tires than small receptacles, the numbers of the two that are infested are equal.

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# OBSERVATIONS ON THE BIOLOGY AND ECOLOGY OF *ORTHOPODOMYIA CALIFORNICA* BOHART (DIPTERA:CULICIDAE)

H. C. CHAPMAN

Entomology Research Division, Agric. Res. Serv., U.S.D.A.[1]

INTRODUCTION. Very little biological or ecological information has been published on *Orthopodomyia californica* since its description by Bohart in 1950. This species is recorded in California from Fresno, Kern, Kings, Riverside, San Bernardino, and Yolo Counties (Freeborn and Bohart, 1951) and additionally from Butte, Contra Costa, Lake, Los Angeles, San Joaquin, San Mateo, Sutter, Tehama, and Tulare Counties by Loomis *et al.* (1956). The *Field Guide to Common Mosquitoes* of California (Loomis, 1963) also lists this species from Plumas and Shasta Counties. Most of these records are based on adult collections.

Rigby and Ayers (1961) reported *O. californica* from Arizona, but my examination of their larval material indicated their specimens were *O. kummi* Edwards. Hence, *O. californica* is still known only from California.

I observed *O. californica* both in the field and in the laboratory over a period of several years (1961–1964) and some of my observations are presented below.

The aquatic stages of *O. californica* have been reported only from cottonwood, willow, and oak (Reeves, 1941; Grant, 1953; Bohart, 1950). The last author mentions this species occurring wherever suitable holes are found in cottonwood and willow trees. Bohart (1953) also states: "Competition may also be the influence which makes *Orthopodomyia*

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[1] In cooperation with the California State Department of Public Health, Bureau of Vector Control, Fresno, California.

*californica* a rare and local species. In most types of treeholes it is discouraged or crowded out by the more vigorous *Aedes varipalpus* (Coq.) (=*sierrensis* (Ludlow)). It is only in certain types of soupy cottonwood treeholes that *O. californica* holds its own year after year. As these special holes are not common, the species is correspondingly restricted." Competition between mosquito species is difficult to demonstrate. Evidence presented below, however, indicates that there is little competition between these two species because of their different biological characteristics.

The writer observed the aquatic stages of *O. californica* from the following areas: 6 cottonwoods at the type locality near Elkhorn Ferry in Yolo County; 5 willows near Lemoore Naval Air Station in Kings County; 6 willows, 3 cottonwoods, and a live oak near Piedra in Fresno County; and 11 cottonwoods and 5 willows near Friant in Fresno County. Periodic observations were made at many of these treeholes in Fresno and Kings Counties, but Yolo County was visited only infrequently. The information obtained, therefore, will apply more to the San Joaquin Valley than to other areas of California.

FIELD OBSERVATIONS. All of the general areas in the San Joaquin Valley with trees containing *O. californica* larvae are located in or adjacent to water. Cottonwoods and willows are unusual in that they are deep rooted and obtain their water from the water table or from the overlying capillary fringe and hence are referred to as phreatophytes (well plants). Sycamores and various species of oaks are also found in these areas but are not phreatophytes. Many observations revealed that the water level in most of these cottonwood and willow treeholes showed little discernible change throughout the dry months despite the fact that only 0.61 and 0.24 inch of precipitation fell in Piedra and Friant, respectively, from April–October 1962. Some of these treeholes are located in the trees in such a manner as to make it virtually impossible to obtain moisture from precipitation, except from a driving horizontal rain. During this period of time, water was absent in treeholes in sycamores and oaks, and also in many cottonwoods and willows not in close proximity to water. It was obvious that the water lost by evaporation from some tree holes was being replaced with ground water by the tree. There appears to be a surge of moisture in many of the treeholes and wounds, especially in cottonwood, in late fall during and following the dropping of the leaves. One would think that the water level of these treeholes would recede in the winter, in the absence of precipitation, because of the reduced demand for water by the leafless trees; but such was not observed. Apparently the tree generally provides sufficient water to maintain the balance required to offset that lost by the very reduced evaporation that occurs in the cooler months.

I have been unable to find any reference in the literature to this phenomenon of water replenishment in treeholes by phreatophyte trees. It is possible that the treehole is intercepting some of the flow of liquids through the xylem. Most of the treeholes which possessed *O. californica* were never dry but contained water the year around. This maintenance of water in holes of certain tree species in or adjacent to bodies of water undoubtedly occurs in other states and certainly presents potential mosquito producing sites during periods of nonprecipitation not heretofore recognized.

*Aedes sierrensis* (Ludlow), although a multivoltine species, is often one-brooded in the San Joaquin Valley. Hatching of the eggs in the treeholes occurs during the initial fall rains. Overwintering is in the larval stage or in the egg stage if the holes have not been flooded. Additional rains during the spring may cause hatching of eggs not previously reached by past rains or hatching of recently deposited eggs. *A. sierrensis* eggs are deposited above the water line in the moist holes in late spring and remain there until flooded in the fall. Therefore, it can be said that the principal breeding season of *A. sierrensis* is in the late fall, winter, and spring. Host trees include almost any tree species capable of holding fall, winter, or spring precipitation.

Since the water level of many of the phreatophyte treeholes remains relatively constant, the noticeable lack of the aquatic stages of *A. sierrensis* is not unexpected. On several occasions *A. sierrensis* and *O. californica* were found together in numbers and these collections always occurred in late fall, winter, or early spring. Most of these mixed collections were from deeper holes that apparently were not filled to capacity naturally by the tree or from treeholes which were placed most advantageously for trapping fall or winter precipitation with the potential for maximum evaporation in the spring.

The larval stages of *O. californica* were noted every month of the year. The species overwinters chiefly as 2nd to 4th instar larvae. Pupae have not been observed before early May. Apparently, adults emerge and breeding is continuous in these holes as long as water is present. Deposition of eggs has been noted as late as November. No pupae were collected after September except for small numbers from several treeholes in early November, 1963. Eggs were laid above the water line on partially submerged leaves in the treeholes. The egg, deposited singly, is typical of *Orthopodomyia* with a membranous sheath that probably holds the egg to a surface. The newly hatched larvae wriggle down to the water surface. Bohart (1950) speculated that the egg serves as an overwintering phase of this species. Since I also found some first instar larvae in January, the eggs may overwinter; but it is just as easy to postulate that these small larvae came from eggs recently deposited by overwintering females. Many examinations indicated that first instar larvae occurred only rarely early in the year and that the larval stage represents the important overwintering stage. I never obtained large numbers of eggs from the field. All of the eggs collected in late fall demonstrated no diapause since they hatched in 4 days at 30° C. and 7 days at 20° C.

The water from 14 cottonwood and 11 willow treeholes was analyzed in November, 1963, for pH with a pH meter and for total soluble salts with a Wheatstone bridge. These data, presented in Table 1, indicated that the treehole water in willow was slightly more alkaline and had a higher salt content than that in cottonwood. The water in willow treeholes was always very dark whereas in cottonwood treeholes the water was sometimes rather colorless, especially

Table 1.—Range and mean of pH and total soluble salts in willow and cottonwood treehole waters possessing either *Orthopodomyia californica* or *A. sierrensis* or both.

| Number of treeholes | pH | | Total soluble salts (p.p.m.) | |
|---|---|---|---|---|
| | Range | Mean | Range | Mean |
| | | Willow treehole water | | |
| 11 | 8.4–9.4 | 8.9 | 4,539–8,000 | 5,963 |
| | | Cottonwood treehole water | | |
| 14 | 8.0–9.6 | 8.8 | 3,950–8,889 | 5,695 |
| | | Treeholes with *Orthopodomyia californica* | | |
| 21 | 8.0–9.6 | 8.8 | 4,000–8,889 | 5,669 |
| | | Treeholes with *Aedes sierrensis* | | |
| 12 | 8.0–9.6 | 8.9 | 3,950–8,102 | 5,950 |

in those treeholes that obtained all of their liquid from the tree and none from precipitation. Larvae occurring in these holes were generally covered with a whitish encrustment. The exclusion of either mosquito species from a tree could not and should not be correlated with the chemical constituents of the water. Rather, the presence or absence of either *O. californica* or *A. sierrensis* should be correlated with the past history of the treehole. If the treehole remained relatively full of water, especially in spring and early summer, it would contain *O. californica* and no or very few *A. sierrensis.* If the water level of the treehole dropped in spring or early summer, the treehole contained fair to large numbers of *A. sierrensis* when the water level was raised by the tree or by precipitation in the fall. If a dry treehole were filled with water late in the year, after the ovipositional period of *O. californica* females, the treehole would contain only *A. sierrensis* larvae.

As shown in Table 1, *A. sierrensis* larvae were present in 12 of the 25 treeholes and *O. californica* in 21. *A. sierrensis* was the dominant species in 4 of the treeholes and in each instance it was also the only species present Had these collections been made in the summer rather than in late fall, these four treeholes very probably would have been dry and *A. sierrensis* would have been absent in more of the treeholes.

The pH range of treehole water in willow was 8.4–9.4 and 8.0–9.6 in cottonwood. Bohart (1950) reported a pH range of 8.0–8.5 in cottonwood treehole water from the Yolo County area. The pH of treehole water from some sycamores ranged from 7.6–7.9 (mean 7.8) whereas live oak treehole water ranged from 6.9–8.0 (mean 7.2). Some of the darker waters gave both the highest and lowest pH readings; hence color is no criterion of acidity or alkalinity of treehole water. All of these data are contrary to the statement by Usinger (1956) that "water in rotten hole limbs and other parts of plants gives an acid reaction." Our results certainly indicated that treehole water in this part of California is usually alkaline and only very infrequently slightly acidic.

Three of the 21 lots of *O. californica* collected during November possessed the flagellate *Crithidia fasiculata* Leger and this flagellate represents the only parasite noted in this mosquito.

*O. californica* is not crowded out of treeholes in other tree species by *A. sierrensis* because *O. californica* does not normally occupy these sites. *O. californica* is primarily restricted to cottonwood and willow because these species contain water and maintain the water level during the principal breeding season of *O. californica* and during the time the females are seeking egg laying sites. Normally no other tree species possess water during this time.

I observed the aquatic stages of *O. californica* once in live oak treeholes in late summer near Piedra. These treeholes would not have held water at this time nor at the time the eggs were deposited in the treeholes except that the holes were flooded and maintained in this condition from June on by investigators working with *A. sierrensis.* The report by Grant (1953) of *O. californica* larvae occurring quite abundantly in oak trees in the vicinity of San Mateo in 1952 undoubtedly can be correlated with the extreme precipitation that occurred from October, 1951, on through the remainder of the year. This abundant precipitation presented a greater variety of host trees for fall oviposition by *O. californica* than during a year with normal precipitation. The very different life cycles and habitat preferences of *A. sierrensis* and *O. californica* certainly prevent competition between these species.

LABORATORY OBSERVATIONS. *Orthopodomyia signifera* (Coquillett), *O. kummi* Edwards, and *O. californica* are unique in that the fourth instar larva may bear dorsal sclerotized plates on segments VI, VII, and VIII. *O. alba* Baker never possess these plates. Reeves (1941) found that these plates were added during the fourth instar in *O. californica* without molting and were apparently a hypodermal deposition. He examined 671 fourth instar larvae and noted the following: 9 percent of the larvae had dorsal sclerotized plates on segments VI, VII, and VIII; 63 percent of the larvae possessed dorsal sclerotized plates on segments VII and VIII; and 28 percent had these plates only on segment VIII. Thus one would conclude from the literature that these dorsal sclerotized plates might occur only on segment VIII, or on segments VII and VIII, or on segments VI, VII, and VIII.

An examination of the fourth instar exuviae of 355 *O. californica* from four different localities showed the following distribution of dorsal sclerotized plates: none on segment VIII alone; 62 percent with plates on segments VII and VIII; and 38 percent with plates on segments VI, VII, and VIII. Thus the more accurate picture obtained when studying the exuviae clearly indicated that mature fourth instar larvae probably never have the dorsal sclerotized plate on segment VIII alone.

Relative to this dorsal sclerotized plate, the literature contains no mention of fourth instar larvae of *O. californica, O. signifera,* or *O. kummi* without dorsal sclerotized plates. My observations indicated that all freshly molted fourth instar larvae of *O. californica* possessed no plates. The initial plate is added on segment VIII within a day at 30° C. whereas it may take from 2–3 days for the initial plate to be formed on segment VIII at 20° C. There is a great discrepancy in size between freshly molted and mature fourth instar larvae of *O. californica.* If one did not check the head capsule or the completely ringed anal segment, one might think that these freshly molted fourth instar larvae were late third instars. Mature fourth instar larvae were 1.6 times as long (total length of specimen) and 1.6 times as wide across the thorax as freshly molted fourth instar larvae.

In the only report on the biology of *O. californica* Bohart (1950) stated that the aquatic stages develop more slowly than any other mosquito species in the West. His laboratory observations indicated that 4 months was required for this species to reach the fourth larval stadium and the pupal stage lasted 7 to 8 days.

While the growth of this species appears to be quite slow in the field, observations in our laboratory conflicted with the above information. The minimum time at 20° C. for newly hatched larvae to become fourth instars was 15 days, 14 days for completion of the fourth instar to pupation, and 7 days for the pupal stage. At 30° C. the minimum times required were 8 days for the first three instars, 6 days for the fourth instar, and 3 days for the pupa. Thus the minimum aquatic cycle in the laboratory at 20° and 30° C. was 36 and 17 days, respectively. These larvae were reared in aerated treehole water diluted with distilled water which contained leaves and debris from treeholes.

Notes on Diapause. Larval diapause was reported in various treehole mosquitoes by several investigators. From experiments conducted under somewhat uncontrolled conditions, Baker (1935) concluded that increased photoperiod was the dominant factor in terminating diapause in larvae of *Anopheles barberi* (Coquillett) and *Orthopodomyia signifera.* In some carefully planned and controlled experiments, Love and Whelchel (1955) reported that diapausing *Aedes triseriatus* Say fourth instar larvae were stimulated to pupation by a combination of 29° C. temperature and constant light. No pupation occurred in their experiments under the following conditions: natural winter light variations and 27° C. temperature; constant darkness and 27° C. temperature; natural winter light variations and 7° C. temperature; constant light and 7° C. temperature; and natural winter light variations and 39° C. temperature.

Field observations in the Central Valley of California showed that *A. sierrensis* pupae first appeared in February and were very abundant in March. When field collected *A. sierrensis* larvae were brought into the laboratory during late fall or winter and provided food and a suitable room temperature, they resumed growth and shortly pupated. Cold temperatures appeared to be the only factor which kept *A. sierrensis* in larval diapause in the winter.

Pupae of *O. californica* were not noted in the field until May, even though large, robust fourth instar larvae were present the previous November. Periodical observations during the winter months on field collected *O. californica* larval material brought into the laboratory and held at room temperature, occasionally indicated a reluctance of some populations to completely pupate. So as to ascertain whether an increased photoperiod was important in breaking the larval diapause, the following tests were performed:

Large numbers of fourth instar *O. californica* were collected from treeholes and pooled in the laboratory. Two replicates of 25 fourth instar larvae were placed in 600 ml. of cottonwood leaf infusion in a plastic container (5 x 5 x 2.5 inches) and exposed to different conditions. All replicates were aerated and the leaf infusion replaced when necessary. The temperatures used were as follows: High temperature (28° to 29° C.) controlled with a water bath; room temperature (18° to 22° C.) as ambient in the room; and outside temperature (—1° to 7° C.) as ambient outdoors. The light conditions used were as follows: Constant darkness (larvae were in darkness except when samples were checked for pupae); regular day length (about 10 hours in December and January); and regular day length plus 6 hours of artificial light (produced by a 150-watt light bulb placed about 1½ ft. away from larval containers). The samples were usually checked daily or every other day for pupae. Results are presented in Table 2.

Pupation occurred under all the different conditions except those maintained outdoors. Pupae appeared first in the containers kept at the highest temperature (28° to 29° C.). Complete pupation never occurred, owing mainly to mortality in many of the samples.

In some concurrent tests, 25 second and third instar larvae of *O. californica* were placed in leaf infusion water kept at 28° to 29° C. and exposed to both normal day length and normal day length plus 6 hours of artificial light. Although some larvae died, others completed development in each situation.

Since pupae appeared in samples maintained in the normal short photoperiod of winter as well as in the absence of light, day length did not seem to be important in terminating diapause. Temperature appeared to be the principal factor responsible for keeping the larvae in or bringing them out of diapause. Pupation in the fourth instar larvae tests occurred over a period of slightly more than 1 month. It is my belief that this extended time was probably due to our lack of knowledge of the optimum nutritional requirements of this species or of other requirements.

HABITS OF ADULTS. Heretofore nothing

TABLE 2.—Effect of light and temperature on the pupation of field collected fourth instar larvae of *Orthopodomyia californica* in mid-December, 1963 (two replicates—25 larvae each).

| Temperatures used | Percent pupae in 34 days | | |
|---|---|---|---|
| | Constant darkness | Regular day length (10 hours) | Regular day length plus 6 hrs. of artificial light (16 hours) |
| Outside —1–7° C. | 0 | 0 | 0 |
| Room 18°–22° C. | 82 | 34 | 84 |
| High 28°–29° C. | 96 | 92 | 68 |

has been reported on the feeding of this species. Several thousand *O. californica* adults, reared from field collected larvae, were placed in a cage (82 x 80 x 18 inches) and occasionally offered a chicken, rabbit, frog, toad, human arm, salamander, lizard, and pads soaked with citrated rabbit and chicken blood. Feeding was observed only on chickens and on the pads soaked with either chicken or rabbit blood. Many eggs were deposited above the water line on moist filter paper, paper toweling, or leaves. No eggs were deposited on the water surface. Only one clutch of 50 eggs was viable. Unlaid eggs dissected from 5 females averaged 197 and ranged from 170–220. Many engorged females were reluctant to oviposit in containers placed in the cage; therefore, some of the gravid females were placed in glass vials (20 x 93 mm) partially lined with moist filter paper and with a moist cotton plug at the bottom. All females that lived deposited eggs, apparently because of this "forced oviposition." An average of 88 eggs was deposited by these females with about three-fourths laid on the moist filter paper and the remainder on the moist cotton.

KEYS. With the discovery by McDonald and Belkin (1960) that *Orthopodomyia kummi* occurred in the United States (Arizona), the existing larval keys to *Orthopodomyia* larvae (Bohart 1950; Carpenter and LaCasse 1955) are now incomplete. The only description of the *O. kummi* larva is by Buen (1952). In this description, she erroneously named metathoracic hair 2 as hair 1 and did not illustrate metathoracic hair 1. The following key will separate fourth instar larvae of our four species.

KEY TO FOURTH INSTAR LARVAE *Orthopodomyia* OCCURRING IN THE UNITED STATES

1. Pentad hair 3 of abdominal segment VIII small, less than half as long as saddle of anal segment...... *alba* Baker
   Pentad hair 3 of abdominal segment VIII large, about as long as saddle of anal segment............... 2
2. Metathoracic hair 1 as long as or longer than the distance from its base to base of mesothoracic hair 1.................. *californica* Bohart
   Metathoracic hair 1 much shorter than the distance from its base to base of mesothoracic hair 1.... 3
3. Siphonal tuft about as long as distance from insertion of tuft to apex of siphon; metathoracic hair 1 very short, not conspicuous...... *signifera* (Coquillett)
   Siphonal tuft longer than the distance from insertion of tuft to apex of siphon; metathoracic hair 1 not short, quite conspicuous..*kummi* Edwards

The use of the presence or absence of sclerotized plates should be avoided in future larval keys to *Orthopodomyia* species since, as previously mentioned, freshly molted fourth instar larvae of at least *O. californica* do not possess a sclerotized plate on any abdominal segment but acquire the plates later in their stadium.

Adults of *Orthopodomyia alba, californica,* and *signifera* are very similar and generally inseparable. As mentioned by McDonald and Belkin (1960), *O. kummi* adults are quite distinct from these other three species in that *O. kummi* adults do not possess a conspicuous central pale spot in the wing and the lower line of white scales on the thoracic pleuron appears to meet and form a band across the prosternum in front.

SUSCEPTIBILITY TO INSECTICIDES. Jenkins and Carpenter (1946) mentioned an unpublished report that *O. signifera* larvae tolerated a concentration of 0.3 p.p.m. of DDT. Since many thousand field collected fourth instar larvae of *O. californica* from Elkhorn Ferry, Yolo County, were available in December, 1962, some bioassay tests were conducted with DDT and parathion. DDT gave an $LC_{90}$ of 0.04 p.p.m. with complete mortality at 0.1 p.p.m., whereas the $LC_{90}$ for parathion was 0.09 p.p.m. with only 93 percent mortality resulting from the top dosage of 0.1 p.p.m.

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# *AEDES COMMUNIS NEVADENSIS*, A NEW SUBSPECIES OF MOSQUITO FROM WESTERN NORTH AMERICA (DIPTERA:CULICIDAE)

H. C. CHAPMAN[1] AND A. R. BARR[2]
Fresno, California

Several investigators have suggested that *Aedes communis* (De Geer) in the Americas is composed of more than one species. Hocking *et al.* (1950) reported that there were large and small forms at Churchill, Manitoba; the ratio of lengths of proboscis to wing were different in both males and females of the two forms and it was suggested that they were probably different species. Hocking (1954) reported later that the smaller form was completely autogenous, that he never observed it sucking blood, and that it obtained nourishment for egg production by autolysis of the flight muscles. In Canada, Beckel (1954) found a difference in number of anterolateral and posterolateral mesonotal setae between the large and small forms of *A. communis.* Although Vockeroth (1954) also mentioned that in northern Canada *A. communis* may never feed on blood, many investigators reported this species to be a severe pest in many parts of North America, including the following: Alaska (Jenkins, 1948), Ontario and Quebec (Jenkins and Knight, 1950, 1952), Minnesota (Barr, 1958), northwestern United States (Stage *et al.*, 1952), Utah (Nielsen and Rees, 1961), California (Bohart, 1950; Carpenter, 1962), Nevada (Chapman, 1961), and many others. These differences in feeding habits, type and degree

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[1] Entomology Research Division, Agri. Res. Serv., U.S.D.A.

[2] California State Department of Public Health, Bureau of Vector Control.