Pestycydy, 2008, (1-2), 35-41.
ISSN 0208-8703

# Developmental expression and hormonal regulation of male-specific Yellow Protein mRNA in adults of the desert locust, *Schistocerca gregaria*

Murshida BEGUM, M. Mazibur RAHMAN*,
Roger HUYBRECHTS and Arnold DE LOOF

*Zoological Institute, Laboratory for Developmental Physiology, Genomics and Proteomics, Naamsestraat 59, Catholic University of Leuven, 3000 Leuven, Belgium*
**e-mail: shajal66@yahoo.co.uk*

**Abstract:** Adult males of *Schistocerca gregaria* turn yellow when they become sexually mature. This is due to the deposition in the cuticle of a male-specific Yellow Protein (YP), of which the amino acid sequence is known. Yellowing only happens in crowd-reared (gregarious) males, and results from the deposition of a specific 'Yellow Protein'. If individual males (solitarious) are isolated after the adult emergence, they become sexually mature but they do not turn yellow. On the basis of a partial YP-mRNA sequence, we established a reverse transcriptase polymerase chain reaction (RT-PCR) assay to study the developmental expression of YP in crowd-reared males, isolated-reared males and females. In crowd-reared adult males the transcription of YP gene started from day 5 on, and reached a maximum at day 12. The effects of juvenile hormone (JH), insulin (bovine), corazonin, ecdysone and 20 0H-ecdysone (20E) on the regulation of YP-mRNA synthesis were also investigated. JH made the cuticle turn yellow and, as shown by RT-PCR, YP-mRNA was induced. The effect of 100 μg JHIII was stronger than that of 10 μg. Insulin was only effective in inducing YP-mRNA synthesis at high dose (19 μg) and after more days (18 d). Corazonin and 20E made the cuticle turn black, but no YP-mRNA synthesis was observed. Ecdysone (10 and 100 μg) showed no effect on body coloration and YP-mRNA. Thus, JH was found to be the most potent inducer among the hormones tested.

**Keywords:** sex determination, sex hormones, Yellow-Protein, body color polymorphism, phase transition, juvenile hormone, insulin

## INTRODUCTION

Body colour polymorphism is one of the most conspicuous phase-related phenomena in locusts [1]. Adult male *Schistocerca gregaria* turn yellow when reared in crowded conditions (gregarious) [2], but this never happens upon rearing in isolation (solitarious). Neither do isolated or crowded females ever turn yellow. This means that this differential and inducible yellow body coloration is a good model to study the endocrine control of sexual differentiation in adult insects [3] and as for identifying not yet known factors controlling phase polymorphism. Allatectomy prevented yellow coloration, while external introduction of JH on allatectomized males resulted in yellowing in *S. gregaria* [4]. The JH analog methoprene also caused yellowing in *Locusta migratoria* [5]. The role of other hormones has not yet been investigated.

The beta-carotene binding protein responsible for the yellow coloration was isolated and characterized [6]. This enabled the partial sequencing of the YP-mRNA [7] and the development of a YP – Polymerase Chain Reaction (PCR) bioassay. We used this assay to study the developmental expression of YP and the role of JH, insulin (bovine), corazonin, ecdysone and 20 OH-ecdysone and in the regulation of YP-mRNA synthesis in *S. gregaria*.

## MATERIALS AND METHODS

### Rearing of locusts, dissection and tissue collection

The desert locust *Schistocerca gregaria* (Forskal) was raised either under crowded (gregarious) or in isolated conditions (solitarious) at 32 ±1 °C with a light-dark cycle of 13:11 h [8]. Per developmental stage examined, four pieces of abdominal cuticle were dissected in *S. gregaria* Ringer solution, immediately placed in liquid nitrogen, and stored at -70 °C until use.

### RT-PCR study

cDNA was prepared [9] and PCR was performed using the Clontech protocol. The YP gene-specific forward and reverse primers (Eurogentech, Belgium) were 5’ CCAGACGACAGATGCGGAGAT3’ and 5’AGGTACGTCTGGCCGTTTGGA3’ respectively. For the controls, two primers based on the actin gene of *Locusta migratoria* were used (forward: 5’ AGGATGGCTACTGCTGCA3’ and reverse: 5’ GAACAGTGCCCT CAGGGTACC3’) primers (Eurogentech, Belgium). Thermal cycling consisted of 30 cycles with a denaturation step for 1 min at 94 °C, an annealing step for 1 min at 60 °C and an extension step for 1 min

at 68 °C. PCR material was analysed by 1.5% agarose-ethidium bromide gel electrophoresis. Band intensity was measured by Image Master ID software.

**Hormonal induction**

Juvenile hormone III (JHIII) (Sigma) dissolved in soybean oil was injected at 48 hr intervals, starting at day 6, by a Hamilton syringe through the dorso-lateral membrane between the 2nd and 3rd abdominal segment at doses of 10 or 100 $\mu$g JHIII per animal. Per condition 25 animals were treated. Soybean oil served as control. All experiments were repeated 4 times. A similar protocol and injection scheme were used for Bovine Pancreas insulin (1.9 and 19 $\mu$g) (Sigma) and corazonin (1.35 and 13.5 $\mu$g) (Eurogentech, Belgium). Ten $\mu$g and 100 $\mu$g of 20-OH ecdysone (20E) (Sigma) were dissolved in 4 $\mu$l of DMSO-Ringer per injection. Controls received only DMSO-Ringer.

## RESULTS

**Developmental expression of YP**

The presence of YP-gene in adult locusts of increasing age was determined by RT-PCR. Results clearly demonstrated that YP-mRNA synthesis started when males became 6 d old and continued until day 18 (Figure 1). Band intensity revealed that the synthesis of YP-mRNA increased with sexual maturation. However, in females and in solitarious males no traces of YP-mRNA could be seen, while the actin signal was clear (results not shown). Thus, the expression of the YP gene is restricted to gregarious males only.

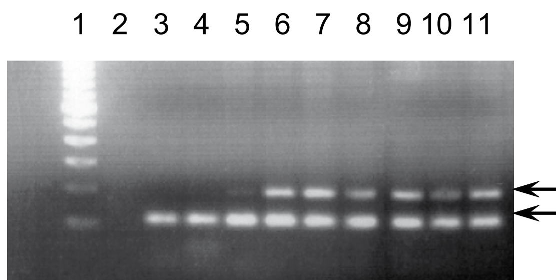


**Figure 1.** RT-PCR analysis confirming the presence of YP-mRNA at different ages of gregarious male *S. gregraria.* Lane 1: molecular size marker (100 bp), Lane 2: $H_2O$ control, Lane 3-11 indicates the ages: 0 d, 3 d, 6 d, 9 d, 10 d, 11 d, 12 d, 15 d and 18 d, respectively. Upper and lower arrows indicated the YP cDNA and actin bands respectively.

**Hormonal regulations**

JH injections potently induced YP-mRNA synthesis (Figure 2 A, B) but the brightness of the yellow color was lower than in normal gregarious males. Actin mRNA levels were not influenced by JH. The effect of the 100 $\mu$g JHIII was stronger than of 10 $\mu$g.

Repeated injections of 1.9 µg of bovine insulin showed no visible yellowing or YP-mRNA synthesis (Figure 3 A). Repeated injections of 19 $\mu$g (Figure 3 B) of bovine insulin resulted in a clear YP-mRNA band at 18 days. Injections of either 1.35 $\mu$g or 13.5 $\mu$g corazonin in oil caused melanization of the cuticle, a typical feature accompanying gregarization, in respectively 60 and 100 percent of the treated animals. However, RT-PCR revealed that corazonin did not induce YP-mRNA synthesis (Figure 4 A, B). Two repeated injections of 20E made the cuticle of the animals turn black. However no increased YP-mRNA synthesis was observed.

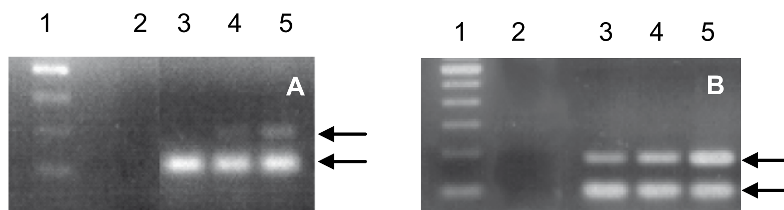


**Figure 2.** RT–PCR results from the samples of JHIII-treated animals A: 10 $\mu$g dose and B: 100 $\mu$g dose. Lane 1: 100 bp DNA ladder, Lane 2: $H_2O$ control, Lane 3-5: samples from different ages: 12 d, 15 d and 18 d of JHIII-treated animals, upper and lower arrow indicate YP-mRNA and actin bands, respectively.

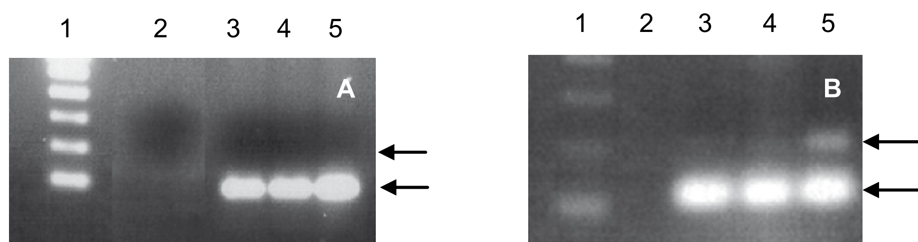


**Figure 3.** RT-PCR results from the samples of insulin-treated animals A: 1.9 $\mu$g dose and B: 19 $\mu$g dose. Lane 1: 100 bp DNA ladder, Lane 2: $H_2O$ control, Lane 3-5: samples from different ages: 12 d, 15 d and 18 d of insulin-treated animals, upper and lower arrow indicate YP-mRNA and actin bands, respectively.

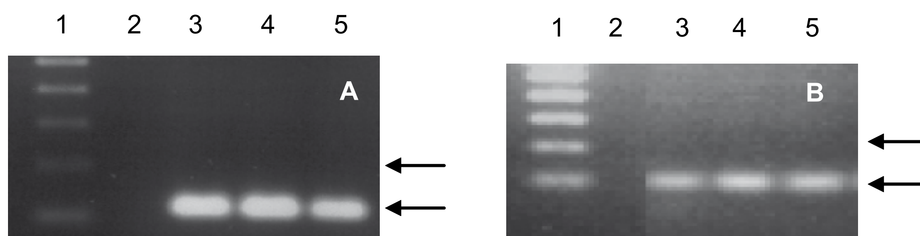


**Figure 4.** RT-PCR results from the samples of corazonin-treated animals. A: 1.35 $\mu$g dose and B: 13.5 $\mu$g dose. Lane 1: 100 bp DNA ladder, Lane 2: $H_2O$ control, Lane 3-5: samples from different ages: 12 d, 15 d and 18 d of corazonin-treated animals, upper and lower arrow indicate YP-mRNA and actin bands, respectively.

## DISCUSSION

Textbooks of Developmental Biology and Insect Physiology still forward the view that "in insects sex is strictly genetic", thus that insects do not have sex hormones. This – from the standpoint of physiology is highly questionable [3] – view is mainly based on a misinterpretation of the mechanism underlying the occurrence of gynandromorphy in a *Drosophila* mutant (described by Morgan, 1923, for Figure see [3]) of which the right side was XX and female, while the left side of the body was XO and male.

This resulted in the interpretation that in insects the ratio autosomes:sex chromosomes determines sex, without a role for hormones. The discovery of the *complementary sex determiner* gene in the honey bee *Apis mellifera* [10], a gene that occurs in 2 isoforms, shows that other explanations are possible. The allelic composition of *csd* is the primordial genetic mechanism of sex determination. A plausible explanation is that when both isoforms of csd protein are present, a heterodimeric (splicing) factor is formed that ensures correct mRNA splicing needed for bringing about the female phenotype. Homodimers and monomers result in the male phenotype. This system does not rule out the existence of sex hormones in insects, of which the major role is to inform the body about the developmental state of the gonads. The counterpart of csd in locusts (and other species) remains to be identified. The search for insect sex hormones has been hampered by the lack of good bioassays in males. We think that YP synthesis is a good model. Indeed, YP is male-specific and its synthesis can be manipulated. JH was the most potent hormonal inducer, in line with former results of [4]. In [4] allatectomy was shown to prevent yellow coloration in crowded male

*Schistocerca americana gregaria*. External introduction of JHI and JHIII induced yellowing. The effect was dose-dependent. JHI was more potent than JHIII, and a mixture of both had a synergetic effect. However, because JH produced by the CA also occurs in females it cannot be a true classical sex hormone unless some part of the male genital system, e.g. the accessory glands, would be an additional site of JH synthesis. JH probably acts as a synergist. Ecdysteroids may be the overlooked sex steroids of insects [11]. While 20E has undoubtedly a role as a female sex ecdysteroid, a male sex ecdysteroid – if present - remains to be discovered and is momentarily being searched for. The stimulating effect of insulin might perhaps be due to a stimulating effect on ecdysteroid synthesis, like in *Aedes* [12]. Our results suggest that if this is the case, insulin does not act via 20E, but via another ecdysteroid [3] because 20E does not stimulate YP mRNA synthesis. Another possibility is that it stimulates JH synthesis like in *Drosophila* [13]. The YP bioassay also paves the way to identify the brain factor that is also involved in controlling yellowing [9]. Yellowing in locusts coincides with the ripening of the accessory glands. So, it is probable that the male accessory glands are a major source of JH synthesis like in mosquitoes [14].

## REFERENCES

[1] Uvarov B.P., Grasshoppers and Locusts - A handbook of general Acridology, Vol. 1, Cambridge University Press, London 1966.
[2] Norris M.J., Anti-Locust Bull., 1954, 18, 1-44.
[3] De Loof A., Gen. Comp. Endocrinol., 2008, 155, 3-13.
[4] Pener M.P., Lazarovici, P., Physiol. Entomol., 1979, 4, 251-266.
[5] Hasegawa E., Tanaka S., Jap. J. Entomol., 1994, 62, 315-324.
[6] Wybrandt G.B., Andersen S.O., Insect Biochem. Mol. Biol., 2001, 31, 1183-1189.
[7] Begum M., Ph. D. dissertation: Search for possible bioassays in unraveling the brain-gonad axis in two model insect species, 2004, Katholieke Universiteit Leuven, Belgium, 103 pp.
[8] Rahman M.M., Hoste B., De Loof A., Breuer, M., Gener., 2002, 26, 161-172.
[9] Sas F., Begum M., Vandersmissen T., Geens M., Claeys I., Van Soest S., Huybrechts J., Huybrechts R., De Loof, A., Peptides, 2007, 28, 38 – 43.
[10] Beye M., BioEssyas, 2004, 26, 1131-1139.
[11] De Loof A., Insect Sci., 2006, 13, 325-338.
[12] Riehle M.A., Brown M.R., Mol. Biol., 1999, 9, 855-860.
[13] Tatar M., Kopelman A., Epstein D., Tu M.P., Yin C.M., Garofalo R.S., Science, 2001, 292, 107-110.

[14] Borovsky D., Carlson D. A., Hancock R. G., van Handel E., Insect Biochem. Mol. Biol., 1994, 24(5), 437-44.