Overwintering of *Culex pipiens pallens* (Diptera: Culicidae) in Shandong, China[1]

Lijuan Liu[2], Benguang Zhang, Peng Cheng, Huaiwei Wang, Xiuxia Guo, Chongxing Zhang, Haifang Wang, Yuqiang Zhao, and Maoqing Gong[3]

Department of Medical Entomology, Shandong Institute of Parasitic Diseases, 11 Taibai Middle Road, Jining, 272033, Shandong Province, P.R. China

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Abstract *Culex pipiens pallens* Coquillet can spread various mosquito-borne infectious diseases. This study identifies the overwintering sites and physiological condition of *Cx. pipiens* during hibernation in Shandong Province of P.R. China. Results show that female adult *Cx. pipiens* overwinter in habitats characterized by warm temperature, high humidity, and calm winds. Cellars yielded the highest densities, followed by air-raid shelters, caliducts, sewers, mountain caves, wells, stone caverns, etc. The adult density among overwintering sites differed significantly ($F = 45.88$, $P < 0.01$). Larvae and pupae were not captured after late December. In early winter, multiparous and bloodsucking mosquitoes accounted for a large proportion of the observed populations. However, their numbers gradually decreased as winter progressed, mainly due to their relatively high death rate in winter. Most mosquitoes overwinter in a state of diapause, and females have usually mated. Collections in midwinter showed that of those collected, 99.8% were in diapause and 100% had mated. Overwintering mosquitoes suffered few deaths under natural conditions and, upon emerging from overwintering diapause, could take blood meals and oviposit. The time taken for oviposition by mosquitoes emerging from diapause was longer than that observed with females continuously reared in the laboratory; however, there was no significant difference in the numbers of eggs ($F = 0.571$, $P = 0.467$) oviposited.

Key Words *Culex pipiens pallens*, overwintering sites, physiology condition, fecundity

Culex pipiens pallens Coquillet belongs to the *Cx. pipiens* complex and is an important vector of Bancroftian filariasis, caused by *Wuchereria bancrofti* (Cobbold), as well as Type B Epidemic Encephalitis. In China, *Cx. pipiens* thrives in regions north of 33° latitude, and is the dominant mosquito species complex in those regions. Outbreaks of filariasis in the Shandong Province from the 1950s to 1970s were vectored by *Cx. pipiens*, and the species remains an important vector of Type B Epidemic Encephalitis in that area (Cao et al.1994, Li et al. 2014).

Shandong is a coastal region in eastern China (N 34°22.9′–38°24.01′, E 114°47.5′–122°42.3′) with a monsoon climate characteristic of medium latitudes. It

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[2]Department of Medical Entomology, Shandong Institute of Parasitic Diseases, 11 Taibai Middle Road, Jining, 272033, Shandong Province, P.R. China, and Department of Entomology, South China Agricultural University, 483 Wushanlu, Guangzhou, 510640, China.

[3]Corresponding author (email: maoqingg@yahoo.com).

has abundant surface waters that provide favorable habitats for mosquito breeding. Population densities of *Cx. pipiens* are high, thus complicating mosquito control efforts. In regions with cold winter temperatures, mosquitoes reportedly overwinter in a state of diapause (Reisen et al. 1986, Shin et al. 2013). Diapausing mosquitoes can host pathogens and, after emerging from diapause, exhibit a high capacity for vectoring those pathogens to susceptible hosts (Bugbee et al. 2004, Farajollahi et al. 2005, Halouzka et al. 1999, Nasci et al. 2001). Therefore, overwintering mosquitoes are significant components in the population dynamics of the species and in the spread and prevalence of mosquito-borne diseases.

Overwintering mosquitoes are often relatively concentrated in their overwintering sites, and they are relatively immobile. Targeting overwintering mosquitoes to reduce their populations and, subsequently, the potential incidence of mosquito-borne diseases appears to be a viable control tactic. To effectively control overwintering mosquitoes, however, necessitates knowledge of their overwintering sites and their physiological condition in those sites. Only a few studies have been reported on the physiological states of overwintering *Cx. pipiens* (Nelms et al. 2013, Russell 1987, Strickman and Fonseca 2012); thus, this study was undertaken to identify primary overwintering sites in Shandong Province of China and to characterize mortality, physiological state, and fecundity rates of overwintering *Cx. pipiens*. The results of this study may serve as a basis to control *Cx. pipiens* by targeting overwintering mosquitoes.

Materials and Methods

Overwintering sites. Surveys for possible overwintering sites were conducted in 11 districts of Shandong Province in China from October 2013 to March 2014. These areas included Heze, Jining, Zaozhuang, Linyi, Jinan, Zibo, Weifang, Liaocheng, Tai'an, Rizhao, and Qingdao (Fig. 1). Urban and rural areas were surveyed in each district.

Each site surveyed was sampled for *Cx. pipiens* adults using battery-powered aspirators (Phoenix Red Science and Education Equipment Factory, Ningbo, China) as described by Silver (2008). Any water present in the site was sampled for larvae and pupae using a sampling spoon (Silver 2008). Sites sampled included different types of rooms, livestock sheds, cellars, bushes, caves, tunnels, bridge openings, air-raid shelters, and sewers. Temperature, humidity, and wind speed at each site were recorded. Mosquitoes were collected for 20 min per shelter using aspirators, and numbers were converted to density collected per man-hour.

The mosquitoes were identified in the Medical Entomology Laboratory, Shandong Institute of Parasitic Diseases. Morphological identification was based on taxonomic keys (Dong 2010, Lu and Chen 1997).

Physiological condition, survivorship, and fecundity rate. Female adult mosquitoes were randomly selected from the samples each month and dissected to detect the presence of a blood meal, the state of diapause, and multiparity. The midgut was examined for presence of blood residue as an indication of the female having taken a blood meal prior to sampling. The ovaries also were examined to determine diapause state. Mosquitoes in diapause are characterized by small ovaries and the cessation of development of the first follicle at the N-stage so that it

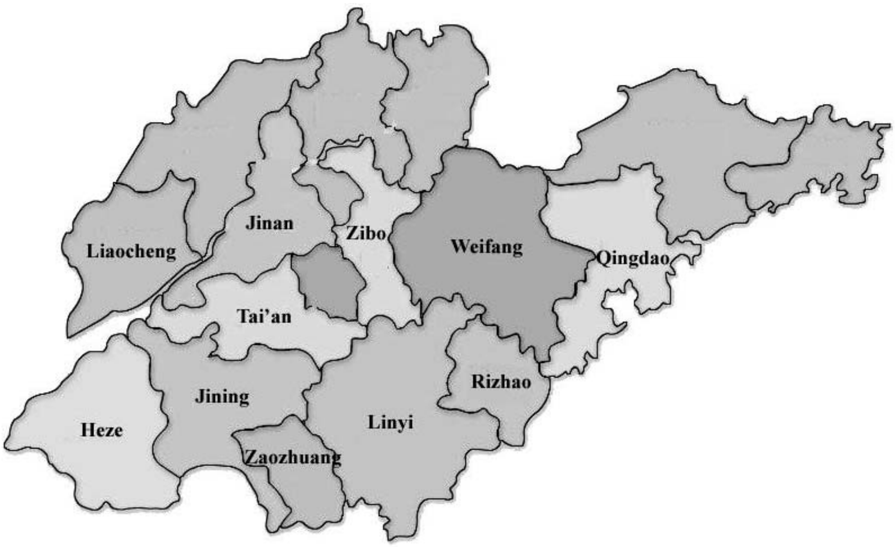

Fig. 1. Map of Shandong Province and study area.

is approximately the same length as the germarium (Hudson 1979). Multiparity was indicated by the ovaries having a large ampulla, an extended ovarian tracheole, and a diverticulum or by the presence of residual eggs in the ovarian canaliculus petioles (Polovodova 1949).

Overwintering *Cx. pipiens* collected on 15 November 2013 from cellars using a battery-powered aspirator were placed in cages (30 × 30 × 50 cm) covered with gauze. Three cages, each containing 100 adult females, were placed in a cellar. Mosquito mortality was recorded until 10 March 2014. In March 2014, 50 female adult *Cx. pipiens* were collected and maintained in the laboratory at 26°C with 14 h of daylight. After 3 d of acclimatization, mice were provided as blood sources. Feeding and subsequent egg-laying of *Cx. pipiens* were recorded.

Data analysis was conducted using SPSS 11.5 (IBM, New York, NY, USA) using analysis of variance to compare adult density in different overwintering sites.

Results and Conclusions

Overwintering sites. During this survey, 1,150 rooms (e.g., bedrooms, guest rooms, vacant rooms, woodsheds, storage rooms), 179 livestock sheds (including cow, donkey, sheep, and pig), 106 cellars, 31 wells, 198 cavities (e.g., mountain caves, air-raid shelters, soil caves, culverts, bridge openings, sewers), and several plants (e.g., shrubs, reeds) were sampled. Sites containing a mean density of >2 *Cx. pipiens* per man-hour were primarily cellars, air-raid shelters, caliducts, sewers, mountain caves, wells, and stone caverns. Cellars yielded the highest densities, followed by air-raid shelters and urban caliducts. The adult density at seven sites (including cellars, air-raid shelters, caliducts, sewers, mountain caves, wells, and stone caverns) showed significant differences ($F = 45.88$, $P < 0.01$) (Table 1). A

Table 1. Conditions of overwintering sites surveyed for *Cx. pipiens pallens* in Shandong Province, China.

Sites	*n*	Mean Density (mosquitoes/ man-hour)*	Avg. Air Temperature (°C)	Relative Humidity (%)	Illumination (lux)	Wind Speed (m/s)
Cellar	106	128.5 a	11.7	92	2.2	0.0
Air-raid shelter	19	59.3 b	7.5	81	3.4	0.1
Caliduct	63	31.6 bc	9.0	74	5.3	0.2
Sewer	78	25.7 bc	4.5	65	4.6	0.3
Mountain cave	43	7.2 c	2.3	55	3.2	0.3
Well	31	6.5 c	4.1	63	5.5	0.2
Stone cavern	26	2.5 c	0.3	52	3.5	0.3

* Means followed by the same lowercase letter are not significantly different ($P = 0.05$).

total of 20,183 female adult *Cx. pipiens* were collected in the survey. Only a few male adult *Cx. pipiens* were collected in early winter, and none were captured after mid-January. We also found two female adult *Anopheles sinensis* Wiedemann in a livestock shed and a cellar; other mosquito species were not collected throughout the winter. A limited number of *Cx. pipiens* larvae and pupae were collected in early December, but none were collected after late December as water in the larval and pupal habitats freezes with temperatures decreasing below 0°C. Therefore, control of overwintering mosquitoes should focus on management of overwintering adults primarily in cellars, sewers, caliducts, and mountain caves.

Physiological condition, survivorship, and fecundity rate. Of the 4,572 overwintering adult female *Cx. pipiens* dissected in this study, >99% were found to be in a state of diapause and >75% had mated prior to overwintering. These results are consistent with those of Sulaiman and Service (2007) in their study of overwintering *Cx. pipiens* in England. It is, thus, apparent that with appropriate environmental conditions, female mosquitoes that have mated before overwintering can emerge from diapause, acquire a blood meal, and immediately deposit their eggs. This is a public health concern in terms of vectoring diseases of humans in early spring when people are taking few preventive measures for mosquito attacks (Yang et al. 2010).

Only a small percentage of multiparous mosquitoes or females that had taken a blood meal survived through the winter. Based on the dissections, 25.7% of females were multiparous in early winter, while 33% had taken a blood meal. The proportion of multiparous and having taken a blood meal females declined significantly by midwinter, the percentage having taken a blood meal had decreased to 7.5% while multiparous mosquitoes were not observed (Fig. 2). These results are consistent with those of Andreadis et al. (2010) in their study of overwintering *Cx. pipiens* in New York. However, in late winter, the proportions of multiparous adults and

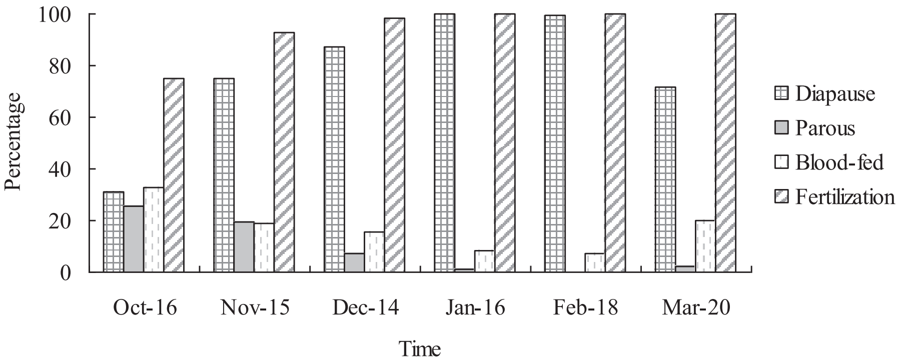

Fig. 2. Physiological state of overwintering *Cx. pipiens pallens* in Shandong Province, China.

females that had taken blood meals increase, presumably as temperatures increased.

From November 2013 to March 2014, mortality of overwintering *Cx. pipiens* in the three treatment groups was 5%, 12%, and 7% (mean mortality of 8%). After termination of diapause, 41 of the 50 females took blood meals and eventually produced offspring. In fact, females emerging from diapause can oviposit several times. In our study, 60.5% oviposited four times, and 18.6% oviposited six times. However, the females continuously reared in the laboratory can oviposit five times (Table 2). We found no significant difference in the amount of eggs produced per female between the females reared in the laboratory and those that had overwintered ($F = 0.571$, $P = 0.467$).

Bloodsucking and multiparous mosquitoes account for a large proportion of mosquito populations in the early winter and then gradually decrease in number with high mortality rates during the winter. This phenomenon has been reported in previous studies (Jaenson 1987, Mitchell and Briegel 1989). On the contrary, Bailey

Table 2. Frequency of oviposition by *Cx. pipiens pallens* females (overwintering versus laboratory-reared).

Oviposition Frequency	Overwintering Females		Laboratory-Reared Females	
	Number	Percentage (%)	Number	Percentage (%)
1	41	95.35	49	98.00
2	38	88.37	36	72.00
3	31	72.09	22	44.00
4	26	60.47	10	20.00
5	18	41.86	2	4.00
6	8	18.60	0	0.00

et al. (1982) captured large numbers of bloodsucking mosquitoes in the winter, probably in response to local weather conditions.

In late winter, the proportion of the population of bloodsucking mosquitoes increases. While many overwintering mosquitoes remain in overwintering sites at this time, they are active, and some warm-blooded animals are apparently present to serve as hosts for the mosquitoes (Buffington 1972). Indeed, female adults not in a state of diapause are captured throughout the winter. Although only a few adult females not in diapause were collected in midwinter, their sources and their apparent tolerance or resistance to low temperatures must be studied further.

In conclusion, we observed an overall high survivorship of mosquitoes collected from their overwintering sites in Shandong Province with an observed mean mortality of $<$10%. The actual mortality rate of these overwintering populations could be even higher because of natural predation and other interactions with abiotic and biotic factors in the overwintering sites. These factors will be explored further in future studies.

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