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SUMMARY

Studies on development of female reproductive system in insects have shown its utility in physiological age grading. The irreversible character of ovarian alterations correlated with mandibular wear and presence of yellow body, were applied to estimate the physiological age of *Passalus convexus* and *Passalus latifrons* females, giving results comparable to other studies and indicating the value of these methods in determination of physiological age.

INTRODUCTION

In recent years knowledge of the bionomics of some beetles has progressed rapidly with the publication of works that have clarified details of development of these insects. With the appearance of techniques that permit estimates of physiological age based on ovarian morphology (Tyndale-Biscoe, 1978), cuticular growth (Neville, 1963; Shlein & Gratz, 1972), presence of yellow body (Tyndale-Biscoe & Hughes, 1969), coloration of the integument and presence of meconia (Corbert, 1960) and ovarian tracheation (Detinova, 1945), understanding of reproductive behavior of some groups of insects has become clearer.

The association of these techniques has been employed to verify estimates of age. Tyndale-Biscoe (op.cit.) studying the physiological age of female *Euoniticellus intermedius* (Reiche) employed ovarian morphology, associated with the degree of wear of tibial teeth and the presence of yellow body to obtain satisfactory results for Coleoptera.

(*) Part of thesis presented to the Graduate School of Instituto Nacional de Pesquisas da Amazônia (INPA) and Fundação Universidade do Amazonas (FUA) by the first author, in partial fulfillment of requirements for Master of Science Degree

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Saunders (1962) made estimates based on ovarian development and hardening in *Glossina pallipides* Austen; Nelson (1966) estimated the age of *Culex tarsalis* Coquillett, based on ovarian development and tracheation; Tyndale-Biscoe & Kitching (1974) used cuticular growth together with ovarian development to calculate age in *Lucilia cuprina* (Wildemann).

In the present study, a combination of several methods was used to estimate the physiological age of female *Passalus convexus* Dalman, 1817 and *Passalus latifrons* Percheron, 1841. Modifications arising in the ovaries during development of the follicles constitute characteristics that permit recognition of nuliparous and parous females. These characteristics together with mandibular wear and the presence of yellow body are useful in the estimates of the age of females in the studied species.

MATERIAL AND METHODS

Adults of *Passalus convexus* and *Passalus latifrons* were captured from rotting tree trunks in primary forest, located at Km 14, on the Manaus - Caracaraí Federal Highway (BR-174), State of Amazonas, Brasil.

The female individuals encountered were placed in glass dishes and maintained alive in the laboratory to examine alterations in their ovaries. Wood fragments from the trunks where beetles had been encountered were also placed in the dishes to serve as food.

In the laboratory, adults were killed in petri dishes with chloroform. Immediately afterwards, a dissection was performed to observe the ovaries, using the method of Kamm & Ritcher (1972).

With fine-pointed scissors, two lateral incisions were made in the pleural membrane of the exoskeleton, from the anal aperture to the fourth abdominal sternon. Then, opening the exoskeleton ventrally, the sternites were removed, exposing the ovaries (Fig. 1). These were then placed on a glass slide with several drops of 0.9% saline solution. Under a dissecting microscope, and with the aid of forceps, tracheae that remained adhering to the ovaries were removed to facilitate clear observation of ovarian morphology.

The specimens collected have been deposited in the Systematic Entomology Collection of INPA, Manaus.

RESULTS

After emergence of adults, the ovaries are of a reduced size, with transparent white coloration and the ovaries have approximately 1/3 of their final volume. (Fig. 2A, Fig. 3A).

At completion of adult sclerotization, males and females come together, no doubt to initiate reproductive activity.

Examination of the spermatheca shows that soon after hardening of exoskeleton, copulation is effected, and if this does not occur, maturation of oocytes remains suspended; however, it is very difficult to find adults that have not mated.

Copulation probably stimulates the hormonal mechanisms and noticeable modifications in ovarian morphology begin to be recognized. The oocytes begin a maturing phase in successive order, and the ovarian volume increases, while the oviducts pass through a process of wall thickening, a type of reinforcement that even modifies the color of the oviducts. This oviduct reinforcement is evident, in such manner that, during ovulation, it is apt to support the tension provoked by passage of the ovule in the direction of the distal third of common oviduct, where it is fertilized and chorion added (Fig. 2A, B, C).

The modifications seen in the ovaries during the life of the females permits their classification into two categories: nulliparous and parous. Nulliparous are those which have not oviposited and whose ovaries show no oocytes developing. Parous are those which have already oviposited or whose ovaries show oocytes developing or vestiges of past activity. The ovaries of these females are well developed and very characteristic, as they are twice as large as nulliparous ovaries, and the oviducts are stronger with walls that show well marked distension, and also differentiated by milky white color, without the initial transparency (Fig. 3 C, D). When the period of oviposition terminates, the parous females have oviducts extremely dilated, similar to very stretched elastic, that by the force exerted, remains distended (Fig. 2C).

The yellow body is not encountered very frequently because it is always torn by the following ovule during ovulation.

The ovarioles also have volume altered during maturation of oocytes. There is a sequential order in maturation that is noted in the different sizes of follicles in ovarioles (Fig. 3D). One egg is produced and laid each time. In all ovaries examined, never was more than one egg encountered in the distal third of common oviduct, awaiting oviposition. The large volume taken up by the egg does not leave space for more than one egg at the site destined to receive the chorion and final intraovarian maturation.

In the species studied, the difference between nulliparous and parous females can be easily established by direct observation of the ovaries, mandible and exoskeleton (Table 1).

DISCUSSION

Modifications presented by the ovaries during development of the follicles, together with the degree of mandibular wear and hardening of the exoskeleton were utilized as characters to estimate the ages of female *P. convexus* and *P. latifrons*. Ovarian alterations, distension and milky color are of irreversible nature, thus allowing recognition of nulliparous and parous females through a simple observation of the oviducts.

The isolated utilization of one character offers little security in the estimate of female ages. For example, mandibular wear can give false information as to age, by virtue of being related to wood hardness where the insect is living. If the wood is softer, mandibular wear will be less, and vice-versa, if the wood has greater resistance. Tyndale-Biscoe (1978) thought that the rate of wear by parts of exoskeleton subject to attrition is reasonably constant, for insects originating from the same area; thus passalids taken from the same trunk will show parallel mandibular wear. However, for a population located in various trunks, whole wood content is of a variable nature, and it becomes prudent to use only mandibular wear as a character to determine age.

Tyndale-Biscoe & Kitching (1974) studying age in **Lucilia** based on cuticular growth and ovarian development, noted delays in development of ovaries and oviposition, when they compared the ovaries with rings of cuticular growth. Thus, age as measured only by ovarian development showed a younger insect than when estimated by cuticular growth rings. In this way, the associations of characters brings the estimation closer to reality.

Estimates of age based on presence of the yellow body have been valid for all species of coprophagous beetles examined from Africa and Australia (Tyndale-Biscoe, 1978). However, for passalids, this character has not been safe for age determination, because the yellow body is torn by the following ovule during ovulation, not leaving any vestige after the last ovulation. The number of yellow bodies encountered does not correlate with the number of ovipositions that have occurred.

ACKNOWLEDGEMENTS

We would like to acknowledge Dr. Norman Penny for reviewing and translating this paper.

RESUMO

*Estudos sobre o desenvolvimento do aparelho reprodutor feminino em insetos tem demonstrado utilidade na determinação da idade fisiológica. As alterações ovarianas de caráter irreversível, associadas ao desgaste mandibular e a presença do corpo amarelo, foram aplicáveis para estimar a idade fisiológica em fêmeas de **Passalus convexus** e **P. latifrons**, obtendo-se resultados comparáveis com outros estudos, indicando o valor destes métodos na determinação da idade fisiológica.*

Table 1. Parameters used to estimate the physiological age in *Passalus convexus* and *Passalus latifrons*.

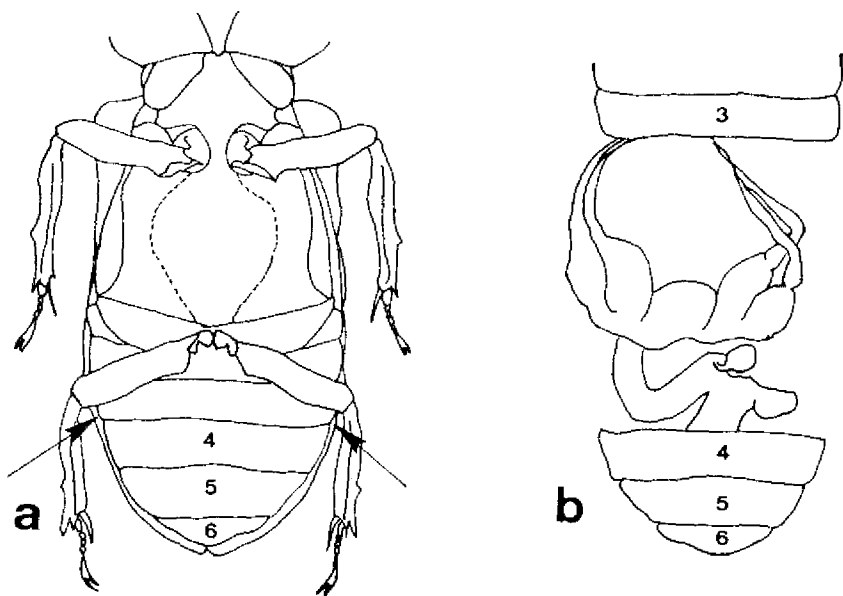
	OVIDUCTS	MANDIBLES	EXOSKELETON	YELLOW BODY
Nulliparous	Transparent and thin	not worn*	hardened, or not	absent
Initial Parous	Starting to thicken with little trans <u>u</u> parency	partially worn**	hardened	present***
Advanced	Much thickened, widened and not transparent Elastic	much worn	hardened	present***

(*) There were cases of nulliparous females with mandibular wear.

(**) There were encountered cases of initial parous females without mandibular wear.

(***) The yellow body is almost always torn by succeeding ovulations making it difficult to encounter it before the last ovulation.

Figure 1. Passalidae. a. Ventral view of the body without prothorax and head. For rapid removal of the female reproductive system apply lateral cuts to abdomen at the level marked by arrows. b. The last three sternites separated with the adhering ovaries.



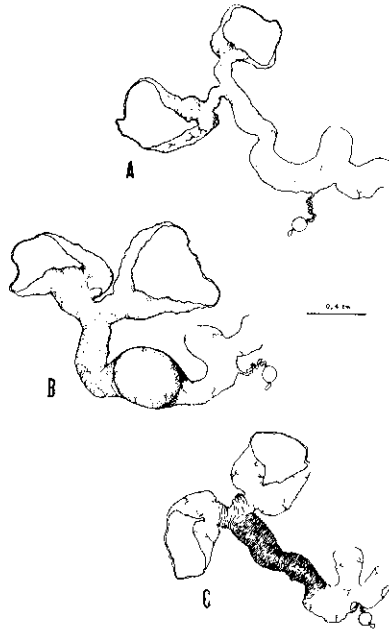


Figure 2. Schematic drawing showing the ovarian changes during adult phase.
 A. Ovary of nulliparous female; B. Stretching of common oviduct during ovulation; C. View of the oviducts after a complete ovarian cycle. Parous female.

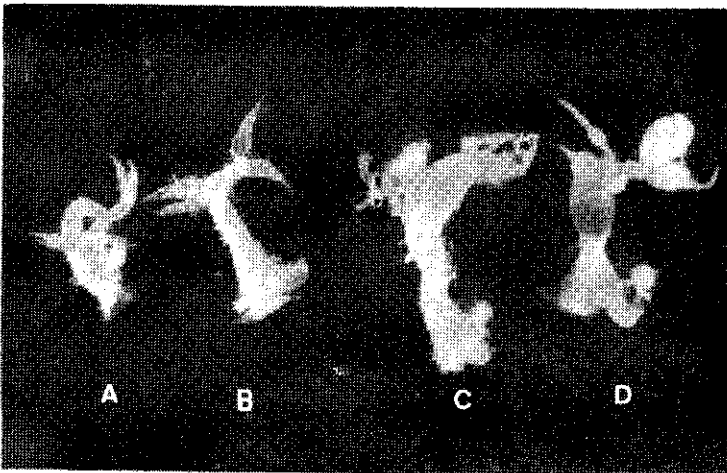


Figure 3. Ovaries of *Passalus convexus* Dalm.; A. Nulliparous ovaries; B. Nulliparous ovaries initiating activity; C. Initial parous ovaries; D. Parous ovaries with an egg descending the common oviduct; the sequence of maturing follicles can be seen.

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(Aceito para publicação em 09/11/84)