Oikos 121: 2072–2080, 2012
doi: 10.1111/j.1600-0706.2012.20582.x

Subject Editor: Matthew Symonds. Accepted 26 March 2012

What are the possible benefits of small size for energy-constrained ectotherms in cold stress conditions?

Mohannad Ismail, Philippe Vernon, Thierry Hance, Jean-Sébastien Pierre and Joan van Baaren

M. Ismail, J.-S. Pierre and J. van Baaren (joan.van-baaren@univ-rennes1.fr), Univ. de Rennes 1, Campus Scientifique de Beaulieu, UMR 6553 CNRS - EcoBio, France. – P. Vernon, Univ. de Rennes 1, Station Biologique de Paimpont, UMR 6553 CNRS - EcoBio, France. – MI and T. Hance, Univ. Catholique de Louvain, Earth and Life Institute, Biodiversity Research Centre, Place Croix du Sud, 4-5, BE-1348 Louvain-la-Neuve, Belgium.

In stressful environments, two main hypotheses have been proposed to explain the consequences of body size: (1) the absolute energy demand hypothesis (AED), which predicts that larger individuals are at a disadvantage under stressful conditions; (2) the relative efficiency hypothesis (RE), which predicts the reverse. We compared the effects of cold stress on different fitness traits of large and small individuals of the parasitoid wasp *Aphidius ervi* (Hymenoptera: Aphidiinae). For that, we exposed nymphs of this wasp to 5 treatment conditions as follows (control at 20°C; 7C1 and 7C2: constant cold temperature of 7°C for 1 and 2 weeks respectively; 4C1 and 4C2: constant cold temperature of 4°C for 1 and 2 weeks respectively).

After cold stress, only the large females that emerged in the 7C2 and 4C2 treatments displayed a reduction in the fitness traits studied (longevity, egg load at emergence, life-time fecundity). The decrease in lipid content in large adults may have been responsible for their lower fitness. Our results thereby supported the AED hypothesis. Furthermore, the small females in these treatments produced more eggs at emergence than the control females. This highlights the fact that in stressful environments, small females switch their reproductive strategy from a synovigenic strategy (in which females mature new eggs after emergence) to a more pro-ovigenic one (in which females emerge with more mature eggs).

Temperature is one of the abiotic environmental factors that most strongly affects behaviour, physiology, and life history in animals (Hoffmann et al. 2003, Blanckenhorn and Henseler 2005) and the relationships between different species (Bale 2002). Since negative effects are expected, temperature changes can be considered to be stressful. Indeed, stress is defined as 'any environmental change that drastically reduces the fitness of an organism' (Hoffmann and Parsons 1997). The intensity of the negative effects of temperature depends on the type and duration of the thermal stress and of the characteristics of the exposed population (Hoffmann et al. 2003, Ismail et al. 2010).

Body size is a key feature in ecology and evolution theories (Angilletta et al. 2004), since it affects all aspects of animal physiology and life history, and consequently, fitness (Thorne et al. 2006, Chown and Gaston 2010). Temperature and nutrition are the most important factors affecting phenotypic size variation (Chown and Gaston 2010). Laboratory studies support the notion that animals grow larger at lower temperatures according to the temperature–size rule (Atkinson 1994, Angilletta and Dunham 2003) and its field counterpart, Bergmann's rule (endotherms and some ectotherms tend to be larger in colder environments) (Atkinson 1994, Arnett and Gotelli 2003, Angilletta et al. 2004). The benefits of large body size have been widely demonstrated. Larger body size is associated with greater fitness in many animal species (Stearns 1992, Andersson 1994, Chown and Gaston 2010), particularly for males. For example, Lacoume et al. (2006) found that small males of *Dinarmus basalis* (Hymenoptera: Pteromalidae) had a reduced size and sperm stock as compared to large ones, and that they can fertilize less females in competitive situations. It has also been shown that larger individuals generally have greater resistance than smaller ones to stresses linked to energy reserves, such as starvation stress (Cushman et al. 1993, Rivero and West 2002, Arnett and Gotelli 2003). As earlier emphasized by Forrest (1987), the possible benefits of small body size were not so specifically investigated.

Two physiological hypotheses have been proposed for comparing the resistance of individuals to energy constraints in function of their size under stressful conditions. The first is the absolute energy demand hypothesis (AED), which predicts that larger individuals require more energy reserve to sustain their body functions (Blanckenhorn et al. 1995, Reim et al. 2006), probably because they have a proportionally higher metabolic rate than smaller individuals (Calder 1984, Gillooly et al. 2001), and so they will be at a disadvantage in a stressful environment. The second,

the relative efficiency hypothesis (RE), predicts that larger individuals will be at an advantage in an energetically stressful environment (Cushman et al. 1993, Arnett and Gotelli 2003, Blanckenhorn et al. 2007) since they use their reserves more efficiently, probably because of a proportionally lower metabolic rate (Bokma 2004, Glazier 2005, Savage et al. 2007). However, no study, to our knowledge, has explored the link between the amount of energy at emergence, body size and stress resistance.

Insect parasitoids are a good candidate model to assess the impact of size on temperature resistance, and find out which of these two hypotheses applies. These insects have an immature life stage that develops on or within a single insect host (Godfray 1994), ultimately killing the host. Consequently, parasitoid resources are often limited by nutrition, as in most species, the lifetime's total amount of lipids is acquired during this larval instar only (Olson et al. 2000). These insects are used in biological control programs, i.e. methods of controlling and limiting the development of pest populations by natural enemies that include predation, parasitism, disease or competition, in order to reduce or eliminate the use of chemical pesticides in agriculture and forestry (Boivin 2001). To provide successful biological control, high quality emerged individuals are required. It is known that quality varies among individuals within a population, as a result of genetic variation, and this could further affect their adaptive phenotypic evolution (Wilson and Nussey 2010). In parasitoids, body size is generally considered to be an indicator of the quality of an individual (West et al. 1996, Rivero and West 2002). Indeed, large females are expected to live longer, to have higher fecundity, greater mating success, and better dispersal capacity than smaller ones (Ellers et al. 1998, Sagarra et al. 2001, Doyon and Boivin 2005). Similarly, it has been found that smaller males are rarely able to fertilize females in the face of competition from larger males (Lacoume et al. 2006), and that larger males have larger reproductive organs which contain more sperm than smaller ones (Martel et al. 2011). The amount of lipids available is the main factor mediating the relationship between fitness and size (Rivero and West 2002), and it has been found that lipid content is positively correlated with fitness parameters and with body size during adult life (Ellers 1996, Ellers and van Alphen 1997). The amount of lipids available was assumed to constitute a resource for withstanding the starvation and the desiccation stresses in *Drosophila* species (Djawdan et al. 1998), but almost nothing is known for parasitoids. One of their specificities is that the quantity of lipids is limited, because most parasitoid species are unable to synthesize lipids during adult life (Ellers et al. 1998, Olson et al. 2000, Visser et al. 2010); this leads to tradeoffs between various different functions such as longevity and fecundity (Ellers 1996).

In parasitoids, a particular form of energy stress is the period of exposure to cold applied to parasitoid immature instars for storage after mass production to insure the availability of large numbers of individuals for inundative release biological control programs. The immature instars (nymphs inside the dead host) are stored at a low temperature for a few weeks (van Lenteren and Tommasini 2003, Colinet et al. 2006b). It is known that adult size is determined by the size of the nymph, because at this stage the host is dead and no further nutrition can occur (Godfray 1994, Ellers 1996, Olson et al. 2000). An increase in the consumption of lipid reserves as a function of the duration of cold exposure has been demonstrated, and may explain the decline in fitness parameters, such as longevity and fecundity, after cold exposure (Colinet et al. 2006a, Ismail et al. 2010). In parasitoids, many studies have investigated the allocation of fat reserve to survival and reproduction (Ellers 1996, Ellers and van Alphen 1997), but few have explored the link between fat reserve at emergence, body size and stress resistance (Rivero and West 2002). Generally, starvation is the most studied stress in insects to determine the benefit of size (Ballard et al. 2008). Contrary to our previous study (Ismail et al. 2010), which addressed the problem of physiological costs of cold storage, this paper focuses on the impact of size on cold stress resistance, a more fundamental ecological issue. In this paper we aim: 1) to find out whether different life history traits linked to fitness (longevity, fecundity at emergence, total fecundity) are affected by cold storage to a greater extent in large or small individuals of our model species, the parasitic wasp *Aphidius ervi*, in order to find out whether this species is subject to the absolute energy demand hypothesis (AED) or the relative efficiency hypothesis (RE). 2) if fitness traits are affected differently in large and small individuals, the tradeoffs between these traits should also be affected differently depending on size. As previous studies on *A. ervi* have shown that in the context of stress linked to cold storage, energy tends to be invested in fecundity to a greater extent than in maintenance, which means that the first trait to be affected by energy limitation should be longevity (Ismail et al. 2010). Consequently we would expect to see a size-linked difference in the strategy of energy allocation, as either small or large individuals will be constrained by energy to a greater extent. 3) in parasitoids, there is a high degree of sexual size dimorphism, with males usually being smaller than females. Moreover, gamete production is considered to be less costly for males than for females (Ernsting and Isaaks 2002). It has also been shown that females oviposit a higher proportion of daughters in large hosts than in smaller ones, as small hosts contain less energy than larger ones (Charnov et al. 1981, Seidl and King 1993), and because the advantage of large body size is greater for females than for males (Colinet et al. 2006b). Lastly, low quality hosts affect females relatively more than males (Charnov et al. 1981, Sequeira and Mackauer 1992). For all these reasons showing that males seem to be less constrained by energy than females, we can predict that the stress induced by cold storage should be less intense for males than for females. As far as we are aware, no study has so far focused on distinguishing the effects of this type of stress on large and small individuals of both sexes.

Material and methods

We chose the parasitoid *Aphidius ervi* (Hymenoptera: Braconidae) as our model, because it is commercially produced and widely distributed in several European countries for mass release programs. It is a common cosmopolitan solitary endoparasitoid (Marsh 1977) of several aphid

species of economically-important crops such as legumes and cereals (Stilmant et al. 2008). A large batch (more than 300 mummies) of *A. ervi* reared on the grain aphid *Sitobion avenae* (Homoptera: Aphididae) was provided in January 2007 by Viridaxis S.A. in Belgium. Another batch of mummies was supplied in January 2008, to refresh the rearing. This company also regularly refreshes their own rearing stock. In our laboratory, the aphids were reared on an SA1 clone (INRA collection) of the same host species, which was collected from wheat crops around Rennes in France in 1990. The aphids were reared under laboratory conditions in small pots of wheat (*Triticum aestivum* cv. Boston). Parasitoids and hosts were maintained in plexiglas cages ($50 \times 50 \times 50$ cm) in air-conditioned rooms at 20 ± 1°C, 60 ± 10% RH, and with a photoperiod 16L: 8D. *Aphidius ervi* was reared for three generations before the experiments.

Aphid parasitoids stop feeding to form a 'mummy' and pupate when the larva spins its cocoon inside the empty cuticle of the aphid. One-day-old nymphs inside mummies (about nine days from laying the egg) are considered the best instar for the storage of aphid parasitoids (Levie et al. 2005). We used two storage temperatures (7°C and 4°C) respectively, which are higher and lower, respectively, than the mummy-adult development threshold (i.e. the temperature at which an organism is able to develop), which is 6.6°C for *A. ervi* (Sigsgaard 2000). These two temperatures were chosen because parasitoid cold storage is usually performed at temperatures close to the threshold development, and the best temperature (above or below the threshold) seems to vary depending on the species studied (Pandey and Johnson 2005, Colinet et al. 2006a). For each of these low temperatures, we tested storage periods of two durations (one and two weeks, respectively), since higher mortality is expected for longer periods. Consequently we used the following five treatments: control (Ctrl) at 20°C; 7C1: constant cold temperature for one week at 7°C; 7C2: constant cold temperature for two weeks at 7°C; 4C1: constant cold temperature for one week at 4°C; and 4C2: constant cold temperature for two weeks at 4°C.

For both temperatures, mummies were acclimated by exposing them progressively to temperatures from 20°C to 7°C (with 2 h at 17, 14 and 11°C), or to 4°C (with 2 h at 16, 12 and 8°C), as described by Levie et al. (2005). They were then kept in a thermo-regulated incubator with a thermal precision of ±1°C, with a photoperiod of 16L: 8D. After cold storage, mummies were progressively transferred to 20°C, with 2 h at 11, 14, and 17°C for the mummies stored at 7°C, and with 2 h at 8, 12, and 16°C for the mummies stored at 4°C. To avoid a potential incubator effect, the incubators used for each treatment were regularly switched. For all the treatments, we measured the following life history traits: longevity without food of the emerged adults, their egg load at emergence, their lifetime fecundity and their lipid content at emergence.

To correlate body size with fitness parameters, we measured the length of the left hind tibia of each individual using the digital image analysis software Pegasus Pro V4 under a binocular (×3.15) linked to a video camera. Tibia length is the most commonly-used indicator of body size in parasitoid wasps, and this measure is strongly correlated to others, e.g. dry mass (Godfray 1994).

Emergence proportion

The emergence proportion was measured by placing 100 mummies per treatment group individually in a small gelatin capsules (Ø = 0.5 cm, L = 1.5 cm). The adult emergence proportion was expressed as the number of individuals that emerged from these 100 mummies.

Time to emergence

Emergences of adults were checked daily, allowing us to measure the time taken for each adult to emerge after cold exposure: Ctrl: n = 112; 7C1: n = 95; 7C2: n = 97; 4C1: n = 102 and 4C2: n = 107.

Longevity

After emergence, we recorded the adult longevity without food (n = 20 males and 20 females), since it is representative of the amount of energy reserves remaining inside the body after storage in both sexes. It has been shown in several parasitoid species that there is a positive relationship between the amount of lipid reserves accumulated from the host during the development and the longevity without food (see for example Ellers et al. 1998). Very likely, there is also a positive correlation between survival time under starvation and the amount of global energy reserves obtained from the host (Hoffmann et al. 2001, Rivero and West 2002, Chippendale et al. 1996). Individual adults without hosts were put into small tubes (1.5 cm in diameter and 10 cm long), and their lifespan was measured by observing them daily until they died.

Egg load at emergence

Thirty females of about 2 h old per treatment were dissected in a drop of water on a microscope slide under a microscope (×4) to count the number of mature eggs in the ovaries at emergence. Mature eggs could be distinguished from immature eggs because they were fully formed (Le Ralec 1991).

Lifetime fecundity

One-day old males and females were mated randomly with one individual issued of the same treatment. If males were affected by the treatment, it could affect the sex ratio, but not the lifetime fecundity as this species, as most of Hymenoptera species exhibit arrhenotokous parthenogenesis, meaning that unmated females still produce progeny, but only male progeny. Moreover, as no evidence of nuptial gift during mating has been recorded for this species, the effect of the male quality on lifetime fecundity is very improbable. Mated females were transferred to a small pot containing a wheat plant with about 100 aphids of 3rd and 4th larval instars (control: n = 18; 7C1: n = 13; 7C2: n = 13; 7F1: n = 15; 7F2: n = 13; 4C1: n = 13; 4C2: n = 13; 4F1: n = 15; 4F2: n = 13 individuals). These pots were renewed every day for five days, and then once every three days until the female died. The females were provided with honey ad libitum. This experiment was carried out at 20 ± 1°C with a 16L: 8D photoperiod. Lifetime fecundity was evaluated by counting the number of mummies obtained.

Lipid content

To estimate the lipid content at emergence (n = 20 males and 16 females per treatment), we first measured the dry mass by exposing the adults to 60°C in an air oven for three days. To evaluate the lipid mass, each dried adult was placed in an Eppendorf tube containing 1 ml of a chloroform/methanol (2:1) extraction solution for two weeks (Vernon and Vannier 1996, Terblanche et al. 2004). The adults were then dried in an air oven at 60°C for 12 h to remove the extraction solution so that the lean dry mass could be measured. The body lipid mass was obtained by subtracting the lean dry mass from the dry mass. Adults were weighed using a micro-electronic balance (sensitivity 0.1 μg). To correlate the lipid content to body size, we used the lean dry mass as an indicator of the body mass. Results were available only for control individuals and for individuals that emerged after exposure to 7°C for one or two weeks (the individuals sampled at 4°C were accidentally destroyed before being measured).

Statistical analyses

Emergence proportions (i.e. proportions of adults that emerged successfully) were compared between treatments using χ^2-tests. The time lag before emergence (log-transformed), was compared using analysis of variance (ANOVA), and was followed by Bonferroni's post hoc multiple comparison tests when the treatments were significantly different ($a = 0.05/k$, where k is the number of comparisons).

The relationships between fitness traits and size were analyzed by a nested variance-covariance linear model (analysis of covariance, ANCOVA). Two nested factors were taken into account: the storage temperature, and the duration of storage. As the control level has a single modality for the duration of storage (none), we treated the design as being nested, the duration of storage or the absence of storage being nested inside the storage duration (nesting factor with three levels). As our purpose was to compare the regression slopes under each of the five sublevels (Ctrl, 7C1, 7C2, 4C1, 4C2), we declared the linear model as being fully interactive. This meant that each sublevel has its own slope and that the slopes can be compared between sublevels. The responses of longevity, egg load at emergence, lifetime fecundity to size were studied by this way, as well as the responses of male and female lipid content to the body mass. Nesting, however, had the advantage of making it possible to detect any additive effect of the temperature of storage. The effect of size thus appears to be a doubly-nested covariate: size is nested within storage time, which in turn is nested within storage temperature. The statistical model depends on the following equation: $y_{ij}k = \mu + \alpha_i + \beta_{ij} + \delta_{ij}.\ S_{ijk} + \varepsilon_{ijk}$, where μ is the intercept, α_i the effect of the storage temperature, β_{ij} the effect of the storage duration nested within the effect of storage temperature. δ_{ij} is the slope of the size in each sublevel, y_{ijk} is the response, S_{ijk} the tibia length, and ε_{ijk} the error term.

When the effect of size, as defined above, was found to be significant at the threshold $\alpha = 0.05$, we compared the slopes two-by-two by a pairwise *t*-test (Draper and Smith 1998) subject to the Bonferroni correction. As the procedures provided by *R* do not easily include slope comparisons, we wrote our own *R* function (*compslope*). The analysis was done separately for females and males without any attempt to compare the sexes, since females were significantly larger than males (Godfray 1994) and the interaction would unnecessarily complicate the analysis. All statistical analyses were done using R ver. 2.8.0 (R Development Core Team).

Results

Emergence proportion

The emergence proportion of *Aphidius ervi* did not decline with storage duration, and was similar to the control ($\chi^2 = 0.50$; DF = 4; $p = 0.974$). All treatments showed a high emergence proportion (T = 96.55 ± 1.82, 7C1 = 95.96 ± 1.97, 7C2 = 95.10 ± 2.16, 4C1 = 91.07 ± 2.85, and 4C2 = 86.29 ± 3.44%).

Time to emergence

The time taken (in days) for adults to emerge after cold exposure (mean ± SD: Ctrl = 6.00 ± 0.71; 7C1 = 4.50 ± 0.65; 7C2 = 2.50 ± 0.65; 4C1 = 4.50 ± 0.65 and 4C2 = 3.50 ± 0.65), was significantly affected by the storage temperature at 7°C ($F_{1,16} = 4.11$; $p = 0.03$), and significantly affected by the storage duration after two weeks ($F_{1,16} = 6.06$; $p = 0.02$). No interaction was found between the storage temperature and the storage duration ($F_{1,16} = 1.08$; $p = 0.31$). This proved that the development continues slowly during the fluctuating treatments and above 7°C, as already discussed in Ismail et al. (2010).

Longevity

The storage temperature significantly reduced the longevity of both females ($F_{2,90} = 4.06$, $p = 0.02$) and males ($F_{2,90} = 5.12$, $p = 0.007$). Storage duration also significantly reduced the longevity of females ($F_{2,90} = 5.68$, $p = 0.004$), but not of males ($F_{2,90} = 1.68$, $p = 0.19$). The effect of the size (tibia length), in interaction with the storage duration (nested within storage temperature), was highly significant for both females ($F_{5,90} = 6.19$, $p < 0.001$) and males ($F_{5,90} = 9.85$, $p < 0.001$).

The relationship between starved female longevity and size is shown in Fig. 1 (a, b, for 7° and 4°C respectively). Longevity increased significantly with body size in the control ($F_{1,18} = 21.37$, $r^2 = 0.54$, $p < 0.001$) (it lies in a mean range of 2.4 to 3.6 days for the smallest to the largest individuals), and the 7C1 ($F_{1,18} = 6.04$, $r^2 = 0.25$, $p = 0.02$) (The range of survival is from 2.4 to 3.4 days for the smallest to the largest individuals) treatment groups, whereas no significant relation was found for the 7C2 ($F_{1,18} = 2.50$, $r^2 = 0.12$, $p = 0.13$), 4C1 ($F_{1,18} = 1.76$, $r^2 = 0.08$, $p = 0.20$) or 4C2 ($F_{1,18} = 0.09$, $r^2 = 0.005$, $p = 0.76$) treatment groups, where the longevity lies in the mean range of one to three days for all individuals of these three treatments. The pairwise slope comparison tests indicated that there were significant differences between the control and 7C2

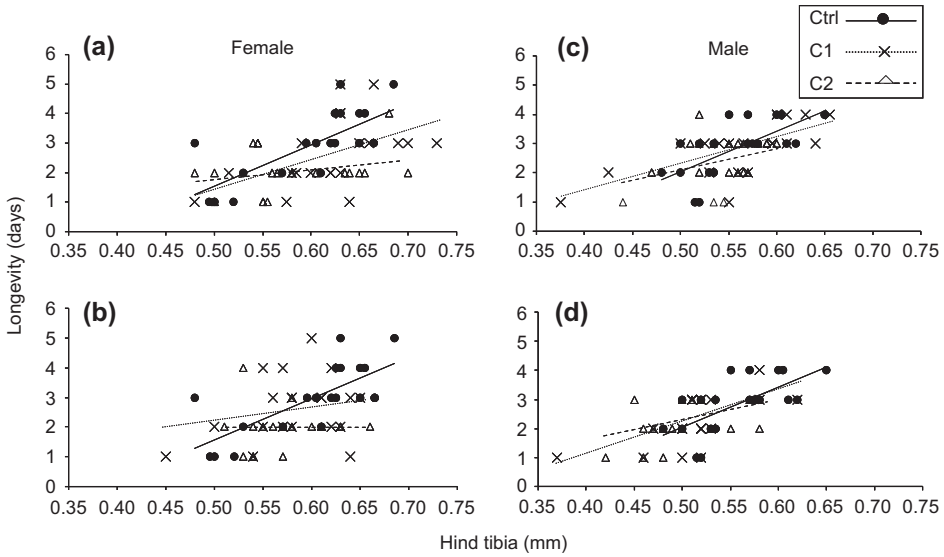

Figure 1. Regression of longevity on body size measured as tibia length for both sexes: females (Ctrl: $y = 14.02\ size - 5.46$) and males (Ctrl: $y = 13.67\ size - 4.79$). (a) females stored at 7°C (C1: $y = 10.03\ size - 3.58$, 7C2: $y = 4.28\ size - 0.47$), (b) females stored at 4°C (C1: $y = 6.51\ size - 1.15$, C2: $y = 1.21\ size + 1.25$). The p values (Bonferroni, $p = 0.008$) of the pairwise slope comparison tests between the different treatments on females are: Ctrl~7C1: $t = 0.75$, $p = 0.23$; Ctrl~7C2: $t = 2.08$, $p < 0.007$*; Ctrl~4C1: $t = 1.38$, $p = 0.08$; Ctrl~4C2: $t = 2.09$, $p < 0.007$*; 7C1~7C2: $t = 1.21$, $p = 0.11$; 4C1~4C2: $t = 1.11$, $p = 0.13$. (c) males stored at 7°C (C1: $y = 9.39\ size - 2.42$, C2: $y = 7.10\ size - 1.43$). (d) males stored at 4°C (C1: $y = 11.15\ size - 3.438$, C2: $y = 7.75\ size - 1.69$). The p-values (Bonferroni, $p = 0.01$) of the pairwise slope comparison tests between the different treatments on males are: Ctrl~7C1: $t = 0.84$, $p = 0.20$; Ctrl~7C2: $t = 1.16$, $p = 0.12$; Ctrl~4C1: $t = 0.49$, $p = 0.31$; Ctrl~4C2: $t = 1.12$, $p = 0.13$; 7C1~7C2: $t = 0.72$, $p = 0.24$; 4C1~4C2: $t = 0.58$, $p = 0.29$.

and between the control and 4C2 groups (Fig. 1). We also observed that the longevity of smaller females in the 7C2 and 4C2 treatment groups was equivalent to that of small control females, whereas that of larger females in these treatment groups was shorter than in the control group.

The relationship between starved male longevity and size is shown in Fig. 1c–d. Longevity increased significantly with body size in the control ($F_{1,18} = 13.10$, $r^2 = 0.42$, $p = 0.001$), 7C1 ($F_{1,18} = 16.83$, $r^2 = 0.48$, $p < 0.001$), 4C1 ($F_{1,18} = 12.55$, $r^2 = 0.41$, $p = 0.002$) and 4C2 ($F_{1,18} = 5.66$, $r^2 = 0.24$, $p = 0.02$) treatment groups (The longevity lies in a mean range of 1.5 days for the smallest individuals to 3.5 days for the largest ones), whereas no relationship was found in the 7C2 treatment group ($F_{1,18} = 2.39$, $r^2 = 0.12$, $p = 0.14$), where the average survival is 2.45 days for all individuals. The pairwise slope comparison tests indicated that there were no significant differences between any of the treatments (Fig. 1).

Egg load at emergence

No significant effect of storage temperature ($F_{2,140} = 1.32$, $p = 0.27$) or duration ($F_{2,140} = 0.17$, $p = 0.85$) was observed on female egg load. However, the effect of size, in interaction with the storage duration (nested within storage temperature), was highly significant ($F_{5,140} = 9.27$, $p < 0.001$). Egg load at emergence increased significantly with body size in the control ($F_{1,28} = 21.35$, $r^2 = 0.433$, $p < 0.001$), 7C1 ($F_{1,28} = 18.88$, $r^2 = 0.40$, $p < 0.001$) and 4C1 ($F_{1,28} = 10.45$, $r^2 = 0.27$, $p = 0.003$) treatment groups, with a mean egg load in the range of 20 for the smallest individuals to 100 for the largest ones, whereas no relationship was found in the 7C2 ($F_{1,28} = 0.08$, $r^2 = 0.002$, $p = 0.78$) or 4C2 ($F_{1,28} = 0.09$, $r^2 = 0.003$, $p = 0.76$) treatment groups, where the mean egg load is 71 for all individuals (Fig. 2a–b). The pairwise slope comparison tests indicated significant differences between all the treatments, except between the control and 7C1 and between the control and 4C1 groups. We observed especially that the egg loads of the small females in the 7C2 (mean 82) and 4C2 (mean 78) treatment groups were higher than those of the small control females (mean 54), whereas the egg loads of the large females in these same treatment groups were 76 and 77 respectively for 4C2 and 7C2 against a mean of 92 for the large control females.

Lifetime fecundity

The storage temperature significantly reduced female lifetime fecundity ($F_{2,60} = 3.90$, $p = 0.02$), whereas storage duration did not ($F_{2,60} = 0.22$, $p = 0.85$). The effect of the size, in interaction with the storage duration (nested within storage temperature), was highly significant ($F_{5,60} = 7.11$, $p < 0.001$). Lifetime fecundity increased significantly with body size in the control ($F_{1,16} = 15.15$, $r^2 = 0.49$, $p = 0.001$), 7C1 ($F_{1,11} = 11.82$, $r^2 = 0.52$, $p = 0.005$) and 4C1 ($F_{1,11} = 6.59$, $r^2 = 0.38$, $p = 0.03$) treatment groups, with a mean lifetime fecundity in a mean range of 40 for the smallest individuals to 120 for the largest ones, whereas no relationship was found between fecundity and size in the 7C2 ($F_{1,11} = 4.42$, $r^2 = 0.29$, $p = 0.06$) and 4C2 ($F_{1,11} = 2.16$, $r^2 = 0.16$, $p = 0.17$) treatment group, where the mean lifetime fecundity is 60.4 and 58.0 eggs for individuals of

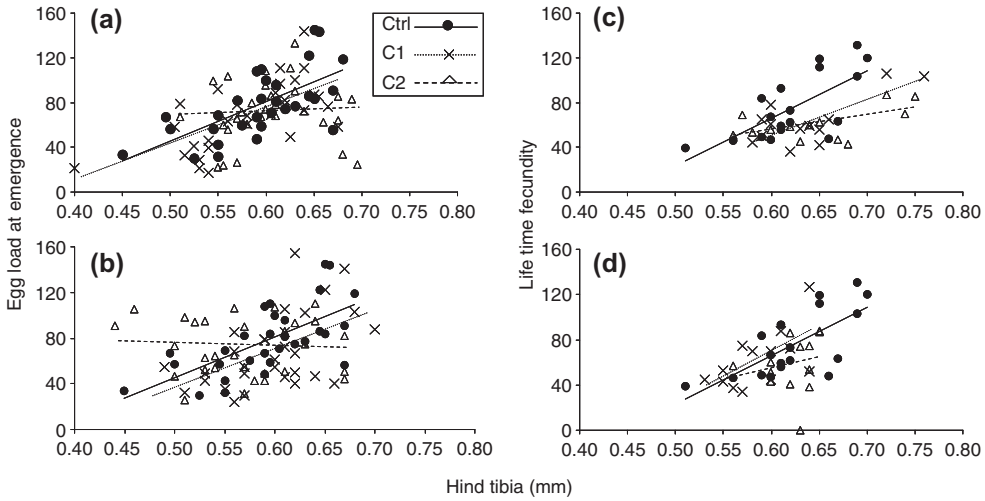

Figure 2. Regression of egg load at emergence on body size measured as tibia length (Ctrl: y = 354.03 size − 131.65) and lifetime fecundity (Ctrl: y = 426.65 size − 190.01). (a) egg load of individuals stored at 7°C (C1: y = 306.08 size − 109.74, C2: y = 31.14 size + 52.51). (b) egg load of individuals stored at 4°C (C1: y = 342.79 size − 136.17, C2: y = −24.11 size + 84.99). The p-values (Bonferroni, p = 0.008) of the pairwise slope comparison tests between the different treatments are: Ctrl~7C1: t = 0.75, p = 0.23; Ctrl~7C2: t = 2.08, p < 0.007*; Ctrl~4C1: t = 1.38, p = 0.08; Ctrl~4C2: t = 2.09, p < 0.007*; 7C1~7C2: t = 1.21, p = 0.11; 4C1~4C2: t = 1.11, p = 0.13. (c) lifetime fecundity of individuals stored at 7°C (C1: y = 290.71 size − 122.15, C2: y = 119.61 size − 16.19). (d) lifetime fecundity of individuals stored at 4°C (C1: y = 443.34 size − 197.48, C2: y = 278.75 size − 110.21). The p-values (Bonferroni, p = 0.008) of the pairwise slope comparison tests between the different treatments are: Ctrl~7C1: t = 1.22, p = 0.11; Ctrl~7C2: t = 2.24, p = 0.006*; Ctrl~4C1: t = 0.50, p = 0.31; Ctrl~4C2: t = 0.38; p = 0.35, 7C1~7C2: t = 0.92, p = 0.18; 4C1~4C2: t = 0.75, p = 0.23.

the treatments 7C2 and 4C2 respectively (Fig. 2c–d). The pairwise slope comparison tests indicated a significant difference between the control and the 7C2 treatment groups (Fig. 2). We observed that the lifetime fecundity of the small 7C2 and 4C2 females was equivalent to that of the small control females (51 eggs), whereas lifetime fecundity of the large females in these same treatment groups was 65 ad 64 eggs, i.e. 50% lower than that of the large control females (118 eggs).

Lipid content

Storage temperature had no significant effect on lipid content in females ($F_{1,42} = 2.88$, $p = 0.09$), but did have a significant negative effect in males ($F_{1,54} = 10.75$, $p < 0.001$). Storage duration had no significant effect on lipid content in both females ($F_{1,42} = 1.84$, $p = 0.18$) and males ($F_{1,54} = 0.24$, $p = 0.62$). The effect of the size, in interaction with the storage duration (nested within storage temperature), was highly significant for females ($F_{3,42} = 14.03$, $p < 0.001$) as well as for males ($F_{3,54} = 19.32$, $p < 0.001$). The lipid content for both sexes was positively correlated to the body mass in the controls (Female: $F_{1,14} = 15.12$, $r^2 = 0.52$, $p < 0.001$; Male: $F_{1,14} = 26.33$, $r^2 = 0.59$, $p < 0.001$), and in the 7C1 treatment groups (Female: $F_{1,14} = 22.47$, $r^2 = 0.61$, $p < 0.001$; Male: $F_{1,18} = 17.40$, $r^2 = 0.49$, $p < 0.001$), from 0.010 to 0.060 mg for the smallest individuals to the largest ones. In contrast, we found no relationship in the 7C2 treatment groups for either sex (Female: $F_{1,14} = 0.71$, $r^2 = 0.05$, $p = 0.42$; Male: $F_{1,18} = 2.76$, $r^2 = 0.13$, $p = 0.11$), with values about 0.015 mg for all individuals (Fig. 3). The pairwise slope comparison tests indicated significant differences between treatments in both control and 7C2 females and males (Fig. 3). Our results showed that small females and males in the 7C2 treatment group had the same lipid content as small individuals in the control group (0.034 mg for males, 0.024 mg for females), whereas we observed that large females (0.013 mg) and males (0.018 mg) in this group had lower lipid content than controls (decrease of roughly 50%) (Fig. 3).

Discussion

Our work emphasizes that the fitness traits we measured (longevity without food, egg load at emergence and lifetime fecundity) were positively correlated with body size in control treatments, which means that fitness is higher in larger individuals. In contrast, after cold storage at either temperature for two weeks, this positive correlation between size and the different life history traits studied was no longer found for most of the traits studied, indicating that larger individuals are affected to a greater extent than smaller ones by cold storage. In other words, we found that larger females survived for a shorter period without food, had fewer eggs in their ovaries at emergence, and oviposited less eggs into hosts during their lives in the 7C2 and 4C2 treatment groups than in the control group. Larger males survived for a shorter period in the 7C2 treatment group only. For a shorter duration of stress (treatments 7C1 and 4C1), we observed intermediate results, showing that the energy constraint increased with the duration of the stress. This lack of correlation was not an accidental phenomenon, as it was detected at both storage temperatures tested.

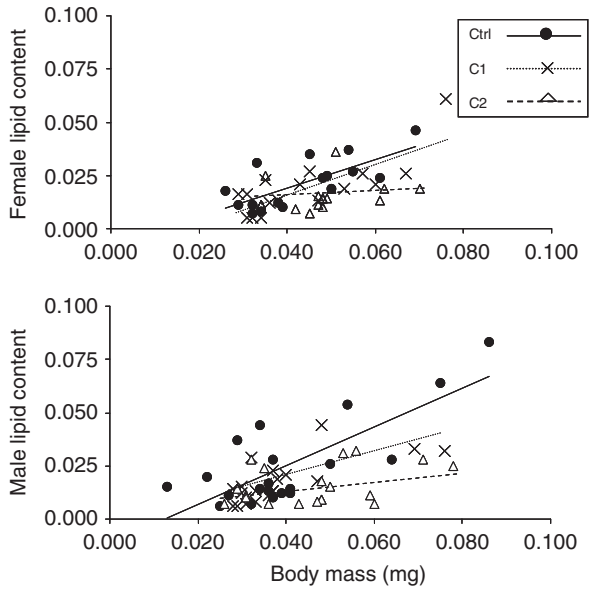

Figure 3. Regression of lipid content with body mass for both sexes. Female: Ctrl: $y = 0.672$ body mass $- 0.008$, 7C1: $y = 0.735$ body mass $- 0.014$, 7C2: $y = 0.158$ body mass $+ 0.008$. The p-values (Bonferroni, $p = 0.02$) of the pairwise slope comparison tests between the different treatments are: Ctrl~7C1: $t = 0.29$, $p = 0.39$; Ctrl~7C2: $t = 1.86$, $p = 0.01*$, 7C1~7C2: $t = 2.19$, $p = 0.01*$. Male: Ctrl: $y = 0.907$ body mass $- 0.011$, 7C1: $y = 0.556$ body mass $- 0.004$, and 7C2: $y = 0.285$ body mass $+ 0.004$. The p-values (Bonferroni, $p = 0.02$) of the pairwise slope comparison tests between the different treatments (Ctrl~7C1: $t = 2.12$, $p = 0.02$; Ctrl~7C2: $t = 3.55$, $p < 0.001**$; 7C1~7C2: $t = 1.16$, $p = 0.12$).

These results support the AED hypothesis, which predicts that under constrained conditions, large individuals need to use more energy to sustain their body functions and so they suffer more (Blanckenhorn et al. 1995, Reim et al. 2006). The hypothesis is that larger individuals have larger organs, which are energetically costly to produce and maintain (Suarez 1998). Consequently, small adults could be better adapted to coping with cold stress than larger ones because they need less energy to sustain themselves, and consequently they may live longer (starvation resistance) in particular for males and have better fecundity for females. In other words, the AED hypothesis from an adaptive point of view, can be a factor selecting for small body size in nature (Blanckenhorn et al. 1995). These results are also in agreement with those of another study in *Aphidius ervi*, indicating that after an extensive period of stress (four weeks of storage at 0°C), only the smallest individuals survived and performed as well as the control individuals (unpubl.). Contrary to other studies that show a tradeoff between resistance to cold and starvation (i.e. individuals that stand cold stress, do not stand starvation stress and vice versa, Hoffmann et al. 2005), our results showed that small individuals stand and can adapt better to both unfavorable conditions than the larger ones. This could be important in natural conditions, since insects generally suffer cold sress and food limitation at the same time (Hoffmann et al. 2005).

So, why were larger individuals more affected than smaller ones? We analyzed the lipid content of newly-emerged adults to find out whether the amount of lipids used to resist the cold storage stress was proportionally the same in large and small individuals. Our results showed that in the 7C2 treatment group, larger adults had significantly lower lipid content than smaller ones when compared to control individuals at emergence. This implies that during cold exposure, larger individuals consumed their lipids proportionally more rapidly than the smaller ones. Larger individuals did not have enough lipids after emergence to sustain their fitness functions, whereas the smaller ones did. This study indicates that higher lipid levels in emerged small individuals were the main reason for stress resistance. This higher consumption rate may be related to the higher amount of energy needed by the larger individuals to resist exposure to cold, which is responsible for the decrease in fitness traits of the larger adults. This energy could be related to a higher rate of biological repair during stress in larger individuals. Evaluating the metabolic rate of immature instars could help to explain why larger individuals consumed more energy during exposure to cold than smaller ones. However, this is difficult to evaluate since the immature instars are located inside the mummies of aphids. Furthermore, the metabolism of emerged individuals after exposure to cold did not differ from that of the controls (unpubl.). After subjecting adult parasitoids to starvation, Rivero and West (2002) found that starved large individuals consumed their lipid reserves more rapidly than starved smaller ones, although in their study survival was lower in the smaller ones, because they had used up all their lipid reserves, which were smaller. Our results therefore confirm the importance of lipid availability in parasitoids. Indeed, it has previously been demonstrated that lipid availability can be considered to be a principal resource required to cope with a variety of stresses (Cushman et al. 1993, Djawdan et al. 1998, Ballard et al. 2008). This could be due to the absence of lipogenesis in adult parasitoids and could probably not be generalized to all ectotherms (Visser and Ellers 2008). Indeed, some studies showed that there is no correlation between lipid availability and starvation resistance in *Drosophila* species (Hoffmann et al. 2001, Baldal et al. 2005).

As parasitoid adults are unable to synthesize lipids (Visser et al. 2010), limited lipid reserves allocation to the various fitness traits result in tradeoff (Arrese and Soulages 2010). For example, females of *Asobara tabida*, which have invested a larger amount of energy in their egg load, have a shorter lifespan than others (Ellers and van Alphen 1997). In our study, we observed that all fitness traits (longevity, egg load at emergence and lifetime fecundity) were reduced in larger females, but it was not possible to identify which trait was proportionally the most affected. On the other hand, we observed that smaller females in the 7C2 and 4C2 treatment groups had a larger egg load at emergence than the control females, whereas they had approximately the same lifetime fecundity. We found here that these small females invested more energy in producing and maturing eggs to be used soon after emergence. We had previously shown that cold exposure induced a reduced longevity (Ismail et al. 2010), and so it is probable that a greater investment in fecundity at the beginning of life is responsible for this potentially reduced longevity. In other words, in stressful environments, females invest their energy in early reproduction, and so switch

their reproductive strategy from a synovigenic strategy (in which females mature new eggs after emergence) to a more pro-ovigenic one (in which females emerge with all their eggs mature) (Jervis et al. 2003, Thorne et al. 2006). Denis et al. (2011) have developed a model showing that a stress inducing energy consumption (in that case, an increase of temperature during adult life) should generate an increase of the ovigeny index, meaning a greater investment in early fecundity. Ellers and Jervis (2003) showed that larger females tend to have a lower ovigeny index than the smaller ones, which increases their lifetime reproductive success. Thorne et al. (2006) showed in the solitary parasitoid wasp *Aphaereta genevensis*, that small body size can favor a developmental shift in juveniles that favors early reproduction, but which has adverse late-life consequences. As in our study, the developmental conditions are at the origin of this shift towards early investment in reproduction.

In parasitoid species, females are generally larger than males. It is well known that mothers choose larger hosts in which to oviposit the eggs that will produce their daughters, and smaller ones in which to oviposit eggs that will produce their sons (Charnov et al. 1981, Seidl and King 1993). Consequently, the larger size of females is generally due to larger energy reserves, and males require less energy to maintain their activities. In *Aphidius ervi*, it is probable that producing eggs for females is more expensive in terms of energy than producing sperm for males (Ernsting and Isaaks 2002). This could explain why the longevity of larger females was reduced more than that of larger males.

To summarize, no significant mortality was found after two weeks of cold storage, but the emerging larger individuals were significantly more affected and displayed lower fitness than the smaller ones by exposure to both the temperatures tested. In contrast, we found that smaller females changed their reproductive behaviour by reproducing earlier in their lives than controls. We found that a decrease in lipid content in the emerged larger adults is probably the most important factor responsible for the reduction in fitness traits. This decrease in lipids might be related e.g. to a higher metabolic rate in the larvae (Blanckenhorn 2000) or to differences in the rate of growth between individuals (Gotthard et al. 1994).

As ample benefits are associated with a large body size in most of organisms, and as associated costs are seldom observed, the evolutionary question arises as to why these organisms do not evolve a larger body size. Among the costs of being large suggested by Blanckenhorn (2000), our study has highlighted the importance of thermal stress resistance. These findings could also have consequences for biological control programs. Indeed, to be an effective biological control agent, after cold storage the parasitoids have to meet several requirements regarding their quantity, quality, and efficiency for inundative releases (van Lenteren 2003). The fact that cold storage is less favourable for large individuals than for small ones reveals the need for further studies in this field of research to elucidate this phenomenon and find better methods of insect storage.

Acknowledgements – This study was supported by a scholarship from the Syrian Ministry of Higher Education, and funded by the UMR 6553 CNRS Ecobio (Univ. of Rennes 1, France). We thank Viridaxis S.A. (Gilly, Belgium) for providing the parasitoids *Aphidius ervi*. We are grateful to Damien Denis and Thiago Andrade for their helpful comments on an earlier version of the manuscript. We thank Valérie Briand for technical help.

References

Andersson, M. 1994. Sexual selection. – Princeton Univ. Press.

Angilletta, M. J. and Dunham, A. E. 2003. The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. – Am. Nat. 162: 332–342.

Angilletta, M. J. et al. 2004. Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. – Integr. Comp. Biol. 44: 498–509.

Arnett, A. E. and Gotelli, N. J. 2003. Bergmann's rule in larval ant lions: testing the starvation resistance hypothesis. – Ecol. Entomol. 28: 645–650.

Arrese, E. L. and Soulages, J. L. 2010. Insect fat body: energy, metabolism and regulation. – Annu. Rev. Entomol. 55: 207–225.

Atkinson, D. 1994. Temperature and organism size – a biological law for ectotherms. – Adv. Ecol. Res. 25: 1–58.

Baldal, E. A. et al. 2005. The effects of larval density on adult life-history traits in three species of *Drosophila*. – Mech. Age. Dev. 126: 407–416.

Bale, J. S. 2002. Insects and low temperatures: from molecular biology to distributions and abundance. – Phil. Trans. R. Soc. B 357: 849–861.

Ballard, J. et al. 2008. Starvation resistance is positively correlated with body lipid proportion in five wild caught *Drosophila simulans* populations. – J. Insect Physiol. 54: 1371–1376.

Blanckenhorn, W. U. 2000. The evolution of body size: what keeps organisms small? – Q. Rev. Biol. 75: 385–407.

Blanckenhorn, W. U. and Henseler, C. 2005. Temperature-dependent ovariole and testis maturation in the yellow dung fly. – Entomol. Exp. Appl. 116: 159–165.

Blanckenhorn, W. U. et al. 1995. Time and energy constraints and the evolution of sexual size dimorphism – to eat or to mate. – Evol. Ecol. 9: 369–381.

Blanckenhorn, W. U. et al. 2007. Size-dependent energy reserves, energy utilization and longevity in the yellow dung fly. – Physiol. Entomol. 32: 372–381.

Boivin, G. 2001. Parasitoïdes et lutte biologique: paradigme ou panacée? – VertigO doi: 10.4000/vertigo.4096.

Bokma, F. 2004. Evidence against universal metabolic allometry. – Funct. Ecol. 18: 184–187.

Calder, W. A. 1984. Size, function and life history. – Harvard Univ. Press.

Charnov, E. L. et al. 1981. Sex ratio evolution in a variable environment. – Nature 289: 27–33.

Chippendale, A. K. et al. 1996. Complex tradeoffs and the evolution of starvation resistance in *Drosophila melanogaster*. – Evolution 50: 753–766.

Chown, S. L. and Gaston, K. J. 2010. Body size variation in insects: a macroecological perspective. – Biol. Rev. 85: 139–169.

Colinet, H. et al. 2006a. Water relations, fat reserves, survival, and longevity of a cold-exposed parasitic wasp *Aphidius colemani* (Hymenoptera: Aphidiinae). – Environ. Entomol. 35: 228–236.

Colinet, H. et al. 2006b. The impact of fluctuating thermal regimes on the survival of a cold exposed parasitic wasp, *Aphidius colemani*. – Physiol. Entomol. 31: 234–240.

Cushman, J. et al. 1993. Latitudinal patterns in european ant assemblages – variation in species richness and body-size. – Oecologia 95: 30–37.

Denis, D. et al. 2011. How temperature and habitat quality affect parasitoid lifetime reproductive success – a simulation study. – Ecol. Modell. 222: 1604–1613.

Djawdan, M. et al. 1998. Metabolic reserves and evolved stress resistance in *Drosophila melanogaster*. – Physiol. Zool. 71: 584–594.
Doyon, J. and Boivin, G. 2005. The effect of development time on the fitness of female *Trichogramma evanescens*. – J. Insect Sci. 5: 4.
Draper, N. and Smith, H. 1998. Applied regression analysis. – Wiley.
Ellers, J. 1996. Fat and eggs: An alternative method to measure the tradeoff between survival and reproduction in insect parasitoids. – Neth. J. Zool. 46: 227–235.
Ellers, J. and van Alphen, J. J. M. 1997. Life history evolution in *Asobara tabida*: plasticity in allocation of fat reserves to survival and reproduction. – J. Evol. Biol. 10: 771–785.
Ellers, J. and Jervis, M. 2003. Body size and the timing of egg production in parasitoid wasps. – Oikos 102: 164–172.
Ellers, J. et al. 1998. A field study of size-fitness relationships in the parasitoid *Asobara tabida*. – J. Anim. Ecol. 67: 318–324.
Ernsting, G. and Isaaks, J. A. 2002. Gamete production and sexual size dimorphism in an insect (*Orchesella cincta*) with indeterminate growth. – Ecol. Entomol. 27: 145–151.
Forrest, T. G. 1987. Insect size tactics and developmental strategies. – Oecologia 73: 178–184.
Gillooly, J. F. et al. 2001. Effects of size and temperature on metabolic rate. – Science 293: 2248–2251.
Glazier, D. S. 2005. Beyond the '3/4-power law': variation in the intra- and interspecific scaling of metabolic rate in animals. – Biol. Rev. 80: 611–662.
Godfray, H. C. J. 1994. Parasitoids: behavioral and evolutionary ecology. – Princeton Univ. Press.
Gotthard, K. et al. 1994. Adaptive variation in growth rate life history costs and consequences in the speckled wood butterfly, *Pararge aegeria*. – Oecologia 99: 281–289.
Hoffmann, A. A. and Parsons, P. A. 1997. Extreme environmental change and evolution. – Cambridge Univ. Press.
Hoffmann, A. A. et al. 2001. Levels of variation in stress resistance in *Drosophila* among strains, local populations, and geographic regions: patterns for desiccation, starvation, cold resistance and associated traits. – Evolution 55: 1621–1630.
Hoffmann, A. A. et al. 2003. Low potential for climatic stress adaptation in a rainforest *Drosophila* species. – Science 301: 100–102.
Hoffmann, A. A. et al. 2005. Evidence for a robust sex-specific tradeoff between cold resistance and starvation resistance in *Drosophila melanogaster*. – J. Evol. Biol. 18: 804–810.
Ismail, M. et al. 2010. Physiological costs of cold exposure on the parasitoid *Aphidius ervi*, without selection pressure and under constant or fluctuating temperatures. – Biocontrol 55: 729–740.
Jervis, M. A. et al. 2003. Body size and the timing of egg production in parasitoid wasps: a comparative analysis. – Funct. Ecol. 17: 375–383.
Lacoume, S. et al. 2006. Effect of host size on male fitness in the parasitoid wasp *Dinarmus basalis*. – J. Insect Physiol. 52: 249–254.
Le Ralec, A. 1991. Les hyménoptères parasitoïdes: adaptations de l'appareil reproducteur femelle. Morphologie et ultrastructure de l'ovaire, de l'œuf et de l'ovipositeur. – PhD thesis, l'Université de Rennes 1.
Levie, A. et al. 2005. Consequences of acclimation on survival and reproductive capacities of cold-stored mummies of *Aphidius rhopalosiphi* (Hymenoptera: Aphidiinae). – J. Econ. Entomol. 98: 704–708.
Marsh, P. M. 1977. Notes on taxonomy and nomenclature of *Aphidius* species (Hymenoptera Aphidiidae) parasitic on pea aphid in North America. – Entomophaga 22: 365–372.
Martel, V. et al. 2011. Phenotypic plasticity in the reproductive traits of a parasitoid. – J. Insect Physiol. 57: 682–687.
Olson, D. M. et al. 2000. Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp. – Physiol. Entomol. 25: 17–26.
Pandey, R. R. and Johnson, M. W. 2005. Effects of cool storage on *Anagyrus ananatis* Gahan (Hymenoptera: Encyrtidae). – Biol. Control 35: 9–16.
Reim, C. et al. 2006. Size-dependent effects of larval and adult food availability on reproductive energy allocation in the yellow dung fly. – Funct. Ecol. 20: 1012–1021.
Rivero, A. and West, S. A. 2002. The physiological costs of being small in a parasitic wasp. – Evol. Ecol. Res. 4: 407–420.
Sagarra, L. A. et al. 2001. Body size as an indicator of parasitoid quality in male and female *Anagyrus kamali* (Hymenoptera: Encyrtidae). – Bull. Entomol. Res. 91: 363–367.
Savage, V. M. et al. 2007. Scaling of number, size, and metabolic rate of cells with body size in mammals. – Proc. Natl Acad. Sci. USA 104: 4718–4723.
Seidl, S. E. and King, B. 1993. Sex-ratio manipulation by the parasitoid wasp *Muscidifurax raptor* in response to host size. – Evolution 47: 1876–1882.
Sequeira, R. and Mackauer, M. 1992. Covariance of adult size and development time in the parasitoid wasp *Aphidius ervi* in relation to the size of its host, *Acyrthosiphon pisum*. – Evol. Ecol. 6: 34–44.
Sigsgaard, L. 2000. The temperature-dependent duration of development and parasitism of three cereal aphid parasitoids, *Aphidius ervi*, *A. rhopalosiphi* and *Praon volucre*. – Entomol. Exp. Appl. 95: 173–184.
Stearns, S. C. 1992. The evolution of life histories. – Oxford Univ. Press.
Stilmant, D. et al. 2008. Host specialization in habitat specialists and generalists. – Oecologia 156: 905–912.
Suarez, R. K. 1998. Oxygen and the upper limits to animal design and performance. – J. Exp. Biol. 201: 1065–1072.
Terblanche, J. S. et al. 2004. Metabolic rate variation in *Glossina pallidipes* (Diptera: Glossinidae): gender, ageing and repeatability. – J. Insect Physiol. 50: 419–428.
Thorne, A. D. et al. 2006. Small body size in an insect shifts development, prior to adult eclosion, towards early reproduction. – Proc. R. Soc. B 273: 1099–1103.
van Lenteren, J. C. 2003. Need for quality control of mass-produced biological control agents. – In: van Lenteren, J. C. (ed.), Quality control and production of biological control agents: theory and testing procedures. CABI, pp. 1–18.
van Lenteren, J. C. and Tommasini, M. G. 2003. Mass production, storage, shipment and release of natural enemies. – In: van Lenteren, J. C. (ed.), Quality control and production of biological control agents: theory and testing procedures. CABI, pp. 181–189.
Vernon, P. and Vannier, G. 1996. Developmental patterns of supercooling capacity in a subantarctic wingless fly. – Experientia 52: 155–158.
Visser, B. and Ellers, J. 2008. Lack of lipogenesis in parasitoids: a review of physiological mechanisms and evolutionary implications. – J. Insect Physiol. 54: 1315–1322.
Visser, B. et al. 2010. Loss of lipid synthesis as an evolutionary consequence of a parasitic lifestyle. – Proc. Natl Acad. Sci. USA 107: 8677–8682.
West, S. A. et al. 1996. The relationship between parasitoid size and fitness in the field, a study of *Achrysocharoides zwoelferi* (Hymenoptera: Eulophidae). – J. Anim. Ecol. 65: 631–639.
Wilson, A. J. and Nussey, D. H. 2010. What is individual quality? An evolutionary perspective. – Trends Ecol. Evol. 25: 207–214.