

# IN VITRO REARING *OESTRUS CAUCASICUS* THIRD-INSTAR LARVAE AND PUPAE (DIPTERA: OESTRIDAE) FROM NATURALLY-INFESTED IBERIAN IBEX, *CAPRA PYRENAICA* (ARTIODACTYLA: BOVIDAE)

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## Summary:

Third-instar *Oestrus caucasicus* larvae ( $n = 236$ ) obtained from Iberian ibex, *Capra pyrenaica*, were reared in a laboratory to obtain adult flies. They were maintained at a temperature of  $21.9 \pm 2.7^\circ\text{C}$  and a relative humidity of  $38.9 \pm 8.0\%$ . In all, 78 imagos emerged (33.1%), with a sex-ratio at emergence not differing significantly from 1:1; 25 larvae did not complete pupariation. A total of 14 adult flies (17.9% of the adults obtained) showed malformations, mainly in their wings. The pupariation period lasted around 30 hours and the pupal stage lasted on average  $29.8 \pm 6.8$  days. The success of pupation in both sexes was mainly determined by the weight of the larvae. Sexual dimorphism, with higher weights in females, was evident in third-instar larvae, pupae and adults. The mean longevity of adult flies was  $224.8 \pm 91.4$  hours and males generally survived for longer than the females.

**KEY WORDS :** *Capra pyrenaica*, Diptera, Oestridae, *Oestrus caucasicus*, culture, biology, pupation, demography.

**Résumé :** CULTURE *IN VITRO* DE LARVES ET NYMPHES D'*OESTRUS CAUCASICUS* (DIPTERA : OESTRIDAE) PROVENANT DE BOUQUETINS IBÉRIQUES *CAPRA PYRENAICA* (ARTIODACTYLA : BOVIDAE) NATURELLEMENT INFESTÉS

Des larves de troisième stade d'*Oestrus caucasicus* ( $n = 236$ ) provenant de bouquetins ibériques (*Capra pyrenaica*) ont été élevées en laboratoire afin d'obtenir des adultes. Elles ont été maintenues à une température de  $21,9 \pm 2,7^\circ\text{C}$  et une humidité relative de  $38,9 \pm 8,0\%$ . 25 larves n'ont pas présenté de nymphose complète, 78 imagos ont achevé leur émergence (33,1%) avec un sex-ratio ne différant pas significativement de 1:1. 14 mouches adultes (17,9% des adultes obtenus) ont présenté des malformations, principalement au niveau des ailes. La durée de la pupariation se situe autour de 30 heures et le stade nymphal dure en moyenne  $29,8 \pm 6,8$  jours. Le succès de la nymphose dans les deux sexes est surtout en relation avec le poids des larves. Le dimorphisme sexuel, qui correspond à un poids plus important des femelles, est marqué pour les larves du troisième stade, les nymphes et les adultes. La longévité moyenne des mouches atteint  $224,8 \pm 91,4$  heures et apparaît supérieure chez les mâles.

**MOTS CLÉS :** *Capra pyrenaica*, Diptera, Oestridae, *Oestrus caucasicus*, culture, biologie, nymphose, démographie.

Larvae of Oestrinae (Diptera: Oestridae) frequently produce cavitary myiasis (nasopharyngeal cavities and frontal sinuses) in perissodactylan and artiodactylan mammals. The sheep bot fly, *Oestrus ovis*, parasitizes sheep and goats worldwide and has been reported from the Asiatic ibex (*Capra ibex sibirica*), argali (*Ovis ammon*), bighorn sheep (*O. canadensis*) and European mouflon (*O. aries*) (Grunin, 1957; Capelle, 1966; Wetzel & Bauristhene, 1970; Moreno *et*

*al.*, 1999). According to Howard (1980) it may also occur in the Nubian ibex (*C. i. nubiana*) and aoudad (*Ammotragus lervia*). Third-instar *Oestrus caucasicus* larvae were discovered in the skull of a Caucasian tur, *C. cylindricornis* and, afterwards, larvae collected from the same host and an Asiatic ibex from central Asia were reared to adults and then described (Grunin, 1957). Later, *O. caucasicus* was found to parasitize Asiatic ibex in Mongolia (Minar *et al.*, 1985) and Iberian ibex (*C. pyrenaica*) in southern Spain (Pérez *et al.*, 1996). As part of a detailed SEM study of different stages in the development of *O. caucasicus*, first- and second-instar larvae have been recently described by Guitton *et al.* (2001), who point out the differential or specific features of this parasite, in addition to important synapomorphies shared with *O. ovis*. Gravid *Oestrus* females are not strictly host specific and so domestic animals could be considered as the main source of infestation for wildlife (Colwell, 2001).

Because adult oestrid flies have rudimentary and non-functional mouthparts and thus do not feed, their larvae must accumulate all the energy reserves they will

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need during the pupal and adult stages. Therefore, larval weight acquires special relevance, particularly for females, which after becoming gravid must seek hosts and carry out larviposition to complete their life-cycles (Zumpt, 1965; Wood, 1987; Kettle, 1990; Cepeda-Palacios *et al.*, 1999). During *Oestrus ovis* larval growth the highest weight increases occur during the early L<sub>3</sub> phase (Cepeda-Palacios *et al.*, 1999), when larvae acquire about 45 % of their average mature weight. Cepeda-Palacios *et al.* (2000) obtained a critical weight of 0.28 g at which mature *Oestrus ovis* larvae become adult flies and also reported sex differences in mature larval weight. These authors predicted a 38 % mean reduction in adult populations of this oestrid if mature larval weight is reduced by 40 %.

The pupal development of this species has also been studied in the laboratory. Rogers & Knapp (1973) found no influence of relative humidity on the number of adults emerging from pupae, although temperature did greatly influence pupae survival. They obtained *O. ovis* adults within a temperature range of 16-32°C. At a constant temperature of 16°C, pupae developed but adults did not emerge. Also, temperatures over 32°C proved fatal for pupae development. Similar results were obtained by Breev *et al.* (1980), who obtained adult emergence within a range of 17-34°C and, in addition, reported that development in female *O. ovis* took significantly longer (30.4 ± 0.5 days for males and 32.3 ± 0.8 days for females). Within the above-mentioned temperature range, these authors obtained high mortality rates; however, minimum mortality rates (41.7 %) occurred when pupae were reared at 20°C.

Aside from scattered data on the prevalence and intensity of *O. caucasicus* parasitism in *Capra pyrenaica*, very little is known about the biology, behaviour or basic demographic parameters of this parasite. The main goal of our study were: a) to characterize the pupal stage of this oestrid in terms of duration and weight dynamics; b) to explore the effects of weight and sex on pupae survival; c) to measure eclosion success, the sex-ratio of emergent imagos, and adult longevity under laboratory conditions; and d) to analyze sexual dimorphism in terms of weight at different phases of pupation. All of these parameters will be very useful in improving our knowledge of the epizootiology of this kind of myiasis.

## MATERIALS AND METHODS

### LARVAE COLLECTION AND EXPERIMENTAL CONDITIONS

Heavily pigmented mature *Oestrus caucasicus* third-instar larvae (L<sub>3</sub>) (n = 236) were collected from the head and horn cavities of 71 Iberian ibexes from the Sierra Nevada mountain range in sou-

Variable	Minimum	Maximum	Mean ± SD
Temperature (° C)	13	30	21.9 ± 2.7
Temperature (minimum)	10	32	19.5 ± 3.1
Temperature (maximum)	18	33	23.9 ± 2.9
Relative humidity (%)	24	62	38.9 ± 8.0
Relative humidity (minimum)	20	56	33.5 ± 7.1
Relative humidity (maximum)	29	70	44.2 ± 8.8

Table I. – Temperature and relative humidity (a total of 227 daily records with minimum and maximum values) at which larvae were reared.

thern Spain (36° 55'-37° 10' N, 2° 34'-3° 40' W), as described in Pérez *et al.* (1996). These animals were randomly removed for management purposes or selectively shot when controlling outbreaks of sarcoptic mange. According to the degree of spiracular and integumental pigmentation, the physiological age of larvae corresponds to L<sub>3</sub>-D<sub>5</sub> (Cepeda Palacios *et al.*, 1999).

The larvae were weighed (to the nearest 0.0001 g) and then transferred to plastic vials containing sawdust as a substrate and reared under laboratory conditions (temperature: 21.9 ± 2.7°C; relative humidity: 38.9 ± 8.0 %) (Table I). Room temperature (current, maximum and minimum) and relative humidity (current, maximum and minimum) were recorded daily throughout the whole experimental period. The larvae were checked every day to assess pupal formation and the duration of this process. If pupae formed, the weights of each pupa were recorded 10 (P<sub>10</sub>) and 20 days (P<sub>20</sub>) after formation on the same scale. The date of adult emergence and their sex and weight were also recorded. After emerging adult flies were individually marked and kept in the laboratory to measure their longevity and observe their mating behaviour. Pupae leading to the emergence of an adult fly without evident anomalies were classified as 'perfect', while those not producing imagos or producing abnormal flies were classified as "unviable". Pupae which did not produce imagos were necropsied to confirm their death.

Pupariation is taken to be the time elapsing from the collection of larvae to the formation of the puparium; the terms "pupation" or "pupal phase" are applied to the insect from the formation of the puparium to the moment of adult emergence (Rogers & Knapp, 1973).

### STATISTICAL ANALYSES

The effect of larval weight on pupation success and sexual dimorphism were analysed by means of a Student's *t* test. The influence of sex and viability ("perfect" versus "unviable") on imago weight was analysed by a two-way ANOVA. The time elapsing before the formation of the pupa was compared between perfect and unviable pupae using a Mann-Whitney test. The

Stage	Females					Males					T-Student	P-value
	Mean	SD	Min	Max	CV (%)	Mean	SD	Min	Max	CV (%)		
Larva III	648.29	103.51	411.00	874.23	15.96	569.23	137.45	285.70	821.70	24.14	2.613	0.011
Pupa (10 days)	441.25	54.12	338.80	532.60	12.26	397.87	58.46	280.10	526.40	14.69	3.101	0.03
Pupa (20 days)	393.79	52.76	293.00	519.90	13.39	358.41	48.99	251.90	473.70	13.66	2.803	0.07
Adult	109.76	37.09	40.50	174.40	33.79	81.61	25.22	38.90	127.80	30.09	3.588	0.001

Table II. – Dynamics of weight (in mg) of pupae producing male (n = 35) and female (n = 41) adults.

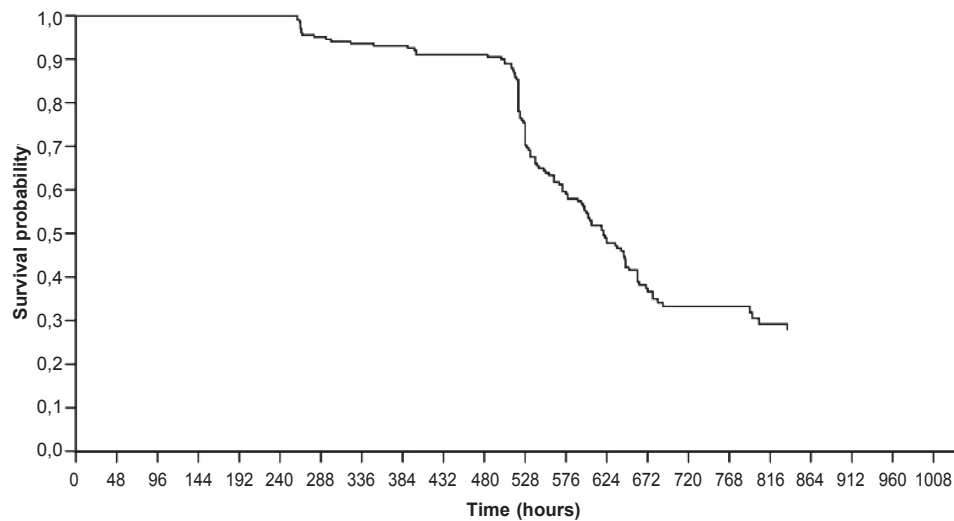


Fig. 1. – Survival curve during *Oestrus* metamorphosis controlled by time-dependent covariables LW and  $P_{10}$ .

influence of pupal weight on the emergence of the imago was analysed by a MANOVA test, with  $P_{10}$  and  $P_{20}$  as dependent variables and “perfect” and “unviable” as factors. The possible differences between male and female flies in terms of time spent in the pupal phase were checked by a Mann-Whitney test. We also used Mann-Whitney and Chi-square tests to analyse sexual differences in longevity and to assess deviations from a 1:1 sex ratio, respectively. A survival analysis (Cox regression) was used for modelling survival based upon the values of given covariates (weight and sex in the case of our study) (Therneau & Grambsch, 2000). Given that it decreased during pupal development (Table II), weight was transformed into a time-dependent covariate in the following way: if time is < than five days, use  $L_3$  weight; if it is > than five days and  $\leq$  than 10 days, use  $P_{10}$  weight; and, finally, use  $P_{20}$  weight if time is > than 10 days.

Data analyses were performed with the aid of the programme SPSS 12.0 (SPSS Inc, 2003) and the null hypothesis was rejected at a  $< 0.05$ .

## RESULTS

Of the 236 larvae reared, a total of 78 adult flies (33.1 %) were obtained: 41 females, 35 males and two specimens from which data regarding

sex were not available (sex-ratio = 1.17, not significantly different from a female: male sex ratio of 1:1,  $\chi^2 = 0.636$ ; 1 df;  $p = 0.425$ ). The remaining individuals died during the different developmental phases (Fig. 1). Larvae producing males took  $31.5 \pm 13.4$  hours to form the puparium and those producing females did so in  $30.0 \pm 17.2$  hours (no significant differences were found:  $U = 597$ ,  $p = 0.207$ , Mann-Whitney test). Mature third-instar larvae weighed, on average,  $594.8 \pm 129.8$  mg (range: 241.5-874.3 mg). Larval weights fitted a normal distribution. 25 larvae (10.6 %) did not form a puparium, 147 (62.3 %) did not complete pupation (that is, no adult emerged,  $n = 133$ , or produced abnormal imagos,  $n = 14$ ) and only in 64 cases (27.1 %; 34 males and 30 females) did adults without evident abnormalities emerge (Table III). Of the 14 abnormal imagos, 13 showed vestigial wings (one of them had its legs

Pupation development	N	Mean weight (mg)	Standard deviation
No pupa produced	25	556.5	160.5
No eclosion reached	133	605.5	109.2
Adult with malformations	14	604.0	91.8
Perfect	64	611.6	129.9

Table III. – Larval weights in relation to success of pupation and production of imagos.

stuck to the inner wall of the puparium and another had a malformation in its abdominal tergites) and the other emerged with its right wing stuck to the puparium.

The success of pupation and adult emergence were influenced by larval weight, since the larvae that produced imagos were significantly heavier than those which did not complete pupation and/or produced unviable flies ( $T = -3.289$ , 231 df,  $p = 0.002$ , T-Student test) (Table II, Fig. 2). Larval and pupal survival depended on the minimum weight obtained and its dynamics according to time (Wald = 0.09, 1 df,  $p = 0.03$ , Cox regression), but was independent of sex (Wald = 0.112, 1 df,  $p = 0.738$ , Cox regression). The most critical period appears to be the last ten days of pupal development, when survival probability is reduced by 60 %. The duration of the pupal stage reached a mean value of  $714.0 \pm 163.7$  hours, or  $29.8 \pm 6.8$  days. No significant differences were found between sexes ( $U = 495$ ,  $p = 0.823$ , Mann-Whitney test), or between perfect and unviable adults ( $U = 2886$ ,  $p = 0.182$ , Mann-Whitney test). As expected, pupal development involved weight losses of up to 85 % of the weight of the post-feeding larvae (Table II).

At room temperature adult males survived  $233.4 \pm 83.0$  hours ( $n = 16$ ; range: 102-462); however, females survived a significantly shorter period of  $219.5 \pm 97.6$  hours ( $n = 26$ ; range: 27-433) (Pearson's chi square test = 5.704; 1 df;  $p = 0.017$ ). Adult females weighed on average nearly 38 % more than males (Table II). Mating behaviour was recorded only once, involving the adults marked as O-94 (male) and O-95 (female).

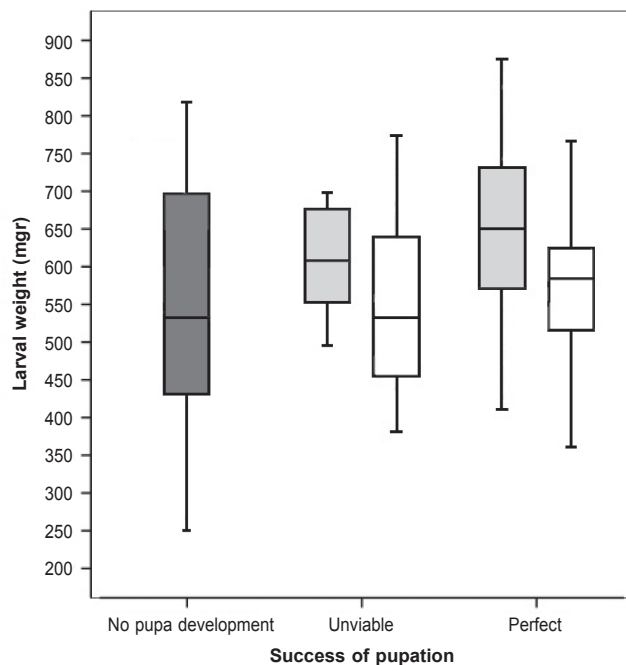


Fig. 2. – Relation between larval weight and success of pupation. Grey boxes: females; white boxes: males.

## DISCUSSION

The percentage of adult flies that emerged was smaller than that reported by other authors for *Hypoderma lineatum* (72 %) and *H. bovis* (55 %) at 20° C. These rates increased up to 86 % and 77 %, respectively, when larvae were reared at 15-25° C and 14-26° C/16-24° C (Pfadt *et al.*, 1975). Rogers & Knapp (1973) obtained 42.8 % adult emergence after rearing *Oestrus ovis* larvae at 16° C-32° C. Breev *et al.* (1980) obtained a hatch rate of 35 % in *Oestrus ovis* adults after rearing larvae at 29.6° C and mortality rates higher than ours when temperature exceeded 34° C. Temperature has a strong influence on pupal development and within the range at which pupation can be achieved, 17-34° C for *O. ovis* (Breev *et al.*, 1980), it appears to be inversely related to the percentage of adults emerging.

In our study, the pupariation period was longer than that obtained by Cepeda-Palacios & Scholl (2000) when reared *Oestrus ovis* at temperatures ranging from 16 to 32° C: 12 hours in heavily pigmented larvae and 22 hours in slightly pigmented larvae. The pupal period of the sheep bot fly can range from 12 to 52 days depending on environmental conditions such as temperature, as reported by Breev *et al.* (1980). The mean value obtained in our study was within this range and very similar to that given by these authors for a temperature of 19.9° C:  $30.4 \pm 0.5$  days; nevertheless, our findings were higher than those given by both Cepeda-Palacios & Scholl (2000) and Cepeda-Palacios *et al.* (2001), who reported that males emerged after 22 days and females after 23 days, and by Rogers and Knapp (1973), who recorded a mean value of  $20.1 \pm 3.3$  days for *Oestrus ovis*, possibly because of a lower mean rearing temperature. The pupal period of cattle grubs averaged 21.7 days in *Hypoderma lineatum* and 28.8 days in *H. bovis* (Pfadt *et al.*, 1975) and was shown to increase as temperature decreased. According to Biggs *et al.* (1998), an extended pupation time may prevent the emergence of adults under adverse climatic conditions. This phenomenon may be considered as an alternative strategy to the overwintering of first-instar larvae and as an external hypobiotic period as well (Tabouret *et al.*, 2001).

Weight decrease from the moment of larvae collection to adult emergence can be considered as a good example of the maximum use of body reserves. After intestinal evacuation, the cuticle of *Oestrus ovis* still loses water during the process of sclerotization and phenolic tanning. Glycogen is also mobilised during the process of metamorphosis (Wigglesworth, 1984; Cepeda-Palacios & Scholl, 2000). Although females were heavier than males during all of the developmental stages studied (all  $p$ -values < 0.05,  $t$ -Student

test) (Table II), maximum sexual dimorphism was reached in imagos (male weight/female weight = 0.74), followed by L<sub>3</sub> weight (0.87) and, finally, by pupal weight (0.9 for P<sub>10</sub> and P<sub>20</sub>). It is also remarkable that weight variability was greater in L<sub>3</sub> and imagos (CV = 20.05 % and 31.94 %, respectively) than in pupae (CV = 13.47 % for P<sub>10</sub> and CV = 13.52 % for P<sub>20</sub>). Regarding larval and adult weight, sexual dimorphism has also been reported in *Hypoderma tarandi* and *Cephenemyia trompe* (Nilssen, 1997).

The longevity of the adult flies obtained was short, not reaching 10 days (9.4 ± 3.8 days), but was similar to that obtained by Biggs *et al.* (1998) in *Oestrus ovis* (mean longevity = 7.7 days). Rogers & Knapp (1973) recorded a mean adult *O. ovis* survival rate in the laboratory of 15.9 ± 6.0 days. Biggs *et al.* (1998) found that both *O. ovis* adult males and females have similar energy reserves (near 27 kJ/g). If adult *O. caucasicus* show a similar pattern, then the activities of host seeking and larviposition by females will explain their shorter longevity.

Rogers & Knapp (1973) estimated that mortality during immature (larval) stages of *O. ovis* in wild populations ranged from 90.2 to 99.1 %. If the wild population of *O. caucasicus* has a similar mortality rate and we add the rate obtained in the laboratory for the pupal stage, then we can estimate that only about a 0.3-3 % of larvae produced by gravid females will become adult flies. *Oestrus ovis* females lay about 500 eggs (Kettle, 1990). For demographic calculations we must take into account a relatively low mean intensity (25.4 ± 27.3 larvae/host) along with a high mean prevalence (74 %) and at least two generations per year (Pérez *et al.*, 1996) within the context of a host population of around 15,000 ibexes (Granados *et al.*, unpublished data).

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