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THE LIFE-CYCLE OF *Dermatophagoides evansi* Fain, 1967 (ACARI : PYROGLYPHIDAE), A MITE ASSOCIATED WITH POULTRY *

BY K. Y. MUMCUOGLU 1 and I. LUTSKY 2

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**LIFE-CYCLE**

**HOUSE DUST**

**MITE**

**SUMMARY**: *Dermatophagoides evansi* was cultured in a medium composed of chicken skin or human skin scales plus powdered bakers yeast at a temperature of 25-27°C and relative humidity of 75-80%. The mean duration in days for each stage of the mite life-cycle was: egg 8.3; larva; protonymph 5.4; tritonymph 6.6; male 28.9 and female 52.9. The average time required for completion of one generation was 28.7 days. The female was oviparous and laid an average of 35.5 eggs during her lifetime; parthenogenesis was not observed. Adults copulated frequently. The male : female ratio was 1.2 : 1.

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**CYCLE DE VIE**

**ACARIENS**

**DES POUSSIÈRES**

**RÉSUMÉ**: *Dermatophagoides evansi* a été cultivé dans un milieu comprenant de la peau de poulet ou des pellicules de peau humaine et de la poudre de levure de boulanger à une température de 25-27°C et une humidité relative de 75-80%. La durée moyenne exprimée en jours de chaque stade de l'acarien est pour l'œuf 8.3; la larve 6.4; la protonymphpe 5.4; la tritonymphpe 6.6; le mâle 28.9 et la femelle 52.9. La durée moyenne pour le développement d'une génération de l'acarien est de 28.7 jours. L'acarien est ovipare et il n'a pas été observé de parthenogénèse. La femelle pond en moyenne 35.5 œufs pendant sa vie. Les adultes copulent fréquemment. Le rapport male : femelle est de 1.2 : 1.

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**INTRODUCTION**

*Dermatophagoides evansi* was first identified as a species from material derived from a feather pillow in England (Fain 1967). It has been occasionally isolated from birds' nests (Fain, 1967), house dust samples (Wharton, 1976) and poultry houses (Brady, 1970).

In a recent survey of 35 poultry houses in Israel, *D. evansi* was found in all of the 35 poultry houses examined. This species was the most prevalent species in dust samples of poultry houses, and accounted for 74.4% of the total mite fauna (Mumcuoglu & Lutsky, 1990).

In view of the possible significance of *D. evansi* as an allergy causing agent, the aim of this study was to culture *D. evansi* and to study its life-cycle under laboratory conditions.

**MATERIALS & METHODS**

1. **Isolation of mites**: Dust samples collected in different poultry houses near Jerusalem were examined for mites by a method described previously

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Samples which contained only *D. evansi* specimens were placed in plastic beakers (5 cm in diameter and 5 cm high) and incubated at a temperature of 25-27°C and relative humidity (RH) of 75-80%. Two days later, 100 mg of culture medium was added to an empty area of the container floor. Seven days later the culture medium which was infested with mites was transferred *in toto* to a new container. Examination of 3-5 samples from each culture container two months later confirmed that the culture was pure.

2. *Culture of the mites*: Two culture media were employed. One culture medium consisted of chicken skin and powdered bakers yeast (*Saccharomyces cerevisiae*) (Sigma Co. St. Louis, Mo. USA) in a ratio of 1 : 1.5 (w/w). After removing the skin from commercially available chickens, the fatty layers were excised with a scalpel; further defattting was achieved by placing the skin in acetone for two days. The acetone was discarded, and the skin dried at room temperature and homogenized in a Waring Blender. The second culture medium contained human skin scrapings instead of chicken skin. These skin scrapings were obtained from a chiropodist’s office and defatted with acetone.

Plastic beakers 8 cm in diameter and 5 cm high were used to maintain the colony. The rim of the beaker was smeared with “Tanglefoot” to prevent the mites from escaping and the invasion of the culture either by other mite species or small insects.

The mites were kept in an incubator at a temperature of 25-27°C and 75-80% RH. Additional growth studies were carried out at 20° and 30°C.

Cultures were examined once a week, and every month 50-250 mg of culture medium was added. In those cases where heavy growth occurred, part of the culture was transferred to an empty beaker and maintained as described previously.

3. *Observation of specimens*: The pre-adult stages were studied in plastic “Terasaki” microplates having 60 wells (Cel-Cult, Sterilin, Feltham, England). Approximately 1 mg of culture medium was distributed into each well to which a freshly oviposited egg was added. Nine wells were covered with one cover glass (22 x 22 mm), which was attached to the surrounding well-edges by vaseline.

This provided sufficient space for exchange of air, but did not permit mites to escape.

Contents of the wells were examined daily, and the duration of each stage from egg to adult was recorded. In order to distinguish between active and resting stages, the mites were exposed briefly to a strong light source, rendering the active mites more mobile.

To facilitate observation of the duration of adult stages, oviposition and copulation, two resting tritonymphs were separated from the main culture and placed in a well of a 96-well flat-bottomed tissue culture plate (Cel-Cult, Sterilin). Approximately 2 mg of culture medium was added to each well prior to the introduction of nymphs. Four colonized wells were covered with a 22 x 22 mm cover glass and examined with a stereo-microscope at a magnification of 30 x.

In order to distinguish the original (tritonymph-derived) adults from those males and females in the same well belonging to the new generation, tritonymphs were identified by a vital dye. This was achieved through the addition of 5 mg of Pararosaniline (basic parafuchsin, Sigma Co.) to 100 mg of mite culture; upon ingestion the mid-gut was stained red for several weeks. This technique enabled the study of adult stages of mites derived from the stained tritonymphs. In cases of overcrowding and/or lack of food, the original adults were removed to new wells together with food. For this purpose the micro-plate was placed on a cold-plate at 5°C and left until all mites were immobile. The mites were then individually transferred under the stereo-microscope with the aid of a mounted needle.

**RESULTS**

The developmental stages of *D. evansi* include: egg, larva, protonymph, tritonymph and adult male and female. Resting larval, protonymphal and tritonymphal stages were observed, whereas deutonymph, hypopi and heteromorphic males were not identified.

Table 1 shows the duration of the different developmental stages of *D. evansi* under laboratory
TABLE 1: Duration of the developmental stages of *D. evansi* under laboratory conditions.

<table>
<thead>
<tr>
<th>Stage</th>
<th>n*</th>
<th>Lifespan (days)</th>
<th>Mean lifespan (days ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>52</td>
<td>4-13</td>
<td>8.3 ± 3.3</td>
</tr>
<tr>
<td>Active larva</td>
<td>23</td>
<td>4-6</td>
<td>4.7 ± 0.7</td>
</tr>
<tr>
<td>Resting larva</td>
<td>48</td>
<td>1-3</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>Active protonymph</td>
<td>18</td>
<td>2-5</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
<td>Resting protonymph</td>
<td>25</td>
<td>1-2</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>Active tritonymph</td>
<td>18</td>
<td>2-8</td>
<td>4.5 ± 1.6</td>
</tr>
<tr>
<td>Resting tritonymph</td>
<td>14</td>
<td>1-3</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>Male</td>
<td>25</td>
<td>21-35</td>
<td>28.9 ± 4.9</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>30-62</td>
<td>52.9 ± 8.6</td>
</tr>
</tbody>
</table>

n*: number of mites examined.

conditions. The average duration of the egg was 8.3 days; the duration of active and resting stages combined was: 6.4 days for larva, 5.4 days for the protonymph and 6.6 days for the tritonymph. The average time of development from egg to adult was 26.7 days. Males lived for an average of 28.9 days and females for an average of 52.9 days.

Preliminary studies on the effect of temperature on mite development indicate that at 30°C; the duration of the different stages was shorter, with a higher overall mortality; at 20°C, longer developmental stages and lower mortality were seen. *D. evansi* is oviparous. In 138 cases it was observed that at 25-27°C females laid 1-6 eggs daily (average 3.0 ± 2.6 SD). The initial oviposition in 15 females studied occurred 1-3 days (average 2.0 ± 0.4 SD) after the first copulation.

In 14 cases the initial mating occurred in the first 24 hrs after the adults had hatched. Among 77 tritonymphs studied, 42 developed into males and 35 into females (ratio 1.2 : 1).

Copulation occurred frequently. In 6 cases males and females were observed in a mating position for 6 consecutive days. During mating the male attached itself to the posterior part of the female with the help of two anal suckers and the fourth pair of legs, in such a manner that mouthparts of the male and female mites pointed in opposite directions. Males attached to resting tritonymphs were also observed.

Reproduction without copulation was not observed in 16 virgin females maintained in isolation and examined over a period of 30 days. Females which had been separated from males following copulation, oviposited for at least 10 days.

DISCUSSION

The life-cycle of *D. evansi* resembles that of other Pyroglyphid mites, consisting of egg, larva, protonymph, tritonymph, and adult male and female stages (Fain, 1967; Wharton, 1976).

The development of *D. evansi* from egg to adult under laboratory conditions is similar to that of *D. pteronyssinus* (Spieksma, 1967), *D. farinae* (Oshima & Sugita, 1966) and *D. microceras* (Griffiths & Cunnington, 1971), mites normally found in house dust. The lifespan of *D. evansi* (male: 21-35 days and female: 30-62 days) is similar to that of *D. farinae* (Oshima & Sugita, 1966) and *D. microceras* (Griffiths & Cunnington, 1971), in contrast to the lifespan of *D. pteronyssinus* (male: 60-80 days and the female: 100-150 days) (Spieksma, 1967).

The mating behaviour of *D. evansi* resembles that of *D. pteronyssinus* and *D. farinae* (Spieksma, 1967; Bronswijk & Sinha, 1971). Egg deposition in *D. evansi* and in other species follows copulation; the female *D. evansi* lays 25-50 eggs during life-time, as compared to *D. farinae*, 40-80 eggs, and *D. pteronyssinus*, 70-100 eggs.

*D. evansi* is relatively mobile and in its behaviour is more similar to that of *D. farinae* (Bronswijk & Sinha, 1971) than to *D. pteronyssinus*, which tends to hide under the food substrate (Spieksma, 1967).

The cultivation of *D. evansi* in vitro has facilitated the preparation of diagnostic antigens. Prick skin testing and provocative bronchial challenge tests utilizing these antigens are now being carried out on poultry workers in order to determine the possible clinical relevance of *D. evansi* in poultry-house related hypersensitivity diseases.

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