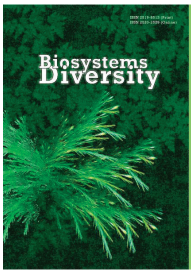

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Physiological age of female blood-sucking midges (Diptera, Ceratopogonidae) in the south of Tyumen oblast

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Despite the fact the fact that biting midges (Diptera, Ceratopogonidae) are abundant blood-sucking insects in a number of areas of Siberia and the Far East, their biology in Russia and abroad is poorly studied. The objective of our research was physiological age of female midges in the subzone of southern taiga of Tyumen Oblast. Physiological age of female midges was determined using the method of V. P. Polovodova and T. S. Detinova, suggested for mosquitoes, modified for midges, in relation to the number of the yellow bodies – enlarged ovarioles. The conducted studies focused on physiological age of females of three species of midges of the subgenus *Avaritia* (*Culicoides punctatus*, *C. fascipennis*, *C. grisescens*), which occur in the forest zone of the southern taiga subzone. The species are abundant blood-sucking insects. Mostly, they complete one gonotrophic cycle, but by the end of the season, the number of females which had laid eggs twice reached 20–30%. Part (10–22%) of the female population does not succeed in completing even a single gonotrophic cucle. In spite of colder weather in the end of summer, a rejuvenation of the population was observed, which occured due to death of physiologically old females. Comparison of changes in number and age composition of females allows us to state that the first two species have two, and the third – one generation during a season. Determination of physiological age of females or the number of gonotrophic cycles they complete, and therefore, the extent of their biting, is a subject of not only theoretical but also practical interest for assessing the epidemiological situation in the areas where midges are vectors of a number of infectious and invasive diseases, such as tularemia, onchocerciasis, bluetongue virus or febris catarrhalis ovium, and Schmallenberg virus. This indicates the relevance of studying density of population vectors of infections. In the Russian Federation, such research unfortunately has only been conducted within the scope of narrow studies, and only in certain regions.

Keywords: *Culicoides*; gonotrophic cycle; southern taiga subzone.

Introduction

Biting midges (Diptera, Ceratopogonidae) are characterized by gonotrophic harmony, a distinctive feature of which is that one blood meal is sufficient for development of a portion of eggs (Gluhova, 1989). Internal morphology of Ceratopogonidae is described in a wide range of studies (Gluhova, 1958; Amasova, 1959; Linley, 1985; Mullens & Schmidtmann, 1982). Ovaries of biting midges are similar to all Diptera of polymorphic type, i.e. each follicle, except for oocytes, includes specialized cells (Filimonova & Brodskaya, 1998; Lusuk, 2007, Lysyk & Danyk, 2007). Gonotrophic harmony of Ceratopogonidae is clearly seen in a range of species from different geographic zones (Gluhova, 1989).

Determination of physiological age of females and determination of the number of their gonotrophic cycles, and therefore blood meals, is a subject of not only theoretical but also practical interest for evaluating the epidemiological situation in the areas where biting midges are vectors of a range of infectious and invasive diseases (tularemia, onchocerciasis, bluetongue virus or febris catarrhalis ovium, Schmallenberg virus) (Gluhova, 1989; Sprygin et al., 2015, 2016). The physiological age of female midges allows one to make assumptions about the abundance of these insects.

The literature available to us, both Russian and foreign, does not provide a complete picture of analytical research on physiological age of female biting midges, since these studies were conducted in the 1960–1980s. Despite the fact that biting midges are abundant blood-sucking insects in a number of regions of Siberia and the Far East, their biology in Russia (Amasova, 1957; Buianova, 1966; Gornostaeva, 1969; Mirzaeva, 1974) and abroad (New York State, USA), is still poorly studied (Linley, 1966; Edwards, 1982; Birley & Boorman, 1982; Schmidmann & Washino, 1982; Mullens & Schmidmann, 1982; Zimmerman & Turner, 1984). The objective of our research was physiological age of females of biting midges (Diptera: Ceratopogonidae) in the subzone of Southern Taiga of Tyumen Oblast.

Materials and methods

The study was conducted in the outskirts of Malye Velizhany village in Nizhnetavdinsky district of Tyumen Oblast, located in the forest zone of the subzone of the southern taiga (Zapadnaja & Sibirj, 1963; Tarasenko, 1964; Atlas Tjumenskoi oblasti, 1971) in 2017. Physiological age of the female midges was determined using the method of Polovodova (1949) and Detinova (1949), suggested for mosquitoes, in modification for midges, according to the number of yellow bodies – enlarged ovarioles (Gluhova, 1956, 1958).

Autopsy of the female midges was conducted according to the method of Pavlovsky (1935), which consists of a number of rules for dissection of small insects and mites. The instruments used consisted of a set of standard laboratory tools (dissecting needles, tweezers, scissors, etc.), and also a spade-shaped dissecting needle created according to the model of Pavlovsky (1935), which has a wide end with semi-round free edge. Autopsy of the females was conducted in physiological solution on a watch glass under MBS-1 microscope. According to the highest number of yellow bodies per 1 ovariole, we assessed the number of ovipositions and completed gonotrophic cycles of the females. Also, for dividing the midge females into those which had laid eggs and those which had not (parous and nulliparous), the method of Dyce (1969) was used. This method is based on visual detection of pigment in the epithelium layer of the female's abdomen, which accumulated over oogenesis. The pigment is stable even during prolonged retention of females in 96 degrees spirit.

The midge imagoes were caught using a butterfly net with replaceable sacks (Detinova et al., 1978). Midge imagoes were caught in the morning from 4 to 7 am and in the evening from 20 pm till midnight. The interval between the counts was 1 hour. The species of midge imagoes were identified according to the tables composed by Mirzaeva (1963). We analysed the data according to the scheme of gonotrophic cycle, accepted in the literature (Fig. 1).

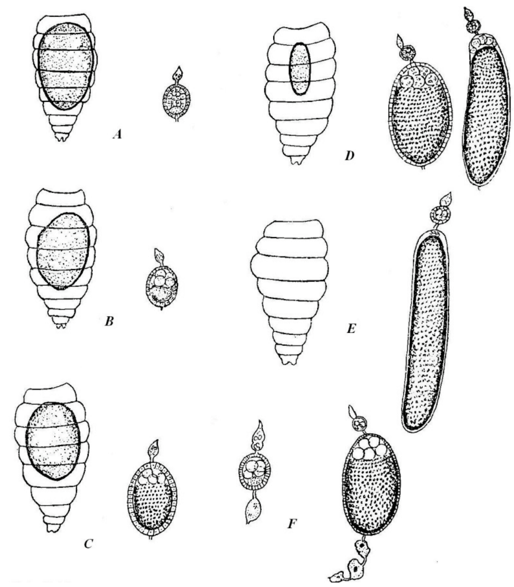

Fig. 1. Gonotrophic cycle of *Culicoides obsoletus* Mg. (according to Gluhova): *A* – the abdomen of a female which has just eaten, the ovary is not developed, the egg cell is not separated from follicular; *B* – the second stage of blood digestion, the egg cell is seen clearly; *C* – the third stage of blood digestion, the grains of yolk are clearly seen; *D* – the blood digestion process is close to completion, the egg becomes elongated; *E* – the blood is completely digested; the egg is developed and ready to be laid; *F* – the ovarioles with one and three enlargements.

Results

In the conditions of the southern taiga forest zone, the flight of midges began in the third decade of May, lasting to September – early October, depending on the snowfall. The total period of midges' activity during favourable meteorological conditions equaled 3.5–4.0 months. Duration and number of midges depends on meteorological factors.

The analysis of the species composition of the midges in relation to the seasonal dynamics revealed that the dominant species was *C. punctatus* Mg., and the subdominant species were *C. fascipennis* St. and *C. grisescens* Edw. Physiological age of the females of these three most numerous species of biting midges was studied in the southern taiga forest zone in the territory of Nizhnetavdinsky district (Velizhany village) over the summer of 2017. In total, 1,450 females were examined for systematic determination of the age composition. The results of these studies are presented in Table 1.

Table 1
Phenology and duration of the flight of midges in the forest zone of the southern taiga subzone

No	Species of blood-sucking midges	Extreme catch dates		Duration of summer, days
		the first	the last	
1	*C. obsoletus* Meigen, 1818	22.05	1.09	101
2	*C. chiopterus* Meigen, 1830	22.05	23.08	92
3	*C. gornostaevae* Mirzaeva, 1984	24.06	10.09	88
4	*C. pulicaris* Linnaeus, 1758	–	4.08	–
5	*C. punctatus* Meigen, 1804	22.05	5.10	137
6	*C. grisescens* Edwards, 1939	2.06	10.09	100
7	*C. reconditus* Campbell et Pelham-Clinton, 1960	16.06	–	–
8	*C. fascipennis* Staeger, 1839	2.06	10.09	100
9	*C. subfascipennis* Kieffer, 1919	13.06	23.08	71
10	*C. pallidicornis* Kieffer, 1919	2.06	26.08	85
11	*C. circumscriptus* Kieffer, 1918	2.06	27.07	56
12	*C. salinarius* Kieffer, 1914	3.06	–	–
13	*C. manchuriensis* Tocunaga, 1941	–	19.08	–
14	*C. nubeculosus* Meigen, 1830	13.06	19.08	67
15	*C. stigma* Meigen, 1818	22.05	29.08	99
	The overall period of summer	22.05	5.10	137

Table 2
The ratio of age groups of female midges during the season of 2017

Decades of the months	*C. punctatus*				*C. fascipennis*				*C. grisescens*			
	number of examined females	uniparous			number of examined females	uniparous			number of examined females	uniparous		
		nulliparous		biparous		nulliparous		biparous		nulliparous		biparous
II decade of June	100	100/100	0	0	50	50/100	0	0	–	–	–	–
III decade of June	100	88/88	12/12	0	100	94/94	6/6	0	10	10/100	0	0
I decade of July	100	74/74	26/26	0	100	79/79	21/21	0	15	12/80	3/20	0
II decade of July	100	57/57	41/41	2/2	100	44/44	46/46	10/10	20	9/45	11/55	0
III decade of July	100	29/29	58/58	13/13	100	32/32	53/53	15/15	20	5/25	14/70	1/5
I decade of August	100	56/56	34/34	10/10	100	51/51	42/42	7/7	10	3/30	6/60	1/10
III decade of August	100	22/22	48/48	30/30	20	2/10	11/55	7/35	5	1/20	3/60	1/20
I decade of September	100	46/46	40/40	14/14	–	–	–	–	–	–	–	–

Notes: numerator – number of females; denominator – the percentage of autopsized females.

C. punctatus, the most numerous species, was caught during our study from mid June till the first decade of September. The first maximum was observed in the III decade of June, and the second – in mid August. In total, 800 females were examined. In the first selection, among the examined females, only nulliparous females were found. Uniparous females were found in the third decade of June, equaling 12%. In July, in the conditions of general decrease in the number, we observed a corresponding decrease in the share of nulliparous females, which had fallen to 29% by the end of month. At the same time, the number of parous females increased to 71%, and in the middle of July, single females appeared, which had completed two gonotrophic cycles, and by the end of the month they equaled 3%. In early August, we observed an increase in the number of midges, which was accompanied by a significant increase in the number of nulliparous females, the number of which in the first decade equaled 56%. Among the females which had laid eggs, 34% were uniparous, and 10% – biparous. By late August, the number of the females which had oviposited increased to 78%, including those that had laid eggs once – 48%, and those that had laid twice – 30%. Due to unfavourable weather conditions and the limited period of daily activity in the first decade of September the population became generally younger due to the physiological death of old females. The number of parous females decreased to 54%, among which those which had laid eggs twice equaled 14%.

The comparison of the seasonal course of number and age composition of midge females of *C. punctatus* indicated that the second rise in numbers was accompanied by a rejuvenation of the population, which is explained by the beginning of the flight of second generation of this species.

C. fascipennis, the subdominant species, was recorded from mid June to late August. A total of 570 females were examined. The females which had laid eggs once appeared at the end of the third decade of July (10%). By the end of the third decade of July, the percentage of nulliparous females equaled 32%, and the number of those which had completed one and two ovipositions equaled 53% and 15% respectively. A rejuvenation of the population was observed in early August, when the number of nulliparous females increased to 51%. By late August, the population had significantly decreased, and out of 20 examined females, 10% were nulliparous, 55% – uniparous and 35% – biparous. The rejuvenation of the population in the early August, which coincided with increase in the number of midges of this species, indicates the presence of two generations.

C. grisescens is a low abundance species which was recorded from the III decade of June to late August. Because of the low abundance of this species, we examined only 80 females. Females which had laid eggs once appeared in the I decade of July, and those which had laid twice – in the III decade of July. The number of parous females increased till the end of the flight of this species, the highest number of the females which laid eggs once equaled 60, and those which laid eggs twice – 20%. The obtained data indicate that *C. grisescens* in southern Tyumen Oblast is univoltine.

Thus, the research conducted on the determination of physiological age revealed that in the conditions of southern Tyumen Oblast, the females of the most abundant species – *C. punctatus*, *C. fascipennis* and *C. grisescens* – mostly complete one gonotrophic cycle (55–60%), but by the end of the season, the number of females which had laid eggs twice reached 20–30%. A significant part of population of females (10–22%) did not succeed in completing a single gonotrophic cycle. As it became colder, at the end of the period of their activity, a rejuvenation of the population was observed due to death of physiologically old females. The comparison of changes in numbers and age composition of the females allow us to state that the first two species have two generations, and the latter – one generation over a season.

Discussion

Natural conditions in Tyumen Oblast contribute to massive breeding of blood-sucking Diptera insects of the gnat complex. They are broadly distributed in the area and cause significant damage. The territory of Tyumen Oblast is characterized by a ramified river network, a large number of lakes of different size and swampiness, the region has different biotopes for development of midges with wide ecological flexibility in choosing sites for breeding. Apart from natural water bodies, pre-imago phases of midge development can take place in water bodies of anthropogenic nature (for example, puddles and dips near livestock complexes), rich in organic matter. The currently used classification of sites of development of pre-imago phases of midges was suggested by Gluhova (1989), who divided the breeding sites of midges into two main groups – aquatic and ground habitat. Ground habitat consists of decomposing wood and bark, and also decomposing organic matter of animal or plant origin. Aquatic habitat includes subgroups of completely aquatic and semi-aquatic environment. Completely aquatic habitats are constant water bodies: lakes, ponds, rivers, streams, thermal water bodies and mineral springs. The semi-aquatic habitats are considered to be non-constant water bodies, swamps, marshy meadows. Most midge imagoes attack near their breeding sites, the distance of their flight is related to the relief of the area, pattern of vegetation, direction and force of wind.

The studies on the fauna and ecology of blood-sucking midges in the south of the Oblast were performed mostly in the 1960–1980s, which means that they had not been studied for over that 40 years, and research on physiological age of female biting midges in the of southern taiga subzone had not been conducted at all. This issue remains relevant today, being a subject of theoretical and practical interest for assessing the epidemiological situation in the areas where midges are vectors of a number of infectious and invasive diseases.

The seasonal course of changes in the abundance of midges is characterized by rises in the number of midges, caused in large measure by two generations of the dominant species *C. punctatus*, while only one species dominates throughout the summer. The first increase in the number took place in the second-third decades of June or the first decade of July, and the second – in August. In other regions of Russia, researchers have noted that the rise of number of midges during the season is formed not by one species, but by several species. For example, the dominant species in southern Karelia are *C. impunctatus*, *C. obsoletus* and *C. grisescens*, in central Karelia – *C. obsoletus*, *C. punctatus*, *C. grisescens*, and the latter species determines the second peak in the number of midges (Gluhova, 1962). In the conditions of central and southern part of Perm Oblast, the first maximum in abundance occurs due to *C. obsoletus*, and the second – due to *C. obsoletus*, *C. punctatus* (Burylova, 1992). In the southern taiga of the Ob region, due to the environmental conditions, the ratios of the dominant species (*C. obsoletus*, *C. punctatus*, *C. grisescens*) which cause two peaks of abundance can change.

Over the period of monitoring, we determined 3 types of 24-hour rhythm of midge activity. The first type is characterized by evening, night and morning increases in the number and absence of midges over the day. The second type is characterized by rises in abundance only in evening and morning hours, with no activity for the rest of the time. The third type is characterized by the activity of midges from morning to evening, the number of the insects being low during the day, and absent in the night. Over the period of study, the highest number of midges (63.3%) was observed in the morning. The highest activity of midges was observed within the temperature range of +7.0…+18.9 ºC. Research on the daily activity of midges in Karelia allowed V. M. Gluhova to determine 5 types of rhythm. In other regions of Russia, where no "white nights" occur, the researchers more often observed 2–3 types, among which the most common is a 24-hour rhythm characterized by morning and evening increases in number and absence of night and daily activity of midges (Mirzaeva, 1963). In Perm Oblast, Burylova (1992) 4 types of 24-hour rhythm of activity have been found.

Regarding the distribution of attacking midges over 24-hours, the results of our studies do not coincide with the data of the abovementioned authors, according to which the increase in the number of attacking midges can be higher in the evening than in the morning. A. G. Mirzaeva explains higher evening abundance of midges by favourable conditions for them to attack at this time thrughout the season in southern Tomsk Oblast. In particular, such conditions are optimum illuminance for evening activity, a temperature not lower than +7 ºC, and also the subsequent darkness which reduces, but does not stop, the attacks of midges. The higher morning peak of the number of midges can be explained by the fact that our studies were conducted in the conditions of constant enclosure of animals in pens from evening to morning. At the same time, the midges which attacked in the evening did not fly away from the territories of pens, and in the morning, there was a concentration of the insects due to the arrival of new insects at sunrise.

During the search for objects for blood-sucking, the main orientation of midges is olfactory, in contrast to horse-flies, mosquitoes and black flies, which mostly orientate visually. Only for midges, did we observe flight towards an object from the lee side.

As a result of study of physiological age of females of abundant species, we determined that *C. punctatus*, *C. grisescens* in the conditions of the studied region complete no more than two gonotrophic cycles. A significant number of females (10–22%) failed to complete even one gonotrophic cycle. Due to the fall in temperature at the end of season of activity, a rejuvenation of the population was observed, which occurred due to death of physiologically old females. According to the literature data, the repetition of gonotrophic cycles depends not only on the species of insect or geographic conditions of the region, but also on weather conditions of the season (Gluhova, 1989; Shevchenko, 1977). In Karelia, *C. grisescens* females complete up to 4, and *C. punctatus* – two gonotrophic cycles (Gluhova, 1956). In the territory of Ivanovo Oblast, *C. grisescens* females are also able to complete 4 cycles, and *C. punctatus* and *C. fascipennis* – 3 (Isaev, 1972). In the conditions of Perm Oblast, among *C. grisescens* and *C. punctatus*, there were also observed females with two yellow bodies, i.e. which had completed two gonotrophic cycles (Burylova, 1965). At the temperature of +18…+22 ºC, the cycle was completed in 4–6 days, and at +23…+26 ºC – 2–3 days (Gornostaeva, 1965). Therefore, their number depends on the life expectancy of individuals, temperature and other conditions.

Conclusion

Females of the most numerous species of midges in the conditions of southern Tyumen Oblast complete mostly one gonotrophic cycle, but by the end of the season in August – September, the number of females among these species which had laid eggs two times reaches 20–35%. The comparison of changes in number and age composition of females allows us to state that *C. punctatus* and *C. fascipennis* have two generations, and *C. grisescens* – one generation during a season.

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