RATE OF EMBRYONIC DEVELOPMENT IN THE TSETSE FLY, GLOSSINA MORSITANS ORIENTALIS

BY

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Histological examination of a large sample of the tsetse fly, Glossina morsitans orientalis, killed at timed intervals after emergence, showed that embryogenesis takes about 4 days at 25°C during the first intra-uterine cycle. This is considerably longer than is usually stated. Use of these data to obtain an average rate of development for the sample suggested a new method of age determination for the adult female fly which can be used in conjunction with "ovarian" determination of age.

During this study, tsetse flies were obtained from a colony maintained under closely controlled physical and biological conditions. A constant rate of development would, therefore, be expected. The sample of flies revealed a range of embryonic stages at the same age, however, and an investigation of this phenomenon indicates a new method of age determination.

MATERIAL AND METHODS

A sample of 85 females of G. morsitans orientalis Vanderplank was obtained from the Tsetse Research Laboratory, Langford, Bristol. The flies had been maintained at a temperature of approximately 25°C and a relative humidity of 60 to 70%, with subdued diurnal illumination (5 to 27 lux for 12 hours of the day) as described by Nash, Jordan & Trewern (1971). The emergence cages are examined each morning, except Sunday, at Langford and newly emerged flies are removed. This day of collection is termed day one for each fly. The females of this sample were mated individually on day 3 of life with approximately 15-day old males. Batches of flies were then killed and fixed at 0930 hours on each of days 8—13 of life before sectioning to determine how far development had proceeded, using the anatomical time-scale given in Table I.

RESULTS

In G. palpalis (Hoffmann, 1954), and in several other tsetse species, fertilisation occurs between days 8 and 10, and the first-instar larva hatches by day 12. The period covered by the sample was, therefore, expected to include the complete period of embryogenesis.

Examination of the G. morsitans sample showed a regular progression of

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TABLE I

Criteria for the developmental stages of Glossina morsitans used in Figs 1 and 2

<table>
<thead>
<tr>
<th>Stage No</th>
<th>Description of embryonic or larval stage</th>
<th>Saunders' Categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>Preovulation. Empty uterus. Egg in ovariole or oviduct</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>Egg in uterus. Fertilised but not more than a few signs of cleavage.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Cleavage well under way.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Syncytial blastoderm.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Cell walls formed. Thin layer of cells.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Late blastoderm. Cells appear tall and columnar.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Surface furrows appear.</td>
<td>1a</td>
</tr>
<tr>
<td>6</td>
<td>Gastrula with proctodaeal invagination. Group of stomodeal cells.</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Late gastrula with deep stomodeal and proctodaeal invaginations.</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Early organogenesis. Midgut epithelial cells enclose yolk.</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Stomodeum and proctodeum curved due to extension. Malpighian tubules appear.</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Late organogenesis. Cephalopharynx formed.</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Newly hatched first-instar larva. Little or no food in midgut.</td>
<td>1b</td>
</tr>
<tr>
<td>12</td>
<td>First-instar larva. Midgut half full.</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>First-instar larva. Midgut full.</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Small second-instar larva.</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Stage of development reached by 85 female *G. morsitans* killed at 0930 hours on day number 8, 9, 10, 11, 12 or 13 of life.
development which is summarised in Fig. 1, showing that no flies contained developing embryos on day 8 but that by the time the day 9 sample was taken, some of the embryos had begun development. By the time the last sample was taken, some of the embryos had hatched and produced first-instar larvae although this is the furthest that any of the specimens had developed (with the exception of the one second-instar larva in the sample from day 11 which is discounted in this discussion).

Even if it is assumed that fertilisation and the initiation of embryogenesis did not occur in the most advanced individuals until just before the day 9 sample was taken, then at least 4 days must have elapsed before the stage (stage 12) midway through the first instar was reached. No larvae had hatched 3 days (day 12) after the first embryos began development and, therefore, embryogenesis must take at least 3 days (72 hours).

The average stage of development of the sample can be obtained by plotting the average stage of embryogenesis reached in each sample against time (Fig. 2). A mean age of 12 hours less than the nominal day value was plotted against the average stage in Fig. 2. This is the average age if eclosion occurs with equal probability at any time, during the 24 hours previous to collection.
In most recent accounts of dipteran embryology the actual timing and duration of the various events of embryogenesis have been at least partially recorded. This is more difficult for Glossina where both embryogenesis and subsequent larval development are concealed within the maternal uterus. An indirect method of timing embryonic events is described before. This shows that in a sample of 85 females of *G. morsitans orientalis* examined over the first cycle of embryogenesis to take place in the fly, embryogenesis took at least 72 hours. If the estimation by Roubaud (1909 a, b) that the first-instar larva lasts only a few hours is correct, then embryogenesis must take at least 93 hours since the uterine stages were shown in Fig. 1 to require at least 96 hours for development to stage 12, or mid-first-instar larva (0930 hours on day 9 to 0930 hours on day 13). In fact, the duration of the first instar may be significantly longer than the accepted “few hours” (Roberts, 1971, 1972) but the duration of embryogenesis must be much greater than 72 hours because at this time (stage 10) the embryos still have a considerable degree of development to undergo before completion of the larva. This estimate agrees well with the suggestion made by Saunders (1971) that the egg is present in the uterus for 4 days, following his observation that no significant increase in the interlarval period is caused by deprivation of blood-meals during this period.

Even if the duration of embryonic development is considered to be somewhat less than 93 hours, this is still much longer than the corresponding estimates made by Hoffmann (1954) for *G. palpalis*. He states that the blastoderm has formed by 20 to 22 hours, but that germ band formation begins after 24 to 26 hours, whilst embryogenesis is completed and the larva hatches after 50 to 60 hours. He does not state, however, how he derived these figures and they may have been based on few specimens. His sample of flies was maintained at 26° and 80% relative humidity (Geigy, 1948). Of other accounts which consider embryonic development, Hagan (1951) did not give any original data for the timing of embryonic events but quoted Newstead, Evans & Potts (1924) as stating that the egg hatches within a day or so of reaching the uterus. Mellanby (1937) stated that the embryonic period lasts 3½ days in *G. palpalis* during a 9.9-day interlarval period at 24°.

The rate given by the graph (Fig. 2) is not that of individual embryos but of the whole sample, of which different embryos began development at varying ages. Thus, we can say that, for instance, an embryo should have reached stage 7 on average on day 12 of life of the fly. Conversely, a real age for samples of flies can be predicted from their anatomical data, by reference to this type of graph.

"Wing fray" (Jackson, 1946, 1950) and modifications of the “ovarian dissection” (Saunders, 1960, 1962; Challier, 1965; Itard, 1966) methods for adult age determination have produced progressively more precise age categories. Saunders (1962) and Challier (1965) used the size and position of developing follicles in the ovaries to determine how many interlarval periods had passed in the life of
the fly. These interlarval periods of 8.5 days at 25° in *G. morsitans* (Saunders, 1971) were then further split by Saunders (1960, 1962) into three unequal categories based on the presence of an "egg", "small larva" or third-instar larva within the uterus. The results obtained above show that uterine data allow a more detailed and significant interpretation than this. Extension of these data to include larval development and to compare intra-uterine periods after the first larvi-position, would enable the day or part of day which development had reached, in each eight-and-a-half-day period, to be pinpointed.

The rate of emergence was assumed to be constant during the 24 hours previous to collection, for Fig. 2, but this may not be so. Nash & Trewern (1972) have shown a marked diurnal periodicity in larviposition by *G. morsitans* and *G. austeni* maintained at the Langford colony, whilst Brady (1972) has shown an equally marked diurnal pattern of spontaneous activity for the adults of *G. morsitans* maintained under similar laboratory conditions. It seems likely, therefore, that the emergence of the flies will also be found to be governed by a physiological clock system although the puparia (and flies) are kept under constant laboratory conditions. Phelps & Jackson (1971) found that the eclosion of *G. morsitans* from field-collected puparia was not influenced by the photophase but that it was dependent on temperature changes. From this evidence, they argued that any differences in the number of flies emerging at different times of the day in the insectary must have been due to small alterations in temperature. They did not discuss the possibility of an endogeneous rhythm, however, and have apparently assumed that only an exogeneous rhythm could operate, entrained by light or temperature. On this basis, they stated that the process of eclosion is not rhythmical. Examination of their histograms, however, shows a clear rhythm of eclosion with a peak of emergence in late afternoon at about the same time of day as the peak of larviposition. This is an interesting correlate of the situation at Langford where there is evidence (Langley, P. A. & Curtis, C. F., pers. comm.) for a peak of eclosion for *G. morsitans* shortly before noon, which is also at about the same time as the larviposition peak found in this colony (Nash & Trewern, 1972). If a well-defined circadian rhythm of eclosion is definitely shown, the time of emergence could be more accurately estimated.

An alternative method of ageing the test sample of flies would be to stimulate their emergence by subjecting them to gamma-irradiation. Curtis (1970) has shown that a large proportion of *G. morsitans* emerge within a few seconds of treatment if the puparia are subjected to two consecutive 700 rad doses. The stimulated flies appear to develop normally (Curtis, loc. cit.).

Using these more-precisely aged samples, the probability of an individual fly being at the age indicated by its stage of uterine development could be estimated from the standard deviation of the sample. The samples should also be taken at more frequent intervals for the basic table. In this initial study, six time intervals were used to cover fourteen anatomical stages. The temporal occurrence of anatomical stages intermediate to any adjacent pair of these time intervals can be
determined decisively only by taking intermediate time samples, not by extrapolation. The number of time samples used, should, therefore, be greater than the number of anatomical stages which is chosen, even if this level of accuracy is not finally required for age determination.

The uterine method takes longer to perform than the wing-fray determination but the more detailed data provided would be invaluable in laboratory investigations such as those envisaged by Saunders (1962, 1971) and Mews (1969) and the utilisation of an automatic slide processor capable of taking 136 slides simultaneously (such as the Shandon-Elliott linear slide stainer) would reduce the operation to a routine procedure.

The results can be temperature-corrected, if each stage is assumed to be equally affected by temperature, and little difference has been claimed (Glasgow, 1970) between the rates of development of different species at the same temperature. The data should not, therefore, need modification before being applied to other species although the results of some studies (e.g. Harley, 1968) indicate that this requires further verification.

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REFERENCES


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