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# AUTOGENY EXPRESSION IN *CULEX PIPIENS* COMPLEX POPULATIONS IN SERBIA

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## Abstract

From a public health perspective, *Culex pipiens* (the house mosquito) is the most important mosquito species in Serbia. Its confirmed vector competence has enabled the sustained transmission of West Nile virus (WNV) to humans and animals in Serbia for over a decade. Despite this, the species' biology and ecology remain underexplored: this study aims to help fill that knowledge gap.

This research examined autogeny in female mosquitoes and assessed whether the presence of males affects its expression. Larvae of the *Cx. pipiens* complex were collected from three ditches and two urban street catch basins and reared to adult stage. Experimental subjects included adults emerging from field collections and individuals originating from an autogenous laboratory colony. Female mosquitoes were maintained in cages either with or without males and fed solely on sugar solution (no blood meals), and left undisturbed for five to seven days to permit egg development. No oviposition site was provided. Subsequently, females were dissected and their ovarian development evaluated.

Results revealed that, under laboratory conditions, male presence did not affect autogeny expression. After 10-12 days, females contained no eggs in their abdomens, suggesting egg resorption due to the absence of an oviposition opportunity. In field-collected breeding sites, autogeny was rare: only two catch basins and one ditch yielded autogenous females. The highest rate observed was 45% (in a catch basin), while other positive sites recorded rates of 12.5% (catch basin) and 2.15% (ditch).

Given the documented coexistence of the *pipiens* and *molestus* biotypes within the same breeding sites, hybridization is likely to occur. Hybrids, feeding on both avian and mammalian hosts, are recognized for their role as bridge vectors in transmitting WNV to humans and other mammals, and thus hold considerable public health importance.

KEYWORDS: *Culex pipiens*, vector, autogeny, oogenesis, mosquitoes

## Introduction

The mosquito species of the highest significance for Serbia is *Culex pipiens* Linnaeus 1758 (Diptera: Culicidae) due to its vectorial role. It is a primary vector of West Nile virus (WNV) in the country, where WNV is considered endemic. Many studies reported positive human and animal cases every year (Petrić *et al.*, 2017; Dimitrijević *et al.*, 2014). Besides WNV, *Cx. pipiens* is a vector of various pathogens significant to human and animal health, such as *Dirofilaria* sp. (Šiljegović *et al.*, 2024), Rift Valley fever virus (RVFV) (Turell, 2012), Usutu virus (USUV) (Cvjetković *et al.*, 2017), Sindbis virus (SINV) (Turell, 2012), and others (Becker *et al.*, 2012). Regarding its significant vectorial role, it is crucial to explore the biology and ecology of *Cx. pipiens* to better understand its behavior and role in transmission cycles of the mentioned pathogens. Pathogen transmission occurs when the female mosquito takes a bloodmeal from an infected bird and subsequently feeds on an uninfected host. The natural hosts of WNV are birds, and mosquitoes serve as vectors, while humans are incidental hosts and cannot act as reservoirs for further transmission (McLean *et al.*, 2001). There are two biotypes in the *Cx. pipiens* complex: the *pipiens* biotype, which feeds on birds (ornithophilic), and the *molestus* biotype, which feeds on mammals (mammophilic), including humans. Hybrids of these biotypes can act as bridge vectors, facilitating the transmission of WNV from birds to other animals, including humans, due to their intermediate host preferences (Ciota *et al.*, 2013; Vogels *et al.*, 2016; Fritz *et al.*, 2015). These hybrids have opportunistic feeding regimes and feed on both birds and mammals, which may result in successful virus spill-over to the human population. Besides differences in feeding preferences, these two biotypes differ in other ways that help distinguish them. The *pipiens* biotype overwinters in diapause, is eurygamous, and is anautogenous, while the *molestus* biotype does not hibernate (it develops continuously), is stenogamous, and is autogenous (Becker *et al.*, 2012).

Autogeny, the ability to produce the first egg batch without a bloodmeal (Clements, 1992), is a useful indicator for distinguishing *Cx. pipiens* biotypes. Because the biotypes are morphologically indistinguishable, studies of their biology require substantial investment in both equipment and funds. Biotype research mainly involves molecular analyses (Vereecken *et al.*, 2022; Biom *et al.*, 2024; Bell *et al.*, 2024). Ovary dissection provides insight into the developmental process influenced by various factors and offers a reliable, low-cost method for identifying autogenous specimens.

Given the importance of *Cx. pipiens* and the lack of knowledge about its behavior and biology, this study aimed to provide a deeper insight into autogeny in the *Cx. pipiens* biotype *molestus* and to improve understanding of oogenesis and the factors that influence it. In this research, we evaluated the impact of male presence on autogeny and ovarian development. A second question addressed whether females can resorb developed oocytes when conditions are unfavorable for oviposition. Finally, mosquitoes from several breeding sites were assessed for autogeny as an indicator of locations where both biotypes may occur together and thus provide conditions suitable for crossbreeding.

## Materials and Methods

Both field-sampled *Cx. pipiens* complex individuals and laboratory-reared autogenous *Cx. pipiens* biotype *molestus* were reared in the Laboratory of Medical and Veterinary Entomology (LME) at the Faculty of Agriculture, University of Novi Sad, Serbia.

## Maintenance of the autogenous colony

The autogenous colony has been maintained for six years in the LME. Females from this colony oviposit their first egg batch without the blood meal. Adults are fed only a 10% sugar solution.

Oviposition cups were placed inside the adult colony cages, and the eggs were then collected and transferred to white plastic trays containing 5 L of conditioned tap water for hatching. Hatched larvae are fed with cat-food pellets (ZooveT, Novi Sad), with 4-6 pellets added daily depending on the amount of uneaten food left from the previous feeding. The film that appears on the water surface as the food pellets break down is removed daily to allow proper larval respiration. When larvae molt into pupae, they are transferred to adult cages for emergence. Once sexually mature, adults mate and continue the life cycle through oviposition and subsequent larval rearing.

## Experimental design: Laboratory tests

For the experiments, pupae were pipetted daily from the larval trays and transferred to test tubes containing a small amount of water. The tubes were sealed with cotton wool to prevent adults escaping (Fig. 1). Pupae were placed individually in tubes (one per tube) to ensure that only virgin females were used in the experiment, meaning they had no opportunity to mate before the start of the experiment. Although male mosquitoes emerge approximately one day before females to allow rotation of their reproductive apparatus (Becker *et al.*, 2020), any potential contact between sexes, and thus any influence of male hormones on nulliparous females, had to be prevented.

After eclosion, adult sex was determined based on sexual dimorphism, specifically differences in the antennae and maxillary palps (Becker *et al.*, 2010). After sex separation, adults were transferred into cages where they were maintained until dissection. All adults were provided *ad libitum* access to a 10% sugar solution, supplied through adapted sugar feeders. The same rearing and sex-separation procedure was followed in all three experiments.

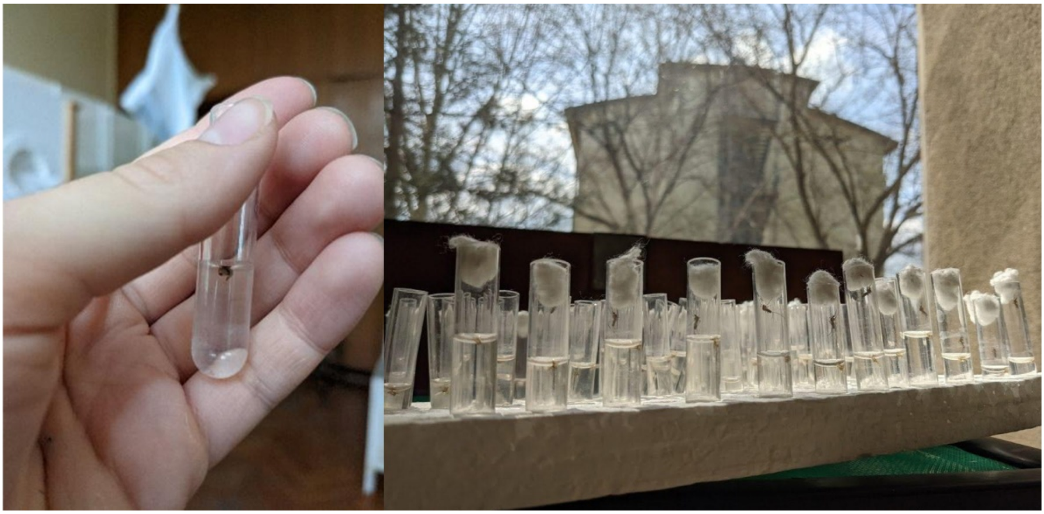

Figure 1. Tubes containing individual pupae, each sealed with cotton wool.

## Experiment 1: Impact of males on autogeny expression

Adults of the *Cx. pipiens* biotype *molestus* were introduced into cages for this experiment. Twenty cages were used. Two treatments were compared: females housed with males (10 females and 10 males per cage; sex ratio 1:1) and females housed without males (10 females per cage). The experiment included 10 replicates, with 200 females tested in each treatment. Seven days after eclosion, females were collected using an automatic aspirator and anesthetized by shaking the tube before counting and dissecting the ovaries. Dissections were conducted under a binocular stereoscope on a Petri dish containing a drop of physiological solution, using fine entomological forceps. The dissection procedure involved gently pulling the tip of the female's abdomen with entomological forceps, thereby extracting the ovaries onto the Petri dish. The extracted ovaries were examined visually and classified according to the degree of oocyte development. They were assigned to categories 0 to 3 as follows: category 0: ovaries without formed oocytes (Fig. 2a), category 1: early oocyte formation with fine round oocytes clearly delineated within the ovaries (Fig. 2b), category 2: small round oocytes present, but without clear delineation (Fig. 2c), and category 3: elongated oocytes (Fig. 2d).

Only the females collected alive from the cage were dissected and included in the data analyses, as those that died several hours before dissection may dry out and give an incorrect indication of the ovarian status.

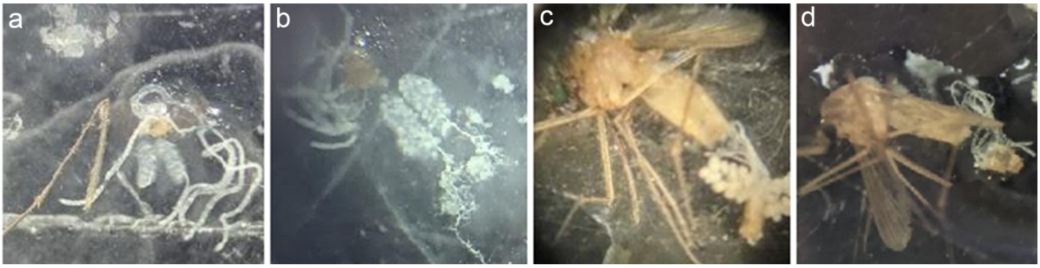

Figure 2. Classification of oocyte development: a) category 0 – ovaries without formed oocytes, b) category 1 – early oocyte formation with fine round oocytes clearly delineated within the ovaries, c) category 2 – small round oocytes present, but without clear delineation, and d) category 3 – elongated oocytes.

## Experiment 2: Ability of autogenous females to resorb oocytes or eggs

This experiment aimed to determine the duration of oogenesis in autogenous females and to assess whether females are able to resorb eggs when oviposition is not possible.

Adults were placed in cages under two conditions: females with males and females without males. Each variant was performed in four replicates with 50 females per replicate (per cage), totaling 200 females per variant. In cages with males, 50 males were added to each replicate (sex ratio 1:1). All adults were fed as previously described. Females were dissected in the following order.

Randomly selected females were collected using a mouth aspirator. They were anesthetized by shaking the aspirator before dissection. As in the previous experiment, only alive females were dissected and included in data analyses. The first dissection was performed two days after eclosion (2 females per cage were selected). A two-day interval between dissections was kept over the 15 days post-eclosion, during which two females per cage were dissected at each interval. After the first 15 days post-eclosion, dissections were carried out at five-day intervals, with five females dissected per cage at each interval. Dissections continued for 25 days in total, and the experiment concluded once all remaining live females had been dissected.

After dissection, tracheae in ovaries without formed eggs were examined: nulliparous females displayed a curled tracheal tip, whereas females that already formed eggs had straight tracheae. During both laboratory experiments, temperature and relative humidity were monitored using a Kestrel meteorological station. The study was conducted under ambient conditions, with a recorded temperature of 25.2±2ºC and relative humidity of 43±10%.

Experiment 3: Field study

For the field experiment, two types of breeding sites were selected: ditches (three breeding sites) and catch basins (two breeding sites). All breeding sites were within the territory of Vojvodina Province, Serbia.

The ditches were located in Petrovaradin (D1, 45.2487123, 19.8961799), Novi Bečej (D2, 45.596679, 20.140853), and Novi Sad (D3, 45.236291, 19.802561).

Both catch basins were in the urban zone of Novi Sad: catch basin 1 (CB1; 45.255501, 19.827679) and catch basin 2 (CB2; 45.259438, 19.818149).

Sampling was conducted as follows: D3 and CB2 on 22.07.2019; D1 on 04.06.2021; D2 on 09.06.2021; CB1 on 16.08.2021, and from 22.07.2019 to 16.8.2021. Only larvae and pupae were collected. Sampling was performed using the standard WHO dipper. Sampled juvenile forms were transported to the LME. Larvae were reared following the previously described procedure until pupation. Pupae from the same breeding site were put in a small plastic cup, which was placed in the cage to enable adults to eclode. After eclosion, females and males were kept together in the same cage to mate. Mosquitoes from a laboratory colony were also utilized for this research as a control group.

Five days after eclosion, females from each breeding site were divided into four groups, representing four replicates. Each replicate consisted of 25 randomly selected females, all dissected on the same day. Dissections followed the previously described procedure. Based on ovarian condition, females were classified as autogenous and anautogenous.

Data analysis

Data were analyzed using the Statistica package TIBCO Software Inc. (2020). Mean percentages and standard deviations were calculated during descriptive statistics. Percentages of autogenous females in the cages with males and without males were compared using a one-way ANOVA (univariate test, $P \leq 0.05$). The percentages of autogenous females per cage (females that formed the eggs; status ≠ 0 category) were arcsine square-root transformed and compared between the two categories of females (M50 and M0).

## Results

In the first experiment, all females were dissected at the same time (7 days post eclosion). In total, 95.65% females were autogenous when they were together with males, while 96.81% of them were autogenous when they were without males. Six out of ten cages where females were with males had 100% of autogenous females. Females in cages without males were 100% autogenous in seven out of 10 cages. In all cages with males, the percentage of females that expressed autogeny ranged from 87.5% to 100% (average 95.63±5.68%). Females without males demonstrated similar results, showing 87.6% to 100% of autogenous females (average 96.63±5.46).

Females in the cages with males had a more synchronized development of oocytes compared to females without males. All autogenous females kept with males had oocytes in the third category of development, while females without males showed slower development in two cages. In most cages, oocytes of females without males were also in the third developmental category. The exception was one female in one cage belonging to the second developmental category, and one in another cage belonging to the first category.

When the percentages of autogenous females in the two groups were compared using a univariate test, no significant difference was detected (SS=5; df=1; MS=5; F= 0.16; P=0.69). This indicates that males had no effect on the expression of autogeny in females.

In the second experiment, dissections revealed the presence of all oocyte/egg developmental categories in both variants, regardless of whether females were kept with males or without males.

In all cages where females were without males, 50% were successfully dissected, while the remaining 50% died before dissection. The same pattern was observed in three cages with males. In one cage. 47.06% of females were successfully dissected, and 52,94% died before dissection. *Culex pipiens* biotype *molestus* females kept with males expressed autogeny two days after adult emergence, when egg development in the ovaries was first recorded (Fig. 3). In cages with males, the proportion of autogenous females ranged from 80% to 96% (on average 88.0±6.53).

In four cages with males, a total of 100 females were dissected. Over the 25-day period, 12% did not form eggs, 4% were classified in the first developmental category, 7% in the second, and 77% in the third. Two days after eclosion, dissections showed that among eight sampled females, one (12.5%) did not develop eggs, two (25%) were in the first category, and 5 (62.5%) were in the second category. Egg development progressed rapidly after eclosion. By day 4, most females (62.5%) had reached the third developmental category, with the remainder distributed across the first (12.5%), second (12.5%), and no-egg (12.5%) categories. By day 6, all dissected females (100%) were in the third category. On day 8, 87.5% of females remained in the third category, while 12.5% were in the second. On day 10, 87.5% were still in the third category, and 12.5% had no egg development. Among females dissected on day 15, 85% were in the third category, 5% in the first, and 10% had not formed eggs. On day 20, 80% of females were in the third category, and 20% had no eggs. By day 25, 85% of females were in the third category, and 15% had not developed eggs.

In cages without males, the proportion of autogenous females ranged from 88% to 92% (89.9±2,2). *Cxulex pipiens* biotype *molestus* females from cages without males also expressed autogeny two days after adult emergence when the development of the eggs in the ovaries was recorded (Fig. 4). In cages without males, a total of 99 females were dissected. Over the 25 days, 10% had not formed eggs, 1.01% were in the first category, 9.09% in the second, and 79.79% in the third. Egg development progressed steadily in females kept without males. Two days after eclosion, all eight dissected females were in the second developmental category. By day 4, 75% of females had reached the third category, while 25% had not formed eggs, suggesting either anautogeny or delayed development. On day 6, 87.5% of females were in the third category and 12.5% in the second; an identical pattern was observed on day 8. By day 10, 75% of females were in the third category, and 25% had not developed eggs. Among females dissected on day 15, 95% were in the third category, and 5% had not formed eggs. On day 20, 85% of females were in the third category, and 15% had not developed eggs. By day 25, 89.5% of females were in the third category, and 10.5% had not formed eggs.

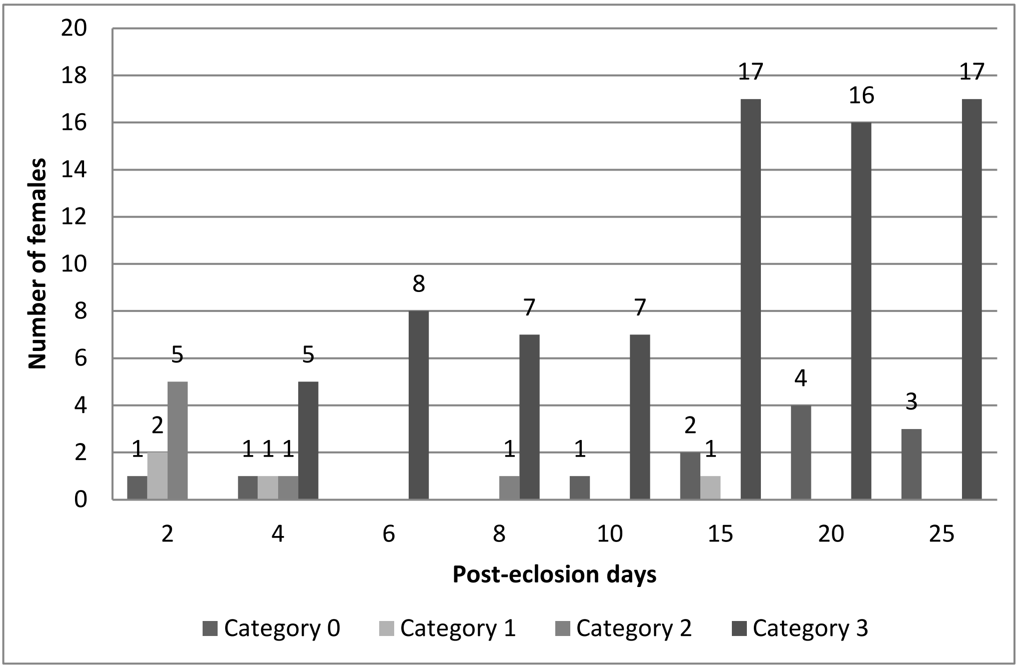

Figure 3. Development of oocytes/eggs in *Cx. pipiens* biotype *molestus* females kept with males over 25 post-eclosion days. Oocytes/eggs were categorized from 0 to 3.

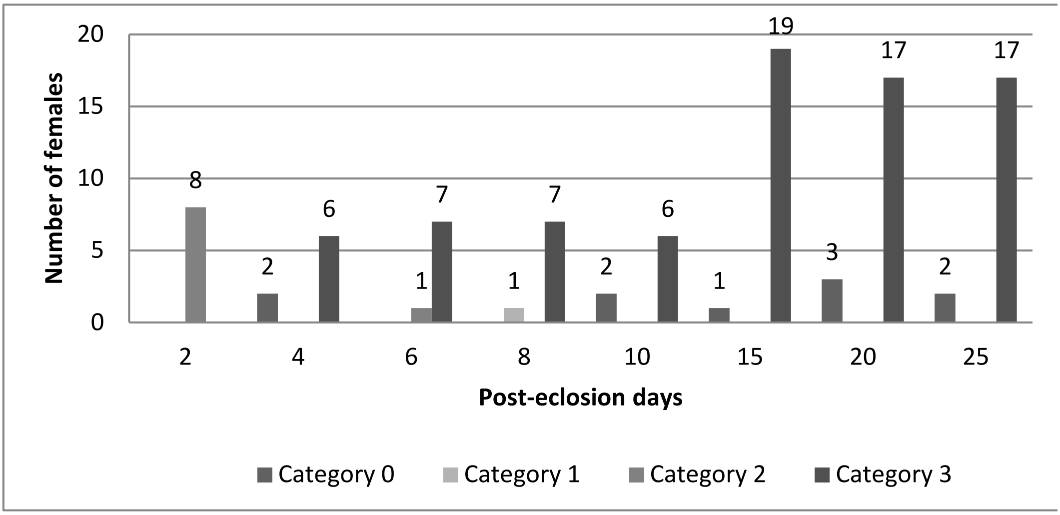

Figure 4. Development of oocytes/eggs in *Cx. pipiens* biotype *molestus* females kept without males over 25 post-eclosion days. Oocytes/eggs were categorized from 0 to 3.

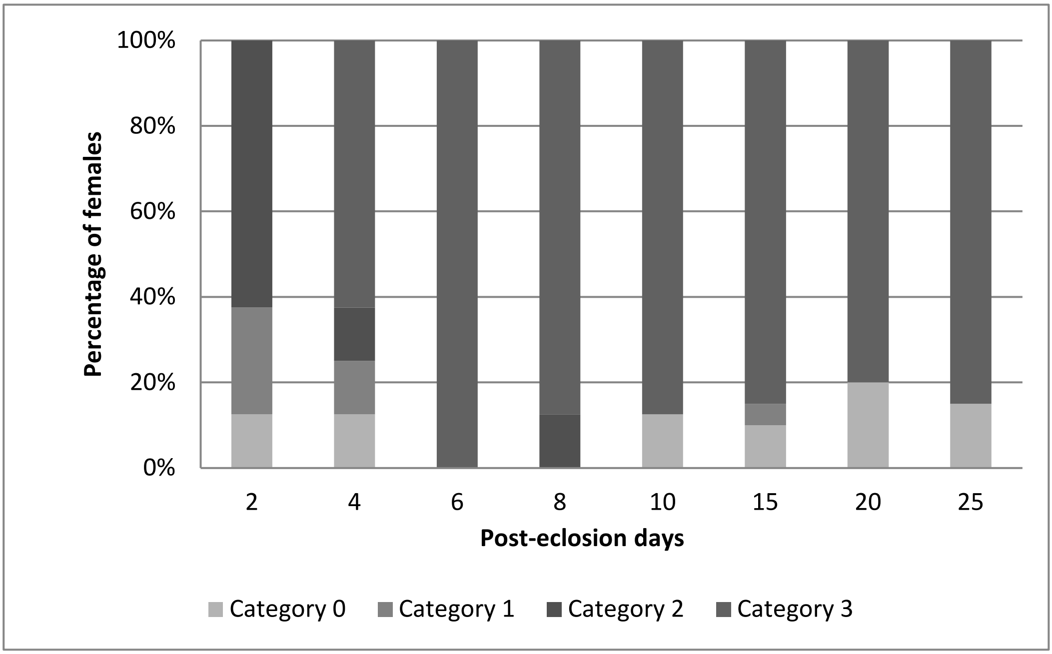

Figure 5. Percentage of females in cages with males whose ovaries/eggs fell into different developmental categories (0 to 3).

Percentages of females with males in each developmental category across post-eclosion days. Two days after eclosion, the highest proportions were in category 1 (25%) and category 2 (62.5%). By day 6, all females (100%) were in category 3. On day 20, the highest proportion in category 0 (20%) was recorded (Fig. 5).

Two days after eclosion, 100% of females were in category 2 (Fig. 6). On days 4 and 10, the largest proportion recorded in category 0 was 25%. Eight days after eclosion, the highest proportion in category 1 (12,5%) was observed. By day 15, 95% of the females were in category 3. On day 10, females without formed eggs reappeared, indicating possible egg resorption.

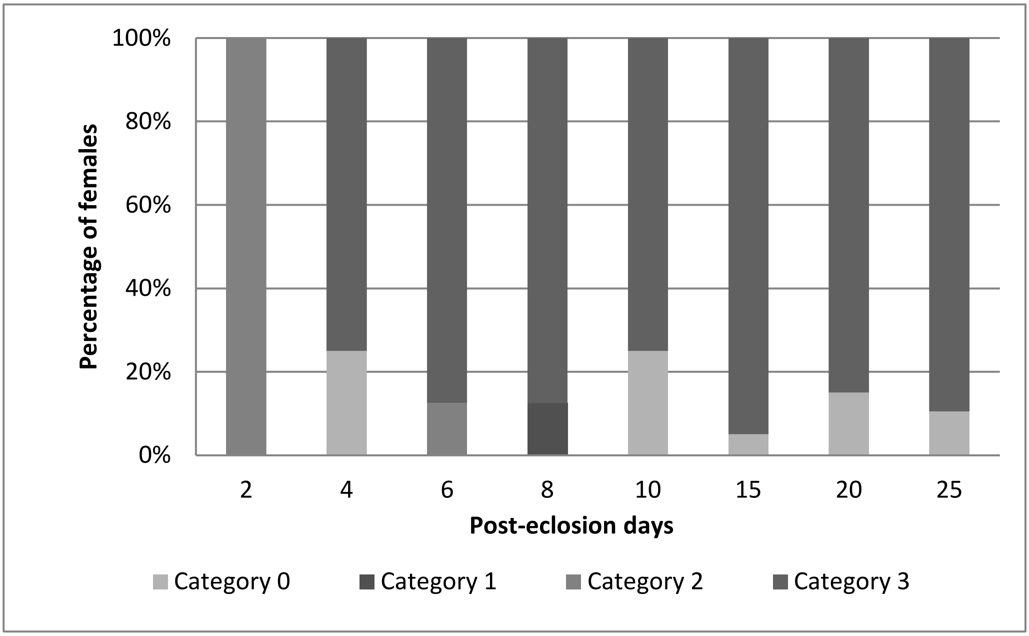

Figure 6. Percentage of females without males whose ovaries/eggs fell into the different categories of development (0 to 3).

When the percentages of autogenous females were compared using a univariate test, no significant difference was found between the two groups (SS=4.5; DF=1; MS=4.5; F= 0.57; P=0.48). This indicates that males had no effect on autogeny expression in females.

Tracheae in the ovaries of the females dissected 20 and 25 days after eclosion were less curly than those observed in females dissected 24 h after eclosion. In addition, ovaries in females dissected on days 20 and 25 showed a less distinct structure and straighter ovarian margins compared to control females dissected 24 h after eclosion (Fig. 7).

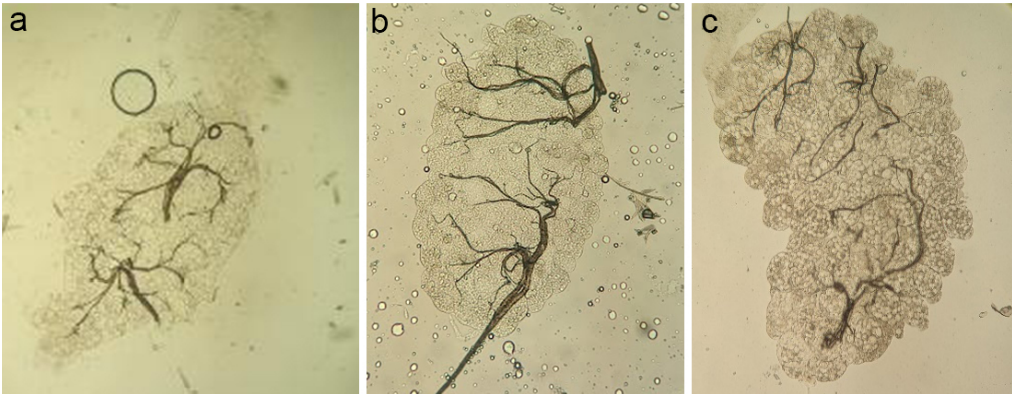

Figure 7. Ovaries of females that did not form the eggs: ovaries 24 h post-eclosion (a), ovaries 20 days post-eclosion (b), and 25 days post-eclosion (c) (Source: Kukić, N. 2021).

The percentage of females without formed oocytes in the ovaries suggests two possibilities: they either failed to form eggs or formed them and subsequently resorbed them.

Table I shows an increase in the percentage of females in category 0 ten days after eclosion. This pattern was observed simultaneously in both test groups and is presumed to indicate oocyte resorption at this time.

Table I. Percentage of *Cx. pipiens* biotype *molestus* females in category 0 each day.

| | Percentage of females in category 0 | |
|---|---|---|
| **Post-eclosion days** | **Females with males** | **Females without males** |
| **2** | 12.5 | 0 |
| **4** | 12.5 | 25.0 |
| **6** | 0 | 0 |
| **8** | 0 | 0 |
| **10** | 12,5 | 25.0 |
| **15** | 10.0 | 5.0 |
| **20** | 20.0 | 15.0 |
| **25** | 15.0 | 10.5 |

In the field study, females collected from the ditches at locations D1 and D2 did not express autogeny; all individuals were anautogenous. At location D3, only 2 out of 93 dissected females were autogenous (2.15%). In catch basins, autogeny rates were 45% in CB1 and 12.5% in CB2. The control laboratory population exhibited a markedly higher autogeny rate of 95%.

For comparison with the colony in the laboratory colony, ovaries of females from CB1 were categorized, as described as described previously. Only autogenous females from CB1 were compared to the control because CB1 had the highest autogeny rate among all field sites. Table II presents the number of females classified by ovarian development category, with category 0 indicating anautogenous females and categories 1-3 indicating autogenous females at different stages of ovarian development.

Table II. Percentages of females categorized by ovarian developmental stage, collected from location CB1 and from the laboratory-reared colony (control group).

| **Mosquito populations** | **% of autogenous females** | | | |
|---|---|---|---|---|
| | Category 0 | Category 1 | Category 2 | Category 3 |
| **CB1** | 55 | 2 | 1 | 42 |
| **Laboratory colony** | 5 | 9 | 5 | 81 |

Figure 8 shows the ovarian development categories and the percentage of females in each category for those collected from CB1 and for the laboratory colony (control). Percentages of autogenous females from the first laboratory experiment (females kept with males) were added to demonstrate differences in ovarian development when dissections were performed only two days later (on day 7 post-eclosion) compared with the timing of dissections in the field experiment.

At CB1, 55% of females were classified in category 0, indicating they were not autogenous. The remaining 45% were autogenous, with 2% in category 1, 1% in category 2, and 42% in category 3. Different outcomes were obtained for the laboratory colonies. On day 5 of dissection, only 5% of females were anautogenous (category 0). In contrast, 9% were categorized in category 1, 5% in category 2, and the majority (81%) fell into category 3. A comparison was also made with females dissected on day 6 in experiment two. By day 7, 96% of females expressed autogeny, while only 4% were anautogenous.

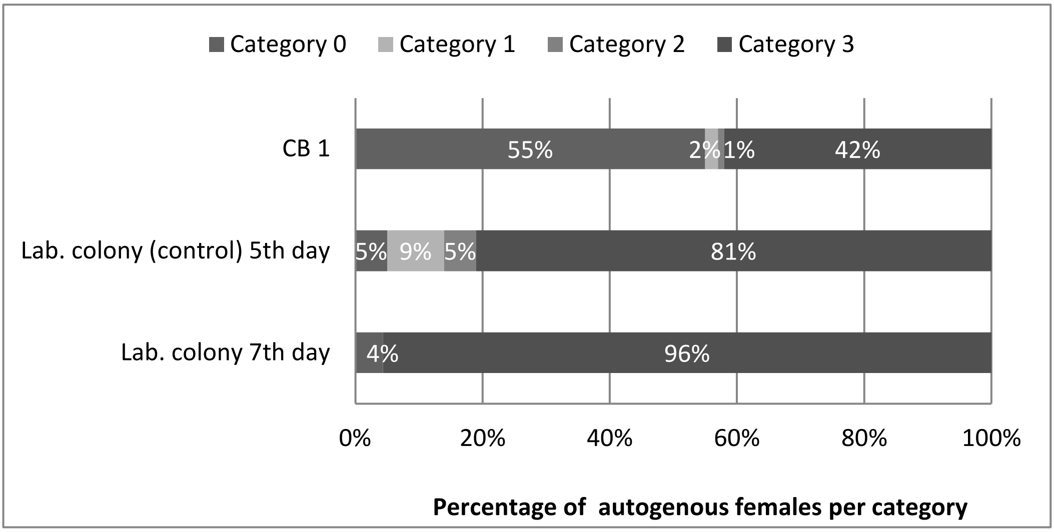

Figure 8. Percentages of females categorized according to oocyte/egg developmental stages (categories 0-3): CB1 population, laboratory colony (control; dissected on day 5 post-eclosion), and females from the first experiment dissected on day 7 after eclosion.

## Discussion

The *Cx. pipiens* complex includes two distinct biotypes, *Cx. pipiens* biotype *molestus* and *Cx. pipiens* biotype *pipiens*. Females of *Cx. pipiens* biotype *molestus* express autogeny, whereas those of biotype *pipiens* do not and are considered anautogenous. Autogeny is the ability of female mosquitoes to develop their first batch of eggs without obtaining a blood meal (Clements, 1992).

Dipteran oogenesis is a highly regulated sequence of development triggered by nutrition and controlled by hormones and neurohormones (Chapman, 2013; Roy *et al.*, 2018). In some mosquito species, mating is a prerequisite for autogeny (O'Meara and Petersen, 1985).

In this study, 90% of *molestus* females kept without males formed eggs, compared with 88% of those kept with males. The absence of a significant difference between the two groups indicates that males had no effect on autogeny expression in females.

In laboratory tests conducted by Su and Mulla (1997), 40% of *Culex tarsalis* females laid autogenous egg rafts without mating. Kassim *et al.* (2012) reported that 73.3% of unmated *Cx. pipiens* biotype *molestus* females also laid egg rafts, although these eggs were not viable.

The influence of mating has been documented in several mosquito species. O'Meara and Evans (1977) reported that populations of the salt marsh mosquito *Aedes taeniorhynchus* (Wiedemann, 1821) include both autogenous and anautogenous females, and that some autogenous females require a mating stimulus to produce eggs. Autogenous egg production associated with mating is triggered by substances from the male accessory glands. Klowden and Chambers (1991) reported that male-derived factors can influence egg development in anautogenous *Ae. aegypti* females that have been stressed by suboptimal nourishment. In *Deinocerites* species, mating can also stimulate the initiation of autogenous egg development. In *Deinocerites cancer* Theobald, 1901, females, autogeny can be triggered by either sugar feeding or mating, whereas in sugar-fed *Deinocerites pseudes* Dyar and Knab, 1909, autogeny is expressed only after mating (O'Meara and Petersen, 1985).

In this study, an increased percentage of females without formed oocytes was observed 20 and 25 days after eclosion in both groups, suggesting that females may resorb their eggs when oviposition is not possible. Rossay (1969) noted that egg resorption in normally developing ovaries of young females can occur when nutritional resources are insufficient for all follicles to reach maturity. In *Anopheles maculipennis* Meigen, 1818, follicular degeneration has been linked to insufficient food, the physiological age of females, unfavorable climatic conditions, and the presence of retained eggs in the ovarioles (Detinova *et al.,* 1962).

Mosquitoes, like other Diptera, have polytrophic meroistic ovaries that contain a basal germarium and multiple ovarioles. Each ovariole houses a series of developing follicles. Each follicle consists of an oocyte, a surrounding follicular epithelium, and nurse cells (trophocytes) connected by cytoplasmic bridges that supply the oocyte with necessary components (Chapman 2013; McLaughlin and Bratu, 2015). Isoe *et al.* (2024) noted that anautogenous female mosquitoes are valuable research models for studying egg development and related processes like follicle resorption and eggshell formation. Species such as *Aedes aegypti* (Linnaeus, 1762) illustrate how multiple hormonal and molecular pathways regulate egg development, with the balance among these pathways governing developmental outcomes. The gonotrophic cycle (the period between blood meal and egg laying) is a key factor in this development. During this cycle, mature eggs are deposited, and the eggshell, a protective layer, is formed through the deposition of specific proteins. The same authors claimed that follicle resorption (oosorption) occurs when oocytes are resorbed under conditions of insufficient nutrient availability. Investigating these processes in mosquitoes provides valuable insights into the molecular mechanisms underlying eggshell formation and follicular resorption.

This study showed that autogenous *molestus* females occur in subterranean developmental habitats such as catch basins, while anautogenous females were predominantly found in open, above-ground developmental habitats such as ditches. This pattern suggests that only *pipiens* females inhabit the ditch environments.

Although the underground habitat contained a higher proportion of autogenous females than the ditches, this study did not show a dominance of autogenous *Cx. pipiens* biotype *molestus* in the catch basins, which can be attributed to the type of catch basins from which the juvenile stages were sampled. The catch basins had an open design with a grid cover, allowing easy access for the eurygamous biotype to obtain an obligatory blood meal. Becker *et al.* (1999) reported similar patterns: in isolated subterranean cisterns with restricted access, 100% of females were autogenous; in underground airshafts and catch basins with less restricted access, only 44% were autogenous; and in above-ground habitats, autogenous females were infrequent, comprising only 0-20% of the population. Analysis of specimens from underground habitats revealed a small proportion of biotype *pipiens*, while most individuals were identified as biotype *molestus*. A considerable number of hybrid forms were also present, which may be significant for WNV epidemiology (Reusken *et al.*, 2010). In research conducted in Amsterdam, biotype *molestus* individuals were detected at a low frequency (4%) in above-ground developmental habitats (Vogels *et al.*, 2015), similar to the 2.15% observed in this study. These findings indicate that biotype *molestus* is not strictly confined to subterranean developmental habitats. Vogels *et al.* (2015) reported not only biotypes *pipiens* and *molestus* in above-ground habitats but also hybrid forms. In this study, individuals of both biotypes were found at the same locations, which may be epidemiologically significant as a potential source of WNV infection, given the possibility of hybridization between the two biotypes (Kim *et al.*, 2018).

## Conclusion

Females mosquitoes kept with males had more synchronized development of oocytes than those without males. However, no significant difference between the two groups was detected when comparing the percentages of autogenous females. This showed that the presence of males did not significantly influence autogeny expression in females. The increased percentage of females without formed oocytes 20 and 25 days after eclosion in both groups suggests that females may resorb their eggs when oviposition is not possible. Females of *Cx. pipiens* in two ditches did not express autogeny, whereas in the third ditch, a small proportion of females were autogenous. These results indicate that mosquitoes found in ditches mainly belong to biotype *pipiens*, or possibly include hybrids, which would require molecular confirmation. In the two analyzed catch basins, fewer than half of the females were autogenous, which may indicate the presence of hybrids, considering that both *molestus* and *pipiens* biotypes occur in the same breeding sites.

# References

Becker, N., Jost, A., & Weitzel, T. (2012). The *Culex pipiens* complex in Europe. *Journal of the American Mosquito Control Ass*. *28*, 53-67.

Becker, N., Jost, A., Weitzel, T., & Rettich, K. (1999). Exploiting the biology of urban mosquitoes for their control. *In Proceedings of the 3rd International Conference on Urban Pests*. Czech University of Agriculture, Prague, Czech Republic Vol. 19, 4259 pp.

Becker, N., Petrić, D., Zgomba, M., Boase, C., Madon, M., Dahl, C., Kaiser, A. (2010). Mosquitoes and their control: Second Edition. *Springer Science & Business Media.* https://doi.org/10.1007/978-3-540-92874-4

Bell, K. L., Noreuil, A., Molloy, E. K., & Fritz, M. L. (2024). Genetic and behavioral differences between above and below ground *Culex pipiens* bioforms. *Heredity*, *132*(5), 221-231.

Blom, R., Krol, L., Langezaal, M., Schrama, M., Trimbos, K. B., Wassenaar, D., & Koenraadt, C. J. (2024). Blood-feeding patterns of *Culex pipiens* biotype *pipiens* and *pipiens*/*molestus* hybrids in relation to avian community composition in urban habitats. *Parasites & Vectors*, *17*(1), 95.

Chapman, R.F. (2013). The insects: Structure and function. *Cambridge University Press* 978-0-521-11389-2

Ciota, A. T., Chin, P. A., & Kramer, L. D. (2013). The effect of hybridization of *Culex pipiens* complex mosquitoes on transmission of West Nile virus. *Parasites & vectors*, *6*, 1-4.

Clements, A. (1992). The biology of mosquitoes: Development, Nutrition and Reproduction. Chapman *and Hall*, 2-6 Boundary Row, London

Cvjetković, I. H., Petrović, T., Petrić, D., Milošević, U., Radovanov, J., Kovačević, G., & Milošević, V. (2017). Usutu virus: an emerging flavivirus in Europe. *Archives of Veterinary Medicine*, *10*(1), 25-35.

Detinova, T. S., Bertram, D. S., & World Health Organization. (1962). Age-grouping methods in Diptera of medical importance, with special reference to some vectors of malaria. *World Health Organization.*

Dimitrijević, D., Milinković, M., Grgić, B., & Ćosić, J. (2014). The current epidemiological situation of WNV in Serbia. *In Proceedings of XVI Epizootiology days of Serbia*

Fritz, M.L., Walker, E.D., Miller, J.R., Severson, D.W., & Dworkin, I. (2015). Divergent host preferences of above-and below-ground *Culex pipiens* mosquitoes and their hybrid offspring. *Med Vet Entomol*. *29*(2):115–23.

Isoe, J., Riehle, M.A., & Miesfeld, R.L. (2024) Mosquito Egg Development and Eggshell Formation. *Cold Spring Harb Protoc*. 10, pdb.top107669. doi: 10.1101/pdb.top107669. PMID: 38190637.

Kassim, N. F. A., Webb, C. E., & Russell, R. C. (2012). Is the expression of autogeny by *Culex molestus* Forskal (Diptera: Culicidae) influenced by larval nutrition or by adult mating, sugar feeding, or blood feeding? *Journal of Vector Ecology*, *37*(1), 162-171.

Kim, S., Trocke, S., & Sim, C. (2018). Comparative studies of stenogamous behaviour in the mosquito *Culex pipiens* complex. *Medical and Veterinary Entomology*, *32*(4), 427–435. https://doi.org/10.1111/mve.12309

Klowden, M. J., & Chambers, G. M. (1991). Male accessory gland substances activate egg development in nutritionally stressed *Aedes aegypti* mosquitoes. *Journal of insect physiology*, *37*(10), 721-726.

McLaughlin, J.M., & Bratu, D.P. (2015). *Drosophila melanogaster* oogenesis: An overview. *Methods Mol Biol* 1328:1–20. doi: 10.1007/978-1-4939-2851-4_1

Mclean, R. G., Ubico, S. R., Docherty, D. E., Hansen, W. R., Sileo, L., & McNamara, T. S. (2001). West Nile Virus Transmission and Ecology in Birds. *Annals of the New York Academy of Sciences*, *951*(1), 54-57. https://doi.org/10.1111/j.1749-6632.2001.tb02684.x

O'meara, G. F., & Evans, D. G. (1977). Autogeny in saltmarsh mosquitoes induced by a substance from the male accessory gland. *Nature*, *267*(5609), 342-344.

O'Meara, G. F., & Petersen, J. L. (1985). Effects of mating and sugar feeding on the expression of autogeny in crabhole mosquitoes of the genus *Deinocerites* (Diptera: Culicidae). *Journal of medical entomology*, *22*(5), 485-490.

Petrić, D., Petrović, T., Cvjetković, I. H., Zgomba, M., Milošević, V., Lazić, G. & Petrić, M. (2017). West Nile virus 'circulation' in Vojvodina, Serbia: Mosquito, bird, horse and human surveillance. *Molecular and cellular probes*, *31*, 28-36.

Petrović, T., Šekler, M., Petrić, D., Lazić, S., Debeljak, Z., Vidanović, D., Ignjatović Ćupina, A., Lazić, G., Lupulović, D., Kolarević, M. & Plavšić, B. (2018). Methodology and results of integrated WNV surveillance programmes in Serbia. *PLoS One*, *13*(4), p.e0195439.

Petrović, T., Šekler, M., Petrić, D., Vidanović, D., Debeljak, Z., Lazić, G., Lupulović, D., Kavran, M., Samojlović, M., Ignjatović Ćupina, A., & Tešović, B. (2021). Intensive West Nile virus circulation in Serbia in 2018—results of integrated surveillance program. *Pathogens*, *10*(10), p.1294.

Reusken, C. B. E. M., De Vries, A., Buijs, J., Braks, M. A. H., Den Hartog, W., & Scholte, E.J. (2010). Scientific Note First evidence for presence of *Culex pipiens* biotype *molestus* in the Netherlands, and of hybrid biotype pipiens and molestus in northern Europe. *Journal of Vector Ecology*, *35*(1), 210-212.

Rosay, B. (1969). Anatomical indicators for assessing age of mosquitoes: changes in ovarian follicles. *Annals of the Entomological Society of America*, *62*(3), 605-611.

Roy, S., Saha, T.T., Zou, Z., & Raikhel, A.S. (2018) Regulatory pathways controlling female insect reproduction. *Annu Rev Entomol* 63:489–511. doi: 10.1146/annurev-ento-020117-043258

Šiljegović, S., Mouillaud, T., Jiolle, D., Petrić, D., Ignjatović-Ćupina, A., Vasić, A., & Kavran, M. (2024). *Dirofilaria* sp. and blood meal analysis in mosquitoes collected in Vojvodina and Mačva, and the first report of Setaria tundra (Issaitshikoff & Rajewskaya, 1928) in Serbia. *Animals*, *14*(9), 1255.

Šolaja, S., Goletić, Š., Veljović, L. & Glišić, D. (2024). Complex patterns of WNV evolution: a focus on the Western Balkans and Central Europe. *Frontiers in veterinary science*, *11*, p.1494746.

Su, T., & Mulla, M. S. (1997). Nutritional reserves, body weight, and starvation tolerance of autogenous and anautogenous strains of *Culex tarsalis* (Diptera: Culicidae). *Journal of medical entomology*, *34*(1), 68-73.

Turell, M. J. (2012). Members of the *Culex pipiens* Complex as Vectors of Viruses1. *Journal of the American Mosquito Control Association*, *28*(4s), 123-126.

Vereecken, S., Vanslembrouck, A., Kramer, I. M., & Müller, R. (2022). Phenotypic insecticide resistance status of the *Culex pipiens* complex: a European perspective. *Parasites* & *Vectors*, *15*(1), 423.

Vogels, C. B. F., Van De Peppel, L. J. J., Van Vliet, A. J. H., Westenberg, M., Ibañez-Justicia, A., Stroo, A., Buijs, J. A., Visser, T. M., & Koenraadt, C. J. M. (2015). Winter activity and aboveground hybridization between the two biotypes of the West Nile virus vector *Culex pipiens*. *Vector-Borne and Zoonotic Diseases*, *15*(10), 619–626. https://doi.org/10.1089/vbz.2015.1820

Vogels, C. B., Fros, J. J., Göertz, G. P., Pijlman, G. P., & Koenraadt, C. J. (2016). Vector competence of northern European Culex pipiens biotypes and hybrids for West Nile virus is differentially affected by temperature. *Parasites & vectors*, *9*(1), 393.

Vujić, A., Stefanović, A., Dragičević, I., Matijević, T., Pejčić, L., Knežević, M. & Veselić, S. (2010). Species composition and seasonal dynamics of mosquitoes (Diptera: Culicidae) in flooded areas of Vojvodina, Serbia. *Archives of Biological Sciences*, *62*(4), 1193-1206.

# АУТОГЕНА ЕКСПРЕСИЈА У КОМПЛЕКС ПОПУЛАЦИЈАМА *CULEX PIPIENS* У СРБИЈИ

НАЂА КУКИЋ, ТАМАРА ПОПОВИЋ, ДУШАН ПЕТРИЋ,
АЛЕКСАНДРА ИГЊАТОВИЋ ЋУПИНА и МИХАЕЛА КАВРАН

## Извод

Са аспекта јавног здравља, *Culex pipiens* (кућни комарац) представља најзначајнију врсту комараца у Србији. Потврђена векторска компетентност ове врсте омогућила је успешан пренос вируса Западног Нила (WNV) на људе и животиње у Србији током више од једне деценије. Ипак, биологија и екологија ове врсте су недовољно истражене. Ова студија има за циљ да допринесе бољем изучавању биологије ове врсте.

Ово истраживање усмерено је на аутогеност код женки *Cx. pipiens* и има задатак да оцени да ли присуство мужјака утиче на појаву аутогености код женки. Ларве *Cx. pipiens* complex сакупљене су из три канала и два урбана улична шахта, и узгајане до стадијума адулта. Експериментални узорци обухватали су одрасле комарце добијене из пољских услова као и из лабораторијске аутогене колоније. Женке су смештене у кавезе са или без мужјака, и храњене су искључиво раствором шећера (без крвних оброка), а потом остављене неометане пет до седам дана како би се омогућио развој јаја. Место за овипозицију није било обезбеђено. Након тога, оваријуми женки су дисековани и вршена је процена њиховог развоја.

Резултати у лабораторијским условима су показали да присуство мужјака није утицало на појаву аутогености. Након 10–12 дана, женке нису имале јаја у абдомену, што указује да је дошло до ресорпције јаја услед немогућности овипозиције. У пољским условима аутогеност је била ретка: само два улична шахта и један канал су дали аутогене женке. Највиши забележени проценат аутогених женки био је 45 % (у шахту), док су остала позитивна места забележила стопе од 12,5 % (шахт) и 2,15 % (канал).

Имајући у виду наведено коегзистирање *pipiens* и *molestus* биотипова у истим акватичним стаништима, хибридизација је највероватније присутна. Хибриди који се потврђено хране и на птицама и на сисарима, сматрају се важним векторима у преносу WNV на људе и друге сисаре, те су због тога значајни са аспекта јавног здравља.

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