

Original Article**The Ecological Aspects of *Culex pipiens* (Diptera: Culicidae) in Central Iran**Hossein Dehghan¹, *Seyed Hassan Moosa-Kazemi², Javid Sadraei¹, Hassan Soleimani²¹Department of Medical Entomology and Parasitology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran²Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

(Received 2 Aug 2012; accepted 3 July 2013)

Abstract**Background:** The aim was to survey the specific factors, which cause to decrease blood feeding of mosquitoes important to succeed vector control.**Methods:** Larval collection was carried out from fixed and variable breeding places of Yazd County, central Iran in 2009. Autogeny-Anautogeny, Stenogamy-Eurygamy, and blood preference of *Culex pipiens* were studied using standard mosquito cages blood meal source for *Cx. pipiens* females considered as the chickens and human and fed females were kept in insectary condition (16:8 L: D, 27±3 °C and 70±10% RH). The data were analyzed using SPSS Ver. 11.5 soft ware.**Results:** Totally, 96 females' mosquitoes were tested for Stenogamy versus Eurygamy and 122 for blood preference assay. In the small cages (20× 20× 20cm) and large cage (60× 40× 60cm), the ability of mating and insemination rates were 60.0 and 67.0%, respectively. In spite of *Cx. pipiens* fed from sucrose 5%, none of them laying eggs in 60× 40× 60 cages during the study. This finding indicated the Anautogeny behavior of this species. This species was found of low tendency to human blood and almost 4 fold fed on chicken.**Conclusion:** The occurrence of Steno-Eurygamy, Anautogeny, and Ornithophilic behaviors of *Cx. Pipiens* was noted. More studies need to be carried out about the bionomics of this species to gain more data about the ecophysiological and behavioral characteristics in other parts of Iran.**Keywords:** Bionomics, Behavior, Mosquito, *Culex pipiens*, Iran**Introduction**

Malaria, West Nile and Sindbis viruses, Japanese encephalitis, Rift Valley fever as well as *Dirofilaria immitis* (dog heart worm) and *D. repens* (dirofilariasis) are important medical and veterinary mosquito-borne diseases in the World (Naficy and Saidi 1970, Saidi et al. 1976, Hayes 2005, WHO 2008, Azari-Hamidian et al. 2009).

Culex pipiens Linnaeus is medically important due to bite humans and animals besides transfer *D. immitis* in Iran (Ataei et al. 2012). Humans and some hosts may have allergy to mosquito bite, and this allergy can

sometimes be very severe (Vinogradova 2000).

Azari-Hamidian (2007) cited sixty four species, three subspecies and seven genera of mosquitoes in Iran. By now, 33 species from two *Anopheles* and *Cellia* subgenera, siblings, genotype and type forms are recorded in the Country (Moosa-Kazemi et al. 2009, Vatandoost and Hanfi-Bojd 2012). Different studies have introduced eight malaria vectors including, *An. stephensi* (type, intermediate and mysorensis forms (Vatandoost et al. 2006), *An. superpictus* as genotypes X, Y, and Z (Oshaghi et al. 2008), *An. culicifacies*

sibling A and B (Vatandoost et al. 2011), *An. fluviatilis* as three cryptic species S, T and U (Mehravaran et al. 2011), *An. maculipennis* group (*An. atroparvus*, *An. maculipennis*, *An. melanoon*, *An. messeae*, *An. sacharovi* and *An. persiensis* (Sedaghat et al. 2003), *An. dthali* (Manoochehri et al. 1972), and *An. pulcherrimus* (Zaim et al. 1993) is detected serologically positive to malaria parasite(s) in Baluchistan malaria foci, Southeastern of the Country. Five species of Anophelinae have been previously reported in Yazd Province, central Iran, including *An. dthali*, *An. marteri* Sogdianus, *An. multicolor* Combouliu, *An. turkhudi* Liston, and *An. superpictus* Grassi (Shahgudian 1956, Saebi 1987). Zaim (1987) mentioned the twelve species and four genera of *Culex*, *Culiseta*, *Ochlerotatus*, and *Uranotaenia* in this area.

Culex pipiens distributed in the Europe, the tropical and subtropical regions of Asia and Africa, the middle part of North America and Southern America and Australia (Vinogradova 2000). This species is distributed in the most part of Iran (Zaim et al. 1985, Koosha 2011). Distribution of this species has close relationship with economic activities and development of new territories. The human activity on natural environment with a change in the land and underground water resources can affect the mosquito fauna and abundance in a region. Some human activities created the irrigation domains marsh, dry land irrigation, channels, irrigation water storage, and various breeding places near the industrial activities (Vinogradova 2000).

Culex pipiens complex has at least two species (Dehghan et al. 2013). *Culex quinquefasciatus* Say distributed in the tropical area with the wide range of hosts whereas, *Cx. pipiens* is found in the moderate area with the limited range of birds nest maker. *Culex pipiens* form *molestus* is Autogenous, and the first laying eggs perform without a blood feeding (Mousakazemi 2000, Vinogradova 2000). *Culex pipiens* was reported as Orni-

thophilous, and intend to blood feeding on birds. Some reports indicated this species feeding on humans. The main host of this species in rural area was reported birds, and animals, whereas in urban area was reported as human, animal, and birds. Vinogradova (2000) described Stenogamy as the mating ability of mosquitoes in small spaces whereas, Eurygamy as the mosquitoes need to large space. Meanwhile *Cx. pipiens* form *molestus*, *Cx. Quinquefasciatus* and possibly *Cx. pipiens pallens* Coquillett were described as Sten-ogamous, whereas *Cx. pipiens*, *Cx. australicus* Dobrotworsky and Drummond, *Cx. vagans* Wiedemann, and *Cx. torrentium* Martini were reported as Eurygamous (Vinogradova 2000).

There are limited studies in relation to physiology, biology, feeding behavior of *Cx. pipiens* in Yazd. The aim of this study was to obtain new data which would be useful for future mosquito control programs.

Materials and Methods

Study area

A descriptive cross sectional study was carried out in Yazd County, Central Iran in summer 2009. The study took place in two randomly selected rural villages with 889 houses and 24358 populations in Yazd County (54°04'N 31°59'E). The study area is located in the Yazd Province and bounded by Isfahan in the west, South Khorasan in the east, Kerman and Fars in the south, Khorasan-e-Razavi and Seman Provinces in the north (Fig. 1). The maximum and minimum mean monthly temperatures were recorded as 40.6 °C in July and -4.4 °C in January In 2009. The total annual rainfall was 62 mm, the minimum of 0.3 mm in May and maximum of 18 mm in March. The mean annual relative humidity was 37%. The main occupation of the peoples is agriculture and husbandry of cow and goats. Based on available epidemiological data from Yazd Health Cen-

tre, there are no villages under the entomological survey.

Sampling methods

The specimens were collected from three fixed villages, Abrand-Abad, Nosrat-Abad and Gerd-e-Faramarz of Shahediyeh District and two variables Villages, Elah Abad and Sarcheshmeh from Zarch District. Mosquitoes larvae were collected by dipping method (WHO 1963, WHO 1992).

Larval habitats present within 500 meter around each village were studied for mosquito larvae collection. Five larval breeding places randomly selected within a 500-m radius of the study site. Up to 10 dipper samples were taken at intervals along the edge of each larval habitat using a standard mosquito dipper (350 ml) depending on the size of the habitat (WHO 1963).

Mosquito identification

The larvae were preserved in lactophenol and the microscope slides of the preserved larvae were prepared using Liquid de Faure's medium. All samples were sent to the School of Public Health, Tehran University of Medical Sciences, where the author identified the specimens using the keys of Shahgudian (1960), Zaim and Cranston (1986), Harbach (1988), Azari-Hamidian and Harbach (2009).

Seta 1 of abdominal segment of III–IV in larval stage and Vein venation including intersection of costa, subcosta and bifurcation of R2+3 veins used to distinguishing of adult females of *Cx. pipiens* complex. Male genitalia shape as dorsal and ventral arms of phallosoma used to distinguishing of adult males of *Cx. pipiens* complex. The mosquito name abbreviations are cited based on Reinert (2001).

Mosquito rearing

The larvae for each habitat were placed separately and transported to the laboratory where they were sorted by genus and instars counted and recorded. *Culex pipiens* larvae

were transferred to the laboratory of Entomology and Parasitology Department, School of Medical Sciences, Tarbiat Modares University for rearing. De-chlorinate tap water used for rearing of larvae and prepared by stand the water in glass jar for at least 24 hours. Larvae were placed in the covet and fed by available diets such as fish powder and milk baby food.

Pupae in batches of 30 samples were transferred to cups container covered by paper funnel and was placed into the cages. Petri dish containing de-chlorinate tap water was placed in to the cages for egg-laying. Cotton wool soaked in sucrose 5% used for feeding of adult mosquito. Mosquitoes were checked and monitored daily.

Behavioral and Physiological study

The experiment was designed to determine the degree Stenogamy or Eurygamy behaviors in a 60× 40× 60cm (Fig. 2A), and 20× 20× 20cm (Fig. 2B) cages. The spermathecae of the females *Cx. pipiens* were dissected for evidence of insemination (Fig. 3). The larvae were reared in insectarium and pupae were separated and transferred into cups inside the cages. Autogeny, Anautogeny and blood preference of this species also were studied with rearing the larvae and transferred the pupae in cages. Sucrose 5% and moisture cotton wool were used for feeding of this species in each cage. Petri dishes with no chlorine water were used for oviposition. Eurygamy, and Stenogamy behaviors were studied 10 days after emerging and mating of species using the dissection of spermathecae. Anautogeny and Autogeny behaviors of non blood feeding mosquitoes were studied at a 16:8 L: D, 27±3 °C and 70±10% RH for 20 days. Adult females were fed on chickens and human forearm for blood meal source in insectary. Fifty eight female mosquitoes were used for feeding on chicken and sixty four for human blood type. (Knight and Malek 1951, WHO

1975, Vinogradova 2000). The data were analyzed using SPSS Ver. 11.5 soft ware.

Results

Results of Stenogamy versus Eurogamy showed that sperm was active and observed in 60% of dissected *Cx. pipiens*. The ability of mating was found in cages in 20×20×20 cm sizes. The same result was found in large cages, 67% of mosquitoes were able to mating in cages 60×40×60 cm sizes (Table 1). In Autogeny and Anautogeny study, the mosquitoes feed on sucrose 5%, none of the mosquitoes laying eggs in 60×40×60 cm cages during the 20 days study. This finding indicated the Anautogeny behavior of this species. The mosquitoes exposed to chicken body almost 4 fold the mosquitoes were contact with the human arm. This finding indicated tendency to bird blood more than human one (Table 2).

Table 1. Result of the Spermatoca dissection of *Culex pipiens* to determination of Stenogamy versus Eurogamy behaviors in Department of Entomology and Parasitology insectary, School of Medical Sciences, Tarbiat Modares University, 2009

| Cage Sizes | Spermatoca Dissection | |
|---------------------------|-----------------------|-----------|
| | Positive | Negative |
| Small 20×20×20 | | |
| 1 | 9 | 4 |
| 2 | 5 | 5 |
| 3 | 7 | 4 |
| 4 | 6 | 5 |
| Total | 27 | 18 |
| Large 60×40×60 | - | - |
| 1 | 7 | 3 |
| 2 | 9 | 3 |
| 3 | 11 | 6 |
| 4 | 7 | 5 |
| Total | 34 | 17 |

Table 2. Frequency and percentage of the blood tendency of *Culex pipiens* on human and chicken hosts in Department of Entomology and Parasitology insectary, School of Medical Sciences, Tarbiat Modares University, 2009

| Replicate | Host | <i>Culex pipiens</i> | | | |
|---------------|----------------|----------------------|------|-----|------|
| | | Unfed | % | Fed | % |
| Cage 1 | Chicken | 4 | 15.4 | 22 | 84.6 |
| Cage 2 | | 8 | 25 | 24 | 75 |
| Total | | 12 | 20.2 | 46 | 79.8 |
| Cage 3 | Human | 24 | 72.7 | 9 | 27.3 |
| Cage 4 | | 20 | 64.5 | 11 | 35.5 |
| Total | | 44 | 68.7 | 20 | 31.3 |



Fig. 1. Map of Iran, highlighting the position of Yazd Province and its study area

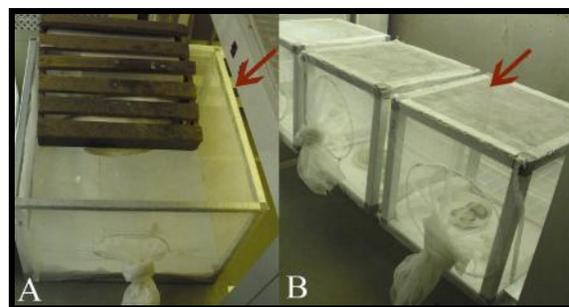


Fig. 2. The mosquito cages including (A) large size, 60× 40×60 cm, (B) small size, 20×20×20 cm. The pictures were taken by a digital camera in the insectary of the Department of Entomology and Parasitology, School of Medical Sciences, Tarbiat Modares University (Original photos)

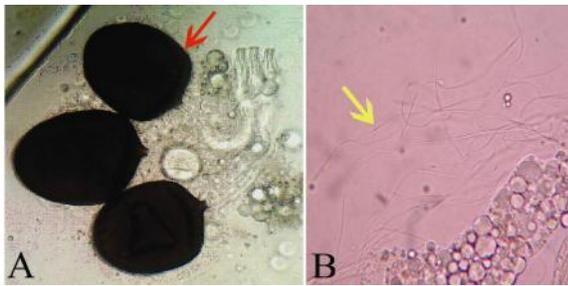


Fig. 3. The spermatheca dissection (A) *Culex pipiens* spermatheca, (B) *Cx. pipiens* spermatozooids, The original photos of spermatheca dissection of *Cx. pipiens* female were taken by a digital camera in the Department of Entomology and Parasitology, School of Medical Sciences, Tarbiat Modares University

Discussion

Culex pipiens larvae were collected from different larval habitats such as subterranean water accumulated with sewage, livestock trough, ponds in residential houses and garden ponds. The flume water contaminated by sewage was found the main breeding places of *Cx. pipiens* inside urban area. Similarity, Vinogradova (2000) cited *Cx. pipiens* larvae found in natural and artificial breeding places with high degree of contamination by organic and industrial materials. Water obtained from sewage treatment plants was created the suitable habitats of mosquito larvae outside the urban area. *Culx pipiens* mosquitoes can be made adaptable with the larval habitats created by human. This species can be considered as an indicator for estimating the environmental conditions in a human habitat. Development of human activity, changed the environment, and increased the abundance indicates ecological and physiological flexibility of this species.

In the present study, 60% and 58% of *Cx. pipiens* were able to mating in small and large cages respectively. The Eurygamy behaviors of *Cx. pipiens* were studied in cages in 100× 100× 100cm, 75× 75× 75cm and 60× 40× 60cm in Russia (Vinogradova 2000). In contrast, some researchers designed cages in

200× 200× 200cm sizes, and was stated a few of this species able to mating (Vinogradova 2000). Vinogradova (2000) stated the several populations of *Cx. pipiens* with Eurygamy behavior never did mating in cages in 20× 20× 30 sizes. We observed both Steno and Eurygamy behaviors. Further support to these results also came from a previous study, reported by Rioux (1965), which described *Cx. pipiens* with Steno-Eurygamous behaviors. It should be considered, at the present, *Cx. pipiens* populations were not observed with the pure form of Eurygamy or Stenogamy behavior.

It seems that there are no agreements among researchers regarding to the maintenance condition of this species in insectariums. Jupp (1987) reported that changed photoperiodic conditions of rearing of this species could be increased or decreased the mating rate.

It should be noted that, *Cx. pipiens* after blood meal was able to laying eggs in the 60× 40× 60cm cages, whereas, none of the individuals which fed only on sucrose 5% were able to lay eggs in the same cages. Our finding indicated the Anautogenous behavior of this species. Similarity, Rioux (1965) and Vinogradova (2003) cited the Anautogenous behavior in *Cx. pipiens* populations.

Tendency of *Cx. pipiens* to bird blood was more than human. Further support to this results also came from a previous study, *Cx. pipiens* was reported strongly Ornithophil behavior in South-central Sweden (Jaenson 1990). Also, Tempelis (1975) cited 28% of blood feeding on human and more tendency of this species to avian blood preference. Rioux (1965) expressed the Ornithophilic behavior of this species. Blood feeding of *Cx. pipiens* on birds and human was reported by Knight and Malek (1951), and Vinogradova (2003).

Culex pipiens has great morphological variations in all life stages and it is not easy to separate this species from *Cx. quinquefasciatus*

and *Cx. torrentium*. Males of this species are distinguishable from the mentioned species using the male genitalia (Harbach 1988). Dehghan et al. (2011) reported the male genitalia was the only reliable character to identification of *Cx. torrentium*, *Cx. quinquefasciatus* and *Cx. pipiens*. The most important diagnostic characters of *Cx. torrentium*, *Cx. quinquefasciatus* and *Cx. pipiens* larvae mentioned the branch of seta 1 of abdominal segment of III–V (Harbach 1988, Dehghan et al. 2010). Based on the mentioned above, we used the recent characters to diagnosis of the *Cx. pipiens* samples.

Conclusion

In situations where frequent blood feeding on birds are common for *Cx. pipiens*, the main complication with respect to estimating the potential for arboviral parasite transmission is that vectorial capacities will be necessary. Therefore, more study about of bionomics of mosquitoes especially among the *Cx. pipiens* needs to be studied rigorously in the future.

Acknowledgements

The authors are grateful to Dr Azari-Hamidian, School of Health, Guilan University of Medical Sciences, Rasht, for reviewing the manuscript, Mr Kalantari, Head of Zarch City Council, Mr Rezaei, Mr Eslami, Environmental Health Director of Zarch and Shahediyeh Health Center, for supporting the investigation. We also would like to express our appreciation to the people of Abrand-Abad, Nosrat-Abad, Gerd-e-Faramarz, Elah Abad, and Sarcheshmeh Villages in Yazd County for their kind cooperation during the study. Many thanks also dedicated to the efforts of the field staff of the Yazd Health Training and Research Center. It is noteworthy that this study has been done by

financial support of Research Committee in School of Medical Sciences, Tarbiat Modares University, ID No 5278604. The authors declare that there is no conflict of interests.

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