Aedes (*Ochlerotatus*) *epactius* Along an Elevation and Climate Gradient in Veracruz and Puebla States, México

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ABSTRACT We report on the collection of immatures of *Aedes (Ochlerotatus) epactius* Dyar & Knab from artificial containers during July through September 2011 in 12 communities located along an elevation and climate gradient extending from sea level in Veracruz State to high elevations (>2,000 m) in Veracruz and Puebla States, México. *Ae. epactius* was collected from 11 of the 12 study communities; the lone exception was the highest elevation community along the transect (>2,400 m). This mosquito species was thus encountered at elevations ranging from near sea level in Veracruz City on the Gulf of México to above 2,100 m in Puebla City in the central highlands. Collection sites included the city of Córdoba, located at ≈850 m, from which some of the first described specimens of *Ae. epactius* were collected in 1908. Estimates for percentage of premises in each community with *Ae. epactius* pupae present, and abundance of *Ae. epactius* pupae on the study premises, suggest that along the transect in central México, the mosquito is present but rare at sea level, most abundant at mid-range elevations from 1,250–1,750 m and then decreases in abundance above 1,800 m. Statistically significant parabolic relationships were found between percentage of premises with *Ae. epactius* pupae present and average minimum daily temperature, cumulative growing degree-days, and rainfall. We recorded *Ae. epactius* immatures from a wide range of container types including cement water tanks, barrels/drums, tires, large earthen jars, small discarded containers, buckets, cement water troughs, flower pots, cement water cisterns, and larger discarded containers. There were 45 documented instances of co-occurrence of *Ae. epactius* and *Aedes aegypti* (L.) immatures in individual containers.

KEY WORDS *Aedes* (*Ochlerotatus*) *epactius*, *Aedes aegypti*, artificial container, climate, elevation

The mosquito *Aedes (Ochlerotatus) epactius* Dyar & Knab (that includes the junior synonyms *Aedes atropalpus nielseni* and *Aedes atropalpus perichares;* Brust 1974, O'Meara and Craig 1970a) was first described in 1908 based on specimens collected in Córdoba, Veracruz State, and Almoloya, Oaxaca State, in México (Dyar and Knab 1908). This mosquito was subsequently collected from six additional states in México — Chiapas, Guerrero, Jalisco, Morelos, Nuevo León, and Puebla (Martini 1935, Diaz Najera and Vargas 1973, Heinemann and Belkin 1977) — as well as from the western and central United States including Arizona, Utah, New Mexico, Colorado, Texas, Kansas, Oklahoma, Nebraska, Louisiana, Arizona, Missouri, and Illinois (O'Meara and Craig 1970a,b; Darsie 1974; Wolff et al. 1975; Munstermann and Wesson 1990; Duhrkopf 1994; Moore 2001; Darsie and Ward 2005; Stevens et al. 2008).

Heinemann and Belkin (1977) reported collections of *Ae. epactius* in México at elevations ranging from 600 to 1,600 m above mean sea level, and a recent study from Arizona reported collections of *Ae. epactius* at elevations ranging from 465 to 1,850 m (Stevens et al. 2008). Previously documented larval development sites for *Ae. epactius* include natural water-holding structures such as rock hole pools, ground pools, borrow pits, tree holes, and water-holding leaves (Dyar and Knab 1908; Johnson 1968; O'Meara and Craig 1970a,b; Heinemann and Belkin 1977), as well as artificial containers including tires, buckets, flower vases, cement basins, metal drums, and fountains (Darsie 1974, Heinemann and Belkin 1977, Munstermann and Wesson 1990, Duhrkopf 1994, Moore 2001). Females reportedly are aggressive blood feeders

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Table 1. General characteristics of study communities in Veracruz and Puebla States, México

State and community	Population estimate[a]	No. of examined clusters/individual premises	Mean elevation of premises (m)	Max/min temp (°C)[b]		Mean annual rainfall (mm)[b]	Time period for surveys of mosquito immatures in 2011
				July	Jan.		
Veracruz State							
Veracruz City	428,000	3/54	11	31.3/23.5	25.2/18.0	1,274	11–13 July
Córdoba	141,000	3/51	853	29.2/18.5	24.6/13.3	2,082	18–19 July
Coatepec	53,000	3/48	1,198	27.8/14.3	21.7/8.9	894	31 Aug–1 Sep
Orizaba	121,000	4/51	1,227	25.9/14.2	21.5/9.5	923	25–27 July
Rio Blanco	40,000	3/54	1,251	ND[c]	ND[c]	ND[c]	2–3 Aug
Ciudad Mendoza	35,000	4/48	1,334	ND[c]	ND[c]	ND[c]	3–8 Aug
Xalapa	425,000	4/51	1,416	25.5/15.2	20.8/10.3	731	23–24 Aug
Acultzingo	7,040	4/50	1,693	24.8/12.4	20.6/7.1	581	11–16 Aug
Maltrata	11,840	3/51	1,713	22.5/11.1	21.5/6.6	606	8–9 Aug
Perote	38,000	4/51	2,417	21.6/5.3	19.2/0.4	476	29–30 Aug
Puebla State							
Puebla City	1,434,000	4/48	2,133	23.0/8.5	19.7/1.7	860	17–18 Aug
Atlixco	87,000	4/50	1,825	25.9/12.4	24.2/7.7	530	19–20 Aug

[a] Based on data for 2010 obtained from Mexico's Instituto Nacional de Estadística y Geografía.

[b] Based on data obtained from Mexico's Servicio Meteorológico Nacional for 1975–2005 (except 1957–1987 for Cordoba, 1961–1979 for Acultzingo, and 1970–2000 for Maltrata).

[c] No long-term data available.

(O'Meara and Craig 1970b). The potential importance of *Ae. epactius* as a pathogen vector is poorly understood. Experimental transmission studies with this species are scarce but have demonstrated that: 1) *Ae. epactius* is a vector of Jamestown Canyon virus (Heard et al. 1991) and 2) female *Ae. epactius* can transmit St. Louis encephalitis virus transovarially to their progeny (Hardy et al. 1980).

We report on the frequent collection of *Ae. epactius* during a study aiming to collect *Ae. aegypti* immatures from artificial containers in communities located along an elevation and climate gradient ranging from sea level to high elevations (>2,000 m) in central México. This includes the first observations of *Ae. epactius* and *Ae. aegypti* immatures co-inhabiting individual artificial containers.

Materials and Methods

Study Environment. Studies were conducted in 12 communities located along an elevation and climate gradient ranging from sea level in Veracruz State to high elevation communities (>2,000 m) in Veracruz and Puebla States (Table 1; Fig. 1). The population size, elevation, and basic climate characteristics of the study communities are given in Table 1.

To facilitate comparison among communities, the study focused on neighborhoods dominated by low- to middle-income homes with small to medium-sized yards. Neighborhoods dominated by the following premises types were excluded from the study: high income premises and low income "fraccionamiento" style premises, which typically are small homes clustered closely together and with very small yards. Based on a survey of the characteristics of the premises that were included in the study (data not shown), the typical home was a one-story house constructed from concrete, brick, or cinder blocks, and with a roof made of concrete or metal. The vast majority of the study homes (>95%) had piped water and regular trash removal services but lacked air conditioning. The average number of rooms per house was 4.6 and the average lot size was ≈340 m^2. Shrubs and trees were common in the yards, and potentially water-holding containers were frequently observed on the premises (averages of 60 potentially water-holding containers and 6.5 actual water-holding containers per premises).

Imagery available through Google Earth (Google, Mountain View, CA), typically <3 yr old, was used to select four clusters within each community to target for surveys of mosquito immatures in artificial containers. A cluster was defined as an area of ≈1 km^2 including blocks (groups of houses surrounded by streets or roads) considered suitable for inclusion in the study. In most communities, with the exception of the small towns of Acultzingo and Maltrata, clusters were separated by a distance of at least 1 km, which exceeds the typical flight range (<100 m) of *Ae. aegypti* (Harrington et al. 2005). The flight range of *Ae. epactius* is not known. The target number of premises to examine per community was 50; these fell within 3–4 different clusters per community (Table 1). No more than five premises were examined within a single block. Survey teams started at the northeastern corner of a block and then proceeded in a clockwise direction, sampling every household for which someone was present to permit entry (up to five per block). Only homes within a targeted block that presented obvious safety concerns for the survey teams were excluded. The locations of sampled premises were recorded with a Global Positioning System receiver (Garmin eTrex Vista H; Garmin, Olathe, KS).

Temporal Sampling Scheme. Because of the intensive sampling effort and the large geographic area covered, we were only able to examine the study premises on a single occasion within the July through September rainy season (peak period for abundance of mosquito immatures in the study area). To minimize the effect of seasonal changes in mosquito abundance occurring over the 11 July through 1 September

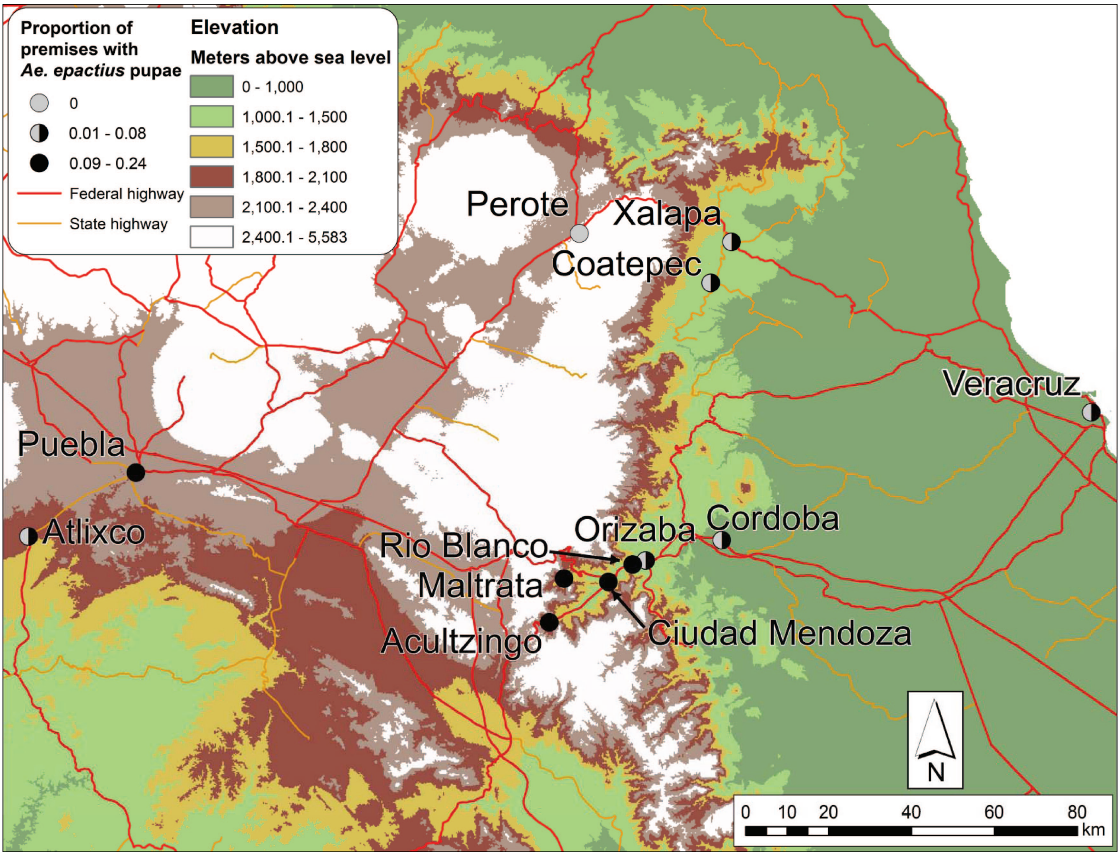

Fig. 1. Locations of study communities in Veracruz State and Puebla State, México, in relation to elevation and the proportion of examined premises with *Ae. epactius* pupae present. (Online figure in color.)

2011 sampling period, we started the sampling effort in the community with the lowest elevation (Veracruz City) and worked progressively upwards in elevation along the core of the transect that also included Córdoba, Orizaba, Rio Blanco, Ciudad Mendoza, Acultzingo, Maltrata, Puebla City, and Atlixco (Table 1; Fig. 1). The sampling in these core communities started on 11 July and was concluded by 20 August. Three additional communities along another elevation gradient further north in Veracruz State (Coatepec, Xalapa, and Perote; Table 1, Fig. 1) were sampled from 23 August through 1 September.

This temporal sampling scheme was designed to minimize the effect of the potential confounder of increasing abundance of container-inhabiting mosquitoes over time within the rainy season, primarily June to October in our study area, on the comparison of mosquito abundance among communities. Within the rainy season, temperature is an important driver for population growth of container-inhabiting species such as *Ae. aegypti* (Focks et al. 1993, Richardson et al. 2011) and presumably also for *Ae. epactius.* By the time sampling occurred in a given community, the cumulative number of growing degree-days (10°C base) from 1 June to the specific sampling period was comparable among the study communities located along the core of the transect (range: 634–779). Thus, these core communities were sampled at points in time, within the 2011 rainy season, that were reasonably comparable with regards to cumulative degree-days. The additional communities to the north (Coatepec, Perote, and Xalapa) exhibited more variation in cumulative growing degree-days because of the later sampling.

Weather Data. Determination of associations between presence of *Ae. epactius* and the local climate focused on weather data for the 30-d period preceding the survey for immatures in a given community. Using shorter (7 or 15 d) or longer (60 d) time periods produced similar results (data not shown). Weather parameters under consideration were: 1) average minimum daily temperature, 2) cumulative growing degree-days (10°C base), and 3) total rainfall.

Temperature and relative humidity (RH) data were obtained from HOBO (Onset Computer Corporation, Bourne, MA) data loggers set up in each community along the transect. Temperature and RH observations from the closest HOBO site were adjusted for elevation to each residence location. Rainfall data were obtained from the 0.07° gridded Climate Prediction Center Morphing technique (CMORPH) dataset (Joyce et al. 2004), which uses precipitation estimates derived exclusively from low orbiter satellite microwave observations and features transported via spatial propagation information obtained from geostationary satellite IR data. CMORPH provides some of the most reliable estimates for tropical summer rainfall compared with other satellite- and model-based rainfall products (Ebert et al. 2007). CMORPH data were bilinearly interpolated from surrounding gridpoints to each residence location. The household-level data were then averaged across clusters and communities.

Surveys for Mosquito Immatures. Water-holding containers on the study premises, including those located inside the house, were examined for presence of mosquito larvae and pupae. The following container types were excluded from the examination based on safety concerns or difficulty of access: plastic roof water tanks, rain gutters, and septic tanks. Container types were classified following García–Rejón et al. (2011) with some minor additions: 1) small discarded containers (bottles, cans, plastic bags, etc.), 2) larger discarded containers (washing machines, refrigerators, etc.), 3) tires, 4) buckets, 5) flower pots, 6) cement troughs for animal drinking water or aquatic plants, 7) large earthen jars, 8) metal or plastic barrels/drums, 9) cement water tanks, 10) cement cisterns, 11) wells, 12) swimming pools, 13) jars and pitchers, 14) toilet tanks, 15) flower vases, and 16) other containers.

All mosquito immatures were collected from small to medium-sized containers (classes 1–7 and 13–16 above). Following the methodology described by Romero–Vivas et al. (2007), a sweep net mounted on a pole was used to sample large containers, including barrels/drums and cement tanks, in which it is difficult to see the immatures without emptying the containers fully. This methodology estimates the total number of pupae in a large container based on those collected with a single sweep of the net and a multiplication factor determined by the container water capacity (less or >1,000 liters) and the water fill level (one-thirds full, two-thirds full, or full) (Romero–Vivas et al. 2007).

Collected immatures were transferred to plastic bags, typically with water from the container from which they were collected, for transport to the laboratory. Immatures were separated by premises of collection, container type, and life stage (larvae or pupae). Recovered immatures were then reared to adults for identification as described below.

Ovitrapping. To complement the surveys for immatures in communities above 1,600 m (Acultzingo, Maltrata, Atlixco, Puebla City, and Perote), ovitrapping was conducted concurrent with the surveys for immatures. We placed 30 ovitrap pairs per community, of which 25 pairs were on residential premises and five pairs in cemeteries. Each ovitrap pair consisted of two black plastic containers (7 cm in diameter and 17 cm high), one filled with 100% hay infusion and the other with 10% hay infusion (Reiter et al. 1991). The hay infusion had been incubated in a closed container for ≈7 d before use. Each ovitrap also was equipped with seed germination paper (Anchor Paper Co., Saint Paul, MN), which lined the inside of the plastic container and provided an egg-laying substrate. Ovitraps were set out for 48 h on a single occasion per community. Recovered eggs were reared to adults for identification as described below.

Rearing and Identification of Mosquitoes. Eggs and larvae were reared to pupae in the laboratory in plastic containers with dechlorinated water. Larvae were fed pulverized dog food or fish food ad libitum and allowed to pupate. Pupae were then placed in emergence chambers (Mini Mosquito Breeder; Bioquip, Rancho Dominguez, CA) and allowed to emerge to adults. Adults were placed in tubes containing 70% ethanol or stored dry in tubes together with a desiccant (t.h.e. Desiccant 100% Indicating; EMD Chemicals, Waltham, MA) before identification. The adults were identified, using the key of Darsie and Ward (2005), as males or females belonging to the following taxonomic entities: 1) *Ae. aegypti*, 2) *Ae. epactius*, or 3) a grouping consisting of any other mosquito species (hereinafter referred to as other mosquito species). Approximately 20% of all observed immatures (16% for larvae and 73% for pupae) were successfully reared to adults and identified. No special efforts were made to identify immatures because only fourth instar larvae are possible to consistently distinguish as *Ae. aegypti* versus *Ae. epactius*, which combined for >90% of identified adults.

Estimation of Percentage of Premises With *Ae. epactius* Pupae Present or Abundance of *Ae. epactius* Pupae Per Premises. Because we were able to identify to species a much larger percentage of field-observed pupae (73%) compared with larvae (16%), abundance estimates focused on the pupal stage and included: 1) percentage of premises in the study communities, and clusters within the communities, with presence of *Ae. epactius* pupae; and 2) abundance of *Ae. epactius* pupae on the study premises.

Percentage of Premises With *Ae. epactius* Pupae Present. Of 607 examined study premises, *Ae. epactius* pupae were present on 53 (i.e., identified as adults resulting from pupae collected from these premises) and absent from 464 (i.e., either with no pupae observed or with all specimens belonging to *Ae. aegypti* or other mosquito species). The remaining 90 premises (14.8% of total premises) produced field observations of pupae that were not identified to species as adults and therefore potentially could include *Ae. epactius.* These premises were proportionally allocated to the presence versus absence categories for *Ae. epactius*, by cluster or community, based on data for premises with definitive presence versus absence within the same cluster or community. For instance, if a community had 10 unassigned premises, 20 premises with *Ae. epactius* pupae present, and 30 premises with *Ae. epactius* pupae absent, the proportion of the 10 unassigned premises for that community classified as likely having *Ae. epactius* pupae present would be: $10 \times (20/(20 + 30)) = 4$.

Abundance of *Ae. epactius* Pupae Per Premises. Field-observed pupae that were not subsequently identified as adults (27% of 3,199 observed pupae) were assigned to *Ae. aegypti*, *Ae. epactius* or other mosquito species in accordance with the data for pupae that could be assigned to these taxonomic classifications. This was based on data from: 1) the same container type on the same premises (if such data were available), 2) other container types on the same premises (if data were not available from the same container type on the same premises but were available for other container types on the same premises), 3) the cluster in which the premises was located (if data were not available from the given premises but were available at the cluster level), or 4) the community in which the premises was located (if data were not available from the given premises or the cluster it was located in, but were available at the community level). Scenarios 2–4 outlined above needed to be used only for 10.2% of premises (62/607) to allocate unassigned pupae by container type on individual premises.

If, using scenario two as an example, a single taxonomic entity was identified from a given premises, then all unassigned pupae from that specific premises were assumed to belong to the same taxonomic entity. If multiple taxonomic entities were identified, then the unassigned pupae were proportionately allocated among them. For example, if a premises yielded two unassigned pupae, 10 *Ae. aegypti* puape, 5 *Ae. epactius* pupae, and 5 pupae of other mosquito species, then the estimate for *Ae. epactius* pupae on that premises would be: $5 + (\text{two} \times (5/(10 + 5 + 5))) = 5.5$. The final step in estimating the abundance of pupae for a given premises and taxonomic entity involved applying a multiplication factor, by the container type that the pupae were observed in, to account for complete sampling of small and medium-sized containers versus partial sampling of very large containers (barrels/drums, water tanks, and water cisterns). Container types with complete sampling were uniformly assigned a neutral multiplication factor of 1, whereas multiplication factors ranging from 1.9 to 3.5 were used, following Romero-Vivas et al. (2007), for very large container types with partial sampling based on their water volume and water fill level.

Statistical Analyses. Statistical analyses were carried out using the JMP statistical package (Sall et al. 2005) and results were considered significant when $P < 0.05$. Statistical tests used are noted in the text. Associations between the estimated proportion of homes with *Ae. epactius* pupae present and natural environmental factors were examined at the cluster level. Only clusters with ≥8 premises examined were included in these evaluations. The general relationships between the estimated proportion of homes with *Ae. epactius* pupae present and natural environmental factors, that is, temperature-related parameters and rainfall, were of a parabolic nature with peak percentages of premises with *Ae. epactius* pupae present occurring at mid-range values for the independent factors. Therefore, we used a regression model based on the following function: $y = \beta_1 x + \beta_2 x^2 + \beta_0$, where β denotes the vector of model coefficients. All variables included in regression model analyses were determined to be normally distributed, or nearly so, using a Goodness-of-Fit test (Shapiro–Wilk test; $P > 0.05$ indicates a normal distribution and all variables included in regression model analyses had P values >0.01).

Despite promising results from the univariate analyses, we refrained from developing more elaborate multivariate models. The rationale for this is that our field sampling included only artificial containers, whereas *Ae. epactius* also can be found in natural water-holding structures. Although the results from the univariate tests are strongly suggestive of climate parameters impacting the local abundance of *Ae. epactius*, future field sampling including both artificial and natural water-holding structures is needed to generate mosquito abundance data that allow for more elaborate modeling.

Results

Outcomes of Survey for Immatures and Ovitrapping. In total, 43,921 immatures were observed in containers on the study premises. This included 40,722 larvae and 3,199 pupae. Approximately 20% (8,833/43,921) of the field-observed immatures were successfully reared to adults and identified, including 15.9% of 40,722 larvae and 73.4% of 3,199 pupae. Identification of adult specimens produced totals of 5,758 *Ae. aegypti*, 2,703 *Ae. epactius* (1,332 females, 1,195 males, and 176 adults not identifiable to sex) and 372 mosquitoes of other species. *Ae. aegypti* thus accounted for the majority (65.2%) of the identified specimens, followed by *Ae. epactius* (30.6%) and other mosquito species (4.2%). Supplementary ovitrapping in communities located above 1,600 m (Acultzingo, Maltrata, Atlixco, Puebla City, and Perote) produced a total of 374 eggs of which 109 were successfully reared to adults and identified: this included 101 *Ae. aegypti* but only eight *Ae. epactius*.

Presence and Abundance of *Ae. epactius* Along the Elevation and Climate Gradient. *Ae. epactius* was collected from 11 of the 12 study communities; the lone exception was the high elevation community of Perote, which is located >2,400 m above mean sea level and represents the highest elevation for any community in the study (Table 2). Supplementary ovitrapping in high elevation communities yielded *Ae. epactius* only from Maltrata and Atlixco (1,710–1,820 m). *Ae. epactius* was thus encountered at elevations ranging from near sea level in Veracruz City on the Gulf of México to >2,100 m in Puebla City in the central highlands of México (Table 2). The estimates for percentage of premises in the study communities with *Ae. epactius* pupae present and abundance of *Ae. epactius* pupae on the study premises (Table 2) suggest that, along our elevation/climate study transect in central México, the mosquito is present but rare at sea level, most abundant at mid-range elevations from 1,250–1,750 m and then decreases in abundance above 1,800 m.

We further examined, at the cluster level, statistical associations between selected weather parameters

Table 2. Collections of *Ae. epactius* from communities in Veracruz and Puebla States, México, during surveys for immatures in artificial containers from July–Sept. 2011

Community (mean elevation of premises)	No. of *Ae. epactius* identified to species[a]	Estimated proportion of premises with *Ae. epactius* pupae present[b]	Estimated mean (SD) no. of *Ae. epactius* pupae per premise[b]	Selected weather data for the 30-d period before the survey for immatures[c]		
				Average daily temp (°C)	Average daily relative humidity (%)	Total rainfall (mm)
Veracruz City (11)	20	0.02	0.04 (0.30)	28.9	79.3	146
Córdoba (853)	103	0.05	0.05 (0.30)	23.5	83.9	321
Coatepec (1,198)	198	0.07	0.32 (1.06)	21.6	82.9	96
Orizaba (1,227)	101	0.08	1.34 (5.65)	20.5	87.2	292
Rio Blanco (1,251)	306	0.24	3.05 (13.31)	20.3	86.0	279
Ciudad Mendoza (1,334)	94	0.15	2.86 (9.25)	19.8	86.0	236
Xalapa (1,416)	40	0.07	0.79 (4.04)	20.6	81.4	73
Acultzingo (1,693)	416	0.16	3.22 (8.63)	18.5	84.7	164
Maltrata (1,713)	773	0.21	21.85 (70.40)	19.4	81.0	190
Atlixco (1,825)	122	0.02	1.18 (7.35)	19.5	72.1	43
Puebla City (2,133)	530	0.15	1.33 (4.32)	17.8	71.6	94
Perote (2,417)	0	0.00	0.00 (0)	13.6	85.6	53

[a] Collected as larvae or pupae from artificial containers and reared to adults prior to species identification. Not all observed immatures (that also included *Ae. aegypti* and other mosquito species) were reared successfully to adults.

[b] See Methods section regarding the process for estimating these numbers.

[c] Calculated based on the specific sampling dates for each community shown in Table 1.

(average minimum daily temperature, cumulative growing degree-days, and total rainfall) during the 30-d period preceding the survey for immatures in a given community and the estimate for percentage of premises with *Ae. epactius* pupae present. This revealed statistically significant parabolic relationships with the estimate for percentage of premises with *Ae. epactius* pupae present for average minimum daily temperature (analysis of variance [ANOVA], polynomial quadratic regression model; $F_{2,35} = 3.71$, $r^2 = 0.175$, $P = 0.03$), cumulative growing degree-days ($F_{2,35} = 3.74$; $r^2 = 0.176$; $P = 0.03$) and total rainfall (based on log-transformed data for total rainfall; $F_{2,35} = 5.53$, $r^2 = 0.240$, $P = 0.008$). The nature of these parabolic relationships, with peak percentages of premises with *Ae. epactius* puape present occurring at mid-range values for the independent factors, is illustrated for average minimum daily temperature in Fig. 2.

Container Type Associations of *Ae. epactius* and Co-Occurrence With *Ae. aegypti* in Individual Containers. We recorded *Ae. epactius* from a wide range of container types including small and larger discarded containers, tires, buckets, flower pots, cement troughs for animal drinking water or aquatic plants, large earthen jars, barrels/drums, cement water tanks, and cement water cisterns (Table 3). The largest numbers of identified *Ae. epactius* adults originated from cement water tanks and barrels/drums (>500 per container type), followed by tires (>300) and large earthen jars, small discarded containers, buckets, and cement water troughs (>200 per container type).

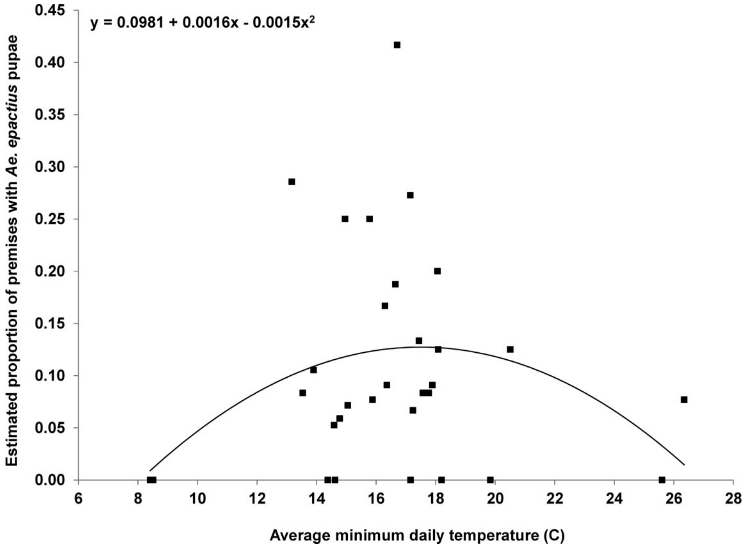

Fig. 2. Relationship between average minimum daily temperature during the 30-d period preceding the survey for immatures and the estimate for percentage of premises with *Ae. epactius* pupae present at the premises cluster level.

Table 3. Collections of *Ae. epactius* by community and container type

Community	No. of identified *Ae. epactius* by container type[a]										
	Small discarded containers[b]	Larger discarded containers[b]	Tires	Buckets	Flower pots	Cement troughs	Large earthen jars	Drums	Cement tanks	Cement cisterns	Other container type
Veracruz City	0	6	0	0	0	0	0	0	0	0	14
Córdoba	91	0	8	0	0	0	0	0	0	0	4
Coatepec	39	0	0	19	2	0	0	39	43	0	55
Orizaba	25	0	0	18	0	0	1	0	0	0	57
Rio Blanco	14	1	12	14	3	16	4	109	1	0	114
Ciudad Mendoza	52	0	0	2	0	0	0	13	4	1	22
Xalapa	3	0	6	5	3	0	2	0	12	0	9
Acultzingo	3	15	10	21	1	115	95	75	74	0	7
Maltrata	0	0	38	9	0	3	141	233	348	0	1
Atlixco	0	0	2	16	0	0	0	48	56	0	0
Puebla City	32	14	232	123	1	83	33	11	0	0	0
Perote	0	0	0	0	0	0	0	0	0	0	0
Total	259	36	308	227	10	217	276	528	538	1	283

[a] Collected as larvae or pupae from artificial containers and reared to adults prior to species identification. Not all observed immatures (that also included *Ae. aegypti* and other mosquito species) were reared successfully to adults.

[b] Small discarded containers include bottles, cans, plastic bags, etc.; larger discarded containers include washing machines, refrigerators, etc.

We recorded 45 instances of co-occurrence of *Ae. epactius* and *Ae. aegypti* immatures in individual containers (i.e., for container types that were represented by a single water-filled container on a given premises with the data thus representing immatures collected from a single individual container) (Table 4). This was most frequently observed for containers falling into the category of "other containers," followed by barrels/drums, small discarded containers, buckets, tires, and cement water tanks. "Other" types of containers yielding both species included discarded toilet bowls, folded plastic tarps, traditional coal stoves, and children's toys. Because only ≈20% of field-observed immatures were reared to adults and identified, co-occurrence of *Ae. epactius* and *Ae. aegypti* immatures in individual containers likely occurred more commonly than the 45 recorded instances, which were based on specimens identified in the adult stage.

Discussion

Our knowledge of the biology of *Ae. epactius* is still very limited, but our study produced new information on environmental and human-related factors which may be involved, directly or indirectly, in determining the geographic range and local abundance of this mosquito species. We also expand the list of types of artificial containers from which *Ae. epactius* immatures have been collected, and provide the first documentation of co-occurrence of immatures of *Ae. epactius* and the dengue virus vector *Ae. aegypti* in individual containers.

The most detailed previous records for collection of *Ae. epactius* immatures in México come from Heinemann and Belkin (1977). They listed numerous collections of this species from rock holes and single collections from a small ground pool and a water-filled

Table 4. Instances with collection of both *Ae. epactius* and *Ae. aegypti* immatures from the same individual container

Community	No. of individual containers from which both *Ae. epactius* and *Ae. aegypti* were collected[a]								
	Small discarded containers[b]	Larger discarded containers[b]	Tires	Buckets	Flower pots	Large earthen jars	Drums	Cement tanks	Other container type[c]
Veracruz City	0	1	0	0	0	0	0	0	1
Córdoba	0	0	0	0	0	0	0	0	0
Coatepec	0	0	0	1	1	0	2	1	1
Orizaba	4	0	0	1	0	0	0	0	7
Rio Blanco	1	0	1	2	0	0	3	0	5
Ciudad Mendoza	0	0	0	0	0	0	1	0	2
Xalapa	1	0	1	0	0	1	0	1	1
Acultzingo	0	0	0	1	0	0	0	0	1
Maltrata	0	0	0	0	0	0	0	0	0
Atlixco	0	0	0	0	0	0	0	1	0
Puebla City	0	1	1	0	0	0	0	0	0
Perote	0	0	0	0	0	0	0	0	0
Total	6	2	3	5	1	1	6	3	18

[a] Collected as larvae or pupae from artificial containers and reared to adults prior to species identification.

[b] Small discarded containers include bottles, cans, plastic bags, etc.; larger discarded containers include washing machines, refrigerators, etc.

[c] Other container types that yielded both *Ae. epactius* and *Ae. aegypti* immatures included discarded toilet bowls, folded plastic tarps, traditional coal stoves, and children's toys.

large agave leaf. Also listed are different types of artificial containers including cement basins in Córdoba, Veracruz State, oil drums and buckets in Santa Catarina, Morelos State, and a fountain in Chapala, Jalisco State. Other mosquito species collected from the same individual containers included *Culex quinquefasciatus* Say and *Culex coronator* Dyar & Knab. We encountered *Ae. epactius* immatures in a wide range of container types, ranging in size from small discarded containers to large water-holding structures such as barrels/drums and cement water tanks. Based on these findings from México, together with records of *Ae. epactius* immatures from seepages, rock holes, ground pools, borrow pits, tree holes, tires, and flower vases in the United States (Johnson 1968; O'Meara and Craig 1970a,b; Darsie 1974; Munstermann and Wesson 1990; Duhrkopf 1994; Moore 2001), it appears that *Ae. epactius* females are willing to oviposit in an exceptionally wide range of water-holding environments. Because we did not examine natural water-holding structures present in or adjacent to the study premises, we cannot assess whether *Ae. epactius* females prefer to oviposit in artificial containers versus natural water-holding structures in the study area. We also note that the study focused on low- to middle-income areas within the study communities, and that additional studies are needed to determine if the findings for *Ae. epactius* are consistent across a broader range of socioeconomic conditions.

Perhaps our most important finding was the cooccurrence of *Ae. epactius* and the dengue virus vector *Ae. aegypti* in individual containers. There is a rich literature on the effects of competition between immatures of *Ae. aegypti* and another container-inhabiting dengue virus vector, *Aedes albopictus* (Skuse) (Macdonald 1956, Moore and Fisher 1969, Chan et al. 1971, Sucharit et al. 1978, Black et al. 1989, Ho et al. 1989, Barrera 1996, Costanzo et al. 2005, Murrell and Juliano 2008, Leisnham et al. 2009, Reiskind and Lounibos 2009, Leisnham and Juliano 2010). In some settings, for example in Florida and Brazil, *Ae. albopictus* immatures can, under certain circumstances, outcompete *Ae. aegypti* leading to reductions in the abundance of the latter species (Juliano 1998, Braks et al. 2004, Juliano et al. 2004, Lounibos et al. 2010). We speculate that a similar scenario may occur for *Ae. epactius* and *Ae. aegypti*, especially at higher elevations with standing water characterized by lower temperatures to which *Ae. epactius* likely is better adapted than *Ae. aegypti*. If this is the case, a naturally occurring mosquito species could potentially present a biological barrier for the spread of the primary dengue virus vector *Ae. aegypti* into higher elevations in parts of México. Additional studies are needed to determine to what extent *Ae. epactius* feeds on humans and whether or not it can serve as a vector of dengue virus under experimental conditions.

Ae. epactius exhibited an interesting spatial abundance pattern along the examined elevation and climate gradient, with the highest abundance at midrange elevations and lower abundances at either low or high elevations despite the presence of abundant larval development sites along the entire transect. Based on the observed association of mosquito abundance with local weather factors, it appears that these mid-range elevations provide the most suitable local climate for *Ae. epactius* to proliferate. We speculate that, under a scenario of climate warming, peak numbers of *Ae. epactius* may shift to occur at higher elevations. Our finding of associations between weather parameters and the likelihood of encountering *Ae. epactius* in artificial containers also presents a first step toward using weather and/or climate data in a Geographic Information System framework to model the spatial distribution and abundance patterns of this mosquito within its known range in North America. This should, however, be based on future efforts that more broadly determine presence and abundance of *Ae. epactius* immatures in natural as well as artificial water-holding structures, and include multiple sampling occasions over the mosquito season, and thus provides more extensive field data to use in the modeling exercise.

We found that *Ae. aegypti* and *Ae. epactius* co-occurred within communities, on individual premises and even in individual artificial containers. This highlights the potential issue of *Ae. epactius* being mistakenly classified as *Ae. aegypti* during surveys for immatures where extensive efforts to identify specimens to species are not possible and the assumption is made that observed *Aedes* early stage larvae or pupae are uniformly *Ae. aegypti*. This could lead to overinflated estimates of abundance of *Ae. aegypti* in certain settings, especially in locations in México falling within the elevation and climate ranges defined herein as most suitable for *Ae. epactius*.

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