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Study of sexual dimorphism in larval stage of Muga Silkworm *Antheraea assama* Ww collected from different altitudes

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ABSTRACT

Sexual dimorphism or gender specific differences were investigated in Muga silkworm Antheraea assama Ww., specifically in terms of cellular immunocompetence/immunological traits, by examining the cellular immune arsenal in both the sexes. Furthermore, experiments were carried out on A. assama larvae collected from four different farms located at different altidues from the sea level. In all the cases, females were found to contain higher haemocyte load as compared to males. Females also recorded higher immunocyte population viz. plasmatocyte (PL) and granulocyte (GR) that suggest stronger cellular defence reactions or immunocompetence.

Key words: *Antheraea assama Ww, plasmatocyte, granulocyte, sexual dimorphism, immunocompetence.*

INTRODUCTION

According to Bateman's principle (1948), sexual dimorphism observed in different species in terms of immunocompetence/ immunological traits is due to the fact that "females invest more in immunity than males"[17]. Males use up much of their energy in gaining fitness for increasing their mating frequency, whilst females increase fitness through longevity because their reproductive effort is much higher. Consequently, females should invest more in immunity than males in order to increase their survival probability i.e. mechanism of immunity is adapted to specific need[14]. Sheldon and Verhulst[19] also reported that by investing more in reproduction the males suppress or downregulate immune functions.

Studies on invertebrates too provide support to sexual dimorphism in immunological traits. Kurtz *et.al.*[11] and Kurtz and Sauer[10] detected higher immunocompetence in females relative to male scorpion flies. Wedkind and Jacobson[21] found higher parasite susceptibility in male copepods than females. Male house crickets showed a higher mortality compared to females when experimentally infested with the bacterium *Serratia liquifaciens*[5]. Studies carried out on gender differences in immunity in *Blatella germanica* by Hazarika and Gupta[8] and on Tse-Tse fly by Nigam *et.al.*[16] also showed a higher female immunocompetence.

The present study intends to explore this sexual dimorphism in immunocompetence in the Muga silkworm *Antheraea assama* Ww, a sericigenous insect native to the state of Assam in the North East region of India[2]. This silkworm produces the unique, world-famous golden-hued Muga silk. Because of

the lustre and high tensile strength of the silk, it is often regarded as the "queen of silk". The *A. assama* being a multivoltine, semi-domesticated variety of silkworm, is exposed to a wide range of ecological and pathological factors. Therefore, identification of gender-specific immunocompetence would help commercial rearers in predicting their yield to a certain extent, because mounting an immune response is energy expensive and as such poses a strain on other physiological traits/developmental processes.

In case of Muga silkworms, we have restricted our study of sexual dimorphism to the cellular immune reactions in the two sexes only. Haemogram, such as Total Haemocyte Count (THC) and Differential Haemocyte Count (DHC) has been considered to determine differences between the sexes in terms of immunocompetence, because high haemocyte loads have been reported to be related to successful immunocompetence in some insects like *Drosophila melanogaster*[9]. DHC has been limited to plasmatocytes (PLs) and granulocytes (GRs) counts only, as they have been reported to be involved in cellular immune reactions in many insects viz. *B. germanica*[6,8], some lepidoptera and other insects studied so far[15].

MATERIALS AND METHODS

Field investigations were carried out at different sericulture farms located at different altitudes during the months of April-May (2014) in order to realize the objective of the present investigation. These farms were randomly selected and include the Khanapara State Sericulture Farm, Assam, situated at an altitude of 55.5m above sea level (ASL); Nongpoh (Central Silk Board farm), Meghalaya, altitude 464m ASL; Tura (Central Silk Board farm), West Garo Hills, Meghalaya, altitude 657m ASL; and Kalimpong (Central Silk Board farm), West Bengal, altitude 1247m ASL.

Insects: Fifth-instar larvae were directly collected from the four different sericulture farms as mentioned above, situated at different altitudes, and transported to the laboratory for conduction of the experiments.

Host Plant: Larvae of *A. assama* which were reared on Som plants *(Machilus bombycina*) were considered for the experiments.

Measurement of Total Hemocyte Count (T.H.C.) and Differential Hemocyte Count (D.H.C.) of circulating plasmatocytes and granulocytes: For this experiment, T.H.C. and D.H.C. were performed on the same insect, and separately in both males and females. These experiments were performed on larvae obtained from each of the above mentioned farms.

Quantitative estimation of hemocytes per cubic millimeter of hemolymph (T.H.C) from healthy well-fed fifth instar larvae (48-hr post moult) were carried out as per the method of Hazarika and Gupta[8], followed by fixation of whole insect in hot water at 56-60°C for 2-3 mins[18]. After heat fixation, the insects were removed and rapidly dried on a filter paper. A metathoracic proleg was severed at the tip and first two-three drops of pale greenish blood were allowed to flow into a clean glass slide. A portion of the blood was quickly drawn to a 0.5 mark of a white blood cell (WBC) diluting pipette, the tip was carefully wiped clean and the blood then diluted to the 11 mark (i.e. 20 times dilution) with physiological saline (NaCl 0.9 gm, KCl 0.041 gm, $CaCl_2$ 0.048 gm, $NaHCO_3$ 0.002 gm, distilled water 100 ml) containing acetic acid (1%). The pipette was then shaken vigorously for several minutes; the first drop was discarded and a hemocytometer was filled. Using a levy double line hemocytometer with improved Neubauer ruling, cells were counted in the four corner squares and total numbers were counted per cubic millimeter by the following formula-

$$\frac{\text{Hemocytes counted in x 1mm squares X dilution X depth of chamber}}{\text{Number of 1mm squares counted}}$$

For D.H.C. of circulating PLs and GRs, hemolymph samples were taken from 5th instar larvae, 48hr post-moult, since at this time the blood (haemolymph) usually carries a full complement of all the haemocyte types[1]. Hemolymph drops were obtained by severing the tip of one of the prolegs of the larvae. Unfixed haemolymph drops were directly collected on clean slides; smear was prepared and then air-dried. Air-dried hemolymph smear was fixed in methanol and stained with Giemsa stain. The stained films were mounted in DPX. A minimum of 200 cells were classified per insect (5th instar larva) and were replicated in a minimum of 3 sets. The percentage of both PR and GR was calculated on the basis of the total number of all the hemocytes which had been obtained in a number of hemolymph smears. The percentage of PLs and GRs were then converted to circulating PLs and GRs[12] from the T.H.C., i.e. no/mm^3.

Means of THC and DHC of PL and GR in both the sexes were compared by unpaired‘t’ test at a confidence level of 5% to find out whether there exists a significant difference in immunocompetence between the two sexes. Statistical analysis of the data was performed using the statistical software OriginPro8.

RESULTS AND DISCUSSION

Cellular immune arsenal of both male and female muga silkworm *Antheraea assama* Ww were investigated to determine gender-specific differences in terms of immunocompetence. From figure 1a, it is evident that females tend to show higher haemocyte load as compared to males, irrespective of altitude. Similarly, females also recorded higher immunocyte population viz. plasmatocytes (PL) and granulocytes (GR) as evident from figure 1b and 1c, respectively. These results suggest stronger cellular defence reactions or immunocompetence in females as compared to males. Sexual dimorphism in *A. assama* was also distinctly observed at all the four different altitudes.

Fig. 1a: Bar diagram showing higher hemocyte load in females compared to males at different altitudes

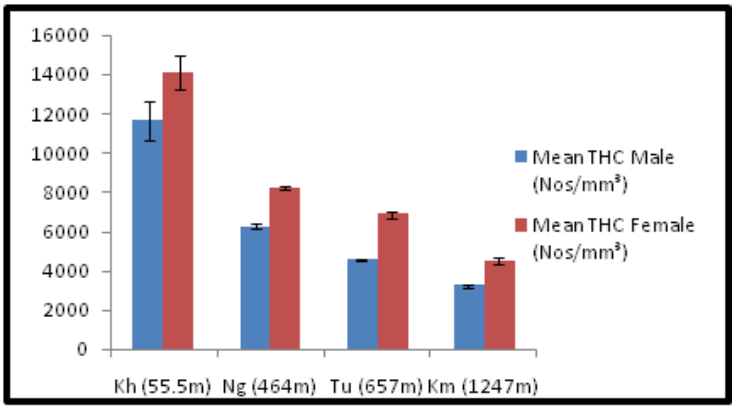


Fig. 1b: Bar diagram showing higher circulating PL in females compared to males at different altitudes

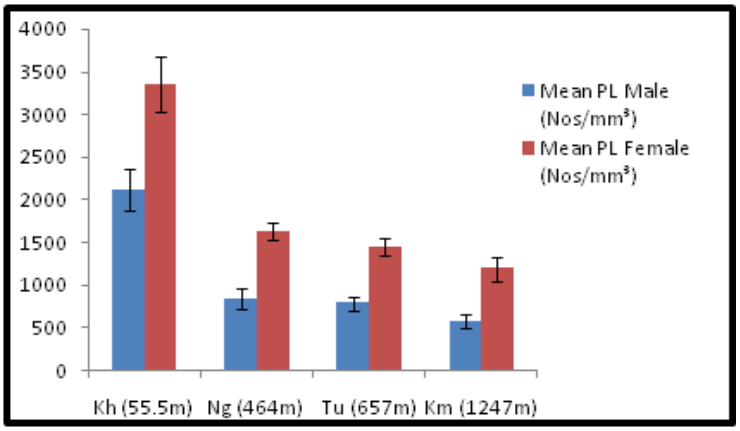
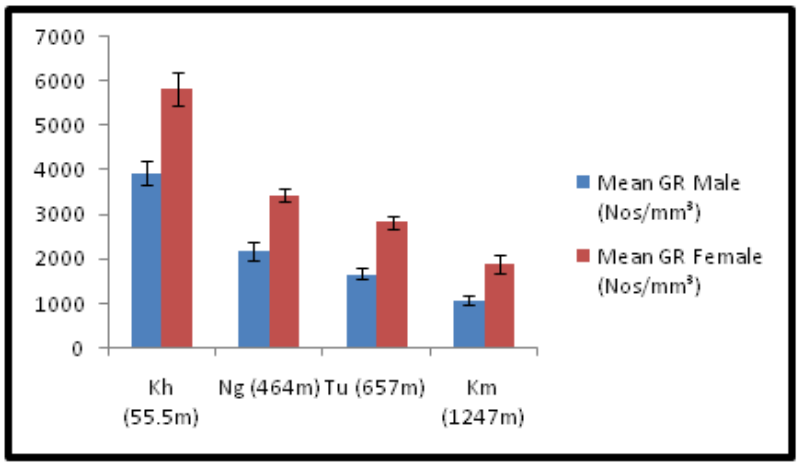


Fig. 1c: Bar diagram showing higher circulating GR in females compared to males at different altitudes



This increased hemocyte load observed in females can be explained in the lines of Bateman's principle, which states that, males gain fitness by increasing their mating success whilst females gain fitness through increased longevity because their reproductive effort is much higher, and as such females need to invest more in immunity than males. Mating rate is a major determinant for fitness in males; whereas longevity is of more importance in females[3,20,4]. Female reproduction depends directly on the resourses gained (quality and quantity), whereas males should invest in obtaining mates[20]. Thus, we can say that males invest more in mating than in immunity (investment in immunity signifies synthesis and production of higher number of hemocytes /immunocytes for protection and survival) but, females invest more in longevity and in order to increase their survival probability, they invest more in immunity. From this observation we can say that females are more immunocompetent than males, thereby establishing sexual dimorphism in terms of immunocompetence.

Similar results have been obtained in case of PLs and GRs, i.e, circulating PLs and GRs have been found to be higher in females than in males, and this trend has been observed consistently in all the different altitudes. Since, PL and GR together constitute the immunocytes[7], i.e, these hemocytes work together to perform the immune related functions, their higher numbers in females suggest superior immunocompetence in females as compared to males. Higher load of immunocytes is reported to be related with higher immunocompetence by various workers viz.[7,8,15] in different insects. Hazarika and Gupta[8] specifically stated that it is reasonable to suggest that larger the PL and GR population, stronger the cellular defence.

The higher PL and GR population in females account for the observed higher THC in the same. The immunosuppressive effect observed in the males is probably due to its increased courtship activities[17] as the male has to seek out the females as has been suggested by Mckean and Nunney[13] in *D. melanogaster*. Nevertheless, the present study suggest that in *A. assama* sexual dimorphism in terms of immunocompetence/immunological traits is evident; the females being more immunocompetent than the male due to higher haemocyte loads.

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