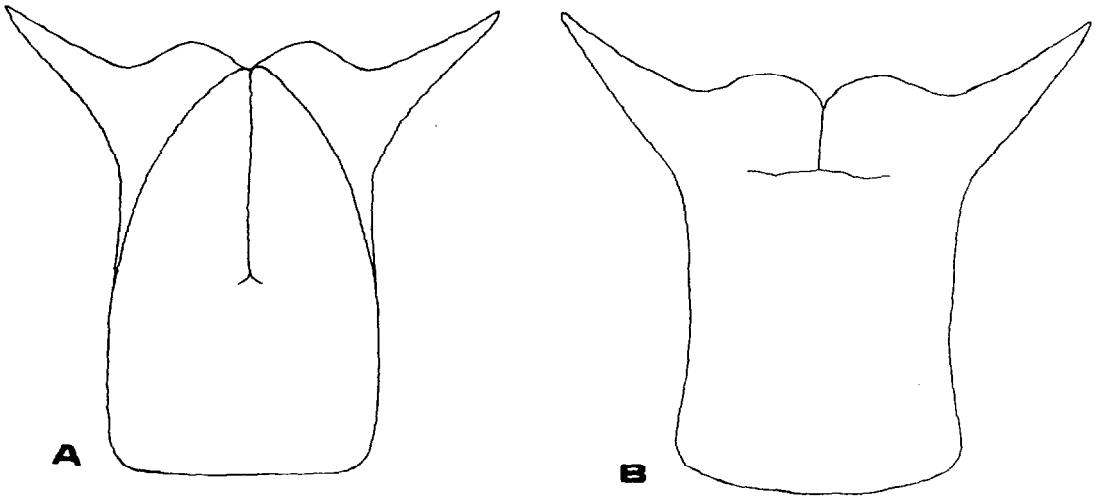

Fig. 1. Pupal terminalia (ventral view) showing difference between male (A) and female (B) of *C. variipennis*.

indication of clutch viability and mating success. Control groups of eggs from decapitated individual AA strain *C. v. sonorensis* females and eggs laid naturally by individual colony females (both groups mated with colony AA males) were treated similarly.

Hybrid larvae (from group-mated crosses) were reared in 10-cm petri plates with polyester batting as a substrate and with nutrient-rich culture water containing algae, fungi, and bacteria provided as food (Jones et al. 1969). Hybrid pupae from a particular cross were pooled for mating and rearing for multiple generations. Males from each of the successful hybrid crosses, as well as field-collected adults of both subspecies and colony-reared AA strain *C. v. sonorensis* specimens were critical-point dried (EtOH dehydration sequence and liquid CO_2 transitional fluid [Tousimis PVT-3, operating manual]), for examination of genitalic characters under a scanning electron microscope. Spicule counts were taken from the photomicrographs; counts of parental material were made using AA- strain *C. v. sonorensis* specimens reared under laboratory conditions and adults reared from larvae collected from *C. v. occidentalis* and *C. v. sonorensis* sites. All statistical tests were done using Minitab statistical software ($\alpha = 0.05$) (Ryan et al. 1985).

Table 1. Statistical significance of seasonal character variability within 2 populations of the *C. variipennis* complex in southern California

Character	Population, *C. v. ssp.*	Sex	*F* (df)	*P*
Wingl.	*sonorensis*	♂♂	38.6 (5, 78)	<0.001
		♀♀	36.7 (6, 96)	<0.001
	occidentalis	♂♂	114.8 (5, 84)	<0.001
		♀♀	132.5 (5, 77)	<0.001
Mteeth	*sonorensis*	♀♀	8.5 (6, 94)	<0.001
	occidentalis	♀♀	14.2 (5, 75)	<0.001
Palp ratio	*sonorensis*	♀♀	3.0 (6, 96)	0.010
	occidentalis	♀♀	1.7 (5, 77)	0.153
Ant ratio	*sonorensis*	♀♀	1.3 (6, 95)	0.246
	occidentalis	♀♀	4.5 (5, 77)	0.001
Palp ratio 2	*sonorensis*	♀♀	2.2 (6, 96)	0.052
	occidentalis	♀♀	1.7 (5, 77)	0.144

Wingl, wing length; mteeth, number of mandibular teeth; palp ratio, ratio of length to width of maxillary palp segment 3; ant ratio, ratio of distal 5/basal 8 flagellomeres; palp ratio 2, width of maxillary palp segment 3/length of segment 5.

Results

Seasonal Variability Within Populations. With the exception of a few selected characters, our analysis applies to females only. Seasonal variability within a population was not statistically significant ($P > 0.05$) for palp ratio (*C. v. occidentalis*), ant ratio (*C. v. sonorensis*), and palp ratio 2 (both populations) (Table 1). Seasonal variability was significant for palp ratio in *C. v. sonorensis* ($F = 2.98$; df = 6, 96; $P = 0.010$) and ranged from a low of 2.21 (July) to 2.36 (September). Only those 2 mo were significantly different from each other and from other times. Antennal ratio in *C. v. occidentalis* varied ($F = 4.47$; df = 5, 77; $P = 0.001$) from 0.77 and 0.76 (June and July, respectively) to 0.85 (May). June and July differed significantly from May, but not from other times.

The characters with the most distinctive seasonal variability were wing length and number of mandibular teeth (Table 2). Wing lengths were longer in cooler than warmer months for both populations. Mandibular teeth mirrored this trend, with fewer teeth in smaller than larger individuals. In fact, the number of mandibular teeth was significantly and positively correlated with wing length in both *C. v. sonorensis* ($r = 0.273$, df = 101, $P < 0.01$) and *C. v. occidentalis* ($r = 0.365$, df = 82, $P < 0.01$).

Table 2. Seasonal female variability in wing length (wingl) and number of mandibular teeth (mteeth) within 2 populations of the *C. variipennis* complex in southern California

Character	Month	Population, *C. v.* ssp.	
		sonorensis	*occidentalis*
Wingl, mm	Feb.	1.85 ± 0.07a	1.54 ± 0.06b
	Mar.	1.61 ± 0.10b	1.80 ± 0.05a
	Apr.	1.67 ± 0.09b	1.58 ± 0.04b
	May	1.63 ± 0.07b	1.41 ± 0.04c
	June	1.45 ± 0.05c	1.32 ± 0.10d
	July	1.58 ± 0.04bc	1.37 ± 0.04cd
	Sept.	1.55 ± 0.11bc	—
Mteeth	Feb.	13.2 ± 1.3a	13.3 ± 1.1a
	Mar.	13.1 ± 0.8a	13.2 ± 0.7a
	Apr.	13.1 ± 1.4a	12.5 ± 1.1a
	May	11.9 ± 1.3b	10.5 ± 1.1b
	June	11.1 ± 0.6b	12.2 ± 1.0a
	July	12.7 ± 1.0ab	12.5 ± 0.8a
	Sept.	13.3 ± 0.8a	—

Monthly means within a population for that character followed by different letters differ significantly ($P < 0.05$) according to the Tukey honestly significant difference (HSD).

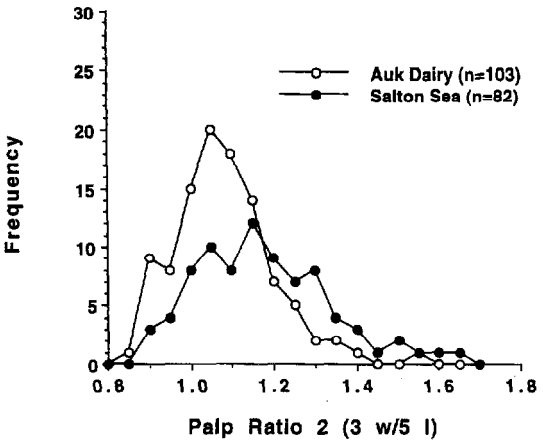

Fig. 2. Frequency distributions of palp ratio 2 (ratio of width of maxillary palp segment 3/length of segment 5) in 2 populations of the *C. variipennis* complex (*C. v. occidentalis* from the Salton Sea and *C. v. sonorensis* from a dairy wastewater pond).

Comparisons Between Populations. Both populations displayed a wide range of spermathecal shapes, from a deep to moderate U-shape (common) to short, straight forms (rare). Attempts at quantification were complicated by the lack of clear reference points for measurement. There also was potential for considerable measurement error associated with the spermatheca, in slide-mounted specimens often not being presented in a perfectly flat plane. Qualitatively, however, it did not appear that the 2 populations differed sufficiently for morphological separation.

The 2 populations did not differ significantly with regard to the number of mandibular teeth ($F = 0.78$; df = 1, 180; $P = 0.378$) or palp ratio ($F = 3.96$; df = 1, 184; $P = 0.702$). However, *C. v. occidentalis* females had significantly longer ($F = 5.72$; df =1, 184; $P = 0.18$) and wider ($F = 3.96$; df = 1, 184; $P = 0.048$) 3rd palpal segments (*C. v. occidentalis* length 0.122 ± 0.013 mm (mean ± SD) and width 0.0531 ± 0.0055 mm; *C. v. sonorensis* length 0.118 ± 0.009 mm and width 0.0516 ± 0.0044 mm). The antennal ratio differed ($F = 5.64$; df = 1, 183; $P = 0.02$), (0.785 ± 0.066 mm for *C. v. sonorensis*, 0.808 ± 0.065 mm for *C. v. occidentalis*). Females of *C. v. sonorensis* had significantly longer wing lengths (1.62 ± 0.14 mm) than did *C. v. occidentalis* (1.52 ± 0.17 mm)($F = 21.12$; df = 1, 184, $P < 0.001$). Only 1 character, palp ratio 2, was useful by itself in separating females taken at random times from the 2 populations (Fig. 2). There was substantial overlap between the populations, but the ratio of palpal segment 3 width/segment 5 length differed significantly ($F = 21.86$; df = 1, 184; $P < 0.001$); females of *C. v. sonorensis*, 1.08 ± 0.12 mm, *C. v. occidentalis* 1.18 ± 0.18 mm.

A discriminant analysis was conducted to contrast the 2 populations using antennal ratio, palp ratio, and palp ratio 2. Using the 3 ratios simultaneously still did not allow satisfactory separation of females. Only 67.6% of females were assigned correctly, although correct assignment was higher for *C. v. sonorensis* (74.5%) than for *C. v. occidentalis* (59.0%).

The single morphological feature which distinguished the forms reliably was the presence or absence of spicules on the male aedeagus (Table 3). Of 84 male *C. v. sonorensis*, 83 had at least some visible spicules on the aedeagus, and most were obvious. Exact counts of spicules on slide-mounted males was difficult, but only 1 male had <10 visible spicules. A single male totally lacking visible spicules was reared from the dairy pond on 27 June. Of 90 males reared from the *C. v. occidentalis* site, 88 lacked spicules. Two individuals possessing distinct spicules were reared from mud at the edge of the Salton Sea in April.

Hybridization Experiments. Hybrid offspring of AA strain (colony) *C. v. sonorensis* and Bolsa Chica *C. v. occidentalis* were produced in each of 3 reciprocal crosses. Hybrid progeny were fertile

Table 3. Numbers of spicules on male aedeagus of different populations and crosses of *C. v. sonorensis* and *occidentalis*

Line	*n*	Mean ± SD (range)
Cvs (AA)	11	50.9 ± 11.4a (37–69)
Cvo male × Cvs female (AA)(F_5)	10	47.6 ± 12.6a (27–69)
Cvo female × Cvs male (AA)(F_5)	17	33.8 ± 16.0b (11–62)
Cvs (Auk)	11	36.6 ± 11.8b (20–58)

Cvo, *C. v. occidentalis* from Bolsa Chica Marsh (Orange County), CA. Cvs, AA, AA strain colony material; Auk, Aukeman dairy wastewater pond, Chino (Riverside County), CA (September). Means followed by the same letter are not significantly different ($P > 0.05$) using the Tukey HSD. Counts were taken from scanning electron photomicrographs.

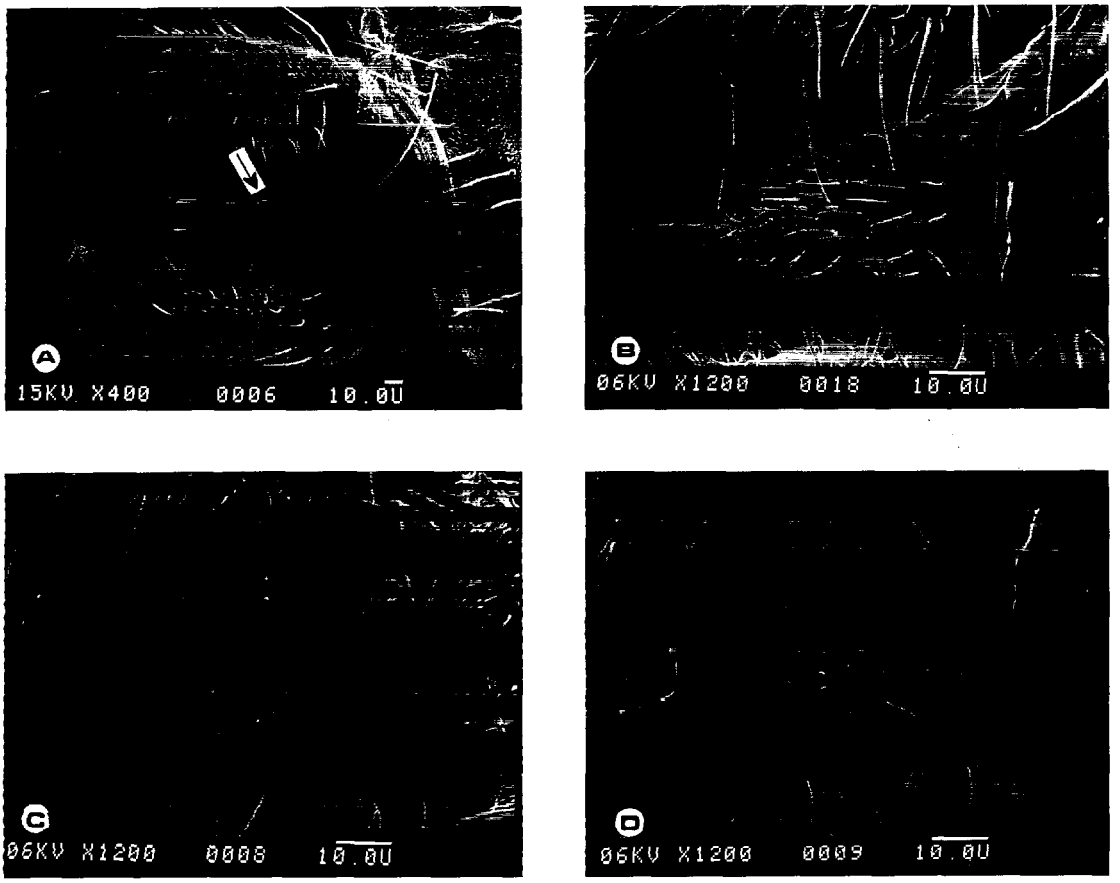

Fig. 3. Spicules on male aedeagus of members of the *C. variipennis* complex. (A) Lack of spicules in perspective photo of *C. v. occidentalis* (Bolsa Chica Marsh, Orange Co.)(400×), (B) Numerous spicules (1,200×) in *C. v. sonorensis* (AA laboratory strain from Texas); (C) Fewer spicules in wild *C. v. sonorensis* from southern California (Aukeman dairy, September), (D) Greatly reduced spicule number and size in hybrid male (F_5) of cross between AA colony *C. v. sonorensis* males and Bolsa Chica *C. v. occidentalis* females.

and each was maintained in culture continuously for 6 generations. An initial cross using Salton Sea *C. v. occidentalis* females and colony *C. v. sonorensis* males also yielded viable hybrid progeny and was maintained for 3 generations.

There was significant variation in viability between reciprocal crosses as indicated by differential hatching success seen in individual clutches. In the 2nd cross, 11 *C. v. sonorensis* females produced F_1 hybrid clutches. Four clutches had no hatch. The remaining 7 had a hatch of 7.4 ± 6.5% (range, 1–19%). Eight *C. v. occidentalis* females laid clutches, 1 of which had no hatch. The remaining 7 had 75.6 ± 12.6% hatch (range, 57–90) from clutches of similar size. A *t*-test on arcsine $\sqrt{}$ proportion-transformed data showed the difference in hatching was highly significant ($t = 11.04$, df = 11, $P < 0.001$). Seven of 11 *sonorensis* females and 7 of 8 *occidentalis* females were decapitated to induce oviposition; no difference was noted in hatch from decapitated females versus those that laid eggs naturally. Egg hatch was quantified for colony *C. v. sonorensis* females inseminated by the colony males as a control. Nine of these laid eggs on their own, whereas 10 were decapitated. There was no difference in egg hatch (transformed to arcsine $\sqrt{}$ proportion), which averaged 81.8% for decapitated females and 85.7% for intact females ($t = 1.05$, df = 12, $P = 0.32$).

Intergradation of Subspecific Characters. The availability of laboratory hybrids allowed us to evaluate evidence of introgression using morphology. Morphometric analyses of the field populations revealed broadly overlapping character states and no useable female characters. We focused on spicules on the male aedeagus as a reliable character which might show evidence of introgression. Males of *C. v. occidentalis* examined using SEM microscopy never showed spicules (Fig. 3A). The largest number of spicules was seen in colony AA *sonorensis* (Fig. 3B; Table 3), whereas significantly fewer spicules were found on wild (Chino Basin dairy) *C. v. sonorensis* (Fig. 3C; Table 3).

The male aedeagal spicules did yield intermediate character states. The number of spicules varied depending upon maternal parent (Table 3).

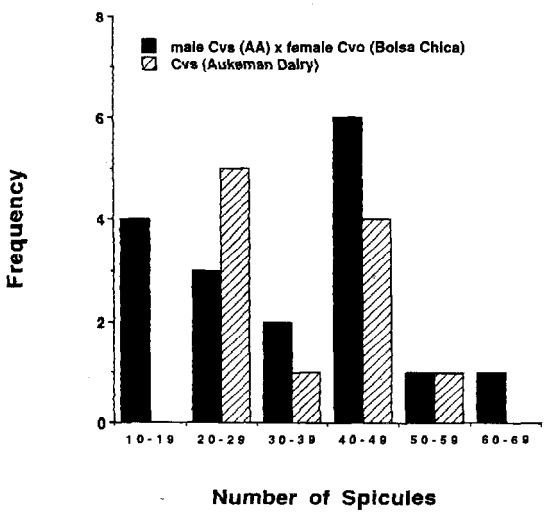

Fig. 4. Frequency distribution of spicule numbers on aedeagus of wild male *C. v. sonorensis* (Aukeman Dairy, September) and F_5 hybrids from cross between AA colony *C. v. sonorensis* males and Bolsa Chica *C. v. occidentalis* females.

Crosses with *C. v. sonorensis* female × *C. v. occidentalis* male parents had progeny with significantly higher spicule counts relative to the reciprocal cross. The spicule counts were bimodally distributed for *C. v. sonorensis* male × *C. v. occidentalis* female, whereas the Chino Basin dairy *C. v. sonorensis* had a similar bimodal pattern which might have been an artifact of smaller numbers (Fig. 4). Some of the male hybrids exhibited as few as 11 spicules (Fig. 3D).

Discussion

Collections of *Culicoides* spp. are dominated by females, because they are easily attracted to hosts or carbon dioxide-baited traps. *Culicoides v. sonorensis* and *C. v. occidentalis* have been considered conspecific based on morphology of field-collected adults, but generally have been considered different morphologically from *C. v. variipennis* s.s. (Wirth and Jones 1957, Jorgenson 1969, Downes 1978, Wirth and Morris 1985). Females of *C. v. sonorensis* and *C. v. occidentalis* commonly have been confused (Jorgenson 1969, Wirth and Morris 1985). Populations of *C. v. sonorensis* and *C. v. occidentalis* are readily distinguishable using electrophoresis and thus are genetically distinct; together with *C. v. variipennis* s.s. they constitute the current concept of the *C. v. variipennis* complex in North America (Tabachnick 1990; 1992a, b; 1996).

The material examined by Wirth and Jones (1957) contained a substantial number of putative *C. v. occidentalis* from cooler coastal climates in California, plus scattered material from cool areas in Oregon, Washington, and British Columbia. Weather in coastal areas, in particular, is influenced strongly by cold water in the Humboldt current, and seldom does temperature exceed 25–30°C. The type locality of *C. v. occidentalis* is Borax Lake (northern California). Specimens of *C. v. sonorensis* seen by Wirth and Jones (1957), on the other hand, were predominantly from warmer inland sites in the southwestern United States. The temperature effect (lower rearing temperature resulting in increased adult body size), (Table 2) combined with allometric shifts in character states would produce an apparent segregation of populations unless sampling was carried out year-round; no doubt this contributed to their interpretation of 4 character states as useful in separating members of the complex, as follows: (1) palpal (maxillary palp segment 3) ratio, (2) wing length, (3) number of antennal flagellomeres bearing sensoria, and (4) number of mandibular teeth.

Variability in Natural Populations. Examining Table 2 in Wirth and Jones (1957), *C. v. sonorensis* and *C. v. occidentalis* were quite close in palpal ratio (2.23 in *C. v. sonorensis*, 2.26 in *C. v. occidentalis*), antennal sensoria (0.83 additional in *C. v. sonorensis*, 0.65 additional in *C. v. occidentalis*), and mesonotal pattern (type C for both forms). The significance of the broader spermatheca in *C. v. sonorensis* also was minimized by the notation that *C. v. occidentalis* often also possessed the broader, shallower U-shaped spermatheca. There remained 3 notable characters in which *C. v. occidentalis* and *C. v. sonorensis* differed significantly—wing length, mandibular teeth, and presence or absence of spicules on the male aedeagus. The first 2 are both size-related and varied significantly within the field populations we examined. Our analyses agree with those of Hensleigh and Atchley (1977) that number of mandibular teeth is an allometric character. The characters designated by Wirth and Jones (1957) for morphological separation of *C. v. occidentalis* and *C. v. sonorensis* females are of little use in separating individual specimens. We were unable to separate females reliably of *C. v. sonorensis* and *C. v. occidentalis* morphologically. Known laboratory hybrid females also were indistinguishable morphologically from either parental form. Therefore, the presence or absence of morphological intergrades among females of *C. v. sonorensis* and *C. v. occidentalis* in the field was of little value for evaluating possible introgression.

Males of *C. v. occidentalis* and *C. v. sonorensis* are readily distinguishable. The 2 specimens reared from the Salton Sea that had spicules on the aedeagus (a *sonorensis* character) were reared in April after winter rains had flooded and probably somewhat diluted the solutes in the pools adjacent to the Salton Sea. This may have allowed *C. v. sonorensis* to use this site briefly. The single male lacking spicules reared from the dairy wastewater pond could be evidence of a polymorphism for this character within this *C. v. sonorensis* pop-

ulation. Alternatively, *C. v. occidentalis* larvae rear well in *C. v. sonorensis*-type habitats, including the nutrient-rich pans used for our laboratory rearing of the AA strain of *C. v. sonorensis* (unpublished data). We are not aware of *C. v. occidentalis* sites near the dairy pond, but incursion from an unknown saline or alkaline site is possible.

Hybridization. Very little is known of the mating systems of either *C. v. sonorensis* or *C. v. occidentalis* populations in nature. Members of the *C. variipennis* complex are thought to form male mating swarms over markers near the larval developmental sites (Zimmerman et al. 1982), and swarms of male *C. v. occidentalis* have been observed near dusk at the Bolsa Chica Marsh (B.A.M., unpublished data). Downes (1978), however, considered the western forms of the *C. variipennis* complex to be facultative swarmers and observed stenogamy in field-collected individuals of *C. v. sonorensis* and, to a lesser degree, in *C. v. occidentalis*. Our observations of field-collected *C. v. occidentalis* from the Salton Sea and Bolsa Chica confirm the ability of some males to mate in confinement, although stenogamy is not as intense as in males of the selected AA strain *C. v. sonorensis*. Our use of presexed groups of males and females (from pupae) was successful in yielding mated females, regardless of the cross. Single females randomly selected from blood-fed, pooled crosses, held to check clutch viability, provided some indication of mating success. Those producing at least some larvae constituted 64% of the *C. v. sonorensts* females and 88% of the *C. v. occidentalis* females.

The 2 subspecies hybridized in the laboratory, and hybrids were maintained for 3–6 generations. Therefore, it is difficult to conclude from these observations that absolutely no gene flow might occur between *C. v. occidentalis* populations and nearby *C. v. sonorensis* populations, particularly if they mate in similar areas. To date, 3 of the 4 characterized *C. v. occidentalis* sites in California (e.g., Salton Sea, Borax Lake, and Bolsa Chica Marsh) are extremely large, and permanent and are several kilometers from the nearest known *C. v. sonorensis* sites. This differs from the typical wastewater pond habitat used by *C. v. sonorensis*. Detection of incidental *C. v. sonorensis* gene introductions into these vastly larger *C. v. occidentalis* populations seems doubtful, but the reverse might be more easily detected. Our studies show that, although female intergrades cannot be detected morphologically, alternate spicule characters in males might allow introgression to be detected. It would be useful to characterize more *C. v. sonorensis* populations in terms of spicule number to determine the inherent polymorphism for this trait. The bimodal frequency distribution in hybrid male spicules from the *C. v. occidentalis* female parent cross reflects genetic control which merits further study. Rare individuals exhibiting a reduced number of spicules, as observed at Chino Basin dairy, might be natural hybrids or merely reflect polymorphism for this trait. For the time being, the lack of recognizable morphological intergrades between *C. v. occidentalis* and *C. v. sonorensis* is not evidence for lack of gene flow.

It must be emphasized that extensive isozyme analyses have shown no evidence of genetic intergrades between *C. v. occidentalis* and *C. v. sonorensis*. Populations of *C. v. occidentalis* exhibit higher interpopulational genetic variability than do *C. v. sonorensis* populations and are clearly identifiable using genetic markers (Holbrook and Tabachnick 1995). It is reasonable to assume that the unique nature of the *C. v. occidentalis* habitats (highly saline or alkaline), coupled with their relatively isolated and disjunct distribution, might isolate the 2 forms reproductively. In addition to the possible geographic isolation (at least over distances likely to be traversed by a *Culicoides* adult), the unique larval habitat would be expected to select strongly against hybridization with a poorly adapted *C. v. sonorensis* genotype. The recent discovery of *C. v. sonorensis* and *C. v. occidentalis* emerging together from smaller larval sites (Holbrook, Tabachnick, Schmidtmann, McKinnon, Bobian, and Grogan, unpublished data), still with no evidence of genetic mixing, seems ample support for their specific status.

The differential viability of hybrid offspring, in clutches with some egg hatch, indicated a postmating isolation mechanism. It would be desirable to examine more crosses using endemic California *C. v. sonorensis* strains. Fertility in decapitated females of some *Culicoides* spp. can be low (B.A.M., unpublished data), but in our studies there was no difference in fertility between decapitated and intact females of *C. v. sonorensis*. If our results using AA strain *C. v. sonorensis* are representative, the very low survivorship in F_1 hybrid offspring of *C. v. sonorensis* females, combined with the small and ephemeral nature of their favored larval habitat, would limit the genetic contribution of even large, nearby *C. v. occidentalis* populations. This may explain in part the lack of genetic introgression evidenced in previous studies (Holbrook and Tabachnick 1995). Alternatively, low egg hatch might reflect sperm depletion in a relatively small number of stenogamous *C. v. occidentalis* males placed together with *C. v. sonorensis* females. Despite our artificial crossing conditions and the extreme stenogamy of the AA strain *C. v. sonorensis* males, the ability to produce viable, fertile, hybrid lines does indicate the close relationship between these taxonomic entities. Other potential isolating factors, including mating behaviors, ovipositional site selection, and preovipositional flight activity (vagility) of females, are in need of investigation.

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