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Visual assessment of morphological changes in female reproductive system of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae)

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ABSTRACT: Reliable methods are needed to ascertain sexual maturity of field captured tephritid fruit flies. To provide such a tool for female Oriental fruit flies, this study documented the changes in ovarian development at different age intervals (1, 3, 6, 11, 15, 18, 20, 21, 23, 26, 30, 35, 40, 45, 52, 55 and 78 day old) of *Bactrocera dorsalis* (Hendel). As fly ages, the differences in morphological characters of female reproductive system were assessed visually and the ovarian maturation process was broadly classified into various developmental stages namely previtellogenesis (stage 1 and 2); vitellogenesis (stage 3 and 4), appearance of mature egg (stage 5) and post-oviposition (stage 6). Based on visual assessment of ovarian characters, from the day 1-6, the flies were in previtellogenic stage; from the day 7-11, the flies were in vitellogenic stage, and from the day 12-15, the flies were in late vitellogenic stage. From the day 15 onwards matured follicles were observed on the terminal end of the fly ovary. At the day 18, presence of opaque mature terminal oocyte (matured egg) was noticed in the flies confirming sexual maturity. This classification based on visual assessment of morphological changes in the ovary would help us determine the age of field collected female *B. dorsalis*.

Keywords: *Bactrocera dorsalis*, Oriental fruit fly, ovary morphology, development indices

INTRODUCTION

The true fruit flies belong to the genus *Bactrocera* which is of great importance as it includes economically important species such as *Bactrocera dorsalis* (Hendel) and *Bactrocera zonata* (Saunders) (Verghese *et al.,* 2002). In India, *B. dorsalis* is a major pest especially on mango, affecting local and export markets. The crop loss due to *B. dorsalis* varies with season and region. The fruit loss in mango ranges from 5-80% and in guava from 10-80% (Verghese *et al*., 2002). The monitoring and management programs for Oriental fruit fly *B. dorsalis* have relied heavily on different types of traps containing either paraphenomone lure namely methyl eugenol or food baits to attract male and female flies respectively. In case of Caribbean fruit fly, *Anastrapha suspensa* Loew traps baited with liquid protein and ammonium acetate-based synthetic lures are used with highly variable female catches (Epsky *et al*. 2004). The mated female flies of *A. suspensa* were found to make choices with respect to protein requirement (Epsky *et al*., 1993), as sexually immature females consumed more protein than sexually mature females (Landolt and Davis-Hernandez, 1993). Thus, the variable trach catches in case of female tephritid flies may be linked to their physiological status. Several studies highlighted that ovarian development could be used as a reliable predictor to estimate the physiological status of female fruit flies (Fletcher *et al.,*1978; Raghu *et al.,*2003; Kendra *et al*., 2006). Literature survey till date is fairly indicative of a relationship between response of female fruit flies to baits and their reproductive development. The extent of bait technique success will depend on proportion of female captures that are still young. In the first place, if a juvenile female prior to egg formation is caught or killed in bait interventions (either trap or spray), which has a potential of 1200-1500 eggs, that otherwise would have been laid is prevented. Secondly if a mature female, prior to mating, indicated by spermatheca examination, is captured will also have positive fallout on IPM by preventing its fertile eggs being laid in a fruit (Fritz and Turner, 2002). Thirdly, if a spent female is captured, the fallout on IPM is negative. However, there is no reproductive development anatomy chart available to track these changes on a temporal scale from day one to about 60 days (considering the average life span of adult female as ~ 2.5 months). So, an attempt was made to develop a pictorial sequence of anatomical progression of reproductive system development of the female *B. dorsalis* as it ages from day one to 78 days.

MATERIALS AND METHODS

Insect cultures

The females of *B.dorsalis* were obtained from a mass-reared laboratory colony maintained at the Fruit Entomology Laboratory, Indian Institute of Horticulture Research, Bengaluru, South India as per the earlier rearing protocol (Kamala Jayanthi and Verghese, 2002). The flies were maintained at a room temperature 28± 1°C.

For the experiment, puparia (N= 100) were removed from the mass culture and placed in Petri plates within a plastic box (20 x 20cm). Each plastic box had the date of eclosion to denote the age of the flies and monitored continuously at 24 h interval for fly emergence. The Petri plate with uneclosed puparia was transferred to another plastic box. This was a continuous process and continued until ~50% of the eclosion. The emergence of ~50% male and female *B. dorsalis* flies collected took place in the first three days, followed by the remaining in about a week's time. Each plastic box served as a repository of known aged fruit flies, which were used in the study.

Female *B. dorsalis* flies were dissected under a Sterio-binocular dissection microscope (Olympus). The attached tissues were slowly removed using hair bristles under the microscope without damaging the reproductive parts. The separated female reproductive part was mounted on a flat microscope slide using a mountant (DPX, SDFCL, Mumbai) for further studies. These slides were used to document visually anatomical changes that were expected as fruit flies matures from day one to its death by about 50-80 days.

RESULTS

The present study depicts the changes in the anatomical characters of the reproductive system in adult female *B. dorsalis* as it ages (Fig. 1). Earlier studies described four different stages of ovarian development namely stage I (previtellogenesis); stage II (vitellogenesis, accumulation of yolk in terminal follicles prior to egg formation); stage III (egg formation) and stage IV (after oviposition) (Raghu *et al*., 2003; Kendra *et al*., 2006).

In the present study, it was visually observed that from the day of emergence to 5th day, the female ovaries were very small in size (Fig. 1). At this stage the ovary contained previtellogenic ovarioles. We observed that the ovaries were in stage I from the first day to 6th day, with no marked variation in size. From the 7th to 10th day, the ovary size was rated bigger than the stage I, and was found to be under late previtellogenic stage (stage II). From the day 11 to 14, the ovary is considered to be in stage III where the accumulation of yolk in follicles was observed. This growth period is called vitellogenesis, and is the stage prior to egg formation. At this stage both length and width of the ovary increased compared to the earlier stages (I, II). When the flies were of 15 to 17 days old, female ovaries showed a transition from the late vitellogenesis to distinct follicles development at the terminal end of the ovary (stage IV).

The first appearance of mature oocytes in ovaries was observed when the flies were of 18 days old. This is categorized as stage V. The largest egg load in the ovary was recorded during this stage. At this stage the ovary size was at the maximum, and seemed to occupy much of the female *B. dorsalis* body cavity in the abdominal as well as the thoracic regions. Matured eggs were found from 15, 18, 21, 23, 26- and 35-days old dissected females. The egg count at 15, 18, 21, 23, and 26 days were 10, 47, 27, 30 and 52 respectively. Later, 58 eggs were found in the 35-day old aged female *B. dorsalis* ovaries.

When females were dissected at 30 days, no egg was found and this confirmed that the fly was actively ovipositing, and confirmed to be in stage VI (after oviposition). At 40, 45, 52, 55 and 78 days age, no eggs were observed in the dissected females. Ovaries of older females are irregular in shape and the size was found to be very small. Considerable variations were noticed at this stage in the ovaries of *B. dorsalis* when compared to the initial stages.

We found that in *B. dorsalis,* females of age 1-10 day old are sexually immature and the current study indicates that the oviposition is initiated from 16th day onwards as mature eggs were noticed from 16th to 26th days. At 30th day, the ovary size was very small and there were no mature eggs. This confirmed the stage 6 (after oviposition). Nevertheless, at the age of 35 days also few of the dissected females were found to have eggs in their ovaries.

DISCUSSION

The specific objective of this study was to develop a reliable method by which sexual maturity of female Oriental fruit fly, *B. dorsalis* can be assessed visually, and thereby approximate age of the female can be fixed.

The results of this study indicated six different stages of the ovarian development in female *B. dosalis*. These six different stages were classified based on the earlier studies by Raghu *et al.,* (2003) and Kendra *et al.*, (2006). Our observations on laboratory reared female *B. dorsalis* shown that there is change in morphology of ovarian

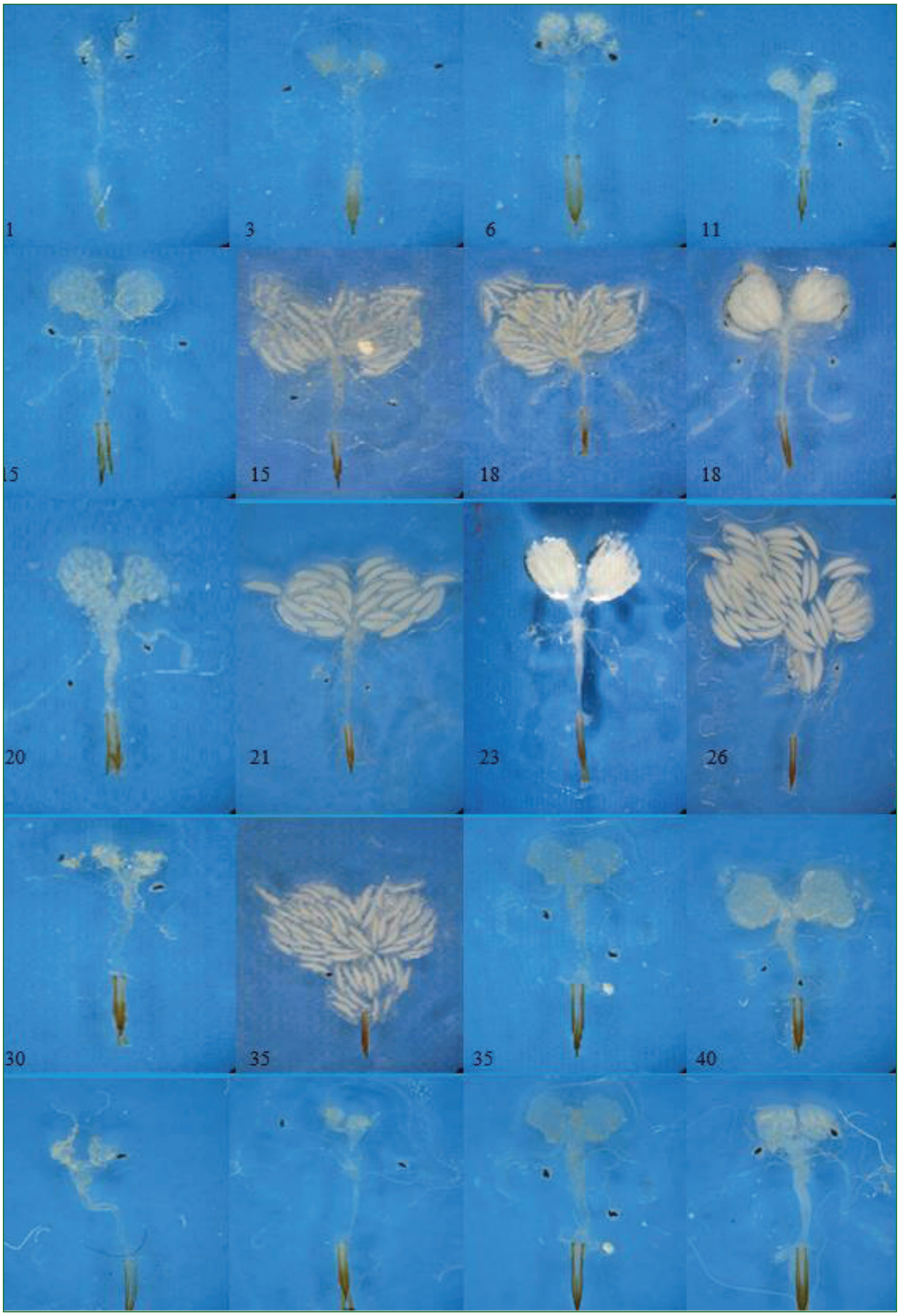

Fig. 1. Progression of morphological changes in female reproductive system of *B. dorsalis* at different age intervals (the numbers on the image depict the age of the fly)

development with age. The results further showed that ovarian development in *B. dorsalis* is not synchronous as reported in other tephritid species like Caribbean fruit fly, *A. suspensa* (Kendra *et al.,* 2006), wild tobacco fruit fly, *Bactrocera cacuminata* (Hering) (Raghu *et al*., 2003) and olive fruit fly, *Bactrocera oleae* (Rossi) (Fletcher *et al*. 1978).

In *B. dorsalis* females, the vitellogenesis (stage III) was observed from the day 11-13. However, in *A. suspensa,* Kendra *et al* (2006) reported that the stage III i.e., the onset of vitellogenesis, the accumulation of yolk in the terminal follicles occurred when the females were of 14-15 days old. In female *B. dorsalis*, stages IV and V of the ovarian cycle were observed when the flies were of 11-15 days old. In case of *A. suspensa,* the adult females attained this stage of the ovarian development when they are of 6-7 days old. (Kendra *et al.,* 2006). Based on our observations, the first appearance of mature oocytes was noticed in 18 days old *B. dorsalis* indicating its reproductive maturity. The presence of mature oocytes in an ovary is regarded as the definitive character for female sexual maturity (Nation, 1972; Aluja *et al.,* 2001). However, in *B. cacuminata* full maturity was reached by day nine itself (Raghu *et al*., 2003) and *A. suspensa* attained this stage when the flies were of 7-9 days old (Kendra *et al*., 2006). Dodson (1982) found that wild *A. suspensa* require at least 14 days to reach sexual maturity, where as laboratory reared strains can mature within 7-8 days (Mazomenos *et al*., 1977; Kendra *et al*., 2005). Further, in addition to the genetic strain differences, the presence of males has been shown to affect the rate of ovarian development in *A. suspensa* (Pereira *et al*., 2006).

The present study showed that in *B. dorsalis,* day 15 is almost the breakpoint between sexually immature and mature females. But in *B. cacuminata* (Raghu *et al.,* 2003) and *A. suspensa* (Kendra *et al.,* 2005), it has been reported as nine and eight, respectively. Marked difference in the period of maturity was found in *B. dorsalis* when compared to *B. cacuminata*. Female tephritid fruit flies are sexually immature at eclosion (anautogenous) and the ovarian maturation process is dependent upon multiple factors, including temperature, photoperiod, diet (especially protein availability) and chemical cues (Fletcher, 1989; Wheeler, 1996; Papaj, 2000; Aluja *et al*., 2001)

For the first time a reliable method to assess the age of the *B. dorsalis* was devised by studying the ovarian development in female *B. dorsalis.* This is deemed useful in assessing the age of trap/bait catches.

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REFERENCES

Aluja, M., Diaz-Fleischer, F., Papaj, D.R., Lagunes, G. and Sivinski, J. 2001. Effects of age, diet, female density, and the host resource on egg load in *Anastrepha luden* and *Anastrepha obliqua* (Diptera: Tephritidae). *Journal of Insect Physiology,* **47**:975–988.

Dodson, G. 1982. Mating and territoriality in wild *Anastrepha suspensa* (Diptera: Tephritidae) in field cages. *Journal of Georgia Entomological Society,* **17**: 189-199.

Epsky N. D., Kendra, P.E., and Heath, R.R. 2004. Development of lures for detection and delimitation of invasive *Anastrepha* fruit flies. pp. 84-89 *In* W. Klassen, W. Colon, and W. I. Lugo [eds.], Proc. of the 39th Annual Meeting of the Caribbean Food Crops Society, July 2003, Grenada.

Epsky N. D., Heath, R.R., Sivinski, J.M., Calkins, C.O., Baranowski, R.M. and Fritz, A.H. 1993. Evaluation of protein bait formulations for the Caribbean fruit fly (Diptera: Tephritidae). *Florida Entomologist*, **76**:626–635.

Fletcher B.S. 1989. Temperature-development rate relationships of the immature stages and adults of tephritid fruit flies. pp. 283-289 *In* A. S. Robinson and G. Hooper [eds.], Fruit Flies-Their Biology, *Natural Enemies and Control*, Vol 3A. Elsevier, Amsterdam.

Fletcher B.S., Pappas, S. and Kapatos, E. 1978. Changes in the ovaries of olive flies (*Dacus oleae* [Gmelin]) during the summer and their relationship to temperature, humidity and fruit availability. *Ecological Entomology,* **3**:99–107.

Fritz A.H. and Turner, F.R. 2002. A light and electron microscopical study of the spermathecae and ventral receptacle of *Anastrepha suspensa* (Diptera: Tephritidae) and implications in female influence of sperm storage. *Arthropod Structure and Development.* **30**:292–313.

Kendra P.E., Montgomery, W.S., Epsky, N.D., and Heath, R.R. 2006. Assessment of female reproductive status in *Anastrepha suspensa* (Diptera: Tephritidae). *Florida Entomologist,* **89** (2): 144-151.

Kendra P. E., Montgomery, W.S., Mateo,D.M., Puche, H., Epsky, N.D. and Heath, R.R. 2005. Effect of age on EAG response and attraction of female *Anastrepha suspensa* (Diptera: Tephritidae) to ammonia and carbon dioxide. *Environmental Entomology,* **34**:584–590.

Landolt P.J. and Davis-Hernandez, K.M. 1993. Temporal patterns of feeding by Caribbean fruit flies (Diptera: Tephritidae) on sucrose and hydrolyzed yeast. *Annals of Entomological Society of America,* **86**:749–755.

Mazomenos B., Nation, J.L., Coleman, W.J., Dennis, K.C. and Esponda, R. 1977. Reproduction in Caribbean fruit flies: comparisons between a laboratory strain and a wild strain. *Florida Entomologist,* **60**:139–144**.**

Nation J.L. 1972. Courtship behavior and evidence for a sex attractant in the male Caribbean fruit fly, *Anastrepha suspensa*. *Annals of Entomological Society of America,* **65**:1364–1367**.**

Kamala Jayanthi, P.D. and Verghese, A. 2002. A simple and cost-effective mass rearing technique for the tephritid fruit fly, *Bactrocera dorsalis* (Hendel). *Current Science,* **82**: 266-268.

Papaj D. R. 2000. Ovarian dynamics and host use. *Annual Review of Entomology,* **45**:423–448.

Pereira R., Teal, P.E.A., Sivinski, J. and Dueben, B. D. 2006. Influence of male presence on sexual maturation in female Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae). *Journal of Insect Behavior,* **19**:31–43.

Raghu S., Halcoop, P. and Drew, R.A.I. 2003. Apodeme and ovarian development as predictors of physiological status in *Bactrocera cacuminata* (Hering) (Diptera: Tephritidae). *Australian Journal of Entomology,* **42**:281–286.

Verghese, A., Madhura, H.S., Kamala Jayanthi, P. D. and John M. Stonehouse. 2002. Fruit flies of economic significance in India, with special reference to *Bactrocera dorsalis* (Hendel). Proceedings of 6^{th} International Fruit fly Symposium, 6 – 10 May 2002, Stellenbosch, South Africa. pp. 317 – 324.

Wheeler D. 1996. The role of nourishment in oogenesis. *Annual Review of Entomology,* **41**:407–431.

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