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DNA regulates foraging physiology and behavior in black ant (*Paratrechina longicornis*) and red ant (*Solenopsis geminata*): A novel molecular approach

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Abstract

Ants prefer variety of food to carry out different physiologically controlled eusocial activities. However, the role of internal biological factors regulating the sensibility in physiological recognition is yet to be explored. Therefore, the present study was conducted to evaluate the possible role of genomic DNA in regulating food preference activity in black ant (BA) (*Paratrechina longicornis*) and red ant (RA) (*Solenopsis geminata*) over a period of time. At first the ant foraging activities were conducted and the observations were recorded over a twelve hour time period keeping the environmental factors like relative humidity and ambient temperature constant. The results indicate that both protein and carbohydrate rewards to lipids in both the ant types. Further, it was observed that the protein rich nutrients were preferred by RA whereas the BA selected carbohydrate rich food. The population size of RA, ascertained through this study, was almost four times greater than that of BA. To verify whether the food preference has any connection with the internal factor like genomic DNA content, both red and black ants' genomic DNA were isolated and then quantified spectrophotometrically before evaluating through DNA gel electrophoretic assay. The data of both the studies further indicated that the net DNA content of RA was greater and more intact owing to clear band intensity as compared to the faint DNA band of BA which could possibly serve the pioneer step towards understanding the molecular basis of food selection physiology and behavior in different ants.

Keywords: Ant, carbohydrate, DNA, foraging physiology and behavior, protein.

Introduction

Ants are known to be one of the most intelligent social animals capable of thriving in wide variety of ecosystems which is often attributed to their superior social organization and their ability to modify habitats, tap resources and defending themselves which parallels them with human societies and have long been an inspiration and subject of study. The

division of labor in eusocial ants needs highly evolved communication skill based on the capability of the odoratous proteins to sense external factors like food abundance, availability, dispersion, predation pressure, type of habit, etc. which controls their food selection (Barbani, 2003).

Carbohydrate sources are abundant and primarily aid in growth and development of all organism. On the other hand proteins are required mainly for reproduction, building blocks of signal molecules and hormones. Ants are highly selective in their food preferences which possibly vary according to the need of their physiology. They are known to regulate their intake preferences of food like carbohydrate and/or protein among suitable available food material (Cook and Behmer, 2010). However, the molecular basis of regulation of internal physiology induced external behavioral changes for selective nutrient preferences in different ant species still remain unexplored.

Genomic DNA has always been the most important factor in every organism as it is the functional unit for cellular growth and differentiation. It may be considered that higher the amount of DNA content in an organism, the more it can be utilized to transcribe mRNA and thereafter synthesize different essential proteins necessary for further production of different enzymes, hormones or any other signaling molecules, etc to carry out different activities. Thus, the possible role of genomic DNA content in regulating any change in the physiological and behavioral parameters in ants may be considered as a novel and pioneer approach in the branch of biology of ant.

Therefore, with the approach of understanding the probable association of DNA with that of ants' physiology and behavior, the present study was structured with the following objectives: i) to evaluate the nutrient selection and specificity in different available ant species like *Paratrechina longicornis* (black ant), *Solenopsis geminata* (red ant), etc ii) to identify whether the food preference vary with different ant species, iii) to evaluate the genomic DNA content and nature in different ant species, iv) to identify any possible co-relation of DNA with the specific nutrient preferences in different ant species.

Materials and methods

2.1 Chemicals, reagents and food items

All the chemicals and reagents for experimental purpose were purchased from Sigma Aldrich, USA. The food items needed for the study were purchased from local market in sealed pack.

2.2 Ant foraging activity

To study the activity of the ant three different sites were chosen in and around the premises of Dum Dum Motijheel College, Kolkata, India. The mean number of ants which were observed on each food item was recorded by several digital images captured daily using a camera (Sony Alpha58), three times every 5 minutes at 2h interval for seven days in the month of April' 2016, May' 2016 and June' 2016. The temperature and the relative humidity were relatively constant as measured in thermometer and hygrometer. The experiments were re-peated thrice and all the data were recorded independently and individually.

The black ant (*Paratrechina longicornis*) and red ants (*Solenopsis geminata)* were identified at first and then the numbers of each type of ants on each food items and on each site were photographed for counting from the captured images in a giant computer screen.

2.3 Assessment of total ant count and specific food preference in red and black ant

The natural diet of black ant (BA) and red ant (RA) reported earlier were seeds, human food products, rubbish, etc. Food choice tests were conducted in the natural environment using foods items (2 g of each item) in the categories of carbohydrates, proteins and lipids. The carbohydrate items were sucrose (20%), rice and honey. The proteinaceous food items were raw fish, boiled egg and dead insect. Butter, ghee and sunflower oil served the purpose of lipid sources. Water was kept as control in each case. One item from each food category of carbohydrates, proteins or lipids was placed in a 6 cm diameter of thermocol bowl. Every three item were positioned near one another in randomized order at each site. Digital images were captured to keep an account of number of ants for statistical analysis. Total number of RA and BA involved in foraging activity were counted individually from all the digital images of food preference tests and statistically analyzed.

2.4 Assessment of DNA in red and black ant

The DNA from equal number of RA and BA were isolated using the standard protocol of phenolchloroform isolation method for small- to medium-sized ant species described by Gadau (2016). Further, the extracted DNA from RA and BA were measured spectrophotometrically at 260nm and arbitrary density was quantified. The band intensity from both types of ants was also assessed through DNA gel electrophoresis as per standard practice (Samadder et al., 2011).

2.5 Statistical analysis

All the data that were collected from three independent experiments were presented as mean values to statistically analyze the level of significance of the differences by Student's t-test.

Results

3.1 Assessment of total red and black ant count and evaluation of their specific food preferences

Different food items used during the course of the experiment for analysis of ants' foraging activity were showed in Figure 1a. As revealed from our data the total number of BA were found to be ~7081 which were almost one fourth of the total number of RA which were observed to be ~27015 (Table 1). For evaluating the specificity in the food preferences, the digital images of ants taken at the time of the experiment, were counted individually. Data revealed that carbohydrates were more preferred by BA (64.69% of BA) than that of RA (12.74% of RA). On the contrary protein preferences of RA (84.1% for RA) were comparatively higher than that of BA (32.44% for BA). As for lipid food assessment, neither the BA (2.87 % lipids by BA) nor the RA (3.16% lipids by RA) showed much preference when compared to

Table 1. Representation of different food items selected by red ant (RA) and black ant (BA).

Nutrition content	Mean number of BA ± S.E.	Mean number of RA ± S.E.	Mean % BA ± S.E.	Mean % RA ± S.E.
Protein	2297 ± 20.08	22720 ± 90.89	32.44 ± 0.30	84.10 ± 0.19
Carbohydrate	4581 ± 54.28	3442 ± 41.62	64.69 ± 0.27	12.74 ± 0.15
Lipid	203 ± 7.36	853 ± 12.34	2.87 ± 0.08	3.16 ± 0.04
Total	7081	27015		
Representation of different kinds of food selection by different types of ant				

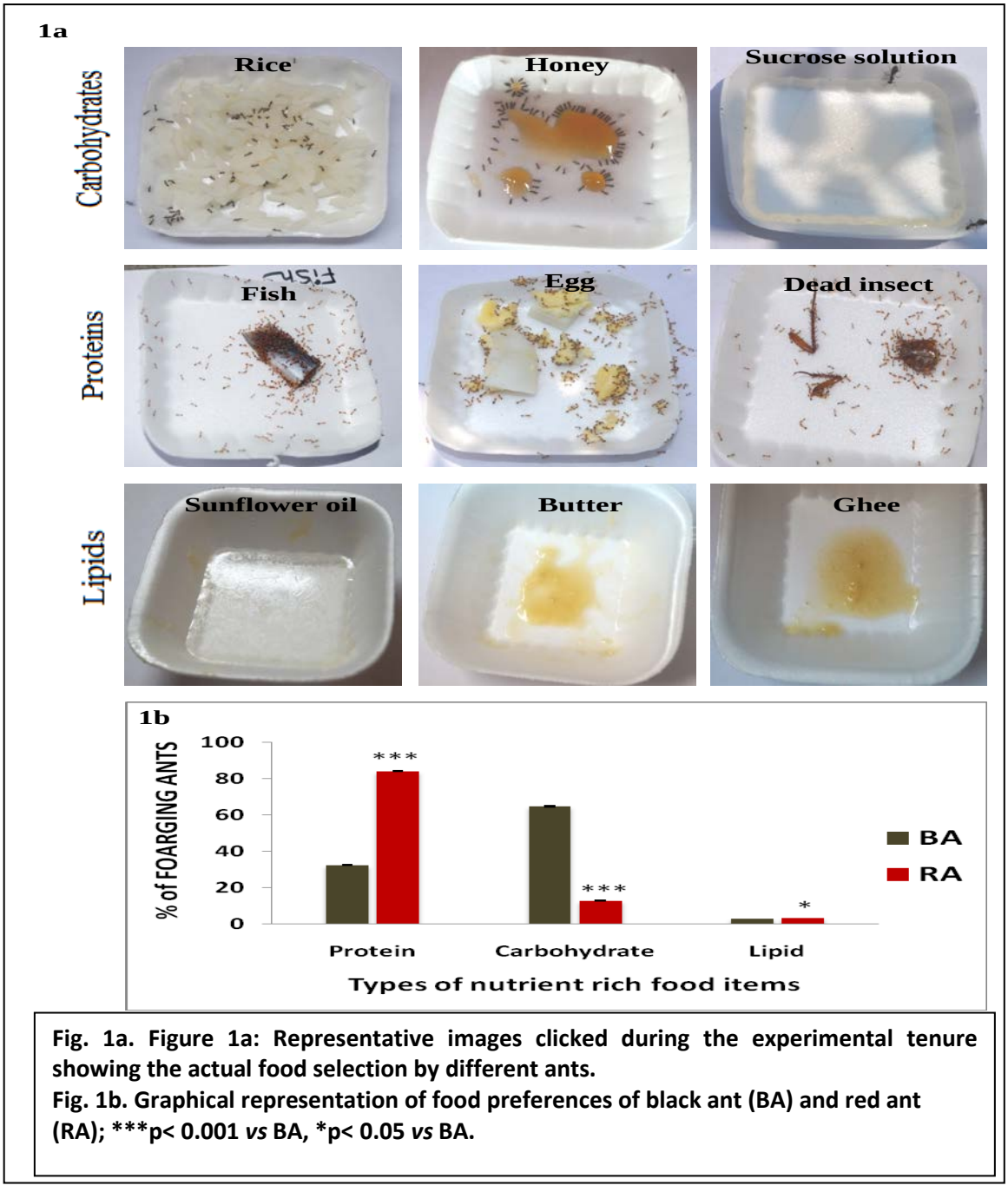

Fig. 1a. Figure 1a: Representative images clicked during the experimental tenure showing the actual food selection by different ants.
Fig. 1b. Graphical representation of food preferences of black ant (BA) and red ant (RA); ***p< 0.001 *vs* BA, *p< 0.05 *vs* BA.

carbohydrate or proteins (Table 1, Figure. 1b). Further, both the types of ants showed hyperactivity in the late afternoon, from 12pm to 5pm for BA and from 12pm to 6pm for the RA, showing no significant differences between the two types.

3.2 Assessment of DNA band of red and black ant

The DNA content in RA was higher, as much as almost double, than that of BA as observed from both spectrophotometric study (Fig. 2a). The DNA gel electrophoresis band assessment also revealed a clear and distinguishable DNA band for RA but a very feeble band was observed in case of BA (Fig. 2b) indirectly indicating differential nature of food selection of each of ant.

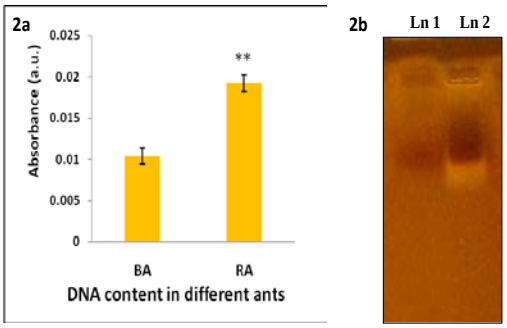

Fig. 2a. Spectroscopic analysis of genomic DNA content in black ant (BA) and red ant (RA); ** p< 0.01 *vs* BA.
Fig. 2b. Evaluation of genomic DNA band fragmentation in black ant (BA) and red ant (RA): Ln1= BA. Ln2= RA.

Discussion and conclusion

The overall result of the present study (Fig. 3) showed that red ants (RA) mostly preferred protein containing food items (raw fish, boiled egg, dead insect) in comparison to other type of available nutrients like carbohydrate or lipids. On the other hand black ants (BA) favoured food source rich in carbohydrate (rice, honey, sucrose solution) which was completely contrasting when compared to that of red ants. This difference in physiological biasness for nutrient preference led us to verify further the possible association of internal factors, if any, in regulating the event of food selection.

Proteins have always been an essential factor in all organisms for building body tissues (Dietary Reference Intakes). Apart from this, in many other organisms including insects, proteins play some additional vital roles like maintaining effective population size by increasing the reproductive fitness/potential (fecundity), enhancing gonadal development for maintaining genetic constituency, synthesizing several regulatory signalling molecules like trail making pheromone, sexual pheromone, etc. which aid in sustaining the eusocial life of ants (Mashaly et al., 2013). Therefore, the preference of protein rich food items by all types of ants should have been a common phenomenon. However, the results of our present study indicated a different aspect altogether, where protein rich macronutrient were particularly preferred by RA only, whereas BA selected carbohydrate source. Therefore, it became evident that there should be a possible link between external protein food preferences and internal synthesis and storage of proteins in ants which plays a pivotal swing in regulating the food selection process in ants.

To further investigate the factors involved in the event of food selection the idea of ascertaining the role of genomic DNA, which is known to serve the fundamental functional units for protein synthesis, came

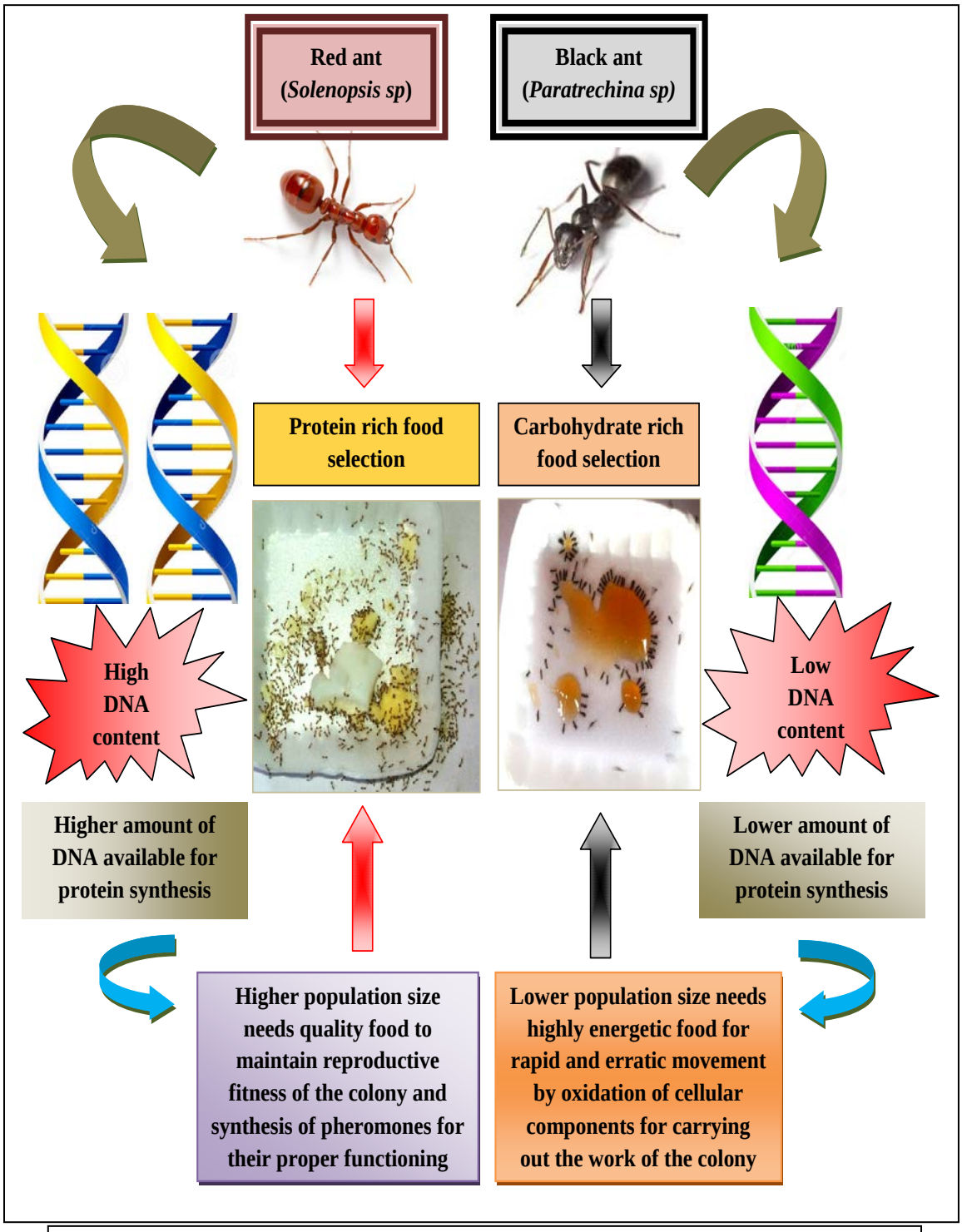

Figure 3. An overview of the present study.

into focus. Therefore, the need to ascertain the role of genomic DNA content, if any, in regulating the physiology and behaviour for foraging preferences became essential to corroborate our own findings. Evaluation of the study of genomic DNA content and DNA band fragmentation assay by DNA gel electrophoresis clearly revealed that the RA had high amount of genomic DNA content than that of BA with a distinguishable DNA band in the gel. This particular observation could perhaps provide an answer to the biasness in food selection physiology in different ant species. It may possibly be stated that the internal DNA content being high could aid in higher conversion from DNA to protein in RA. Owing to their high preferences for external protein food source, the data also revealed that the average number of RA consuming protein nutrient was almost four times greater than that of BA thereby signifying a greater population size and more organized eusocial life in RA than that of BA. BA, on the other hand, having only one-half of net genomic DNA content as compared to the RA and an almost faint and indistinguishable DNA band seen in the gel electrophoresis assay, preferred only carbohydrate rich nutrient. This biasness in food selection could possibly be explained on the basis that BA having very low DNA content, the building block of proteins, needed food source which would serve them instant energy required for rapid and erratic movement by oxidation of cellular components during territory protection, defence and food storage with comparatively much smaller population size, thus, playing a compensatory role altogether. However, observations also revealed that lipid rich food items were completely neglected by both the ant types which perhaps may be due to avoidance of fat deposition in their body which might hinder their daily foraging activities.

Additionally, we also checked whether DNA content has any direct role in the duration of foraging activity of ants but the data revealed that both the ant types exhibited similar hyper foraging activity under constant temperature, humidity and availability of resource or profitable food items.

Therefore, the overall findings of our pioneer study serve a preliminary but vital evidence for close association of genomic DNA content with that of foraging physiology and nutritional preferences in eusocial ants to maintain an optimum balance in their day to day activity. This would presumably accentuate the molecular understanding of signalling factors associated with foraging physiology and behaviour in ants in future.

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Conflict of interest

None to declare.

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