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Identification of female specfic protein by immunoelectrophoresis in the house cricket *Gryllodes sigillatus* walker (Gryllidae)

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Abstract
Using immunological tools an analysis was made on the identification of female specific protein during the first reproductive cycle of House cricket *Gryllodes sigillatus*. The reproductive cycle of the insects were divided into three phases based on the accumulation of vitellogenin, namely vitellogenic, previtellogenic and postvitellogenic phases. The oocyte antigens were purified and inoculated into rabbits to raise the whole oocyte antigens. The antiserum was collected and absorbed with male haemolymph proteins to obtain the antiserum for the female specific proteins. Using immunoelectrophoretic studies, both whole and specific-antisera were made to react with protein extracts of fatbody, haemolymph and ovary during the different phases of reproductive cycle . Immunoelectrophoresis of control vitellogenic fat body and ovary extracts against anti-female haemolymph serum revealed 7 and 8 protein precipitin arcs respectively. While the same antigens agsinst anti-oocyte serum revealed 6 and 7 fractions respectively. Immunoelectropherogram of anti-female haemolymph serum adsorbed with male haemolymph against control vitellogenic fat body and ovary each produced a single procipitin line, thus confirming that the synthesis of FSP was extraovarian. The reaction of anti-oocyte serum adsorbed with male haemolymph against control vitellogenic fat body and oocyte extract showed one and two precipitin protein arcs respectively and thus confirmed that the synthesis of ESP was of intraovarian in origin.

Keywords: vitellogenin, haemolymph, Gryllodes sigillatus, immunoelectrophoresis

Introduction
The insect *Gryllodes sigillatus*, a house hold pest living under the stones and in kitchen was selected for testing the interference of pesticide in the metabolism in general and the reproduction in particular. The insect under study was subjected for 8 days during its first reproductive cycle ie., from the day of adult emergence to the day egg laying. The specific proteins of females were investigated and identified using Poly Acrylamide Gel Electrophoresis (PAGE).The detection of specific proteins were confirmed using immunological studies by immunoelectrophoresis. The ovary is a structurally and metabolically complex system. Raven (1961) has reviewed large amount of cytological data on the accumulation of protein, lipid and carbohydrates reserve by oocytes. It is sometimes possible to distinguish a period of slow development of the egg, when lipid and carbohydrate materials accumulate and this is followed by a period of rapid development in which protein and aminoacid are deposited. Electrophoretic and immunological properties of the fat body proteins similar to those of eggs have implicated that the fat body is the site of synthesis of vitellogenins (Hill, 1962; 1965; Engelmann *et al.,* 1971; Elliott and Gillott, 1979) [12, 7, 6]. *Leucophaea maderae* is reported to synthesize as much as 72 mg of specific protein in a period of 15 days (Brookes, 1969) [2] and deposit the same into the oocytes as yolk, representing an average of 0.21 mg/h (Brookes, 1976) [3]. FSP have been studied in relation to oogenesis in different insect species since its discovery in the haemolymph of female pupae of the moth *Hyalophora cecropia* (Telfer and Williams, 1953; Telfer, 1954) [21]. Haemolymph proteins play a major role in oocyte development and vitellogenins make up the largest amount of yolk proteins, nearly 60-90% (Engelmann, 1979; Hagedorn and Kunkel, 1979) [8, 11]. Laufer (1960) [15] reported in *Phormia regina*, the requirement of a specific haemolymph protein for ovarian growth. Telfer (1961) [10] reported that during oocyte maturation in the saturnid moth, the blood protein concentration particularly that of the FSP drops. Dogra and Kawaguchi (1973) [5] have shown that in *B. mori,* the female protein is synthesized in the fat body and is then transferred to the ovary through haemolymph. These observations strongly suggest that the haemolymph proteins are involved in vitellogenesis. Vitellogenin synthesis in the fat body of *L. maderae* was analysed by Brookes (1986).

Material and Methods
Adult males and females were collected from the storehouses and maintained in the laboratory in glass containers. They were fed *ad libitum* with moist dog biscuits. In *G. sigillatus,* the first instar nymphs hatched out from the eggs on the day 13 or 14 after incubation. The nymphs were also reared with moist dog biscuits. The wing buds appeared in the nymphs, destined to develop into males, arround day 40 after hatching and the ovipositor in the female nymph around day 50 after hatching. The gross anatomy of the female reproductive system was studied from the dissections of the system made in Insect Ringer solution (Ephrussi and Beadle, 1936) [9]. The entire fat body obtained from the abdominal regions of the insect was pooled in a cavity block and washed thoroughly with Insect Ringer solution. Prior to anaesthetizing insects, the haemolymph was collected with the help of graduated

capillary tubes, by cutting the prothoracic leg at the coxal joint. Qualitative immunoelectrophoresis was carried out by the method of Feinstein 1976) [10].

Preparation of plates

Immunodiffusion plates (75 x 50 x 1.4) were pre coated with a thin film of agarose by dipping a sandwich of them into a beaker containing a hot solution (50o C) of 0.5% agarose. 1 mm thick shaped spacer was placed between precoated plate and a silicon-grease coated plate and the plates were held together by metal clamps. 5 ml of hot solution of 1% agarose was poured in the space between the plates and thus immunodiffusion plates of uniform thickness was obtained. Soon after the agar gel solidified, circular wells (1 center and 6 peripheral wells or according to the need) were punched out with a 3 or 4 mm gel punch. The debris in the wells were cleaned with the help of Pasteur pipette removing the small debris Two antigen wells (4 mm diameter) towards one side of the plate and an antiserum trough (65 x 1 mm) in the center were stamped out in the agar gel and the agar plugs were removed from the wells alone.

Preparation of antisera

Maintenance of the animal

Albino rabbits were employed to raise the antibodies. The rabbits of known age (1 year) and known weight were reared in cages. Daily, the rabbits were provided with 30 g of carrot in the morning and 30 g of Bengal gram in the evening along with cabbage leaves and water *ad libitum.* The cages were cleaned daily and the animals were checked for any infection.

Purification of antigens

Antigens were purified according to Cooper (1977). The crude protein extracts of the mature oocytes and haemolymph of vitellogenic female *G.sigillatus* were prepared. Partial purification of the proteins was achived by ammonium sulphate (80% w/v) precipitation. The partially purified proteins were dissolved in 1 ml of 0.15 M phosphate buffered saline (PBS) and dialysed against the same solution. The dialysed proteins were used as antigens for inoculation.

Administration of antigen into rabbits

Immunization schedules were carried out according to Talwar (1992). 5 to 10 mg of partially purified proteins of oocyte or haemolymph of female *G. sigillatus* in 0.5 ml of PBS were emulsified with an equal volume of Freund's complete adjuvant and the emulsions were tested for their integrity. Using a disposable syringe 1 ml of each of the emulsified antigen was injected into rabbits separately through subcutaneous route at multiple sites on the lateral side of the abdomen of the rabbit. The rabbits received similar dosage of injection with Freund's complete adjuvant after 10 days and the third injection after 15 days with Freund's incomplete adjuvant as a booster dose. Then, the blood was collected after 10 days of the injection.

Collection and preservation of antisera

The blood was collected from the rabbit by puncturing the marginal vein of the ear. The hairs over the ear margin were removed by a razor blade. A longitudinal slit was made on the center of the marginal vein and 25 to 30 ml of blood was collected into clean test tubes. After 24 h, the serum was decanted into clean test tubes and centrifuged at 1000 g per min at 4˚C.To the cell free antiserum, 0.1% sodium azide (w/v) was added as a preservative and stored at 20˚**C**

Preparation of specific antisera

To obtain specific antiserum for FSP the adsorption procedure of Talwar (1992) was employed. To 1 ml of whole anti-oocyte serum or anti-female haemolymph serum, 0.2 ml of male haemolymph was added and incubated at 37˚C for 2 h. The common proteins of male haemolymph adn anti-oocyte serum or anti-female haemolymph serum reacted ad precipitated. After a further period of 3 h at 0˚ C, the mixture was centrifuged at 10,000 g for 10 min at 4˚ C. Then, the supernatant was subjected to 2 or 3 similar such treatment until the absorption was complete and a clear solution obtained. This absorbed-female specific anti-serum was used in the immunological experiments.

Antigens and antisera

The plates were placed in the electrophoresis chamber and connected to barbital buffer paper wicks. The chamber in turn was connected to a power pack. Each well was filled 5µl of antigen sample mixed with marker dye and electrophoresis was carried out for 100 min at 7 to 8 v/cm width of the slide. At the end of electrophoresis, the plate were taken out and the central agar strips were removed from the antiserum troughs. The troughs were filled with appropriate antesera and the plates were incubated in a chamber for 48h.

Staining

The immunodiffusion plates were washed extensively in 0.9% Nacl solution (w/v) for 6 h with 2 to 3 changes to remove excess unreacted proteins. Subsequently, the plates wre washed repeatedly with GDW and air dried. The plates were stained with Azocarmine B or Amidoblack or Coomassie brillient blue (w/v) for 15 min and destained in a solution of methanol, GDW and acetic acid (5: 5: 1 v/v). The plates were finally washed once in 7% acetic acid (v/v). The plates were finally washed once in 7% acetic acid (v/v), dried and photographed.

Result

Immunoelectrophoresis of control vitellogenic fat body and ovary extracts against anti-female haemolymph serum revealed 7 and 8 protein precipitin arcs respectively (Fig. 1). While the same antigens agsinst anti-oocyte serum revealed 6 and 7 fractions respectively (Fig. 2). Immunoe lectropherogram of anti-female haemolymph serum adsorbed with male haemolymph against control vitellogenic fat body and ovary each produced a single procipitin line, thus confirming that the synthesis of FSP was extraovarian (Fig. 3). The reaction of anti-oocyte serum adsorbed with male haemolymph against control vitellogenic fat body and oocyte extract showed one and two precipitin protein arcs respectively and thus confirmed that the synthesis of ESP as of intraovarian in origin (Fig .4)

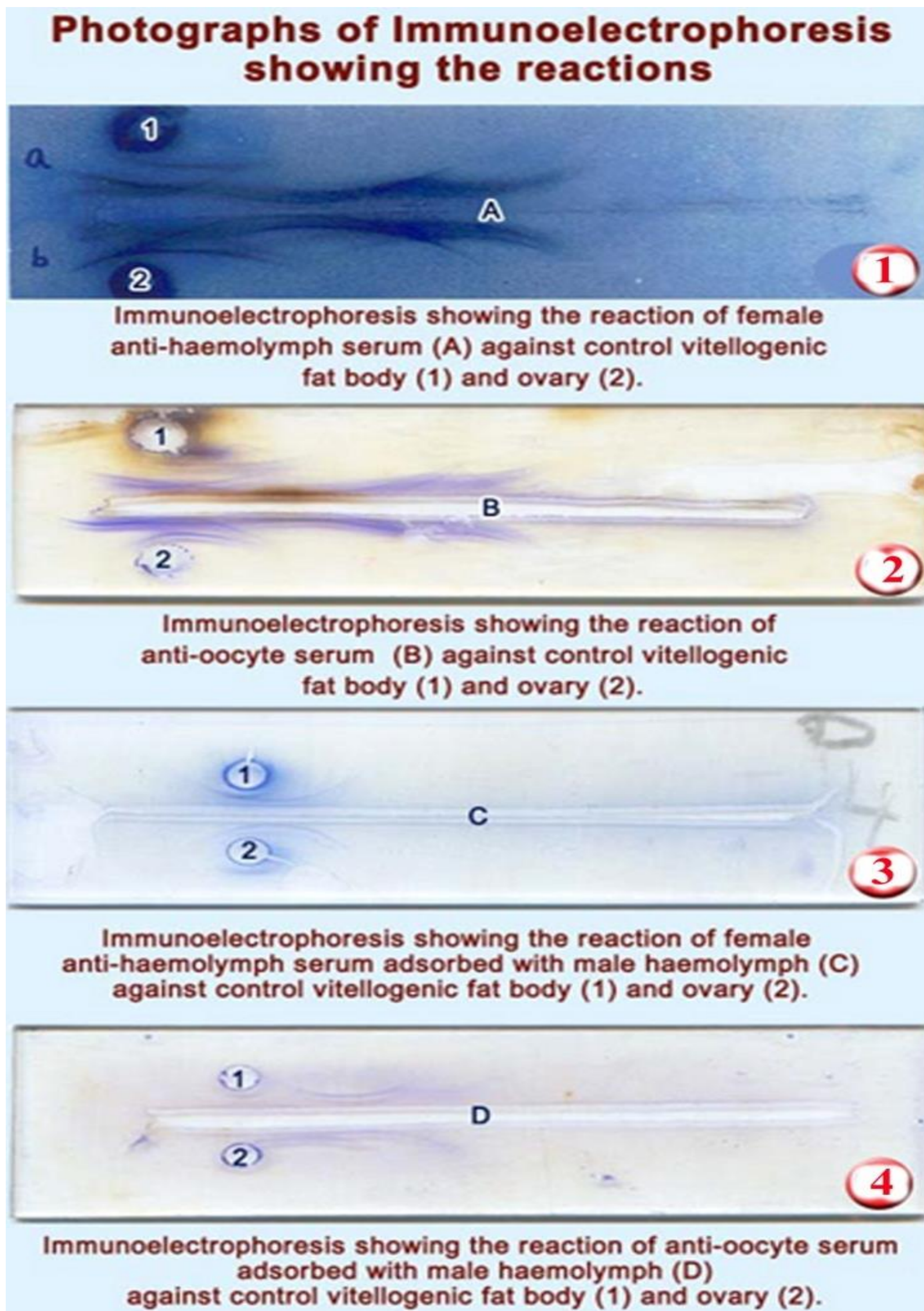

Fig 1

Discussion

Immunoelectrophoretic study also clearly indicates the occurrence of two specific proteins, one from the haemolymph having identical partner in the oocyte and the other one present only in the oocytes. Occurrence of specific proteins was detected by immunoeletrophoresis in the yolk of *H. cecropia* (Telfer, 1960) [19] and *P. americana* (Bell, 1970) [1] that did not appear in the haemolymph suggesting that the ovary is the source of these proteins in these insects. Thus, immunological techniques employed reveal that both vitellin and vitellogenin of *G. sigillatus* are immunologically identical. Since the immunological techniques have a higher specificity and sensitivity than the electrophoretic studies, the results are more reliable and the present obser vations are in agreement with the result observed in *H. cecropia* (Telfer, 1954; 1965) [21]. In *P. americana,* antigenically similar peptides were reported in the haemolymph and terminal oocytes during vitellogenesis (Clore *et al.,* 1978) [4].

Immunological studies in *B. mori* revealed that the antibody prepared against the purified vitellin cross-reacted with the haemolymph vitellogenin suggesting their similarity (Ono *et al.,* 1975; Izumi *et al.,* 1980). Similar identity has been reported for Diptera (Huybrechts and De Loof, 1982; Fourney *et al.,* 1982). In *M. domestica,* three identical subunits of vitellin and vitellogenin were reported (Agui *et al.,* 1985). In several insects, a vitellogenic protein different from vitellogeninof haemolymph is synthesized by the ovary itself and accumulated intothe oocytes (Ono *et al.,* 1975; Irie and Yamashita, 1983) [14]. Egg specific proteins have been confirmed in eggs and the developing ovaries of *B. mori* and is estimated to be the second major protein of the yolk accounting for about 20% to 25% of the soluble proteins of the newly laid egg (Irie and Yamashita, 1983; Zhu *et al.,* 1986) [14, 23]. The follicle specific proteins synthesized by the ovarian follicles are secreted into the intercellular spaces of the follicular epithelium and then taken up by the oocytes by receptor mediated endocytosis (Sato and Yamashita, 1991) [17]. The follicular epithelium of vitellogenic female *D. melanogaster* synthesizes large amounts of yolk peptides (Postlethwait *et al.,* 1980 [16]; Bowens, 1980; 1982; Brennen *et al.,* 1982). Based on the above information, Immunoelectrophoresis of control vitellogenic fat body and ovary extracts against anti-female haemolymph serum revealed 7 and 8 protein precipitin arcs respectively. While the same antigens agsinst anti-oocyte serum revealed 6 and 7 fractions respectively. Immunoelectropherogram of anti-female haemolymph serum adsorbed with male haemolymph against control vitellogenic fat body and ovary each produced a single procipitin line, thus confirming that the synthesis of FSP was extraovarian. The reaction of anti-oocyte serum adsorbed with male haemolymph against control vitellogenic fat body and oocyte extract showed one and two precipitin protein arcs respectively and thus confirmed that the synthesis of ESP was of intraovarian in origin.

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