Post-ecdysis behavior of exarate adults in *Drosophila melanogaster* and *Ceratitis capitata.*

Bochicchio, P.A., D.H. Bodin, L.A. Quesada-Allué*, and A. Rabossi. IIBBA-CONICET, Fundación Instituto Leloir, and Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Av. Patricias Argentinas 435 (1405), Buenos Aires, Argentina. *E-mail: lualque@iib.uba.

Introduction

The life cycle (LC) of cyclorrhaphans follows a well-conserved developmental program in which the different instars and stages within instars show a similar sequence of events (Denlinger and Žďárek, 1994). In spite of the evolutionary distance (around 120 MY), the duration of metamorphosis of *Drosophila melanogaster* and *Ceratitis capitata* seems to represent a similar proportion of time of the whole life cycle, *i.e*., 48.1 and 50.1%, respectively (Bainbridge and Bownes, 1981; Rabossi and Quesada-Allué, 1995). The duration of stages within the puparium expressed as percent of total metamorphosis time also seems to be highly conserved between these two cyclorrhaphans, in spite of the respective slow (600 hs) *C. capitata* and rapid (239 hs) *D. melanogaster* LCs. This might also be true for certain evolutionary and ecologically distant flies, like the blood-sucking fly *Haematobia irritans* (Basso *et al*., 2011) and other muscidae (Denlinger and Žďárek, 1994). In cyclorrhaphans, when the pharate adult inside the puparium opens the puparial operculum, a stage of extrication is initiated, ending when the legs support the body and the insect is able to walk (Žďárek and Denlinger, 1986, 1987). In *D. melanogaster* this stage has been described as Stage P15(i+ii) by Bainbridge and Bownes (1981). Then follows a phase in which the exarate imago acquires the final size, shape, and body coloration. This phase has been described in *D. melanogaster* by Bainbridge and Bownes (1981) as Stages A1 to A3. During these first hours as "unfinished" imago the exarate fly undergoes complex behavioral and molecular processes giving rise to final body maturation. In particular, the ptilinium cuticle region retracts and, after muscular pulsations and body expansion, the wings reach their definitive extension (Johnson and Milner, 1987). Then, the final steps of cuticle sclerotization and pigmentation occur, mediated by catecholamine derivatives (Perez *et al.*, 2002; Hopkins and Kramer, 1992), thus attaining the final external phenotype of the imago. Studies on this phase were reported in muscoids like *Sarcophaga crassipalpis* (Žďárek and Denlinger, 1986, 1987) or *Glossina*-Tsetse (Žďárek and Denlinger, 1992). However, as far as we know, no detailed comparison between *D. melanogaster* and Tephritids post-ecdysis behavior has been published.

Materials and Methods

D. melanogaster (Canton S) was reared in Formula 4.24 Instant Drosophila Medium (Carolina Biological Supply). Wild-type medflies, *C. capitata* (Mendoza), were reared in pumpkin-based medium as described by Pujol-Lereis *et al*. (2006). The flies were kept in a Conviron chamber (CMP 3244) at 23ºC, 60% RH, with a photoperiod of 16:8 h (light:dark). To record extrication and the initial behavior of single male exarate adults, the experimental arenas were plastic petri dishes, where a single puparium was glued to the center with a drop of 2% agar. The arena for each one of 12 *D. melanogaster* males was 35 mm diameter and 4 mm high. The arena for the medflies (24 males) was 90 mm diameter and 5 mm high.

Using these dishes, the flight was avoided and the flies could be recorded in 2-D using Logitech-C-625 cameras. Each camera was able to simultaneously record two arenas, using different zooming for the two different sizes. The final setting, at 23ºC and 60% RH, included 9 LED lamps, that gave 1000 Lux through a translucent paper circular sheet. The recorded data from the onset of extricaton to final body characteristics completion, the phase that we named BMP (see below), were registered and partially processed using a Fly-tracker-β1 experimental program from our laboratory. The timing and events of extrication behavior were analyzed separately. After extrication, the timing and length of the pathway followed by the exarate flies was continuosly recorded, including the stops. The initial position of the exarate fly was recorded as t_0 and the final position (final size and coloration) was t_f (see Figure 2 A,B). *In situ* movements like ptilinum pumping, proboscis bobbing, grooming with the legs, abdominal pulses, expansion of the wings, and so forth, that do not involve body displacement were considered for our purposes as equivalent to immobile stationary behavior. These morphogenetic activities were summarized by Žďárek and Denlinger (1992). Time of immobility was proportional to the radii of circumferences in Figure 2 C,D. These were generated using Microsoft Office Excel.

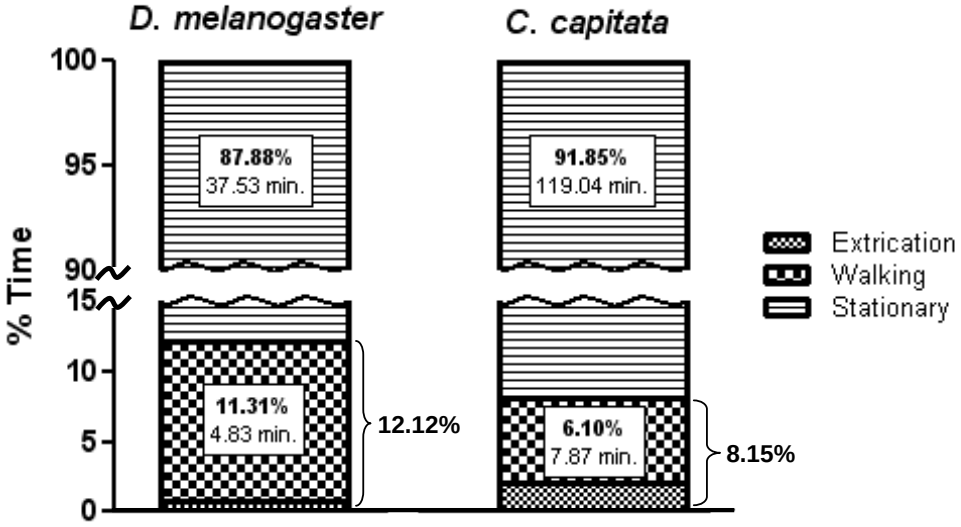

Figure 1. Percentage of time spent in extrication, walking, or stationary behaviors during the total phase BMP of exarate adults. The arrows indicate the percentage of time spent in the active periods: extrication + walking.

Results and Discussion

We have carefully analyzed the stages after ecdysis of male exarate adults of *D. melanogaster* and *C. capitata*, to analyze and quantify the timing of events until full body maturation, when final rigidity and coloration were attained. We defined the total phase of whole body maturation phase (BMP) as starting at the moment of the operculum opening and onset of pharate adult extrication and ending when the definitive features of the body are attained. Thus, this phase of the exarate adult, BMP, represents the transition from the pharate adult to the full imago, in our experimental conditions. In *Drosophila* this roughly corresponds to the P15(ii) and A1-A3 stages described by Bainbridge and Bownes (1981). Extrication in males of *D. melanogaster* required, in average, around 21 seconds (*i.e.*, 0.81% of the BMP), whereas in *C. capitata* it lasted for 2.66 ± 1.03 minutes, *i.e.*, 2.05% of BMP (Figure 1). This difference is probably more related to the anatomy of the puparium than to differences due to insect size or life cycle length. The initial position of the exarate insect standing up on its legs for the first time (t_o, Figure 2 A,B) was the one after complete extrication from the puparium, that was glued to the center of petri dishes used as arenas (see Materials and Methods). From the preliminary results reported in this communication, the total phase BMP including the extrication period required 42.70 ± 11.94 min in *D. melanogaster* and

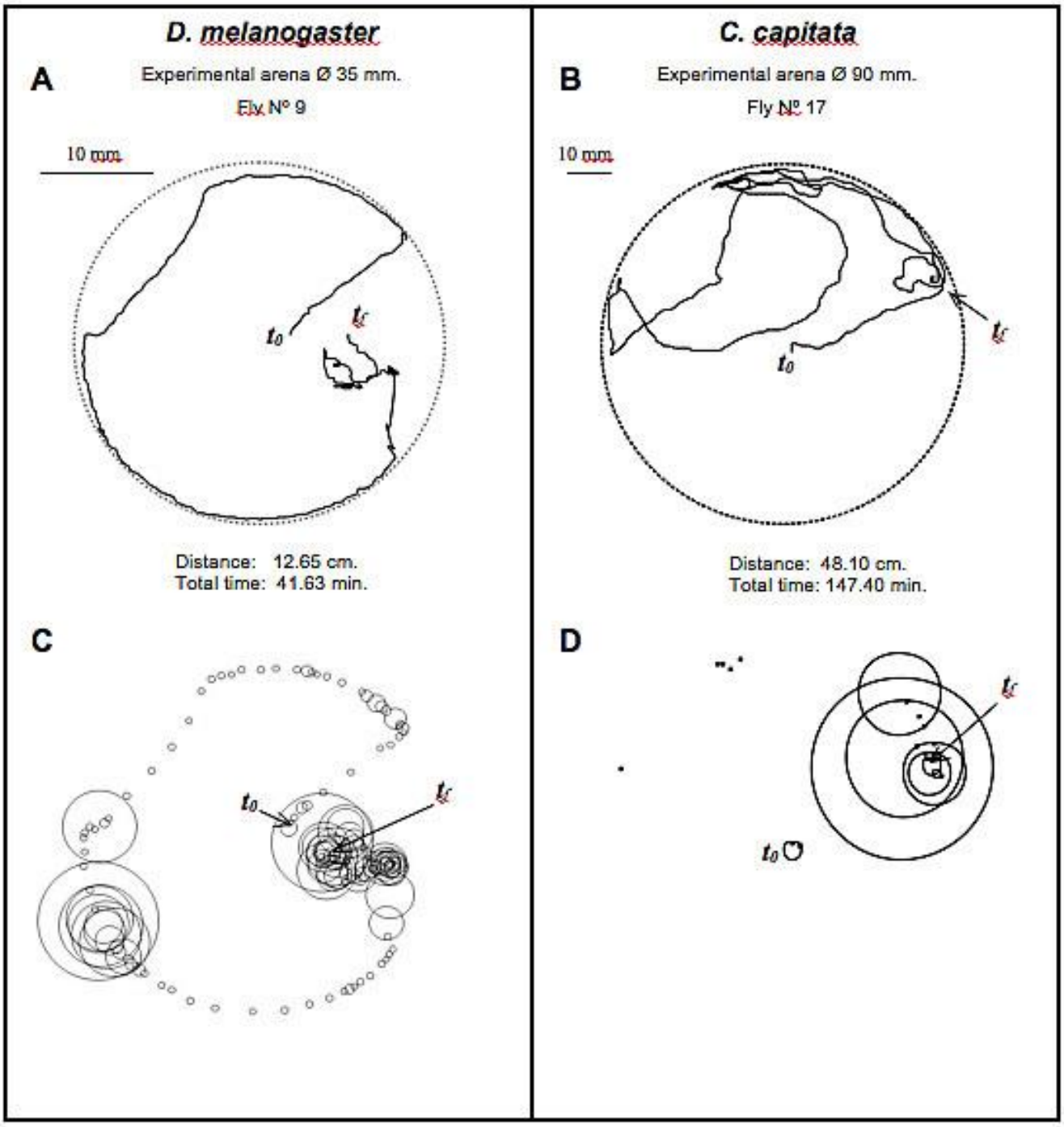

Figure 2. Examples of behavioral patterns in petri-dish arenas. A, B: records of the flies' pathways, starting at extrication point (t_0) and ending when final phenotype is attained (t_f). C, D: diagram of the same paths as in A,B, indicating the position and duration of resting periods. The diameters of the circumferences in the diagrams are proportional to the duration.

126.91 ± 22.60 min in *C. capitata*. The post-extrication behavior of both flies exarate adults was analyzed and compared. Figure 2 A,B show representative examples of the respective pathways followed in a restricted circular arena. During this post-extrication stage *D. melanogaster* shows a walking behavior frequently interrupted by numerous stationary periods of resting (Figure 2 A,C), whereas medflies displayed first a rapid exploratory behavior (on the average 7.86 ± 3.75 min) followed by a single long period of resting (119.04 ± 25.24 min) until the final phenotype of the imago is attained (Figure 2 B,D). The diameters of the circumferences in the diagrams (Figure 2 C,D) are proportional to the duration of the resting time in that position. The length of the path for the examples in Figure 2 was 12.65 cm for *D. melanogaster* and 48.10 cm for *C. capitata*. The average length of the pathways was 11.02 ± 1.91 cm for *D. melanogaster* and 45.40 ± 13.47 cm for *C. capitata*. Total time for *D. melanogaster* periods of walking represented 6.1% of the time of the whole phase and for *C. capitata* the single walking period represented 11.31% of the total BMP (Figure 1). Significantly, when the extrication times were added to the mobility times, total activity time represented 12.12% of the *Drosophila* BMP, whereas the equivalent time for *Ceratitis* represented 8.15% (Figure 1). Comparing these observations with the previously reported equivalent post-ecdysial behavior in other flies like the sarcophagids *Sarcophaga crassipalpis*, *S. bullata*, and *S. argirostoma* (Žďárek and Denlinger, 1986, 1987), all seem to follow a similar pattern to that of *C. capitata*, very different from that of *D. melanogaster* and (probably) other drosophilids. The mobility parameters in our experimental conditions of reduced movement might be proportional to the remainder of the energy resources available for metamorphosis, mainly haemolymph trehalose, muscle glycogen and lipids (Bochicchio, 2012; Nestel *et al.*, 2003) and in this case might be very different in wild conditions. Although behavior heterogeneity among individuals of each species is significant, the post-ecdysial exarate adult behavioral pattern indicates that in both flies around 90% of resting time is required during this period (Figure 1). This seems to indicate that for full completion of exarate adult body features, a similar proportion of resting time is required in both flies. In turn, this suggests that in the wild, a bottleneck for the behavior of cyclorrhaphans during the non-eating BMP might be the availability of energetic reserves to be spent during that phase of the life cycle. This kind of data is also important for the male-sterile programs for pest flies control.

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The effect of pyrogallol on the resistance to starvation in *Drosophila bipectinata.*

Krishna, M.S., and Uchenna U. Waturuocha. Drosophila Stock Centre, Department of Studies in Zoology, University of Mysore, Mysore, India.

Organisms often face stressful environmental conditions in nature, defined as environmental factors that reduce fitness (Koehn and Bayen 1989). Common environmental stressors, desiccation