Body-weight gains in *Blaberus craniifer* cockroaches and the intensity of their infection with gregarines and nematodes

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Abstract
Intestinal parasites are considered to be able to hinder growth of the host animals, reducing the extent of food metabolism, damaging the intestines’ integrity by filling it with products of their metabolism. However, a long co-evolution can mitigate the negative impact of a parasite on the host organism. To study how parasites – nematodes *Cranifera cranifera* (Chitwood, 1932) Kloss, 1960 (Oxyurida, Thelastomatidae) and gregarines *Protomagalhaensia granulosae* Peregrine, 1970 and *Blaberocola cubensis* (Peregrine, 1970) Clopton, 2009 (Eugregarinorida, Blabericolidae) – affect the growth rates of cockroaches, we performed an experiment on 200 larvae of *Blaberus craniifer* Burmeister, 1838 (Blattoidea, Blaberoidea), varying in weight and age. We monitored changes in their body weight, intensity of food consumption, and after the experiment we counted gregarines in the midgut and nematodes in the hindgut. As a result, we found that 100% of the cockroaches were infected with two species of gregarines and one species of nematodes. The intestines of small cockroach larvae (weighing 300–400 mg) contained 16–18 specimens of gregarines on average. Large larvae had a weak tendency towards increase in the intensity of gregarine infestation. Similarly, there occurred changes in the intensity of nematode invasion: young larvae were infected on average by 8–10 specimens of nematodes and large larvae had an average of 12–14 nematodes. At the level of tendency, nematodes were observed to enhance the cockroaches’ growth rates following increase in intensity of the parasitic infection. We found that the two groups of parasites had no effect on one another: the number of gregarines had no effect on the number of specimens of nematodes and vice-versa, the number of nematodes had no effect on the number of gregarine specimens. Perhaps, this is related to different localizations of the parasites: gregarines for most of their life feed in the small intestine, while nematodes feed in the large intestine. Therefore, growth rates of the cockroaches in our experiment have not changed due to the parasites. This indicates minimization of negative effects of gregarines in the midgut and nematodes in the hindgut on the host’s life cycle, developed over long co-evolution.

Keywords: Blattodea; Thelastomatidae; Eugregarinorida; parasitic nematodes; gregarines of insects; body-weight gain; effects of parasite on its host.

Introduction
Organisms with broad diet spectrum consume very diverse food, which the parasites may dislike (for example, secondary metabolites of plants or microorganisms in food of animals can inhibit active centers in enzymes of their parasites). Over thousands of generations of hosts and their parasites, tolerance to certain secondary metabolites of plants and microorganisms in parasites and their hosts has gradually increased. In our studies, we have evaluated the breadth of feeding range of saprophage beetles and phytophages (Brygadyrenko & Reshetniak, 2014; Brygadyrenko & Nazirmov, 2015; Martynov & Brygadyrenko, 2017, 2018) and saprophage millipedes (Svyrydchenko & Brygadyrenko, 2014; Kozak et al., 2020). In the intestines of the species we examined, gregarines and nematodes were very common (Brygadyrenko & Reshetniak, 2016; Brygadyrenko & Svyrydchenko, 2015). A parasite constantly feels changes in composition of its host’s diet and should somehow react to them (Boyko & Brygadyrenko, 2017, 2019a, 2019b, 2021), which would be seen in decrease or rise in its number or release of some metabolism products into the host’s intestines. Influence of a parasite on the host should not lead to the host’s death (because the parasite would die as well), but must nonetheless somehow alter (first inadvertently, and thousands of generations later perhaps purposefully) the metabolism of the host, redirecting it from one diet (unfavourable for the parasite) to another (more favourable for the parasite). Hundreds of thousands of parasite species, combined with millions of host species that consume different diets in different living conditions of the parasite’s range, multiplied by millions of generations of co-evolution should have produced examples of “management” of a host’s metabolism by a parasite. At the same time, unexpected effects of interaction in the parasite-host system are most likely to be found particularly in organisms with broad diet range, for example cockroaches.

Cockroaches are mostly omnivore insects, spread all around the world in various biotopes (Roth, 1983; Fakoorziba et al., 2010). As of now, 3,095 species of cockroaches have been described, and some researchers even consider this number to be up to 4,400, belonging to 500 genera (Beccaloni & Eggleton, 2013; Ameya et al., 2021). Cockroaches have been some of the commonest insects for tens of millions of years, dominating in Paleozoic biocenoses, and to a lower degree in Mesozoic. Despite the fact that the cockroaches had lost their dominance to other groups of animals during the Cenozoic, they remain a comparatively numerous and broadly spread group in current biocenoses, especially in regions with a warm and moist climate. Cockroaches are a significant component of entomofauna, which has adapted to various environmental conditions (Bell et al., 2007). Most of them are decomposers that feed on decaying organic matter, and some have adapted to life with people and are synanthropic (Mille & Peters, 2004; Gore & Schal, 2007; Mikaelyan et al., 2016; Parhomenko et al., 2022). *Blaberus craniifer* Burmeister, 1838 (Blattoidea, Blaberoidea) lives in Mexico, islands of the Caribbean and Central America. Because of unique appearance, possibility to be kept as pet, and other features, this species is
spread all around the globe, often occurring in expositions, private collections, and laboratories studying life of insects. Also, it is cultivated as feed for other exotic animals. This species is often studied for possible use as food for people, because its body contains three times the amount of protein in chicken meat. It is a very convenient object for various physiological, biochemical, and genetic experiments (Goudey-Perrière et al., 2003; Lambiase et al., 2004; Ross, 2012; Kulma et al., 2020).

Many Blattodea (Blaberidae) in many countries, including the USA, Canada, India, Argentina, Bulgaria, and Japan, have been found to be infested by parasitic Oxyuridae nematodes – very ancient parasitic partners of cockroaches, usually living in the hindgut and reproducing by haplodiploidy. Oxyurida are an interesting group of parasites for studying how the parasitism has evolved, because it includes parasites of both invertebrate and vertebrate animals (Ozawa & Hasegawa, 2018). Also, cockroaches are parasitized by many gregarines (Gregarinoidae), which most often inhabit the frontal part of the midgut (Clopton, 2010). Because of humans, this insect has spread around the world, and so have its gregarines and nematodes. Gregarines and nematodes that live in the intestines of B. craniifer cockroaches are well-studied examples of parasitic interactions (Clopton, 2009; Ozawa et al., 2016; Nagae et al., 2021). Parasitic gregarines and nematodes use the intestine of cockroaches as a permanent living location and a source of food resources. Food consumed by a host is the main source of energy for parasites. Parasites use a food resource – their hosts – in their own interests, and therefore should have pronounced effects on the hosts. Studies on other arthropods, including insects and diploponds (Rodriguez et al., 2007; Brygadyrenko & Svyrydchenko, 2015), revealed no negative impact of gregarines.

The objective of our study was evaluating the effects of parasites on growth rates of the larvae of B. craniifer cockroach, accounting for age of the larvae (their body weight), intensity of infection with parasites, and interaction between them.

**Materials and methods**

For the experiment, we selected 200 larvae of Blaberus craniifer Burmeister, 1838 cockroach, varying in age and weight. Before the experiment, all cockroaches were in one 40 L plastic container, i.e. temperature, moisture, feed composition, and probability of gregarine and nematode infections in them were the same. Prior to putting each cockroach larva to a plastic cup, it was weighed on electronic scales with up to 1 mg accuracy. In each 500 mL plastic cup, we made a shelter of porous cardboard (fragment of tray for chicken eggs). To maintain optimal moisture in the cup, we used standard cotton disks, periodically moistened with distilled water. Also, the insects were provided with a complex diet (on average each cockroach received 800 mg of protein, 500 mg of apple, 500 mg of carrot during the experiment). The experiment was conducted at the laboratory temperature of +22…+25 ºС (lower temperature in the night, and higher temperature in the day). Each cup was out of reach of direct sunrays. Light, temperature, and moisture in each cup were controlled daily, all 6 days of the experiment. Prior to and after the experiment, we weighed the larvae, and then dissected them to count the average of parasites (gregarines and nematodes) in the hindgut. To study parasites in the larvae, we removed the intestines, put them on the microscope slide in physiological solution and fragmented them, making 12–15 transversal cuts with a scalpel, depending on length of the intestines (cuts were made at approximately the same distance one from another). The prepared temporal micropreparation of the midgut and hindgut of the larvae’s intestines was analyzed under a light microscope. When discovering gregarines and nematodes, we counted them and performed microphotography using a digital photocamera with 5 megapixel resolution for further identification of parasite species. To do so, we used the methods of microscopic measurement of morphometric parameters of gregarines and nematodes.

**Fig. 1.** Gregarines of in the midgut of B. craniifer larva: a, b – mature associations, c – gametocyst; bar – 200 µm
Earlier studies have reported that the body of the insect host is parasitized by one to three species of gregarines (Chang et al., 2004; Canales-Lazcano et al., 2005), which localize in the midgut. We found that almost the entire laboratory population of cockroaches was infected with parasites. Representatives of the Blattodea (Dictyoptera, Blattaria) order can be infested with gregarines of four families: Gregarinidae (genera *Gamocystis* and *Gregarina*), Blabericolidae (genera *Blabericola* and *Protomaghalhaensia*), Fusionidae (genus *Fusiona*), and Diplocystidae (genus *Diplocystis*) (Desportes & Schrével, 2013). In the examined larvae of *B. craniifer* cockroaches, we found two species of gregarines of the Blabericolidae family Clopton, 2009: *Protomaghalhaensia granulosae* Peregrine, 1970 (Fig. 1) and *Blabericola cubensis* (Peregrine, 1970) Clopton, 2009 (Fig. 2).

The gregarines was identified according to the studies by Geus (1969), Perkins et al. (2000), Clopton (2002, 2010, 2012a, 2012b), and Clopton & Hays (2006). For cockroaches of the Blaberidae family, Geus (1969) mentions four species of gregarines: *Gregarina leucophaeae* Geus, 1969, *G. haasi* Geus, 1969, *G. wolfi* Geus, 1969 and *G. planchlorae* Frenzel, 1892. At the same time, his fundamental monography did not mention that cockroaches of the *Blaberus* genus can be parasitized by gregarines. More complete data on gregarines of cockroaches of the *Blaberus* genus have been given by Peregrine (1970). An in-depth research of gregarines of cockroaches has been conducted by R. E. Clopton over recent ten years. Considering his elucidations of morphology of many species, we identified two species of gregarines infesting *B. craniifer*: *Protomaghalhaensia granulosae* and *Blabericola cubensis*.

![Fig. 2. Gregarines of Blabericola cubensis (Peregrine, 1970) Clopton, 2009 in the midgut of larva of *B. craniifer*: a – solitary trophozoite, b, c – mature associations; bar – 200 µm](image-url)

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Fig. 3. Nematodes of Cranifera cranifera (Chitwood, 1932) Kloss, 1960 in the hindgut of larva of B. craniifer: a – larvae, b, c – females, bar – 100 µm

Of nematodes of the Thelastomatidae family in the species of cockroaches we studied, we detected three species (Adamson & Van Waerebeke, 1992; Guzeeva & Spiridonov, 2009; Carreno & Tuhela, 2011; Morffe et al., 2022): Cranifera cranifera (Chitwood, 1932) Kloss, 1960, Leidyneuma appendiculatum (Leidy, 1850) Chitwood, 1932, and Thelastoma blabericola Leibersperger, 1960. According to the morphological features, we identified the nematode in B. craniifer as Cranifera cranifera (Chitwood, 1932) Kloss, 1960 (Fig. 3).

The materials were statistically analyzed using the regression analysis in the Statistica 8.0 software (StatSoft Inc., USA).

Results

Increment or decrease in the body weight of B. craniifer (Fig. 4a) did not depend on the initial weight of cockroach larvae involved in the experiment. On average for a day, live body weight in the small larvae (body weight of 300–400 mg) increased by 4–5 mg, while body weight in the larger larvae (1,400–1,500 mg) increased on average by 12 mg per day. Consumption of food by the cockroaches – egg white (Fig. 4b), carrot (Fig. 4c), and apples (Fig. 4d) – did not significantly differ between the animals with different body weight. There was seen a weak tendency towards more intensive food consumption by larger insects.

The intestines of the small cockroach larvae (300–400 mg body weight) on average had 16–18 specimens of gregarines. The large larvae had a weak tendency (insignificant change) towards increase in the intensity of gregarine infection (Fig. 5a). Similarly (although more pronounced, but still at the level of tendency, i.e. insignificantly), the intensity of nematode infection of cockroaches changed (Fig. 5b): the young larvae were infested on average by 8–10 specimens of nematodes, and the large larvae had greater intensity of infection – on average 12–14 specimens of nematodes per a cockroach larva.

The answer to the main question – do the parasites affect the growth rates of the hosts? – was negative for both nematodes and gregarines (Fig. 6). At the same time, nematode-infested hosts had a tendency towards increase in growth rates with increase in infection intensity (Fig. 6b). Perhaps, this is related to the fact that the large cockroach larvae had been infected to a greater extent (Fig. 6b), and, as assumed, grew faster than the small larvae (Fig. 6a).

An unexpected discovery was that the two groups of parasites had no effect on one another: the number of gregarines did not affect the number of nematode specimens (Fig. 7a), and vice-versa, the number of nematodes did not affect the number of gregarine specimens (Fig. 7b). Perhaps, this is associated with different localization of the parasites: gregarines live most of their lives in the small intestine (in the section from the stomach to the Malpighian tubule system), while nematodes inhabit the large intestine (the section from the Malpighian tubule system to the rectal opening). On average, in the laboratory population of B. craniifer larvae which we studied, we found 23 specimens of gregarines and 10 specimens of nematodes (Fig. 7).

We found no relationship between changes in the body weight of B. craniifer cockroaches and absolute number of specimens of Blabericola cubensis (Fig. 8a) and Proteomagalhaensia granulosae gregarines (Fig. 8b) in their intestines. We also found no correlation between change in the body weight of the B. craniifer cockroaches and absolute number of specimens of Cranifera cranifera nematodes in their hindgut (Fig. 9).
**Fig. 4.** Correlation between daily weight gain (a), mean-daily change in the weight of egg white (b), carrot (c), and apples (d) in the container and body weight of the *B. craniifer* cockroaches at the beginning of the experiment: the ordinate axis: a – increase in the body weight of cockroaches per day of the experiment (difference between final body weight M2 and initial body weight M1, divided by duration of the experiment, mg/day), b, c, d – change in weight (mg/day) of feed per a day of the experiment: egg white (b), carrot (c), and apples (d)

**Fig. 5.** Intensity of infection (number of specimens of parasite inside one specimen of host) with gregarines (a) and nematodes (b) in the *B. craniifer* cockroaches depending on body weight of insects at the beginning of experiment (M1 on the abscissa axis, live weight, mg): on the ordinate axis – number (spec.) of gregarines (a) and nematodes (b) inside one insect host
Fig. 6. Influence of absolute number of specimens of gregarines (a) and nematodes (b) in the intestines of the *B. craniifer* cockroaches on their body weight per a day of the experiment (mg)

\[ y = 8.0315 - 0.0069x; \]  
\[ r^2 = 0.0004 \]

\[ y = 3.3598 + 0.4528x; \]  
\[ r^2 = 0.0207 \]

Fig. 7. Correlation between absolute number (specimens) of nematodes and number of gregarines (a) and between gregarines and nematodes (b) in the intestines of *B. craniifer*

\[ y = 10.047 - 0.0022x; \]  
\[ r^2 = 0.0004 \]

\[ y = 22.5752 - 0.1655x; \]  
\[ r^2 = 0.0004 \]

Fig. 8. Correlation between the rates of changes in body weight of the *B. craniifer* cockroaches and absolute number (specimens) of gregarines of *Blabericola cubensis* (a) and *Protomagalhaensia granulosae* (b) in their intestines

\[ y = 7.8479 + 0.003x; \]  
\[ r^2 = 0.0000 \]

\[ y = 8.1123 - 0.0299x; \]  
\[ r^2 = 0.0020 \]
Discussion

Gregarines. Obviously, in the parasite-host system, the degree of negative impact of a parasite on vitality of the host depends on invasion intensity. However, according to the results of our experiment, food consumption and growth intensity of the larvae did not depend on the number of parasites (gregarines and nematodes) we found. In the laboratory population of *B. craniifer* which we studied, the cockroach larvae had 23 specimens of gregarines and 10 specimens of nematodes on average, indicating relatively high level of invasion. This was due to the density of infestation of the cockroaches’ container: the substrate contained a large number of oocysts of gregarines and eggs of nematodes, which promoted almost 100% infection of the cockroaches.

Eugregarinida are symbionts, and, depending on their general number, can be mutualists, commensals, and parasites (Maddox, 1987; Brooks & Jackson, 1990; Tanada & Kaya, 1993; Boucias & Pendland, 2012). The main factor that determines the extent of pathogenicity of gregarines is intensity of invasion (number of parasites in one host). Despite the fact that gregarines actually consume food that the host “planned” to use for its own metabolism and damage the intestinal epithelium, this usually does not weaken the host. Invasion intensity, and therefore degree to which the host’s health deteriorates (though term “health” is rarely used in relation to insects, we consider its usage reasonable) depends on the number of gregarine oocysts the host had consumed. Ingestion of oocysts by cockroaches is promoted by (1) increase in density of their population, (2) heightened moisture of the substrate, which favours the survivability of haematoxysts and oocysts, (3) long cultivation of laboratory population of cockroaches on the same substrate, and also other unstudied factors. Gregarine-caused pathologies and effects on the health of the cockroaches, which could be insignificant when the invasion intensity is low or average, should become more pronounced when the number of gregarines in intestines of one cockroach exceeds a reasonable threshold (Lange & Lord, 2012). Then gregarines can cause intestinal obstruction and large lesions of the epithelial wall of the cockroach intestines. We do not know why we did not observe this even in singular cases in 200 cockroaches we examined.

Nematodes. If gregarines live in the midgut where nutrients are intensively absorbed, which is important for metabolism of cockroaches, then nematodes live in the hindgut, in which nutrients are almost unabsorbed. That is, most likely, nematodes cannot negatively affect the cockroaches’ health. The number of nematodes in our experiment was on average 8–10 in the small larvae and up to 12 specimens in the larger cockroach larvae. Obviously, the number of nematodes practically does not depend on length and volume of the intestines, which in our experiment was 5 times greater in older larvae (considering that the volume of the hindgut is proportionate to the body volume). The number of nematodes was found to be unaffected by period of cockroach larva’s life: the heavier the cockroach larva, the longer it spent ingesting the eggs of nematodes in the substrate (theoretically evenly distributed in the container substrate). In our experiment, body weight of the cockroaches was between 300 mg and 1,500 mg, i.e. differed 5 times, and therefore period of life of cockroaches and probability of them eating eggs of nematodes also should have differed 5 times in small and large cockroaches. The number of nematodes did not change as the cockroaches became older. That is, there are some mechanisms maintaining the number of this nematode species at an optimal level, which require further research.

Growth rates of cockroaches in our experiment did not change under the influence of parasites. This indicates that negative impact of gregarines in the midgut and nematodes in the hindgut on the life cycle of the host has minimized as a result of long co-evolution. Our study is another evidence of likelihood of complex balanced interactions between different parasite species and their hosts, which have formed over their long co-evolution in the biosphere.

Conclusion

Analysis of changes in body weight of the cockroach larvae during intensive consumption of various feeds revealed that their growth rates did not depend on the number of parasites (gregarines and nematodes) in the intestines. Also, we determined that in the conditions of laboratory maintenance, having high density of the laboratory population of cockroaches, almost 100% of the *B. craniifer* larvae were infected with two species of gregarines and one species of nematodes. Absence of increase in the infestation intensity in the cockroaches with age indicates an action of a yet poorly studied mechanism of maintaining an optimal number of parasites in the cockroach population, which requires further research.

The authors declare no conflict of interests.

References


