Ageing and thermal performance in the sub-Antarctic wingless fly *Anatalanta aptera* (Diptera: Sphaeroceridae): older is better

L. Lalouette¹, P. Vernon², H. Amat² and D. Renault^{1,*}

¹Université de Rennes 1, UMR CNRS 6553, 263 avenue du Gal Leclerc, 35042 Rennes, France

²Université de Rennes 1, Station Biologique de Paimpont, UMR CNRS 6553, 35380 Paimpont, France

*Author for correspondence (david.renault@univ-rennes1.fr).

Senescence is a progressive biological process expressed in behavioural, morphological, physiological, biochemical and cellular age-related changes. Age-associated alterations in activity are regularly found in insects when examining whole-organism senescence over the adult lifespan. In addition, overall stress resistance usually decreases with senescence. In the present study, we measured the critical thermal minimum (CT_{min}) and the subsequent recovery period over the lifespan of the sub-Antarctic wingless fly, *Anatalanta aptera*. Experiments were conducted on males and females in seven age groups: newly emerged, 1.5-, 5-, 7-, 13-, 15- and 18-month-old adults. Surprisingly, CT_{min} decreased significantly with ageing in *A. aptera*, from $-3.8 \pm 0.5^\circ\text{C}$ just after the emergence to $-5.6 \pm 0.7^\circ\text{C}$ in the 18-month-old flies. The subsequent recovery period remained similar between the seven groups tested. Our unexpected results contradict the previous data collected in other insects. We have demonstrated for the first time that ageing may improve rather than impair locomotor activity during unfavourable thermal conditions. It raises questions and challenges the literature dealing with ageing. These fascinating results also question the underpinning mechanisms involved in the improvement of the thermal performance with ageing in *A. aptera*.

Keywords: senescence; CT_{min}; locomotor activity; fly; chill-coma recovery

1. INTRODUCTION

Senescence is an intrinsic and progressive biological process characterizing nearly all living organisms (Arking 2006). Some of the well-known consequences of ageing include increased homeostatic imbalance, decreased ability to respond to stress and increased risk of ageing-associated diseases. The probability of death exponentially increases as the organism ages

(Tower 1996; Lin *et al.* 1998; Monaghan *et al.* 2008). In insects, most work dealing with ageing has been performed in *Drosophila* species. Whole-organism senescence over the adult lifespan has highlighted age-associated alterations in activity, such as reduced speed of locomotion and flight performance. This is probably a result of myofibril degeneration in flight muscles, as well as a decrease in the functional capacity of the mitochondria, and changes in the activity of several enzymes (see Grotewiel *et al.* 2005 for a review).

Although several aspects of senescence have been detected in insects, functional senescence, i.e. the monitoring of a function or a trait over the whole species lifespan, has been less frequently examined (Grotewiel *et al.* 2005). Unlike most dipteran species, whose adult lifespans usually range from days to months, the sub-Antarctic wingless fly, *Anatalanta aptera* Eaton (Diptera: Sphaeroceridae), represents an exceptional model that is characterized by unusual longevity. Under controlled conditions, adults can live for as long as two years and maintain their reproductive abilities throughout their adult life (Chevrier 1996). *Anatalanta aptera* is active year-round and occurs on several sub-Antarctic islands in the Indian Ocean (Crozet, Kerguelen and Heard Island; Vernon & Vannier 1996). These islands are characterized by a cold and windy climate without large seasonal thermal fluctuations (annual average temperatures *ca* 5°C). Adults of *A. aptera* should thus be characterized by an ability to maintain walking activities at relatively low temperatures.

Senescence of motor activity can be easily measured in insects (Fernández *et al.* 1999; Ridgel & Ritzmann 2005). Locomotor activities are directly or indirectly implicated in many biological functions (Martin *et al.* 1999), and the capacity to maintain motor activity at low temperatures is likely to be a key fitness attribute in *A. aptera*, which experiences low temperatures year-round. Thus, we measured age-related changes in the critical thermal minimum (CT_{min}) to characterize motor activity in this species. CT_{min} is defined as the temperature at which coordinated muscle function is lost, i.e. the onset of chill coma (Klok & Chown 1997), and constitutes a convenient index of insect thermal tolerance (Castañeda *et al.* 2005). Because senescence is characterized by the inevitable and irreversible accumulation of damage leading to loss of functions with age (Monaghan *et al.* 2008), we hypothesized that (i) thermal tolerance would decline with age in adults of *A. aptera* and (ii) the duration of the recovery period after the onset of chill coma would increase with ageing in adults of *A. aptera*.

2. MATERIAL AND METHODS

Imagos of *A. aptera* Eaton (Diptera: Sphaeroceridae) were sampled on Heard Island (53°00' S, 73°00' E) in March 1997. They were maintained under controlled conditions (5.0 ± 0.5°C) in Paimpont (France), with a natural L:D cycle. This saprophagous species was fed *ad libitum* with yolk of hen eggs (Vernon & Vannier 1996). For the present work, newly emerged imagos that had come from different females were isolated in several plastic boxes every day and bred under controlled conditions as described above. Flies of known ages were then randomly sampled in these boxes and CT_{min} was measured in newly emerged adults (less than 3 days) and in 1.5-, 5-, 7-, 13-, 15- and 18-month-old males and females, with *n* = 21, 39, 39, 37, 8, 14 and 11 flies, respectively. Each individual was weighed using a Sartorius M4 microbalance (*d* = 1 µg) and sexed just after CT_{min} was measured. We used the method described

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Figure 1. Critical thermal minimum (CT_{min}) in the seven age groups of *A. aptera*: 0 (newly emerged), 1.5-, 5-, 7-, 13-, 15- and 18-month-old adults, with $n = 21, 39, 39, 37, 8, 14$ and 11 flies, respectively. Box plots with different letters indicate significant differences (Tukey post hoc test, $p < 0.05$). Black squares represent mean values. Stars represent outliers.

by Powell & Bale (2006) to measure CT_{min} . The equipment consisted of an ethylene glycol jacketed glass cylinder (35×5 cm). Flies were placed individually in the inner chamber, and the air temperature was controlled by circulating ethylene glycol from a programmable alcohol bath (Haake F3 Electron, Karlsruhe, Germany) through the outer chamber. White paper was placed inside the inner chamber, so that the flies were clearly visible. Flies were cooled from 4°C to the CT_{min} at $0.50^\circ\text{C min}^{-1}$. Upon entering chill coma (CT_{min}), flies lost the ability to cling onto the column and fell out at the bottom. The temperature within the column was measured with a thermocouple to an accuracy of $\pm 0.1^\circ\text{C}$. Individuals were immediately transferred to a Petri dish placed above ice ($6.0 \pm 1.0^\circ\text{C}$). Two distinct durations were then recorded (i) T_1 = duration needed to observe the first voluntary movement of any appendage and (ii) T_2 = duration needed to recover walking activities with coordinated movements of the legs.

Data are presented as means $\pm$ s.e. The effects of age on CT_{min} were tested using a generalized linear model after ensuring a normal distribution of the data (Kolmogorov–Smirnov test). Variances of CT_{min} among groups did not differ significantly (Levene test, $p > 0.05$). T_1 and T_2 values were log transformed before testing the normal distribution of the values. For T_1 , the distribution deviated from normality, and we thus used a Kruskal–Wallis test. Statistical analyses detailed in the results were carried out using MINITAB Statistical Software Release 13 (MINITAB Inc., State College, PA, USA).

3. RESULTS

When pooling flies of all different ages, males exhibited the lowest fresh mass (10.0 ± 2.0 versus 12.0 ± 2.3 mg for females) ($W = 7014$, $p < 0.05$).

CT_{min} was not significantly different between males ($n = 93$) and females ($n = 76$) ($T_{159} = -0.91$, $p > 0.05$); data from both sexes were thus pooled for all subsequent analyses. CT_{min} differed significantly over the lifespan of *A. aptera* ($F_{6,159} = 7.30$; $p < 0.001$) (figure 1), but the results were opposite to our prediction: youngest flies were characterized by the highest CT_{min} values ($CT_{min} = -3.8 \pm 0.5^\circ\text{C}$), whereas the lowest CT_{min} (i.e. most cold tolerant) were found in the oldest adults (18 months, $CT_{min} = -5.6 \pm 0.7^\circ\text{C}$). CT_{min} did not differ significantly during the first six weeks (Tukey post hoc test $p > 0.05$), and the five- and seven-month-old flies had intermediate positions (figure 1).

The results from the recovery from chill were also unexpected. Neither T_1 nor T_2 differed among the

seven age groups (T_1 : $H_6 = 12.15$, T_2 : $F_{6,160} = 1.78$; $p > 0.05$) and were not correlated with fresh mass (Spearman test = 0.138 for T_1 and 0.100 for T_2 ; $p > 0.05$) or CT_{min} (Spearman test = -0.126 for T_1 and 0.175 for T_2 ; $p > 0.05$). Overall, both T_1 and T_2 were highly variable and ranged from 1 s to 11 min for T_1 and from 3 s to more than 39 min for T_2 (figure 2). In the 0-, 1.5-, 5- and 7-month-old flies, most of the insects (80%) moved during the first minute after they were returned to optimal thermal conditions and almost half of the flies (45%) recovered normal walking during the first 2 min. In the 15- and 18-month-old flies, 66.7 and 54.5 per cent moved during the first minute, respectively, and then recovered walking activities in the range 2–3 min.

4. DISCUSSION

The critical thermal limits characterize the temperature at which an animal loses muscular control (Goller & Esch 1990). In the present work, we found that adults of *A. aptera* were able to maintain walking activities at subzero temperatures, as already observed in other species of the Sphaeroceridae (Soszyńska 2004). *Anatalanta aptera* is a year-round active fly on Ile de la Possession (Crozet archipelago; Vernon & Vannier 1996). The capacity to maintain motor activity at low temperatures is likely to be a key fitness attribute in this wingless fly, which endures constantly cold conditions (mean monthly temperatures range from 0 to 4.2°C on Heard Island). Lower CT_{min} values (-6 to -7) have even been found in another sub-Antarctic arthropod species on Marion Island, the spider *Myro kerguelensis* (Jumban et al. 2008).

Anatalanta aptera is an uncommon biological model, i.e. a wingless dipteran with a strikingly long imaginal life, providing an exciting opportunity to address evolutionary questions on the impact of senescence on insect functional traits. Ageing-related mortality is low during the first year of *A. aptera*'s life, and even 18-month-old imagoes are not extreme surviving individuals (Chevrier 1996; Vernon & Vannier 1996). Thus, the progression of thermal performance did not result from selective mortality. Studies that have examined the biology of ageing and evolution of locomotor function over the adult lifespan highlight physiological changes as a result of impaired locomotor behaviour (Ridgel & Ritzmann 2005). In insects, the fine control of the muscles is achieved by the polyneuronal innervation of the muscles and through neuromodulation; the decline of walking abilities during ageing can be attributed to neural and/or neuromusculoskeletal degeneration (Ridgel & Ritzmann 2005). In that context, our data provide new and exciting findings, which contradict almost all previous conclusions on this subject. The effects of thermal history were controlled (flies were reared under controlled temperature conditions), and the increased ability of old adults of *A. aptera* to maintain walking activities at cold temperatures thus contrasts with the data collected on other flies (e.g. Grotewiel et al. 2005). Taking into account the fact that overall stress resistance usually decreases with senescence in

Figure 2. Frequency distribution of the recovery duration from chill coma in the seven age groups of *A. aptera* (0, newly emerged adults). T_1 , duration needed to observe the first voluntary movement; T_2 , duration necessary to recover walking activities.

flies (Grotewiel *et al.* 2005), the increased ability to sustain muscular control in the oldest *A. aptera* during cold exposure and the underpinning mechanisms are especially curious. Like a recently proposed hypothesis by Bowler & Terblanche (2008), our results both challenge the generality of the prediction that ageing impairs rather than improves performance in thermal biology and highlight the scarcity of studies on the subject.

Another interesting finding was the duration of the recovery period after the flies entered chill coma. Overall, T_1 and T_2 remained constant over the whole adult lifespan, but the oldest *A. aptera* tended to have a slightly higher duration of recovery. The duration of recovery was often linked to the amount of accumulated chill injuries (David *et al.* 1998) and to the level of changes that occur in action potentials of muscles and nerves (Goller & Esch 1990). David *et al.* (1998) found that the recovery period increased with ageing in adults of *D. melanogaster*, even if irregular and uncontrolled variations were found in the oldest flies. Our data suggest physiological changes occurring during ageing in *A. aptera*, which lead to increased thermal limits, thus keeping the amount of accumulated injuries similar among groups. A high variability was found within each age class, probably resulting from the natural physiological variance between the individuals.

To conclude, our work presents novel data on ageing and insect activity at low temperatures, which is of direct significance for senescence research. Importantly, this work showed improved low-temperature tolerance with ageing in *A. aptera*, and highlighted the need for further research on functional traits and ageing.

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- Arking, R. 2006 The biology of aging: observations and principles. Oxford, UK: Oxford University Press.
- Bowler, K. & Terblanche, J. S. 2008 Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? *Biol. Rev.* **83**, 339–355. (doi:10.1111/j.1469-185X.2008.00046.x)
- Castañeda, L. E., Lardies, M. A. & Bozinovic, F. 2005 Inter-population variation in recovery time from chill coma along a geographic gradient: a study in the common woodlouse, *Porcellio laevis*. *J. Insect Physiol.* **51**, 1346–1351. (doi:10.1016/j.jinsphys.2005.08.005)
- Chevrier, M. 1996 Introduction de deux espèces d'insectes aux Iles Kerguelen: processus de colonisation et exemples d'interactions. Thèse de 3^{ème} cycle, Université de Rennes 1.
- David, R. J., Gibert, P., Pla, E., Petavy, G., Karan, D. & Moreteau, B. 1998 Cold stress tolerance in *Drosophila*: analysis of chill coma recovery in *D. melanogaster*. *J. Therm. Biol.* **23**, 291–299.
- Fernández, J. R., Grant, M. D., Tulli, N. M., Karkowski, L. M. & McClearn, G. E. 1999 Differences in locomotor activity across the lifespan of *Drosophila melanogaster*. *Exp. Geront.* **34**, 621–631. (doi:10.1016/S0531-5565(99)00040-6)
- Goller, F. & Esch, H. 1990 Comparative study of chill-coma temperatures and muscle potentials in insect flight muscles. *J. Exp. Biol.* **150**, 221–231.
- Grotewiel, M. S., Martin, I., Bhandari, P. & Cook-Wiens, E. 2005 Functional senescence in *Drosophila melanogaster*. *Ageing Res. Rev.* **4**, 372–397. (doi:10.1016/j.arr.2005.04.001)
- Jumban, K. R., Terblanche, J. S., Deere, J. A., Somers, M. J. & Chown, S. L. 2008 Critical thermal limits and their response in two sub-Antarctic spiders: *Myro kerguelensis* and *Prinerigone vagans*. *Polar Biol.* **31**, 215–222.
- Klok, C. J. & Chown, S. L. 1997 Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic caterpillar, *Pringleophaga marioni* (Lepidoptera: Tineidae). *J. Insect Physiol.* **43**, 685–694.

- Lin, Y.-J., Seroude, L. & Benzer, S. 1998 Extended life-span and stress resistance in the *Drosophila* mutant *methuselah*. *Science* **282**, 943–946. (doi:10.1126/science.282.5390.943)
- Martin, J.-R., Ernst, R. & Heisenberg, M. 1999 Temporal pattern of locomotor activity in *Drosophila melanogaster*. *J. Comp. Physiol.* **184**, 73–84. (doi:10.1007/s003590050307)
- Monaghan, P., Charmantier, A., Nussey, D. H. & Ricklefs, R. E. 2008 The evolutionary ecology of senescence. *Funct. Ecol.* **22**, 71–378.
- Powell, S. J. & Bale, J. S. 2006 Effect of long term and rapid cold hardening on the cold torpor temperature of an aphid. *Physiol. Entomol.* **31**, 348–352. (doi:10.1111/j.1365-3032.2006.00527.x)
- Ridgel, A. L. & Ritzmann, R. E. 2005 Insights into age-related locomotor declines from studies of insects. *Ageing Res. Rev.* **4**, 23–39. (doi:10.1016/j.arr.2004.08.002)
- Soszyńska, A. 2004 The influence of environmental factors on the supranivean activity of flies (Diptera) in central Poland. *Eur. J. Entomol.* **101**, 481–489.
- Tower, J. 1996 Aging mechanisms in fruit flies. *BioEssays* **18**, 799–807. (doi:10.1002/bies.950181006)
- Vernon, P. & Vannier, G. 1996 Development patterns of supercooling capacity in a sub-Antarctic wingless fly. *Experientia* **52**, 155–158. (doi:10.1007/BF01923362)