



Biochemical analysis of the life stages of *Oecophylla smaragdina* (weaver ant)

Sunanda B. Kumre ✉

Department of Zoology, Mahatma Gandhi Arts, Science and late N. P. Commerce College, Armori Dist. Gadchiroli (M.S.), India

ARTICLE INFO	ABSTRACT
<p>Received : 20 September 2023 Revised : 25 October 2023 Accepted : 07 November 2023</p> <p>Available online: 16 January 2024</p> <p>Key Words: Biochemical Analysis Holometabolous Life Stages Protein Weaver Ant</p>	<p>The Asian weaver ant, <i>Oecophylla smaragdina</i>, indeed exhibits fascinating social behavior and a unique nesting strategy. The nesting behavior of weaver ants involves a "leaf-weaving," process where the worker ants use the silk produced by their larvae to bind leaves together, creating a complex and sturdy nest structure. Regarding their life stages and metabolic changes, <i>Oecophylla smaragdina</i> as a holometabolous species undergoes complete metamorphosis, involving distinct developmental stages such as egg, larva, pupa, and adult. Each stage is characterized by significant changes in the insect's physiology and appearance. It is essential to study the metabolic changes in these ants during their life stages to better understand their development, behavior, and adaptability to their environment. The main objective of the present study is a biochemical analysis of the life stages of the weaver ant. During this study, various ants were collected at various stages and analyzed for the estimation of protein, carbohydrate, RNA, and DNA. According to the findings, the concentration of proteins was observed to be highest during all life stages of weaver ants. Following proteins, the concentration of RNA was noted to be the highest, then the concentration of DNA and carbohydrates. It was also noted that the concentration of biomolecules increased from the egg stage to the pupal stage, reaching its maximum level at the pupal stage. However, after reaching the maximum level at the pupal stage, the concentration of biomolecules declines as the ant progresses from the white imago to the adult stage.</p>

Introduction

Among the thousands of social insects, a few deserve to be called classic because certain remarkable features of their behavior have prompted unusually careful and thorough studies. The Asian weaver ant *Oecophylla smaragdina* is a social hymenopteran ant that is found predominantly in tropical and subtropical regions of central India. This species is an arboreal ant that builds nests by binding living leaves together and forming polydomous colonies consisting of multiple nests. According to Crozier *et al.* (2009), *Oecophylla* is an aggressive and generalist predator that can control several pest insects, such as mango, cashew, citrus, and other crops. *Oecophylla smaragdina* (Weaver ants) are holometabolous and undergo diverse changes in the metabolism of proteins, carbohydrates, RNA and DNA during larval-pupal-adultal transformation.

The chemical composition of insect tissue is highly variable among insect species and at different developmental stages of the same species. The quantitative variation in different biomolecules during the postembryonic development and metamorphosis of insects has received some attention in the past few decades. Such variations in relation to different viewpoints in relation to different insects have been interpreted from different viewpoints by Slama *et al.* (2015) and Trumen and Riddiford (2019) as reflecting the balance of synthesis, storage and degradation of the nutrient response to developmental needs. This paper aimed to analyze the quantitative variation in carbohydrates, proteins, DNA and RNA during the embryonic development of *Oecophylla smaragdina*.

Corresponding author E-mail: sunandakumre15@gmail.com

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Materials and Methods

Survey and Collection

Weaver ants were collected from the nests of mango trees in different regions of Nagpur city. All the collected weaver ants were differentiated into eggs, pupae, larvae and adults based on their life stages and used for quantitative analysis of biomolecules.

Reagents and chemicals

All the chemicals and reagents used in this study were obtained from the Zoology Laboratory of RTMN University, Nagpur (M.S.) India. All the chemicals were of analytical grade.

Extract preparation

The developmental stages of weaver ants were homogenized separately using a mortar and pestle. Ringer solution was added to the homogenized mixture, which was allowed to settle for 5 min at room temperature. Afterward, the homogenate was centrifuged at 4500 rpm for 10 min. The resultant precipitate was used to analyze the total soluble protein content, while the supernatant was used to analyze the concentrations of carbohydrates, DNA, and RNA. This process was conducted to assess the biochemical characteristics of the different stages of weaver ant development.

Biochemical analysis

The biochemical composition, including protein and carbohydrate contents, of *Oecophylla smaragdina* during the developmental stages was determined using the methods outlined by Kumar and Michael (2012), whereas DNA and RNA levels were estimated by Swami *et al.* (2021) with slight modifications. The total protein concentration in the development stages of weaver ants was quantitatively estimated via the Lowry method. Standard BSA (5 mg) was dissolved in 5 mL of distilled water (DW) for the standard, and a blank was made with 1 mL of 0.1 N NaOH and 3 mL of DW. The unknown tubes had 1 mL of tissue extract and 3 mL of DW. 5.5 mL reagent solution (50 mL 2% Na₂CO₃ in 0.1 NaOH, 1 mL 0.5 CuSO₄ solution in 1% sodium-potassium tartarate) was added, left undisturbed for 15 min. Then, 0.5 mL Folin-Ciocalteu reagent with equal water was added, vigorously shaken, and left undisturbed for 30 min. Blue color intensity measured at 650 nm using a

spectrophotometer (SL 177). Similarly, the Dubois method was used for the quantitative estimation of carbohydrates during development. Standard carbohydrate solution (1 mg of sugar in 10 mL of DW) and a blank (1 mL of DW + 1 mL of 5% phenol reagent) were prepared. The unknown tubes contained 1 mL of tissue extract and 1 mL of 5% phenol reagent. Five milliliters of concentrated H₂SO₄ was quickly added using a fast-flowing pipette, and the color intensity was measured at 490 nm on a spectrophotometer (SL 177) for a yellowish-brown color. The concentration of DNA was estimated by the Searcy and MacInnis method. Standard DNA solution (1 mg of calf thymus DNA in 10 mL of citrate buffer saline) and a blank (2 mL of buffer saline) were prepared. 4 mL of diphenylamine reagent was added to all the tubes, which were subsequently boiled for 20 min. The color intensity was observed at 595 nm using a spectrophotometer (SL 177) to determine a bluish color. A standard calibration curve was prepared with 5 known tubes of Calf Thymus DNA. Total RNA was assessed by the Disch-Orcinol technique. Standard RNA solution (1 mg of commercial yeast RNA in 5 mL of citrate buffer saline) and a blank (2 mL of buffer saline) were prepared. The unknown tubes contained 1 mL of tissue extract and 1 mL of buffered saline. Three milliliters of acid orcinol reagent was added to all tubes, which were subsequently boiled for 20 min. Standard calibration curves were prepared with 5 known tubes of yeast RNA.

Results and Discussion

During postembryonic development, the weaver ant *Oecophylla smaragdina* passes through the egg, larva, pupa and adult stages. The larvae were classified from first to fourth instars based on the head capsule/body length ratio. The pupae and adults were identified on the basis of morphological features. The concentrations of biomolecules such as proteins, carbohydrates, DNA and RNA were studied in the eggs, larvae, pupae and adults of weaver ants. The present study notice that the concentration of protein, carbohydrate, DNA and RNA was increased gradually from eggs to pupal stage (Protein ranges from 0.0405 ± 0.009 µg/mg to 0.2388 ± 0.02, carbohydrate ranges from 0.0164 ± 0.003 µg/mg to 0.0434 ± 0.001 µg/mg, DNA

ranges from $0.0127 \pm 0.001 \mu\text{g}/\text{mg}$ to $0.0481 \pm 0.0009 \mu\text{g}/\text{mg}$, RNA ranges $0.0335 \pm 0.001 \mu\text{g}/\text{mg}$ to $0.0776 \pm 0.002 \mu\text{g}/\text{mg}$ (Table 1). During the pupal stage, the fungus attempts to reach its maximum level, after which it decreases drastically to the adult stage (Figure 1). Holometabolous insect species undergo diverse metabolic changes in proteins, carbohydrates, lipids, and nucleic acids (DNA and RNA) throughout their transformation from larvae to pupae and then to adults, as observed in studies by Dhumal and Tare (2011). The qualitative variations in different nutrients during the postembryonic

development and metamorphosis of insects have been studied from various perspectives by researchers, such as Konopova and Jindra (2007), Charles *et al.* (2011), Urena *et al.* (2014) and Trumen and Riddiford (2019). These studies highlight the intricate balance between nutrient synthesis, storage, and degradation in response to developmental requirements. Current research has focused on quantitatively analyzing proteins, carbohydrates, DNA, and RNA in different developmental stages of *Oecophylla smaragdina*.

Table 1: The biomolecules observed in the developmental stages of *Oecophylla smaragdina*

SN	Stages of weaver ant	Protein ($\mu\text{g}/\text{mg}$)	Carbohydrate ($\mu\text{g}/\text{mg}$)	DNA ($\mu\text{g}/\text{mg}$)	RNA ($\mu\text{g}/\text{mg}$)
1	Eggs	0.0405 ± 0.009	0.0164 ± 0.003	0.0127 ± 0.001	0.0335 ± 0.001
2	First instar (I)	0.0636 ± 0.004	0.0188 ± 0.002	0.0205 ± 0.0007	0.0374 ± 0.001
3	Second instar (II)	0.0921 ± 0.01	0.0235 ± 0.002	0.0283 ± 0.002	0.0457 ± 0.002
4	Third instar (III)	0.1304 ± 0.04	0.0385 ± 0.001	0.0324 ± 0.001	0.0669 ± 0.001
5	Fourth instar (IV)	0.2042 ± 0.01	0.0343 ± 0.0006	0.0326 ± 0.002	0.0722 ± 0.001
6	Pre pupa (P.P)	0.2388 ± 0.02	0.0434 ± 0.001	0.0481 ± 0.0009	0.0776 ± 0.002
7	Mid pupa (M.P.)	0.1081 ± 0.003	0.0363 ± 0.002	0.0389 ± 0.002	0.0705 ± 0.008
8	Late pupa (L.P.)	0.08467 ± 0.01	0.0336 ± 0.002	0.0321 ± 0.002	0.0539 ± 0.002
9	Adults (A)	0.0919 ± 0.01	0.0233 ± 0.001	0.0323 ± 0.001	0.0524 ± 0.001

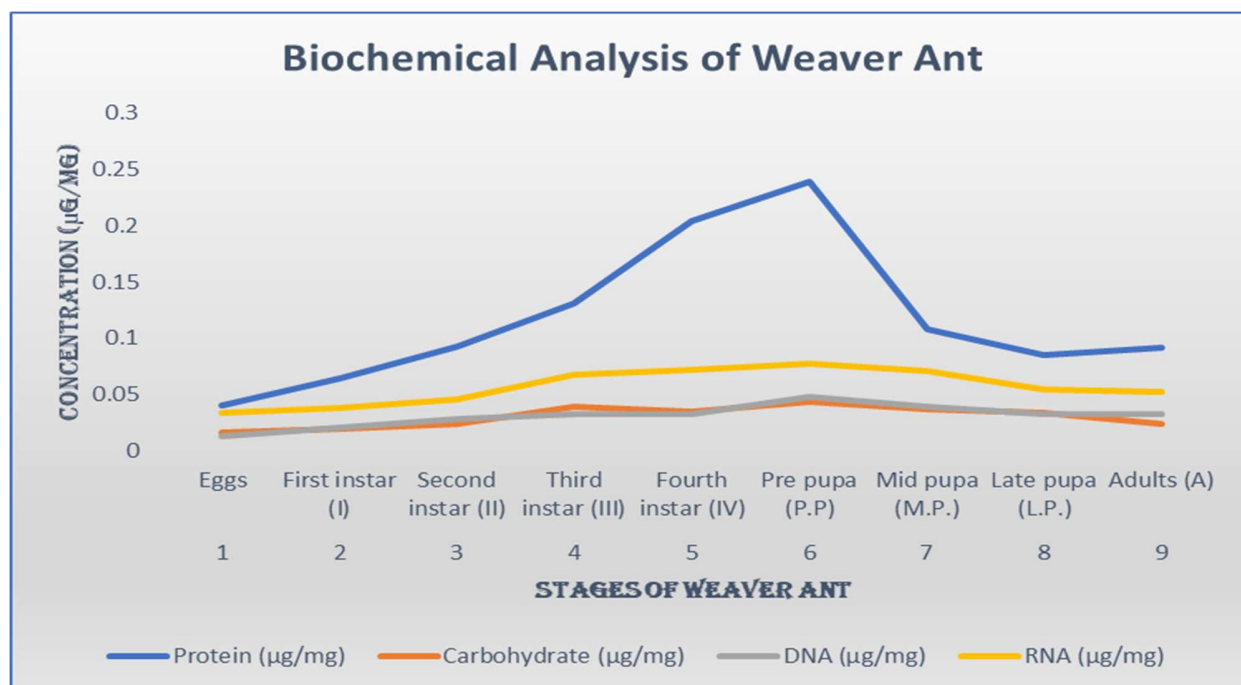


Figure 1: Total concentrations of protein, carbohydrate, RNA and DNA at various stages of Weaver

ant infection

The study revealed a gradual increase in protein, carbohydrate, DNA, and RNA concentrations from the egg stage to the pupal stage, followed by a sharp decrease in the adult stage. Shankar *et al.* (2015) emphasized that proteins, carbohydrates, and lipids are crucial biochemical components that play a significant role in the fundamental biochemical processes governing the growth and development of insects. Overall, the study underscores the vital role of nutrients in various developmental stages, a notion supported by Borphukan and Bhola's (2013) research. These authors highlighted that fluctuations in essential nutrients crucial for morphogenetic changes not only mirror metabolic patterns but also indicate the nutritional requirements of each developmental stage. These fluctuations are influenced by morphological and physiological changes during ontogeny. Additionally, the research noted a rapid increase in protein concentration during the IV instar larval stage of *Oecophylla smaragdina*, reaching its peak at the end of the larval to prepupal stage and declining in the late pupal stage. These findings align with those of Murthy *et al.*, 2014, who observed a progressive increase in the protein concentration in the hemolymph during larval development, peaking in the late fifth-instar larvae of *Bombyx mori* but decreasing in the pupal stage. The estimation of total carbohydrates showed a consistent increase as the larva aged, reaching its highest point during the IV instar larva to prepupal stage. This pattern aligns with previous research findings, such as those of Mishra *et al.*, 2010, in which similar increases in total carbohydrate or reducing sugar concentrations were noted in various silkworm species during the later stages of larval development. These authors suggested that elevated levels of carbohydrates in the hemolymph serve as an energy reserve that is crucial for utilization during metamorphosis, as well as during the pupal and adult

stages of the silkworm life cycle. Insects, especially holometabolous insects, have been underexplored in terms of understanding the crucial role of RNA and DNA in genetic control and protein synthesis during development. To address this gap, a study focused on quantifying the levels of RNA and DNA at different stages in *Oecophylla smaragdina* development. The findings revealed a gradual increase in DNA and RNA concentrations from the IV instar larval stage to the prepupal stage, followed by a decrease during the pupal-to-adult transformation. This pattern is consistent with previous research by Swami *et al.* (2021), who investigated individual honeybees and reported that the highest RNA and DNA yields occurred during the pupal stage and decreased in adulthood. Similarly, Chelex DNA extraction technique was used to analyze the developmental stages of *Aedes aegypti* and discovered significant differences in DNA concentration among stages, with adults exhibiting the highest DNA concentration compared to larvae and pupae.

Conclusion

Overall, in *Oecophylla smaragdina* ant, the expression of biomolecular components such as proteins, carbohydrates, DNA and RNA increased gradually from the egg to the pupal stage and drastically decreased in the adult stage. Overall, these findings indicate that biomolecules are highly utilized in the formation of adult structures. Weaver ants, particularly their ant larvae, can be harnessed as food resources due to their ability to infest humans and their high protein content.

Conflict of interest

The authors declare that they have no conflicts of interest.

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