

The Rate of Food Consumption and Its Relation to Morphometric Parameters in Larvae of Two Malaria Mosquito Species (Diptera: Culicidae)

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Abstract—The rate of consumption of graphite particles and its relation to body length, thorax and head width, and number of teeth on the stigmal plate were studied in fourth instar larvae of two malaria mosquito species, *Anopheles messeae* and *A. beklemishevi* Stegny et Kab. In spite of their narrower head ($p < 0.001$), the larvae of *A. beklemishevi* filled their intestine faster than larvae of *A. messeae*, and males of both species did so more rapidly than conspecific females (in all cases, $p < 0.001$). The rate of food consumption by larvae of both species decreased with an increase in their body length and thorax width. In *A. messeae*, the rate of filling the intestine negatively correlated with the number of pecten teeth on the stigmal plate, whereas in the other species this correlation was absent. Both species displayed negative correlations between the rate of graphite consumption and asymmetry.

INTRODUCTION

Insect feeding is one of the main parameters of their ecology (turnover of substance and energy, interspecific relationships), physiology (metabolic rate and individual development), and pathology (probability of being infected). The feeding behavior of bloodsucking dipterans has been studied not only for adults, but even at larval stages (Beklemishev, 1944; Ivnitiskii *et al.*, 1984; Nikolaeva, 1986; Dadd, 1970; Nilsson, 1987; Dahl *et al.*, 1988; Merritt *et al.*, 1992). Known observations and experiments indicated that the feeding behavior of insects is adaptive and reflects peculiarities of their ecological niche.

The rate of food consumption in larval mosquitoes is one of the acts of feeding behavior that has been studied in relation to the rate of individual development and food digestion (Beklemishev, 1944; Nikolaeva, 1986; Nilsson, 1987; Merritt *et al.*, 1992). This parameter is also interesting because of the sensitivity of mosquito larvae to intestinal drugs produced on the basis of bacteria *Bacillus thuringiensis israelensis* (Bti) and *B. sphaericus* and used to control wild populations of mosquitoes (Rasnitsyn *et al.*, 1991; Aly, 1988; Aly and Mulla, 1986; Aly *et al.*, 1988; Khavaled *et al.*, 1988).

Modes of feeding in mosquito larvae are connected with their morphological traits: head width, head index, and number of teeth on the siphon (Maslov, 1967; Ivnitiskii *et al.*, 1984; Rasnitsyn and Yasyukevich, 1989). Similar studies concerning the rate of food consumption are not numerous (Aly, 1988; Dahl *et al.*, 1988; Sukhanov and Lopatin, 1990). In these studies, the authors relate the rate of food consumption to head

width. Such studies on the forms of the *Anopheles maculipennis* complex are virtually absent because of difficulties in identifying sibling species.

We studied the rate of consumption of graphite particles by fourth instar larvae of two sympatric species, *Anopheles messeae* Fall. and *A. beklemishevi* Stegny et Kab., and its relation to body length, head and thorax width, number of pecten teeth on the stigmal plate, and their asymmetry.

MATERIALS AND METHODS

We used fourth instar larvae collected from two water bodies in the Chulym River floodplain, in the vicinity of Tegul'det, a village in Tomsk oblast. Three hundred larvae from each pond (a total of 600) were placed in square cuvettes (21 × 10 cm) containing 200 ml of water, 100 larvae per cuvette. We used graphite as food, because it is a neutral staining substance. Graphite was ground to a powder in a mortar and evenly distributed over the surface (150 µg per replication). Larvae were fixed 2 h later in an ethanol–acetic acid (3 : 1) mixture. The length of the column of graphite consumed was measured under a microscope in the same way as the morphometric traits of larvae: body and abdomen length and thorax and head width. Unfed larvae were not analyzed. Teeth were counted on both pectens of the stigmal plate. Fluctuating asymmetry was calculated by the formula $R-L$, where R and L are the numbers of teeth on the right and left pectens. Species was identified by the table by Sibataev and Gordeev (1993).

Morphometric parameters of larvae of two *Anopheles* species selected for experiment

Parameter for comparison	Species	
	<i>An. messeae</i>	<i>An. beklemishevi</i>
Body length, mm	3.3–7.95	3.5–7.75
Thorax width, mm	1.319 ± 0.010 (0.9–1.8)	1.269 ± 0.010** (0.9–1.65)
Head width, mm	0.8125 ± 0.0013 (0.74–0.87)	0.7734 ± 0.0014** (0.71–0.84)
Head index	0.9745 ± 0.0018	0.9639 ± 0.0022*
Number of pecten teeth, $M \pm s_m$	49.6 ± 0.03	51.4 ± 0.02**
in females	50.4 ± 0.04	51.7 ± 0.03**
in males	48.6 ± 0.06	51.1 ± 0.04**
Asymmetry	–0.152 ± 0.014	+0.111 ± 0.008**
in females	–0.029 ± 0.027	–0.146 ± 0.019*
in males	–0.247 ± 0.028	+0.298 ± 0.013**

Note: * $p < 0.05$; ** $p < 0.001$.

RESULTS AND DISCUSSION

Species ratios in the ponds differed: the proportion of *A. beklemishevi* in pond 1 was $13.1 \pm 1.95\%$, and in pond 2, $80.1 \pm 2.31\%$. Hence, we identified in the experiment 311 larvae of *A. messeae* and 275 of *A. beklemishevi*. The larvae of *A. messeae* were longer than those of *A. beklemishevi* (Table 1). The rate of graphite consumption inversely correlated with body length in larvae of both species: the longer the individual, the lower the rate of food consumption. The decrease in the rate of food consumption differed: in *A. messeae*, this parameter (by the regression line) decreased from 26 to

14% (by a factor of 1.9), and in *A. beklemishevi* it decreased from 37 to 14% (by a factor of 2.7). Differences in the intestine filling rate concerned only the larvae at the early fourth instar, whose body length was 3.5–5 mm ($p < 0.05$; Fig. 1).

The filling of the intestine in larvae of both species also decreased with an increase in thorax width. The maximal thorax width was greater in *A. messeae*. The distribution of larvae with respect to this parameter was also different in these species (Table 1; Fig. 2a).

The average head width (HW) and head index in *A. messeae* exceeded those in *A. beklemishevi* (Table 1).

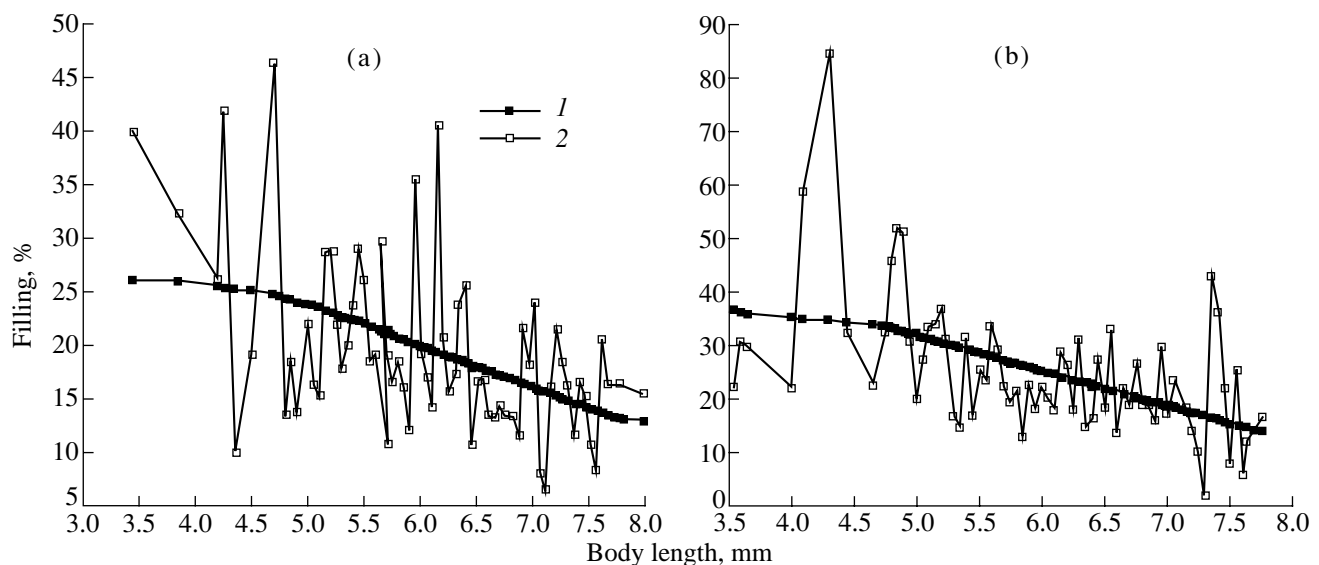


Fig. 1. Regression curve showing the dependence of the rate of intestine filling with graphite on body length in (a) *A. messeae* and (b) *A. beklemishevi* larvae.

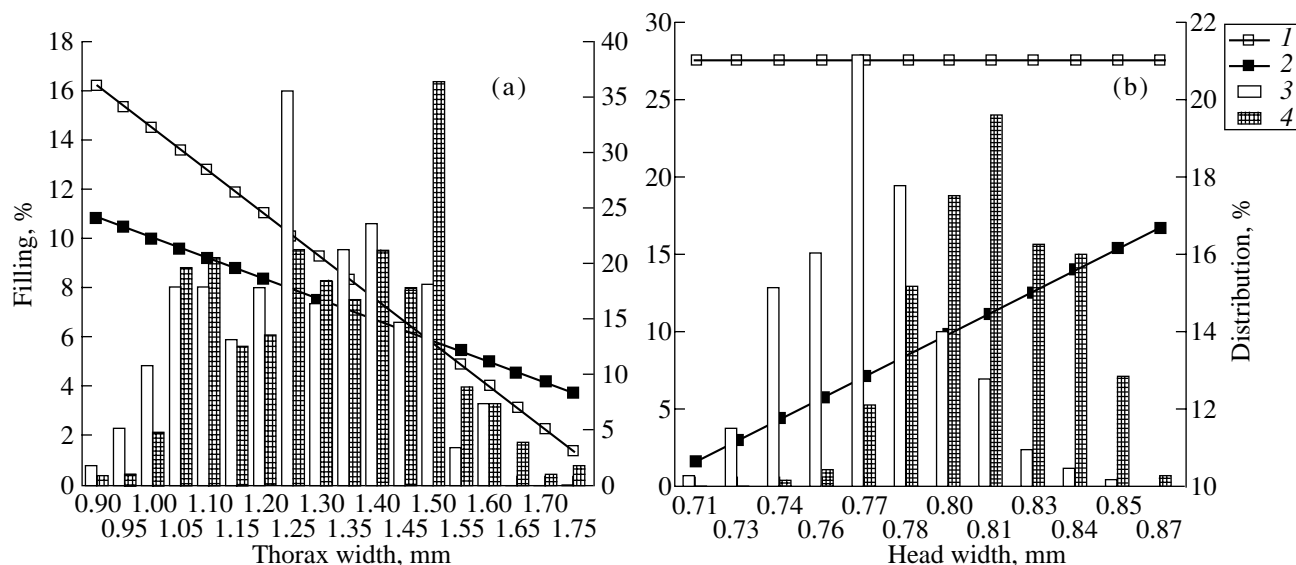


Fig. 2. Dependence of the intestine filling rate on (a) thorax width and (b) head width in larvae of two *Anopheles* species and their distribution with respect to these two parameters: (1, 2) intestine filling: (1) *A. beklemishevi*, (2) *A. messeae*; (3, 4) distribution of larvae: (3) *A. beklemishevi*, (4) *A. messeae*.

The distributions of individuals with respect to HW were also different. Plotting the regression lines indicated that the rate of intestine filling was constant in *A. beklemishevi* and increased with an increase in HW in *A. messeae* (Fig. 2b).

Intestine filling rates in males and females of each species were unequal. Statistical differences were found between all four groups of individuals (Fig. 3).

On average, the number of teeth on pectens of the stigmal plate was greater in *A. beklemishevi* larvae. Sex-related differences in this trait were also detected ($p < 0.001$). The intestine filling rate decreased with an increase in tooth number in larval *A. messeae*, whereas in *A. beklemishevi* it remained constant (Fig. 4).

Fluctuating asymmetry of individuals differed depending on species and sex ($p < 0.001$; Table 1). The regression lines reflecting the dependence of intestine filling on asymmetry revealed that changes in the two species are opposite (Fig. 5).

Our experimental data showed that *A. beklemishevi* larvae consumed graphite much more rapidly than *A. messeae* larvae. The difference concerned mainly larvae at the beginning of the fourth instar, when their length in both species was 3.5–5 mm. No difference was observed at later stages. In this case, it is necessary to take into consideration body length polymorphism of both species, as well as the fact that *A. messeae* larvae in nature usually reach larger sizes (Burlak and Sibataev, 1995). We believe that differences in food consumption may result from the fact that *A. beklemishevi* is an early summer species with higher rates of development and alteration of generations (Novikov and Gordeev, 1983).

As a rule, habitats in the north of the species range are characterized by an excess of food, and there is no full saturation of the environment (Nikolaeva, 1986). Apparently, this should lead to certain adaptations, such as a high rate of food consumption. On the other hand, higher rates of food consumption do not provide evidence for more rapid and efficient food digestion (Beklemishev, 1944). In conditions of food excess, 98.5% of carbon is excreted from the larval intestine (Nilsson, 1987). Deeper adaptations of other kinds are necessary for more efficient food digestion. The low sensitivity of *A. beklemishevi* to entomopathogenic

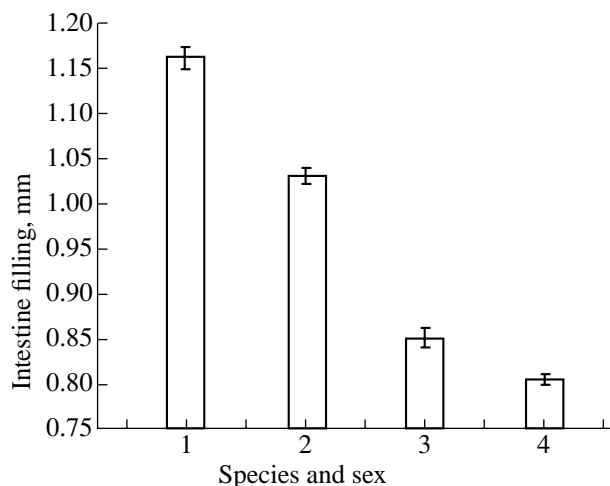


Fig. 3. Intestine filling with graphite in (1, 3) male and (2, 4) female *A. beklemishevi* (1, 2) and *A. messeae* (3, 4); lines on bars show 95% confidence interval.

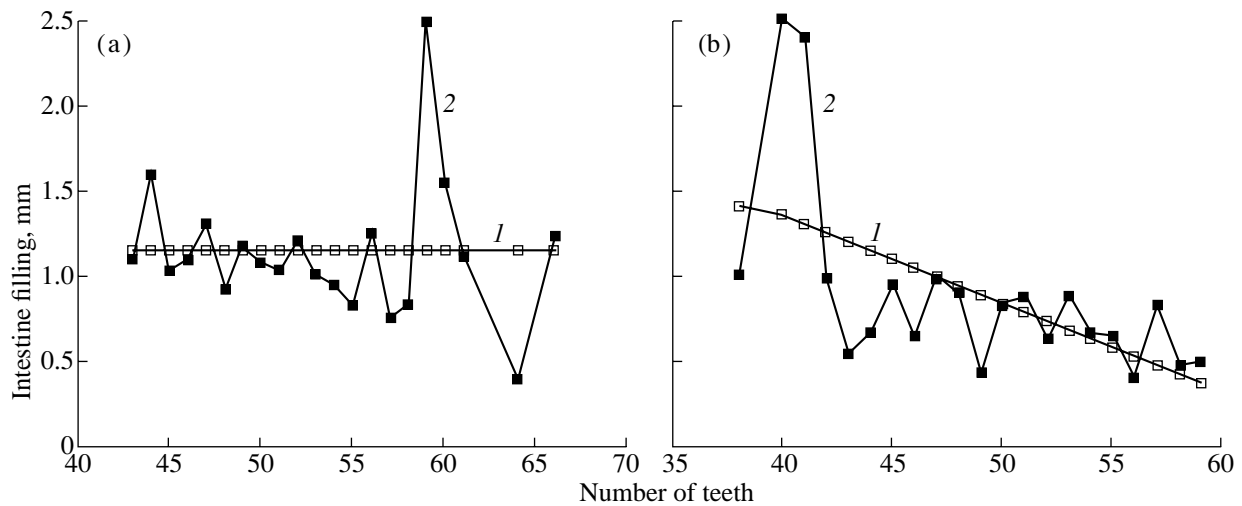


Fig. 4. Regression curve showing the dependence of the larval intestine filling with graphite on the number of pecten teeth on the stigmal plate in (a) *A. beklemishevi* and (b) *A. messeae*: (1) averaged data; (2) regression curve.

bacteria (Bti) and polluted water is evidence for the existence of such adaptations (Stegnii, 1991; Burlak and Sibataev, 1995). In addition, higher fecundity of female mosquitoes in northern populations (Stegnii, 1991) may also be associated with increased rates of food consumption and digestion.

To some extent, this suggestion is confirmed by the fact that feeding rates in males are higher than in females of the same species, because the males are known to develop faster than females (Beklemishev, 1944). At the same time, high feeding rates in males may have another significance, namely, reducing the probability of infection of females by natural pathogens and parasites, such as entomopathogenic fungi and blue-green algae. Studies by Stegnii (1991) provide

indirect evidence for this. He demonstrated that the male : female ratio is shifted from 1 : 1 to 2 : 1 by the end of the fourth instar. Experimental studies of sensitivity to infections in fourth instar larvae did not always demonstrate that males are more sensitive (Boreiko and Kostyuchenko, 1988; Burlak and Gordeev, 1995, 1996; Gordeev and Burlak, 1991, 1992). The cases when males were infected specifically, e.g., by microsporidia (Pankova *et al.*, 1991), are exceptions. We may conclude from the above data that the most sensitive males are eliminated at the first to third instars, whereas surviving individuals have low sensitivity.

In spite of the fact that larval *A. beklemishevi* fill their intestines faster than *A. messeae*, they are less sensitive to Bti infection (Burlak and Sibataev, 1995). The advantage of *A. beklemishevi* is obvious when this infection occurs in conditions of high density (Burlak and Gordeev, 1996). Larval *A. beklemishevi* are also less sensitive to polluted water (Stegnii, 1991; Burlak and Sibataev, 1995). On the basis of these observations, we suggest that the intestinal barrier, one of the main systems of protection against pathogens in insects, is better developed in *A. beklemishevi*. This may be associated with the difference in the period of adaptation of these species to conditions of the northern Palearctic. Since *A. beklemishevi* is a more northern species than *A. messeae* (Stegnii, 1991), it did not undergo such stress transformations of the homeostasis system during its westward dispersal as those that occurred in *A. messeae* during its northeastward migration.

The relation between the intestine filling rate and width of the head, whose size remains unchanged throughout the instar, displays a different trend.

Several authors related the feeding rate in mosquito larvae to the head width (Maslov, 1967; Sukhanov and Lopatin, 1990). Comparative studies on larvae from different genera confirmed this conclusion (Aly, 1988;

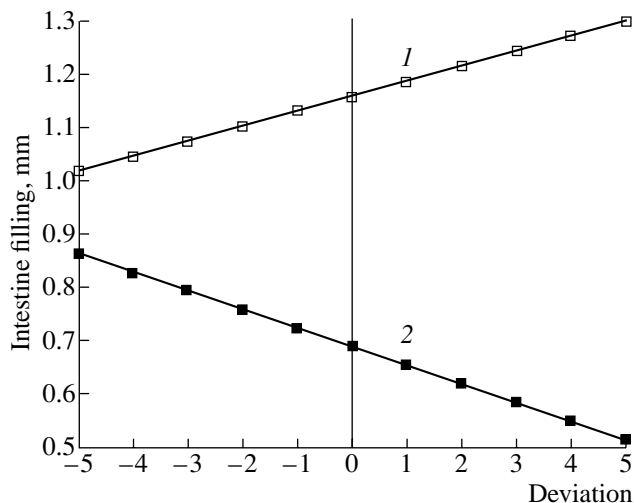


Fig. 5. Regression line showing the dependence of intestine filling with graphite on individual asymmetry in (1) *A. beklemishevi* and (2) *A. messeae*.

Dahl *et al.*, 1988). Our data, on the contrary, provided evidence for a higher feeding rate in larval *A. beklemishevi* with a narrower head. Several points should be outlined here. First, the rate of food consumption depends on the performance of the mouth parts. Similar force applied to swallowing food and filling the intestine will fill it faster in larvae with a narrower head. Second, this may relate to the adaptive prehistory of the species. Third, food quality and the sensory reaction of larvae to the latter may also have an effect. The differences between the two species in the dependence of intestine filling on the width of the head capsule is an interesting fact. This may be determined by the existence of a chromosome polymorphism (in chromosome 2R) in *A. messeae*, which is absent in *A. beklemishevi*.

Relationships between the feeding rate and the number of pecten teeth on the stigmal plate or asymmetry of individuals have not been studied previously. However, there are data that the feeding mode correlates with the number of teeth on the siphon in *Aedes caspius* L. (Ivnitskii *et al.*, 1984) and number of brush scales in *Culiseta* (Maslov, 1967).

Note that the rate of food consumption and the type of feeding are different acts of feeding behavior that seem to display weakly interdependent genetic determination. Larvae of *A. messeae* and *A. beklemishevi* have the greatest number of teeth on pectens of the stigmal plate among all representatives of the *A. maculipennis* complex in the Palearctic (Sibataev and Burlak, 1996). Analyzing variation of pecten teeth in *A. messeae* and *A. beklemishevi* larvae from water bodies of Tegul'det, we found that *A. messeae* always have a lesser number of teeth than *A. beklemishevi* ($p < 0.001$). Taking into account a higher rate of filtration in the latter, we could conclude that the rate of food consumption is higher in "filtrators" *A. beklemishevi* than in "scrapers" *A. messeae*. However, no differences in the tendency toward scraping was found between *A. beklemishevi* and *A. messeae*, despite the wider head in the latter. In addition, we found that it is impossible to extrapolate intraspecific regularities to the interspecific level. First, larvae of *A. sacharovi* display filtering behavior, whereas *A. atroparvus* are more inclined to scraping (Rasnitsyn and Yasyukevich, 1989). The mean number of pecten teeth is greater in *A. atroparvus* than in *A. sacharovi* (Sibataev and Burlak, 1996). Second, *A. sacharovi* and *A. beklemishevi* larvae, which have the minimal and maximal numbers of teeth in the *A. maculipennis* complex, respectively, virtually do not scrape (Sibataev and Burlak, 1996).

The difference between *A. messeae* and *A. beklemishevi* in dependencies of the rates of intestine filling on the number of pecten teeth and asymmetry is probably explained by the divergence of their ecological niches.

Fluctuating asymmetry of animals is regarded as a parameter of instability of their individual development (Zakharov, 1987). This trait has no structural genetic determination, although it is controlled by at least five

modifier genes (Mitrofanov, 1990). Currently, this trait is often used in ecological monitoring. Correlations of asymmetry with genetic and physiological characters are usually studied under the influence of stress. For example, there are works on the influence of temperature, population density, insecticides, and pathogens on the level of asymmetry (Mitrofanov, 1990; Zakharov and Shchepotkin, 1995; Burlak and Sibataev, 1996). Nonrandomly fluctuating asymmetry is regarded as a parameter reflecting the capacity of buffer systems of an organism (Verenich, 1996). Our results provide evidence for differences in intraspecific trends in the species studied. Probably, this can be explained by the interaction between *A. messeae* and *A. beklemishevi* in zones of their sympatry in the course of adaptation to conditions of a particular habitat.

Thus, we can conclude that there are two types of trends: common (unidirectional: the dependence of intestine filling on body length and thorax width) and specific (multidirectional: the dependence of intestine filling on head width, number of pecten teeth, and symmetry of individuals). The latter can also be explained from the standpoint of species interaction in natural habitats.

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