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Influence of Predatory Plant Bladderwort (*Utricularia vulgaris*) on the Process of Selection in Malaria Mosquito Larvae

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Abstract – Influence of the carnivorous plant *Utricularia vulgaris* on the death rate of larvae of the malaria mosquito *Anopheles messeae* of the fourth instar was studied. The death rate of larvae changed at the end of the reproductive season and was dependant on the size, density, and sexual and karyotypic composition of larvae. The survival of the individuals with the inversion XL_0 stable to asphyxia encourages the coexistence of the predator and the prey. Combination of predation and protocooperation between interacting species is discussed.

Research of intraspecific variability, of its role in processes of adaptation and evolution of organisms, is one of the main tasks of population ecology and population genetics. The traditional approach consists of the isolation of the genetical markers or separate phenes, evaluation of their adaptive importance, and analysis of the supporting mechanisms of the polymorphism. However, at present it has become evident that adaptive importance of the genotype is variable, and scientific interests have gradually shifted. Particular attention is given to the factors that influence adaptation, especially to biotic factors. It is generally agreed that the main ways of the biotic interactions are intra- and interspecific competition, and predation and phytophagy are additional mechanisms that regulate specific diversity. Genetical consequences of biotic interrelations are the subject of discussion. It is assumed (Giller, 1988) that the evolutionary importance of competition consists of the divergence of niches, and the evolutionary importance of predation consists of the development of adaptations that permit the victim to coexist with the predator.

It is necessary to conduct analysis of the mechanisms of the interacting species selection in the populations in order to discuss the genetical effects of biotic interrelations. The aim of this work was to study the influence of the predatory plant bladderwort (*Utricularia vulgaris* L.) on the process of selection in the populations of insects that it consumed. Larvae of the malaria mosquito *Anopheles messeae* Fall., 1926 (Diptera, Culicidae), characterized by a high level of chromosome variability, were used as a victim.

MATERIAL AND METHODS

The objects of this study were samples of larvae of the fourth instar of the malaria mosquito *A. messeae*, taken on September 9 and 29, 1992, from a water body in the Kolarovo settlement in the Tomsk region. The plant *U. vulgaris* was taken from the same water body at the same time. The larvae were sorted into five groups by their size. Individuals of the oldest group (prepupae, with a body length of 8.0 - 8.7 mm) were rejected; the rest were used in the experiment (Table 1).

Experiments were conducted in two stages: experiment number 1 – 2 replications and control of 104 larvae (sample of September 9, 1992); experiment number 2 – 6 replications and 4 controls of 50 larvae, 2 replications and control of 100 larvae (sample of September 29, 1992). The larvae were kept in plastic dishes 21 × 10.5 cm with 416 ml (experiment 1) or 400 ml (experiment 2) of water. There was bladderwort together with the larvae in the experimental dishes and hornwort (*Ceratophyllum demersum* L.) in the control (70 cm of the plant stems per dish). Exposure time was 23 h at 22°C.

The surviving mosquitoes were fixed by an alcohol–acetic mixture (3 : 1). Specimens of the polytene chromosomes were prepared from the salivary glands of the fixed larvae by lactoacetorcein procedure (Kabanova *et al.*, 1972). Sex of larvae was determined by the chromosomes. Homo- and heterozygotes were registered in karyotypes according to five paracentric inversions: XL_1, XL_2, $2R_1$, $3R_1$, and $3L_1$. Localization and designation of the inversions were given earlier (Stegnii, 1991). A total of 435 karyotypes of larvae was studied.

RESULTS AND DISCUSSION

Bladderwort *Utricularia vulgaris* (Scrophulariales, Lentibulariaceae) is a carnivorous plant that lives in stagnant water bodies and swamps. Catching bladders of 2 - 5 mm diameter (Fig. 1) are situated on submerged pinnate leaves of the plant. The bladder has a mouth and valve, which opens at slight touch, sucking in animals with water. The victims of bladderwort are small aquatic animals: infusorians, crustaceans – daphnias and cyclopoids, larvae of mosquitoes, worms, and even fish fry (Zhukovskii, 1964; Zemskova, 1981; Kokin, 1982).

The fourth instar larvae of the malaria mosquito are the largest victim for the bladderwort, so far as their

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Table 1. Death rate of the maggots of *A. messeae* in the experiments with bladderwort

Number of replications	Size of larvae (body length), mm	Number of individuals	Perished ($f \pm s_f$), %		
			on the bottom of the dish	consumed by the plant	total
Experiment 1					
1	4.5 - 5.4	104	0	56.7 ± 4.9	56.7 ± 4.9
2	4.4 - 5.4	104	6.7 ± 2.5	37.5 ± 4.7	44.2 ± 4.9
Control					
1 - 2	4.4 - 5.4	104	0	–	0
Experiment 2					
1	7.2 - 7.9	50	4.0 ± 2.8	0	4.0 ± 2.8
Control	7.2 - 7.9	50	0	–	0
2	6.5 - 7.1	50	4.0 ± 2.8	2.0 ± 2.0	6.0 ± 3.4
Control	6.5 - 7.1	50	2.0 ± 2.0	–	2.0 ± 2.0
3	5.5 - 6.4	50	0	6.0 ± 3.4	6.0 ± 3.4
Control	5.5 - 6.4	50	0	–	0
4	4.5 - 5.4	50	8.0 ± 3.8	14.0 ± 4.9	22.0 ± 5.8
5	4.5 - 5.4	50	4.0 ± 2.8	10.0 ± 4.2	14.9 ± 4.9
6	4.5 - 5.4	50	2.0 ± 2.0	16.0 ± 5.2	18.0 ± 5.4
Control					
4 - 6	4.5 - 5.4	50	0	–	0
7	4.5 - 5.4	100	0	22.0 ± 4.1	22.0 ± 4.1
8	4.5 - 5.4	100	1.0 ± 1.0	29.0 ± 4.5	30.0 ± 4.6
Control					
7 - 8	4.5 - 5.4	100	3.0 ± 1.7	–	3.0 ± 1.7

lengths exceed the diameter of the bladders. Swallowing of the mosquitoes always begins from the back end of the abdomen, and the victim rolls up into a spiral inside the bladder (Fig. 2). Most of the bladders are situated in free water, so they catch mainly the larvae that dived from the surface of the reservoir in search of food or disturbed by external irritants. Bladderwort secures the mosquitoes under the water through the use of its catching apparatus, and they die from asphyxia (acute oxygen deficiency). It was shown in our previous studies (Gordeev, Perevozkin, 1995) that mortality of *A. messeae* caused by asphyxia is initiated by 15 min submergence, and its rate reaches 100% value only after 90 min. Consequently, bladderwort must secure living larvae under water for a long time. The largest and vigorous mosquitoes are able to get free of the catching apparatus of the plant and rise to the surface.

In this work, the dependence of mortality of *A. messeae* individuals from bladderwort on their size was investigated (see Table 1, experiment 2, replications 1 - 6). As would be expected, the quantity of mosquitoes consumed by the plant regularly decreased as their size increased, from 13 ± 2.7% in the youngest group (replications 4 - 6) to 0% in the oldest group (replication 1, $p < 0.05$). Some individuals died from asphyxia but were not consumed by the bladderwort. The quota of these individuals in replications 1 - 6 averaged 3.7 ± 1.0% and essentially exceeded mortality rate in the control ($\chi^2 = 3.87$, $df = 1$, $p < 0.05$). Analogous differences were observed in experiment 1.

The total mortality caused by the bladderwort sharply decreased with the increasing size of larvae even in the second and third size groups (experiment 2, replications 2, 3) as compared to the first group (replications 4 - 6, $\chi^2 = 6.53$, $df = 1$, $p < 0.05$). It is evident that the first day after the moult is the critical period in interrelations of the predator and the victim. Having overcame this period, the larvae of the fourth instar become practically unavailable to the carnivorous plant.

The population density of larvae is an important factor controlling the mortality rate from the bladderwort. Following a doubling of the density, the part of the individuals consumed by the plant increased from 13.3 ± 2.7% (replications 4 - 6) to 25.5 ± 3.1% (replications 7, 8, $\chi^2 = 7.11$, $df = 1$, $p < 0.01$). The increase in the number of victims is probably a result of intercollisions and increase in the mobility of larvae, which are characterized by high aggressiveness (Gordeev, Troshkov, 1990).

These facts suggest that bladderwort must be considered as the regulator of the abundance of mosquitoes

Fig. 1. Consumption of the *A. messeae* larvae of the fourth instar by catching bladder of *Utricularia vulgaris*. The pointer shows the mouth of the bladder.

in their breeding places, but the regulatory function of the plant varies during the season and depends upon the climatic conditions. The total mortality of larvae in early September, 1992 (experiment 1), amounted to 50.5 ± 3.5% and was significantly higher than at the end of the month – 26.0 ± 3.1% (experiment 2, replications 7, 8, $p < 0.01$) – under similar density and identical size of the individuals. This may be partly explained by the change in the state of the plants. In the period under study, the average daily temperature of water decreased below 10°C. There are a specific temperature interval and optimum for every vital process, as well as for functioning of the catching apparatus of the bladderwort. Any deviation from the optimum causes functional changes and a decrease in productivity. Lowering of the temperature below the so-called latent boundary decelerates active vital processes, and the protoplasm falls into a cold torpor. Metabolic disturbances in most water plants of the temperate zone begin at temperatures below 10°C, and a further decrease in temperature below 5°C causes irreversible damages (Larcher, 1976).

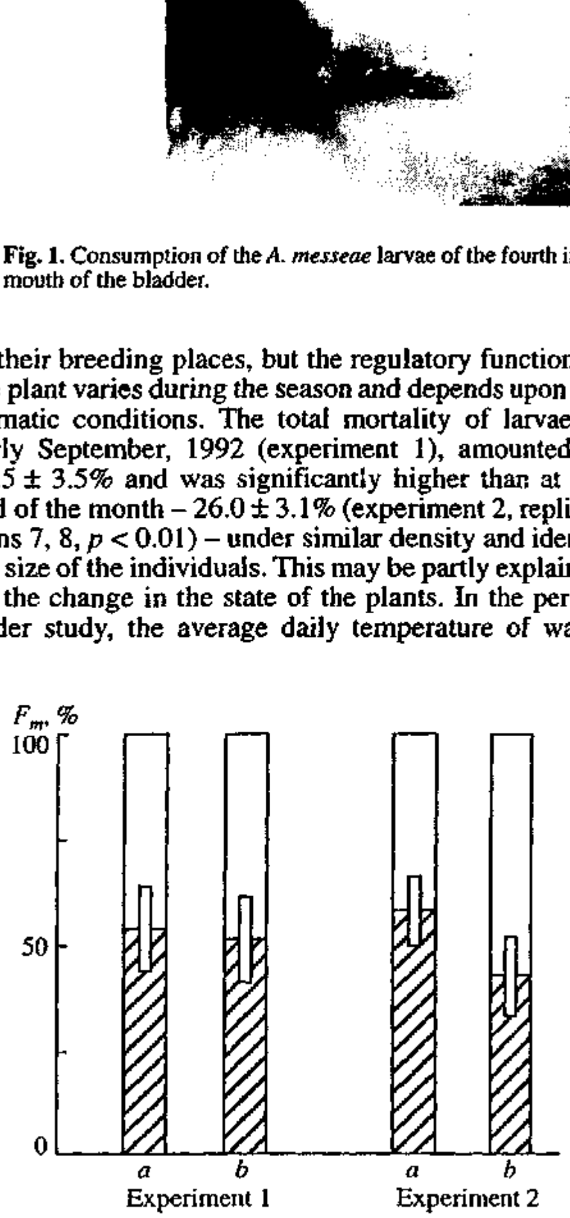

Fig. 2. Sex composition of *A. messeae* larvae in the experiments with bladderwort. Experiment 1: (*a*) replications 1, 2; (*b*) control 1, 2; experiment 2: (*a*) replications 7, 8; (*b*) control 7, 8. F_m – quota of the males (%).

Another explanation of seasonal changes of mortality of larvae from bladderwort may be rearrangement of the population–genetical structure of the mosquitoes. One of the indicators of population structure is the sex ratio of individuals. Samples of September 9, 1992, and September 29, 1992, did not significantly differ in sex ratio but, following interaction with the predatory plant, an increase in the part of the males among the survived larvae was registered in the second experiment (Fig. 3, $\chi^2 = 4.61$, $df = 1$, $p < 0.05$). Analogous but insignificant change in sex ratio was observed in the first experiment, as well. It seems likely that differential mortality is caused by the physiological features of larvae of different sex.

An important parameter of the population structure of *A. messeae* is chromosome variability. Populations of this species are highly polymorphic on five paracentric inversions: XL_1, XL_2, $2R_1$, $3R_1$, and $3L_1$ (Stegnii, 1991). The chromosome composition of mosquitoes in the habitat of Kolarovo settlement has been under control since 1972. Reversible annual changes in the frequency of the inversion depending upon the summer–winter

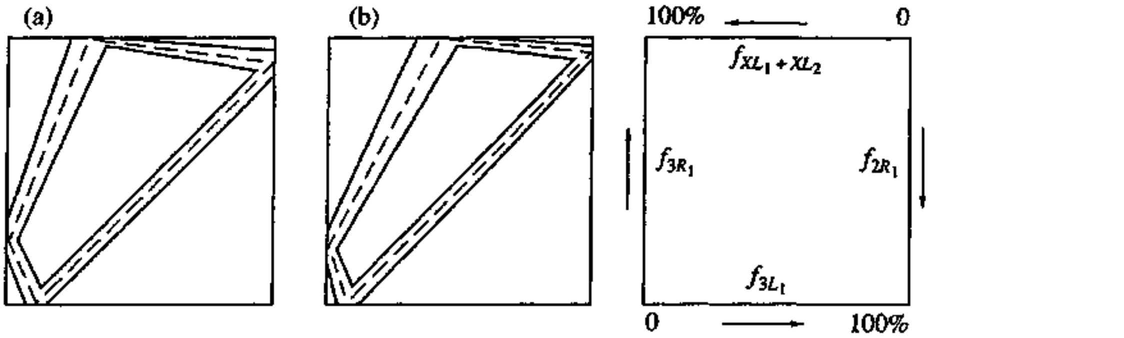

Fig. 3. Chromosome compositions of *A. messeae* larvae in the samples: (a) sample 09.9.92; (b) sample 09.29.92. On the sites of the square frequencies are plotted of inversions of the sex chromosome *XL* and autosomes (%) connected by the dotted line. The corresponding designations of inversions are given on the right square. Unbroken lines record 95% confidence intervals.

drop in temperature were registered (Stegnii, 1991). Warming of the climate in western Siberia causes transformation of the karyo-pool of *A. messeae*. The sharp increase in the part of autosomal sequences $2R_0$, $3R_0$, $3L_0$, which dominate at present in the population under study (Fig. 4), took place after 1982. The frequency of sex chromosome XL_0 increased simultaneously from 5 - 10 to 30 - 40%. All listed chromosome variations dominate in the southwest of the area and are evolutionarily original for the species (Stegnii, 1991).

Besides annual variations, the seasonal variations of the chromosome composition of the mosquitoes were revealed in Kolarovo settlement. It was shown (Stegnii, 1991) that frequencies of inversions XL_1, XL_2, $2R_1$, and $3R_1$, which dominate in the north and east of the area of inversions, increase in winter and decrease during the period of "prosperity" of the population and at the end of the reproductive season. This process was discovered in the samples studied by us (see Fig. 4) and mostly appeared in the variants of the sex chromosome (Table 2). In the control to experiment 2, the concentration of inversions XL_1 and XL_2 essentially decreased and part of the gametes with chromosome XL_0 increased, as compared with the control to experiment 1 ($\chi^2 = 7.92$, $df = 2$, $p < 0.05$).

It is possible that seasonal dynamics of the composition of chromosome *XL* caused the decrease in the mortality of larvae from the bladderwort. Results of the first experiment confirm this assumption. The increase in the frequency of the chromosomes XL_0 with respect to variants XL_1 and XL_2 is recorded among surviving larvae ($\chi^2 = 6.91$, $df = 2$, $p < 0.05$). The change in frequencies is determined mainly by the decrease in the quota of females with variants XL_{11} and XL_{12} against the chromosome composition of the females in the control (frequencies $XL_{11} + XL_{22}$ in the females in replications 1, 2 comprised 30.4 ± 6.8%, in the control 1, 2 – 58.0 ± 7.0%; $\chi^2 = 6.28$, $df = 1$, $p < 0.05$). In the second experiment, mortality was significantly lower, the composition of larvae was different, and frequency of chromosomes practically did not change.

These results suggest that a carnivorous plant is able to influence the processes of selection in the malaria mosquitoes. The selective role of bladderwort is probably connected with low tolerance to asphyxia in larvae with inversions XL_1 and XL_2 (Gordeev, Perevozkin, 1995); as well as higher aggressiveness and mobility of the individuals with these inversions (Gordeev, Troshkov, 1990). It should be stressed that the main differences in mortality are observed between the individuals with the caryotypes combining inversions XL_1 - $2R_0$ - $3R_0$ - $3L_0$ and XL_0 - $2R_0$ - $3R_0$ - $3L_0$. The former dominate in the southeast of the area, the latter in the southwest (Stegnii, 1991). However, both are supported by the *K*-selection (Gordeev, Stegnii, 1987). In fact, bladderwort modifies the action of the *K*-selection, causing an increase in the part of the "southwest" karyotypes. Rearrangement of the karyo-pool favors the coexistence of the plant and the insect.

The selective role of bladderwort appears at a specific stage of the seasonal cycle (the plant vegetates during the period of "prosperity" of the mosquito population) and at specific stages of development of larvae (the 3rd and the beginning of the 4th instars). Consumption of larvae of the junior ages should be considered

Table 2. Frequencies of the inversions of the sex chromosome *XL* in larvae of *A. messeae* in experiments with bladderwort ($f \pm s_f$), %

Variants of gametes in larvae of both sexes	Experiment 1		Experiment 2	
	replications 1, 2	control 1, 2	replications 7, 8	control 7, 8
XL_0	40.4 ± 4.1	29.9 ± 3.7	43.2 ± 3.5	42.2 ± 4.1
XL_1	58.2 ± 4.1	64.3 ± 3.9	55.8 ± 3.5	56.5 ± 4.1
XL_2	1.4 ± 2.0	5.8 ± 1.9	1.0 ± 0.7	1.3 ± 0.9
Number of chromosome	146	154	197	147

Note: When estimating frequencies of inversions, it was taken into account that males have one polytene chromosome *XL* (are hemizygous) and females have two chromosomes (are homo- or heterozygous).

nonselective elimination, since at these stages the individual differences appear only weakly.

It is impossible to reduce the interaction of bladderwort and mosquitoes to the one-side "predator–prey" model. In spite of the death of some individuals in trap bladders, all species of *Utricularia*, as well as *Ceratophyllum demersum*, *Hydrilla verticillata*, and species of *Najas*, especially favor the development of larvae of the malaria mosquitoes (Beklemishev, 1944; Voronikhin, 1953). The plants produce dense submerged aggregations, which reach the surface of the water, form a mooring line, and provide good protection against the mobile predators. They form a barrier for suspended mineral and organic matter, and the sedimented suspension may serve as additional feed for larvae. Intravital secretions of the higher aquatic plants strongly inhibit blue-green algae, which cause the "blooming" of water. Concentration of dissolved oxygen significantly increases in the submerged plant aggregations, and the process of nitrification intensifies. Secretion of organic metabolites is one of the ways of interactions between plants and phytophilous invertebrates, including larvae of malaria mosquitoes (Kokin, 1982). Thus, this suggests that interactions of bladderwort with mosquitoes should be classified as a specific form of the complementary connections in the biocenoses, when predation is combined with protocooperation and depends upon the stage of larvae development.

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