ELSEVIER

# Phenotypic plasticity and variation in morphological and life-history traits of antlion adults across a climatic gradient

Inon Scharf*,1, Ido Filin1, Dafna Ben-Yehoshua, Ofer Ovadia

*Department of Life Sciences, Ben-Gurion University of the Negev, POB 653, Beer-Sheva 84105, Israel*

## Abstract

We report here on two complementary experiments examining the effect of climate on morphological and life-history traits of antlion adults. We first examined whether body size and wing loading of emerging adults are plastic by raising larvae, collected from five antlion populations along Israel's sharp climatic gradient, in two environmental chambers simulating temperature and humidity of desert and Mediterranean climates. The variance in adult morphology was mostly related to body size, with adults of Mediterranean populations being larger than those of desert populations. Wing-to-thorax ratio was negatively correlated with temperature, compensating for the decrease in wing-beat frequency in colder environments. Differences between climatic treatments were significant for body size but not for the wing-to-thorax ratio, suggesting that body size is more plastic than the ratio between different body components. We next investigated how the exposure of antlion pupae to different climatic conditions influences the emerging adults. Adult body mass increased with final larval body mass at a faster rate when exposed to Mediterranean rather than desert conditions. Duration of the pupa stage was positively correlated with final larval mass, but only under Mediterranean conditions. Adult survival increased with initial mass (after eclosion), but was lower under desert conditions. Similarly, adults lost mass at a faster rate when exposed to desert conditions. Notably, the exposure of the pupae to varying climatic conditions had no effect on adult morphology. Climate is a major factor affecting insect life span and body size. Since body size is strongly linked to fecundity and survival, climate thus has a twofold effect on fitness: directly, and indirectly through body size.
© 2008 Elsevier GmbH. All rights reserved.

*Keywords:* Myrmeleontidae; Life span; Body size; Latitudinal clines

## Introduction

The life history of insects is extensively affected by temperature. The most prominent effects are a smaller adult body size and faster development in a warmer climate (Atkinson, 1994; Atkinson and Sibly, 1997;

Speight et al., 1999). These phenomena have been shown to include both a genetically fixed component, independent of the growing conditions (there are differences between populations of the same species, even when exposed to the same conditions) and a plastic component (e.g., Gilchrist and Huey, 2004; Scharf et al., 2008a). Separating between these two sources of variation is crucial for the understanding of the nature of intra-specific geographical variation in morphological and life-history traits (Niewiarowski and Roosenburg,

1993). Doing so requires conducting a common garden or transplant experiment in which individuals of different populations are raised under the same controlled laboratory conditions or are reciprocally transplanted in the field (e.g., Niewiarowski and Roosenburg, 1993; Arnett and Gotelli, 1999; Scharf et al., 2008a). Furthermore, differences in the reaction norms or plasticity levels among genotypes or populations (i.e., a $G \times E$ interaction) are also common (Pigliucci, 2005). For example, reaction norms should be steeper (i.e., stronger plasticity) in populations experiencing higher variation or stronger seasonal fluctuations relative to those inhabiting more stable environments (David et al., 2004).

There is a large body of literature demonstrating the effect of climate on insect body size and growth (for selective reviews see: Atkinson, 1994; Speight et al., 1999, p. 471; David et al., 2004), yet little is known about how climate experienced during only one developmental stage affects the performance of the next stage, for example, how the exposure of only the pupae (and not the larvae) to varying climatic conditions affects the emerging adults. Adults emerging at warmer temperatures are usually smaller, but it is not clear whether their smaller size is simply a consequence of the small larval mass prior to pupation or of a direct effect of temperature on the pupa development.

Temperature is also a major factor affecting the life span of adult insects. Warmer climates usually shorten the life span (e.g., Nilssen, 1997; Dhileepan et al., 2005), but extreme cold weather can also result in fitness loss by reduced body mass, extensive tissue damage and decreased survival (e.g., Renault et al., 2003). Thus, it may be interesting to study whether body mass has the same effect on survival in colder and warmer climates (i.e., to test the interaction between initial mass and climate). Large body size is usually preferable due to several complementary reasons (Blanckenhorn, 2000, 2005), such as an advantage in intra-specific competition (Griffiths, 1992), increased fecundity (Gilbert, 1984; Honek, 1993) and extended survival (Gilbert, 1984; Leather, 1988), although the opposite selective pressure to reduce body size should also be taken into consideration (e.g., Blanckenhorn, 2000; Berger et al., 2006). Larger insects may have an additional advantage in warm climates – they desiccate more slowly owing to their lower surface area to volume ratio. The same argument was used to explain why insects in warmer environments, where they are prone to desiccation, show a less elongated shape and therefore have a lower surface area to volume ratio (Schoener, 1980; Johnson and Strong, 2000).

Moreover, temperature has been previously found to affect wing loading, i.e., the ratio of body mass to wing area in drosophila (Azevedo et al., 1998; Gilchrist and Huey, 2004) and hummingbirds (Altshuler and Dudley, 2002). Lift power, an important criterion of flight performance, is directly related to wing-beat frequency or stroke amplitude, which are, in turn, positively correlated with air temperature (Dudley, 2000, p. 90; Dillon et al., 2006). In other words, for a given insect species, colder or higher altitudes are associated with decreased wing-beat frequency and hence with reduced lift power. To compensate for the diminished flight performance expected in colder environments small flying animals show decreased wing loading as temperature decreases or altitude increases (e.g., Stalker, 1980; Altshuler and Dudley, 2002; Gilchrist and Huey, 2004; Dillon et al., 2006). This pattern was also shown in studies that measured wing length and thorax length (as substitutes for wing area and body mass, respectively) rather than wing loading (e.g., Azevedo et al., 1998; Karan et al., 1998; Petavy et al., 2001).

We used *Myrmeleon hyalinus* (Neuroptera: Myrmeleontidae) as a model system to investigate the effects of climate on morphological (i.e., body size and wing-to-thorax ratio) and life-history traits of antlion adults (i.e., mass at eclosion, time to eclosion and survival). Our study consisted of two complementary experiments. We first examined the extent to which body size and wing loading of emerging adults are plastic by raising larvae, collected from five geographically representative populations along the sharp climatic gradient characterizing Israel (from a Mediterranean climate in the north to a desert climate in the south), in two environmental chambers simulating desert and Mediterranean climatic conditions, until they complete their life cycle. As previously mentioned, the effect of climate on adult body size may be a result of the larval mass prior to pupation or the climatic conditions experienced during the pupa stage. In order to separate between those two distinct scenarios and to pinpoint the dominant factors affecting body mass and size, we selected one typical population and investigated how larval mass and the climatic conditions experienced during the pupa stage translated into adult mass, survival and morphology. Contrary to most previous studies focusing on the behavior or life history of antlion larvae (see Scharf and Ovadia, 2006 for a review) we focused here on morphological and life-history traits of the adults. We also studied the relationship between adult body mass and length using a power function (Rogers et al., 1977). This relationship was shown to be useful, for instance, in estimating insect biomass in a system (Rogers et al., 1977) and in demonstrating the minimization of the surface area to volume ratio in warm environments (Schoener, 1980).

To this end, we hypothesized that antlions either originating from or experiencing colder climate (i.e., Mediterranean climate) should be larger than those experiencing warmer climatic conditions (i.e., desert climate). We also predicted that wing loading (or the

inverse of the wing-to-thorax ratio) should decrease with increasing growth temperature or temperature in their habitat of origin. An additional prediction was that antlions should show distinct body masses and sizes as a response to the climatic conditions experienced during the pupa stage (antlions experiencing a colder climate should be larger). Finally, we predicted that both the pupa stage duration and adult survival rate should be positively correlated with initial adult body mass but negatively correlated with temperature, while the rate of mass loss of adults should be negatively correlated with both initial adult body mass and temperature.

## Methods

### Natural history

*Myrmeleon hyalinus* (Neuroptera: Myrmeleontidae) is the most abundant pit-building antlion in Israel. The larvae are about 10 mm long (Simon, 1988), and may attain body weights of up to 0.06 g before pupating (Scharf et al., 2008a). The larvae are capable of inhabiting different types of soils (Simon, 1988), both in Mediterranean and desert climatic regions, where they mainly occupy shaded areas under trees, bushes, and rocks. *M. hyalinus* larvae develop through three instar stages (this larval phase may last up to one year; Scharf et al., 2008a), pupate, and then short-lived weak-flier adults emerge. Behavioral experiments showed that *M. hyalinus* larvae can actively select shaded micro-habitats (Scharf et al., 2008b) and when exposed to predation risk they substantially decrease pit construction activity (Loria et al., 2008). Previous research focusing on morphological and life-history traits of *M. hyalinus* larvae have illustrated that along Israel's north-to-south climatic gradient there is a gradient in body mass prior to pupation and in pupation rate (Scharf et al., 2008a). Specifically, larvae from desert populations pupate faster and at smaller body masses in comparison with Mediterranean populations. In addition, Mediterranean population larvae suffer a higher mortality rate when exposed to desert conditions, while the mortality rate of desert population larvae is consistent between Mediterranean and desert conditions. These findings indicate that Mediterranean populations are influenced by extrinsic or climatic factors to a larger extent than desert populations (Scharf et al., 2008a).

### First experiment: differences among antlion populations

In July 2006, antlion larvae (mainly 1st and 2nd instars) were collected from five different sites along the sharp north-to-south climatic gradient characterizing Israel: Caesarea (38), Rishon-LeZion (32), Nahal Secher (18), Holot Agur (26) and Hatzeva (27). About 68% of the larvae completed their life cycle and emerged as adults (the exact number of emerging adults is given above in parentheses). The above five sites can be divided into three different climatic regions: Mediterranean (Caesarea and Rishon-LeZion), arid (Nahal Secher and Holot Agur) and hyper-arid (Hatzeva; Goldreich, 2003, p. 13). The north-to-south and west-to-east climate gradients in Israel are roughly characterized by decreasing annual precipitation rates, increased coefficient of variation of annual rainfall, increasing temperatures, and decreasing humidity (Goldreich, 2003, p. 56, 72, 99, 123). Table 1 presents the climatic properties of the study sites; the data are taken from Goldreich (2003); BioGis (2003), and a Hebrew Internet site maintained by the Israel Ministry of Agriculture (http://www.moag.gov.il/csv/index.html). In the laboratory, individual larvae were assigned randomly to either a Mediterranean environmental chamber or a desert environmental chamber maintained under a 24 h cycle of temperature, light and humidity simulating the summer ambient conditions of Tel-Aviv or Eilat, respectively (Tel-Aviv, Mediterranean climate with day/night temperatures of 29.1 °C/20.7 °C and day/night humidity of 60%/82%; Eilat, desert climate with day/night temperatures of 39.0 °C/25.4 °C and day/night humidity of 15%/48%; day:night length 12:12 h). Antlion larvae were kept separately in round plastic cups (5 cm diameter) filled with sand (2 cm) and were fed with flour beetle larvae twice a week until pupation. After emergence, the length of head + thorax and the lengths of all four wings of each of the 141 adults were measured with calipers (accuracy of 0.1 mm). We used the combined length of head and thorax so as to reduce the relative measurement error, since both the thorax and the head are much smaller than the wings.

Our analysis was based on three morphological traits: head + thorax length, average forewing length (average of left and right forewing measurements), and average hindwing length, and 13 environmental variables (Table 1). Because morphological traits displayed a high degree of cross correlation, we used principal component analysis (PCA) to study the structure of morphological variation and to create composite morphological variables that are not correlated with one another (PCA was performed on log-transformed trait values using the covariance matrix). We used two-way ANOVA to test for the effects of population and climate treatment on the two first PCs. We used linear regression to link the morphological variables to each environmental variable individually. In addition, we performed a PCA on the environmental variables (using the correlation matrix), and used linear regression to link the morphological traits and the first PC of the environmental variables.

**Table 1.** Climatic measurements characterizing the five antlion larvae collection sites (from north to south)

| Site | Caesarea | RLZ | NS | HA | Hatzeva |
|---|---|---|---|---|---|
| Longitude (°E) | 34.8945 | 34.7546 | 34.8111 | 34.4241 | 35.2753 |
| Latitude (°N) | 32.8945 | 31.9599 | 31.1033 | 31.0023 | 30.7720 |
| Altitude (m) | 12.4 | 32.3 | 326.9 | 216.8 | −157.8 |
| Annual rain (mm) | 564.6 | 494.8 | 140.2 | 103.7 | 47.4 |
| CV in rain | 25 | 30 | 35 | 35 | 40 |
| Mean humidity (%) | 70 | 70 | 60 | 60 | 40 |
| Mean Jan. temp. (°C) | 12.4 | 12.3 | 10.8 | 11.2 | 14.0 |
| Min. Jan. temp. (°C) | 3.0 | 3.2 | 2.8 | 3.3 | 4.7 |
| Min. June temp. (°C) | 16.1 | 16.1 | 17.4 | 17.4 | 23.9 |
| Max. June temp. (°C) | 29.5 | 31.4 | 36.6 | 32.3 | 39.4 |
| Seasonal temp. range (°C) | 12.9 | 13.3 | 15.1 | 13.5 | 17.4 |
| Dist. to Med. coast (km) | 0.5 | 3.5 | 57.5 | 40.0 | 115.0 |

RLZ, Rishon-LeZion; NS, Nahal Secher; HA, Holot Agur; CV, coefficient of variation.

These analyses were performed with MATLAB v. 6.5 (Mathworks, Natick, MA, USA), and SYSTAT v. 11 (SYSTAT Software, San Jose, CA, USA).

### Second experiment: antlion life history

During May 2007, we collected additionally 70 3rd instar larvae from one of the five representative antlion populations (Nahal Secher; N 31°06′, E 34°49′). We selected this population because of its location in the center of the distribution range of this species in Israel and the intermediate climatic conditions (a semi-arid region at the Negev desert's northern border). Antlion larvae were kept in an air-conditioned room (mean temperature of 25 °C) in round plastic cups. They were fed once a week with flour beetle larvae (mean mass of ~0.015 g). On the day after feeding, we weighed the antlions (CP224S, Sartorius AG, Goettingen, Germany; accuracy of 0.1 mg) and inspected for new pupae. Once a pupa was detected, the whole cocoon was weighed (including the enveloping sand particles) and randomly assigned to an environmental chamber, simulating either Mediterranean or desert climatic conditions (see previous section). Environmental chambers were inspected 6 days a week for emerging adults. We documented time to eclosion and weighed the adults daily till death. We then photographed the adult body and wings using a digital camera (Micropublisher 5.0) connected to a Nikon stereoscope (SMZ 800) and measured the following morphological traits: head length, thorax length, head + thorax length, abdomen length, forewing length, hindwing length, forewing perimeter, hindwing perimeter, forewing area and hindwing area. All these traits were measured using a script written in MATLAB v. 6.5.

We used general linear models (GLMs) to test for: (1) the effect of either larval mass prior to pupation or pupa mass in combination with the climate treatment on time to and mass at eclosion; (2) the effect of body mass at eclosion and climate treatment on the adult life span; and (3) the effect of body mass at eclosion and climate treatment on the adult mass prior to death. We fitted a linearized exponential decay function for the mass loss of each adult as a function of time, and used a GLM to test for the effect of emerging adult body mass and climate treatment on the instantaneous rate of mass loss. Our morphometrical analysis aimed at the following goals: (1) to test for possible differences in body size between environmental chambers; (2) to describe the allometric relations between different parts of the body; and (3) to detect the body measurement which best correlates with adult body mass at eclosion. For the first and second analyses, we used a PCA (Manly, 2005) and a GLM on the PC1 loadings. For the third analysis, we used the usual allometric power-law model, suggested by Rogers et al. (1977)

$$\text{Adult mass} = b_0(\text{Length})^{b_1} \tag{1}$$

By logarithmically transforming this equation we obtained a linear model. We could then find, using simple linear regression, the length measure that best explained the variance in adult body mass (among head length, thorax length, head + thorax length, abdomen length, total length, forewing length and hindwing length). Since sample size was equal across traits, the one which best correlated with body mass should also have the highest $R^2$.

## Results

### First experiment: morphological differences among antlion populations

The first two principal components of the PCA on the three morphological traits, head + thorax length, mean forewing length and mean hindwing length, accounted for more than 99% of the total morphological variation

(of log-transformed trait values). The first principal axis, which accounted for 82.48% of the total variation, described an overall increase in size (i.e., all PC1 loadings were positive: head + thorax: +0.618, forewing length: +0.559, hindwing length: +0.553). This overall increase in size was almost isometric, although the head + thorax increased slightly faster than the two wings (all traits shared a similar relative rate of increase in size, which manifested itself in similar PC1 loadings; recall that the analysis was performed on log-transformed measurements).

The second principal component accounted for 16.85% of the total variation (which was more than 96% of the remaining variation after the overall size component, PC1, had been accounted for). This PC2 axis described variation in the ratio of the wing lengths to the head + thorax length (PC2 loadings of wing lengths were negative: forewing length: −0.407, hindwing length: −0.466, while the PC2 loading of head + thorax was positive: +0.786). Individuals with a high score on this axis had relatively longer thoraxes and shorter wings than similarly sized individuals with lower scores on this axis. Hence, an increase along the second morphological principal component caused a decrease in the wing-to-thorax ratio (for both forewing and hindwing), while the overall size (PC1) remained unchanged. Based on the relative values of the PC2 loadings, we can assert that scores on PC2 are nearly equivalent (up to a fixed multiplicative constant) to the natural logarithm of the following ratio:

$$R = \frac{(\text{thorax} + \text{head})}{\sqrt{(\text{forewing})(\text{hindwing})}} \tag{2}$$

where $R$ is the ratio of head + thorax length to the geometric mean of the forewing and hindwing lengths. Clearly, this ratio is strongly related to wing loading.
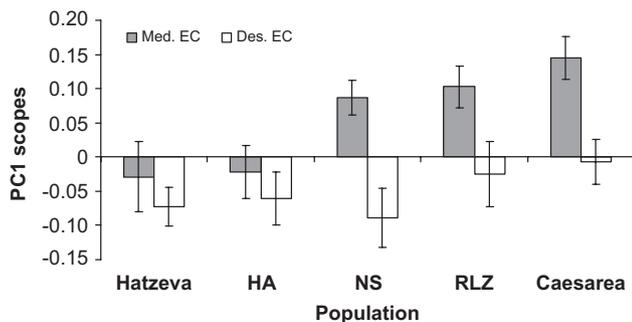
The separation of size and shape (the latter being described by Eq. (2)), corresponding to the orthogonal axes PC1 and PC2, respectively, facilitated the separation of the analysis of overall body size from that of the wing-to-thorax ratio (note that because our size measure, PC1, described isometric size variation, the wing-to-thorax ratio almost did not vary as it moved along PC1). Fig. 1 presents the mean body size (i.e., mean PC1 score) of the five populations and of the two climate treatments. As can be seen, antlions were larger when collected from higher latitudes (Fig. 1; $F_{4,130} = 3.33$, $P = 0.0124$) and when reared in a Mediterranean environmental chamber ($F_{1,130} = 15.93$, $P = 0.0001$), regardless of their population of origin (i.e., non-significant population × climatic treatment interaction term; $F_{4,130} = 0.97$, $P = 0.43$). All five populations were significantly different (Tukey–Kramer post-hoc test).

A two-way ANOVA on PC2 scores, i.e., on our measure of wing loading (Eq. (2)) detected a significant population effect ($F_{4,130} = 4.04$; $P = 0.004$). Fig. 2 presents the mean inverse of the wing-to-thorax ratio or wing loading (i.e., mean PC2 score) of the five populations. The Hatzeva population seemed deviant compared with the other four populations (Fig. 2), and a post-hoc test (Tukey–Kramer) revealed that the Hatzeva population differed significantly from Holot Agur and Rishon-LeZion populations. We could not detect a significant effect of the climate treatment.

Next, we regressed the PC1 and PC2 of all populations against the 13 environmental variables, one at a time. Table 2 presents the environmental variables that were significantly correlated with wing loading. Latitude, annual rainfall and humidity were positively correlated with mean body size (i.e., PC1 scores). The coefficient of variation in annual rainfall, distance to the coast and all variables related to temperature were negatively correlated with body size. When regressing



Fig. 1. Mean body size ± standard error (i.e., mean PC1 score ± SE) for the five populations at Hatzeva, HA (Holot Agur), NS (Nahal Secher), RLZ (Rishon-LeZion) and Caesarea, raised in two separated environmental chambers simulating either Mediterranean (Med. EC) or desert conditions (Des. EC). Antlion adults increased in size along the south-to-north gradient (i.e., with latitude) and were larger when raised under Mediterranean conditions.



Fig. 2. Mean wing loading or inverse wing-to-thorax ratio ± standard error (i.e., mean PC2 score ± SE) of the five populations at Hatzeva, HA (Holot Agur), NS (Nahal Secher), RLZ (Rishon-LeZion) and Caesarea, raised in two separated environmental chambers simulating either Mediterranean (Med. EC) or desert conditions (Des. EC). Wing loading was positively correlated with temperature in the habitat of origin.

**Table 2.** Regression analysis results for each PC separately, representing body size (PC1) and wing loading (PC2)

| Environmental variable | Body size (PC1) | | | Wing loading (PC2) | | |
|---|---|---|---|---|---|---|
| | Slope | $R^2$ | $P$ | Slope | $R^2$ | $P$ |
| Latitude | 0.068 | 0.092 | <0.001 | – | – | NS |
| Longitude | – | – | NS | 0.082 | 0.097 | <0.001 |
| Altitude | −0.0001 | 0.055 | 0.005 | – | – | NS |
| Humidity | 0.004 | 0.070 | 0.002 | −0.001 | 0.056 | 0.005 |
| Rain | 0.0002 | 0.095 | <0.001 | – | – | NS |
| CV in rain | −0.008 | 0.086 | <0.001 | – | – | NS |
| Min. January temp. | −0.046 | 0.037 | 0.022 | 0.026 | 0.059 | 0.004 |
| Max. June temp. | −0.011 | 0.064 | 0.003 | 0.004 | 0.041 | 0.017 |
| Min. June temp. | −0.012 | 0.053 | 0.006 | 0.006 | 0.071 | 0.002 |
| Seasonal temp. range | −0.022 | 0.054 | 0.006 | 0.011 | 0.063 | 0.003 |
| Dist. to Med. coast | −0.001 | 0.074 | 0.001 | 0.0004 | 0.063 | 0.007 |

CV, coefficient of variation.

PC2 against the environmental variables, altitude and humidity were negatively correlated with wing loading and with the inverse wing-to-thorax ratio (i.e., PC2 scores), while longitude, distance to the coast, and all variables related to temperature were positively correlated with wing loading (Table 2).

In addition, we performed a PCA on the environmental variables. PC1 described 71.64% of the variance with the following loadings: longitude: $+0.7376$, latitude: $-0.7097$, altitude: $-0.5578$, rain: $-0.7012$, mean January temp.: $+0.7094$, min. January temp.: $+0.9455$, max. June temp.: $+0.9157$, min. June temp.: $+0.9941$, seasonal temp. range: $+0.9759$, distance to Mediterranean coast: $+0.9507$). It is easily seen that PC1 represents the north–south aridity gradient characterizing Israel (temperatures increase, rain and humidity decrease, etc.). Two separate linear regressions show that body size (PC1 of the morphological traits) is negatively correlated ($R^2 = 0.049$, $F_{1,138} = 7.105$, $P = 0.0086$), while wing loading (PC2 of the morphological traits) is positively correlated ($R^2 = 0.070$, $F_{1,138} = 10.364$, $P = 0.0016$) with Israel's aridity gradient (PC1 of environmental variables).

## Second experiment: antlion life history

### Time to and mass at eclosion

There was a significant effect of larval mass ($F_{42} = 5.519$, $P = 0.024$) on the duration of the pupa stage. The interaction term, larval mass × climate treatment, was also significant ($F_{42} = 5.967$, $P = 0.019$), indicating that it took longer for larger larvae to emerge in the Mediterranean climate (univariate linear regression: $R^2 = 0.304$, $F_{1,21} = 9.177$, $P = 0.006$), while no such pattern was evident in the desert climate ($R^2 < 0.001$, $F_{1,21} = 0.006$, $P = 0.937$) (Fig. 3A). A similar GLM test with pupa mass instead of larval mass showed

similar patterns (pupa mass: $F_{1,42} = 3.857$, $P = 0.056$; pupa mass × climate treatment: $F_{1,42} = 11.860$, $P = 0.001$; Fig. 3B).

There was a significant effect of larval mass ($F_{1,42} = 319.575$, $P < 0.001$) and climate treatment ($F_{1,42} = 5.652$, $P = 0.022$) on adult mass at eclosion. The larval mass × climate treatment interaction term was also significant ($F_{1,42} = 13.616$, $P < 0.001$) (Fig. 4A). Indeed, adult mass
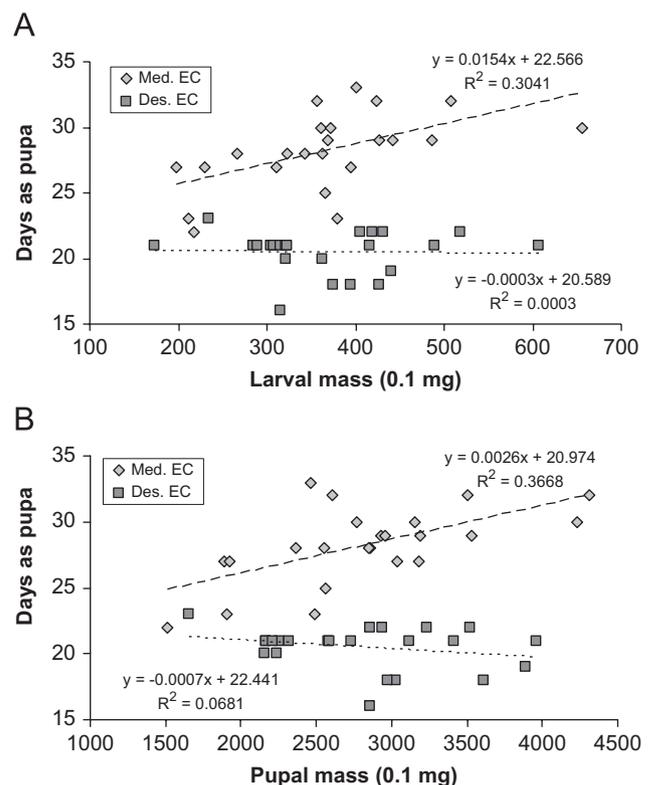


**Fig. 3.** The relationship between larval body mass (A) or pupa mass (B) and the duration of the pupa stage. Pupa stage duration was positively correlated with larval or pupal mass but only in the Mediterranean treatment (Med. EC).
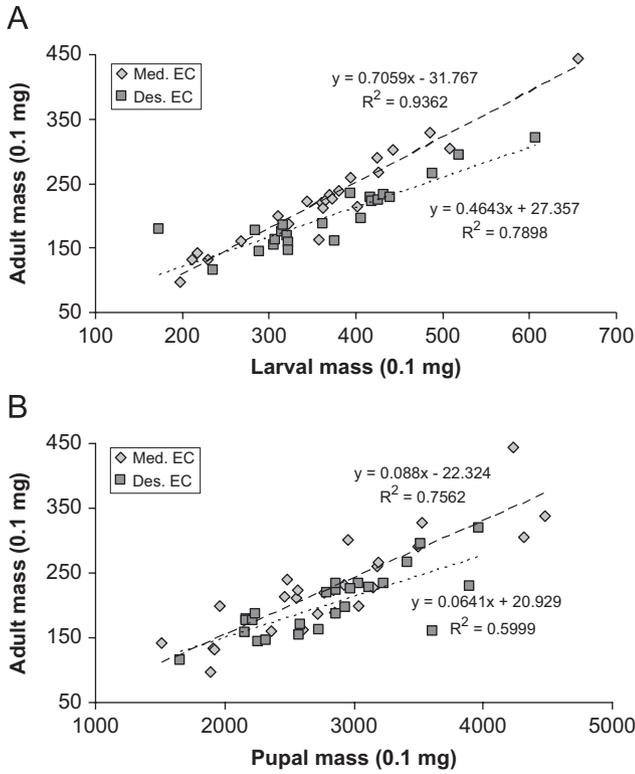
**Fig. 4.** The relationship between larval body mass (A) or pupa mass (B) and the adult body mass at eclosion. Adult body mass increased with larval body mass, but at a faster rate when exposed to Mediterranean conditions (Med. EC). When using pupa mass instead of larval body mass, a similar but non-significant trend was evident.

increased with larval mass, but at a faster rate when exposed to a colder climate (i.e., the Mediterranean environmental chamber). A similar GLM test with pupa mass instead of larval mass showed similar patterns but the interaction term pupa mass × climate treatment was non-significant and the test in general was less powerful (pupa mass: $F_{1,47} = 98.249$, $P < 0.001$; climate treatment: $F_{1,47} = 5.850$, $P = 0.019$; Fig. 4B).

**Adult life span, body mass before death and mass loss rate**

Both climate treatment and adult initial body mass had a significant effect on adult life span ($F_{1,39} = 38.098$, $P < 0.001$ and $F_{1,39} = 8.893$, $P = 0.005$, respectively). Antlions survived fewer days in warmer temperatures but their survival increased with their initial body mass (Fig. 5A). There was no significant adult mass × climate treatment interaction ($F_{1,38} = 0.444$, $P = 0.509$).

Adult initial body mass was positively correlated with final mass prior to death ($F_{1,40} = 158.310$, $P < 0.001$; Fig. 5B). The effect of climate treatment on adult body mass prior to death was marginally significant, indicating that antlions in warmer temperatures died at larger body mass ($F_{1,40} = 3.735$, $P = 0.060$). There was no
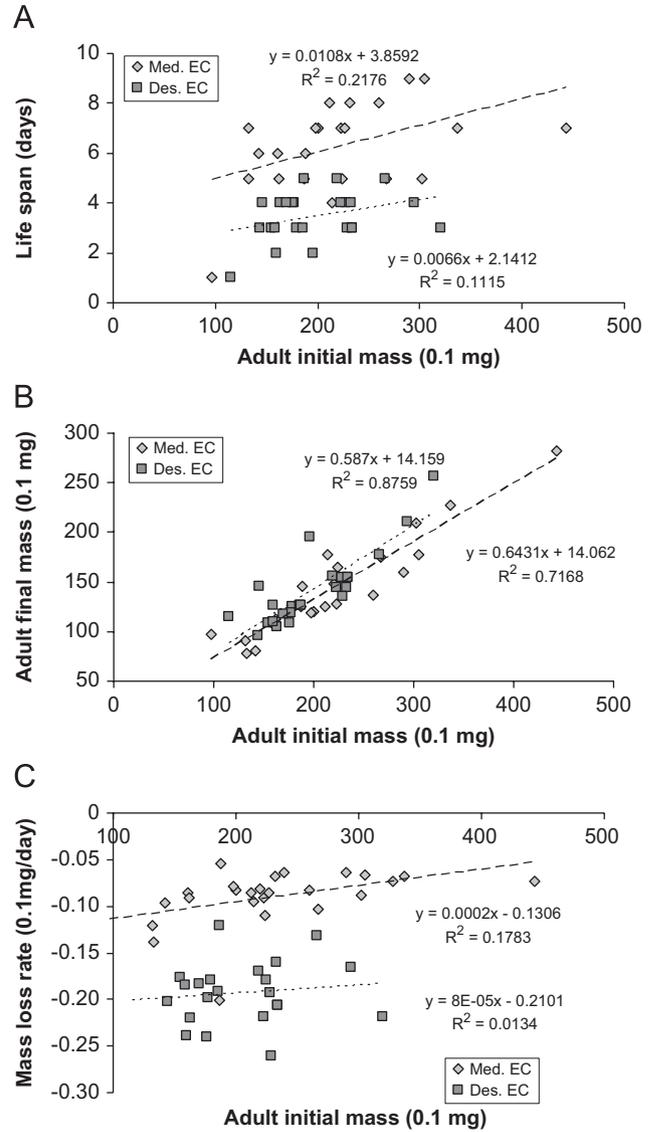


**Fig. 5.** The relationship between adult body mass at eclosion and adult life span (A), adult final body mass prior to death (B), and the rate of mass loss (C). Adult life span was shorter and the rate of mass loss was higher under desert conditions (Des EC). Adult body mass was positively correlated with adult life span and with final body mass prior to death (though only marginally significant).

significant adult initial body mass × climate treatment interaction ($F_{1,39} = 0.040$, $P = 0.842$).

Rates of mass loss were estimated on log-transformed masses. Antlion adults usually showed constant rates of mass loss (i.e., a linear regression described well the decrease in mass vs. time). The climate treatment affected mass loss rate (a faster rate was evident in warmer temperatures: $F_{1,39} = 146.119$, $P < 0.001$). The positive effect of initial mass was considerably weaker ($F_{1,39} = 3.086$, $P = 0.087$; Fig. 5C). The adult initial mass × climate treatment interaction term was not significant ($F_{1,38} = 0.100$, $P = 0.753$).

**Morphological analysis**

We performed PCA on log-transformed body measurements ($n = 45$). One deviant was removed. The first principal axis, which accounted for 84.8% of the total variation, described an overall increase in size (all PC1 loadings were positive: head: +0.2434, thorax: +0.2349, head + thorax: +0.2375, abdomen: +0.1398, forewing length: +0.2447, hindwing length: +0.2488, forewing perimeter: +0.2461, hindwing perimeter: +0.2505, forewing area: +0.5296, hindwing area: +0.5330). PC2 and PC3 explained 6.0% and 5.3% of the variance, respectively, and thus were not further considered. The increase in size was almost isometric for the head, thorax and both wing length and perimeter. The loading of wing area on PC1 was about twice that of wing length (recall that area is equivalent to length$^2$ if growth is isometric), and abdomen length increased more slowly than head and thorax lengths. We performed a GLM (with PC1 scores as the dependent variable) to test for the effect of the climate treatment and adult body mass on morphology. Adult body mass had a dramatic positive effect on body size ($F_{1,41} = 194.604$, $P < 0.001$). In spite of a minor trend showing a slight decrease in body size from Mediterranean to desert climatic conditions, the effect was non-significant ($F_{1,41} = 0.934$, $P = 0.339$). Next, we regressed the log-transformed adult initial mass vs. log-transformed body measurements in order to understand which body parts best correlated with body mass. Both forewing and hindwing lengths showed the best correlation. All other traits also showed a significant and high level of correlation (Table 3).

# Discussion

Climate is a major factor affecting insect life history and body size (Atkinson, 1994; Atkinson and Sibly, 1997; Speight et al., 1999). Since body size is usually positively correlated with different components of fitness, such as longevity and fecundity (e.g., Leather, 1988; Honek, 1993), it is important to study which factors, genetic and environmental, affect body size, and to what extent.

The morphology of *M. hyalinus* adults differs across populations. Most of this variation can be related to body size, with the antlion adults of northern Mediterranean populations being larger than those of southern desert populations. In keeping with previous studies on habitat temperature and insect size (e.g., Atkinson and Sibly, 1997; Karan et al., 1998), we found that either the temperature antlions experienced during our experiment or the temperature in their habitat of origin were negatively correlated with body size. In other words, our results indicated that differences in body size should be attributed to both fixed genetic differences (i.e., northern populations are generally larger than southern ones) and phenotypic plasticity (i.e., individuals grown under desert conditions were smaller). A similar trend was evident in studies of antlion larvae of the same (Scharf et al., 2008a) and a related species (Arnett and Gotelli, 1999). Furthermore, Scharf et al. (2008a) also demonstrated that antlion populations differed in their reaction norms (i.e., northern populations exhibited stronger plastic responses to climatic conditions). Phenotypic plasticity may be extremely important for relatively sedentary animals, such as antlions, which move only short distances as larvae and live only for a short period of time as adults. Similar sessile ("plant-like") animals were indeed found to be extremely sensitive to temperature (Huey et al., 2002).

Annual rainfall and humidity were positively correlated with body size. It is possible that increased rainfall and humidity bring about an increase in the quantity of insect prey, which in turn facilitates antlion growth and development. However, it is also possible that both these abiotic variables are correlated with temperature and have no direct effect on antlion size. In our experiment, antlion larvae were given equal amounts of food regardless of the climate treatment; thus the effect of feeding can be ruled out.

Previous studies on the development of antlion larvae also showed a negative correlation between temperature and final body size (Arnett and Gotelli, 1999; Scharf et al., 2008a). However, these two studies reported different growth rates. Specifically, Arnett and Gotelli (1999) showed that populations reaching higher masses had a shorter developmental period and therefore a higher growth rate compared to populations reaching smaller masses. In contrast, Scharf et al. (2008a) illustrated that *M. hyalinus* larvae reaching higher masses had a longer developmental period and a similar growth rate compared to populations reaching smaller masses (for a more detailed comparison see Scharf et al., 2008a). Those differences between studies emphasize the importance of reporting not only on final mass or size, but also on the developmental period and growth rate.

**Table 3.** Results of the linear regression (data log transformed) of different body measurements (in mm) vs. adult body mass (in mg), arranged by the value of $R^2$

| Measurement length | $R^2$ | $P$ value | $b_0$ | $b_1$ |
|---|---|---|---|---|
| Hindwing | 0.808 | <0.001 | 0.002 | 3.098 |
| Forewing | 0.800 | <0.001 | 0.001 | 3.139 |
| Head + thorax | 0.708 | <0.001 | 0.174 | 2.835 |
| Thorax | 0.538 | <0.001 | 1.676 | 2.063 |
| Total length | 0.429 | <0.001 | 0.016 | 2.356 |
| Head | 0.224 | <0.001 | 9.472 | 1.029 |
| Abdomen | 0.222 | 0.001 | 0.400 | 1.432 |

The regression estimates $b_1$ and $\ln(b_0)$.

Our data indicate that wing loading in *M. hyalinus* adults increased with the temperature in their habitat of origin. This ratio also increased with decreasing altitude and humidity. In fruit flies (Drosophilidae) and hummingbirds (Trochilidae) (e.g., Azevedo et al., 1998; Karan et al., 1998; Petavy et al., 2001), the wing-to-thorax ratio (a measure inversely proportional to wing loading) was found to decrease with temperature. In other taxa, studies of this nature are rare (but see Hepburn et al., 1998 and Dillon et al., 2006 for studies on bees), and to the best of our knowledge, this is the first demonstration of this principle in Neuroptera. At lower temperatures, wing beat is slower and consequently lift power is weaker. Insects, therefore, have to compensate for the loss of lift power in one of two possible ways, either by increasing wing area or by reducing body mass (Gilchrist and Huey, 2004). Since a reduction in body mass confers considerable disadvantages (e.g., female fecundity is usually positively correlated with body mass; Honek, 1993), increasing wing area relative to body mass is the preferred solution. Indeed, wing length usually exhibits higher plasticity levels than thorax length (e.g., Karan et al., 1998; Petavy et al., 2001). In insects, body mass increases faster than wing area, resulting in a positive correlation between body size and wing loading (Dudley, 2000, p. 56, 88; Darveau et al., 2005). Our results provide some support for this correlation, since the PC1 loadings of head + thorax were slightly higher than the PC1 loadings of the two wings. Furthermore, the effect of an increase in altitude on wing loading has previously been found to be similar to the effect of decreasing temperatures (e.g., Altshuler and Dudley, 2002; but see Dillon et al., 2006), and our results are in keeping with this pattern (negative correlation between altitude and wing loading).

It remains to be tested whether antlions with a low wing-to-thorax ratio fly less efficiently and whether their fitness is reduced vis-à-vis adults with higher wing-to-thorax ratios. Studies on other flying animals have reached mixed conclusions. Increased wing loading has been shown to negatively affect the escape success of birds from predators (Burns and Ydenberg, 2002). Furthermore, Renner (2006) has shown that increased wing loading causes birds to have longer take-offs. However, Dillon and Frazier (2006) failed to show an effect of wing loading on flight performance of *Drosophila*, and therefore the significance of wing loading for insect flight and fitness should be further studied. Specifically in antlion adults, wing loading may strongly influence their ability to evade predators. In contrast to the larval stage, antlion adults are probably more prone to predation, because they are weak fliers, relatively large and cannot hide beneath the sand as the larvae do. Therefore, it is possible that increased flight performance may positively contribute to adult fitness, especially owing to the adults' short life span and high susceptibility to predation.

Although body size was negatively affected by temperature during growth (significant effect of the climate treatment on PC1 scores; adults emerging in the desert environmental chamber were smaller), the wing-to-thorax ratio was not affected by temperature or humidity during growth (the climate treatment had no effect on PC2 scores). We showed here that adult body size is a plastic trait which is affected by environmental factors such as temperature. However, we could not detect plasticity in the wing-to-thorax ratio in response to temperature during growth. Most of the previous studies demonstrating phenotypic plasticity in wing loading or wing-to-thorax ratio under laboratory conditions were conducted on *Drosophila* grown at a constant temperature. An exception was the study of Petavy et al. (2001), who suggested that the thermal amplitude is much more important than the mid-temperature. It is possible that our daily fluctuations in temperature and humidity (simulating more realistic weather conditions) imposed a weaker force than those imposed in previous experiments.

Our study showed that body size is highly correlated with temperature during growth, as found in previous experiments on arthropods (e.g., Atkinson and Sibly, 1997; Arnett and Gotelli, 1999), but the change in wing-to-thorax ratio evolves more slowly and is not easily affected in the antlions' life time. The next logical step should be to seek for a factor that is capable of inducing plastic change in the wing-to-thorax ratio of *M. hyalinus*. It is possible that growing antlion larvae at constant temperature will achieve this, but conspecific density and the level of feeding may also affect wing loading, as shown by Karan et al. (1998).

The results of the second experiment indicate that there is an effect of climate and larval body mass prior to pupation on mass at eclosion, duration of the pupa stage, adult survival and rate of mass loss. However, we could not detect morphological differences between antlion adults emerging in the two climatic treatments. The duration of the pupa stage was positively correlated with larval body mass only in the Mediterranean climate. It is possible that a positive (but weaker) correlation exists for the desert climate, but we failed to detect it.

Figure 1 also suggests that the pupa stage lasted longer when exposed to Mediterranean conditions compared with those in the desert, especially for larger antlions. This finding is in accord with previous studies, indicating that higher temperatures usually accelerate development in insects, and specifically the duration of the pupa stage (Nakahira et al., 2005; Castillo et al., 2006). In both climatic treatments, adult body mass at eclosion increased with larval body mass, but the rate was faster when exposed to Mediterranean conditions.

This indicates that smaller antlions are similar in mass at eclosion across climates, but as larval body mass increases, the difference in the emerging adult body mass between the climate treatments increases as well.

Pupa mass showed the same trend as larval body mass, but was in general less powerful and a less successful predictor of the pupa stage duration and adult body mass. Previous studies suggested that pupa mass can be used as a fine predictor of fecundity in insects (Gilbert, 1984; Armbruster and Hutchinson, 2002). However, we here show that larval final body mass prior to pupation is a better predictor of adult body mass, at least in antlions. A probable explanation is the pupa structure in antlions: pupae are round and covered with sand collected from the antlion's surroundings. This explains the increased ratio of pupa vs. larva mass (5.66 in this study). This ratio may change as a function of the sand particle size (pupae may be heavier in coarser sand), and this is another reason why larval mass is a better predictor. Because the sand is used by the antlion as a solid cover attached to the pupa, protecting and separating it from the outer environment, it is impossible to separate between the pupa and the sand particles without causing severe damage.

We also expected to find differences in body size as a function of climate (i.e., smaller body parts in desert climate), but this prediction was not supported by the data. There are differences in adult body size grown in different temperatures (antlions: this study; other Neuroptera: Nakahira et al., 2005). Here we separated the effect on pupae from the combined effect on larvae and pupae. Our results suggest that climatic conditions experienced by larvae affect adult body size, but when exposure occurs only during the pupa stage this pattern disappears (recall that this is not the case when considering adult body mass). It is probable that the larger body size achieved when growing in colder temperatures is mainly a product of the higher mass at pupation and not of a process taking place during the pupa stage. This finding may have implications for seasonal environments, in which larval development may occur in a specific season and the pupa stage in another. In that case, body size will be determined mainly by the temperature experienced by the larva. The difference in body mass between antlion adults emerging under the two climate treatments is probably explained by a faster water loss under desert conditions (a warmer and drier climate; the same phenomenon is described in Nilssen, 1997). This probable conclusion is supported by the shorter life span in desert climate. Adult initial body mass is positively correlated with body mass prior to death. This also supports the conclusion of death from water loss (water is lost, but not other stored materials like fat; therefore, larger adults die at larger mass compared to smaller ones).

Our results suggest an additional advantage of being large: larger antlion adults survive longer under both environmental conditions (it is simply better to be large, at least in this view). This conclusion is similar to that of previous studies showing that larger insects (within the same species) survive longer (Gilbert, 1984; Leather, 1988; Renault et al., 2003; but see Nilssen, 1997), especially when adults do not feed or are naturally short lived. However, the positive correlation between adult mass and life span is similar in both climates (i.e., a non-significant interaction term). Therefore, we could not support our hypothesis that being large is especially important in warmer temperatures (in order to decrease the surface area to volume ratio). Moreover, larger antlions experience a stronger effect of climate on mass at eclosion (Fig. 2). M. hyalinus adults emerge between May and September (Simon, 1988), and are susceptible to warm temperatures. We show here a negative effect of high temperature on survival, and therefore there is probably a strong selective force on fast mating and oviposition. It would be interesting to study the distance moved by adults during their life span as a function of temperature. It is possible that warm temperatures reduce adult dispersal and accelerate reproduction.

Previous studies inspired us to relate body mass to some other body measurements, such as total length (e.g., Rogers et al., 1977; Schoener, 1980), wing length (e.g., Armbruster and Hutchinson, 2002) or others. This relationship is usually described using a power-law formula, such as Eq. (1), and was shown to be important in calculating biomass of some groups of insects (e.g., Johnson and Strong, 2000) or for comparing insect body dimensions in different climates. All body measurements show a significant correlation with body mass, but the best indicators are hindwing and forewing lengths ($R^2$ of 0.808 and 0.800, respectively; Table 3). Head + thorax show a fine correlation too, and the mean slope (or power coefficient without the linear transformation) of these three most adequate measurements is 3.024. Based on these results, we suggest using wing length or head + thorax length to estimate body size and mass in antlion adults and possibly other Neuroptera, too. Wings are easily measured, while the abdomen is a soft tissue which may bend and therefore might introduce bias and lead to inaccurate measurements.

In conclusion, antlion adults are short lived, and therefore their fitness is crucially dependent on larval final body mass prior to pupation. Adults are sensitive to increased temperatures and decreased humidity, which drastically reduce their life span. Antlion adults raised as larvae in warmer temperatures combined with lower humidity had a smaller body size compared to those grown in more comfortable temperatures. However, when isolating the effect of climate on the pupa stage, we failed to show any morphological differences between the antlion adults.

## Acknowledgements

## References

Altshuler, D.L., Dudley, R., 2002. The ecological and evolutionary interface of hummingbird flight physiology. J. Exp. Biol. 205, 2325–2336.

Armbruster, P., Hutchinson, R.A., 2002. Pupal mass and wing length as indicators of fecundity in *Aedes albopictus* and *Aedes geniculatus* (Diptera: Culicidae). J. Med. Entomol. 39, 699–704.

Arnett, A.E., Gotelli, N.J., 1999. Geographic variation in life-history traits of the ant lion *Myrmeleon immaculatus*: evolutionary implications of Bergmann's rule. Evolution 53, 1180–1188.

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

Atkinson, D., Sibly, R.M., 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. Trends Ecol. Evol. 12, 235–239.

Azevedo, R.B.R., James, A.C., McCabe, J., Partridge, L., 1998. Latitudinal variation of wing: thorax size ratio and wing-aspect ratio in *Drosophila melanogaster*. Evolution 52, 1353–1362.

Berger, D., Walters, R., Gotthard, K., 2006. What keeps insects small? – Size dependent predation on two species of butterfly larvae. Evol. Ecol. 20, 575–589.

BioGis (Israel Biodiversity Information System), 2003. 〈http://habitat.bot.huji.ac.il/biogis/static/en/index.html〉.

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

Blanckenhorn, W.U., 2005. Behavioral causes and consequences of sexual size dimorphism. Ethology 111, 977–1016.

Burns, J.G., Ydenberg, R.C., 2002. The effects of wing loading and gender on the escape flights of least sandpipers (*Calidris minutilla*) and western sandpipers (*Calidris mauri*). Behav. Ecol. Sociobiol. 52, 128–136.

Castillo, J., Jacas, J.A., Pena, J.E., Ulmer, B.J., Hall, D.G., 2006. Effect of temperature on life history of *Quadrastichus haitiensis* (Hymenoptera: Eulophidae), an endoparasitoid of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). Biol. Control 36, 189–196.

Darveau, C.-A., Hochachka, P.W., Welch, K.C., Roubik, D.W., Suarez, R.K., 2005. Allometric scaling of flight energetics in Panamanian orchid bees: a comparative phylogenetic approach. J. Exp. Biol. 208, 3581–3591.

David, J.R., Gibert, P., Moreteau, B., 2004. Evolution of reaction norms. In: DeWitt, T.J., Scheiner, S.M. (Eds.), Phenotypic Plasticity: Functional and Conceptual Approaches. Oxford University Press, Oxford, pp. 50–63.

Dhileepan, K., Trevino, M., Raghu, S., 2005. Effect of temperature on the survival of *Aconophora compressa* Walker (Hemiptera: Membracidae): implications for weed biocontrol. Aust. J. Entomol. 44, 457–462.

Dillon, M.E., Frazier, M.R., 2006. *Drosophila melanogaster* locomotion in cold, thin air. J. Exp. Biol. 209, 364–371.

Dillon, M.E., Frazier, M.R., Dudley, R., 2006. Into thin air: physiology and evolution of alpine insects. Integr. Comp. Biol. 46, 49–61.

Dudley, R., 2000. The Biomechanics of Insect Flight. Princeton University Press, Princeton.

Gilbert, N., 1984. Control of fecundity in *Pieris rapae*: I. The problem. J. Anim. Ecol. 53, 581–588.

Gilchrist, G.W., Huey, R.B., 2004. Plastic and genetic variation in wing loading as a function of temperature within and among parallel clines in *Drosophila subobscura*. Integr. Comp. Biol. 44, 461–470.

Goldreich, Y., 2003. The Climate of Israel: Observation, Research and Application. Kluwer Academic/Plenum Publishers, New York.

Griffiths, D., 1992. Interference competition in ant-lion (*Macroleon quinquemaculatus*) larvae. Ecol. Entomol. 17, 219–226.

Hepburn, H.R., Youthed, C., Illgner, P., Radloff, S.E., Brown, R.E., 1998. Production of aerodynamic power in mountain honeybees (*Apis mellifera*). Naturwissenschaften 85, 389–390.

Honek, A., 1993. Intraspecific variation in body size and fecundity in insects – a general relationship. Oikos 66, 483–492.

Huey, R.B., Carlson, M., Corzier, L., Frazier, M., Hamilton, H., Harley, C., Hoang, A., Kingsolver, J.G., 2002. Plants versus animals: do they deal with stress in different ways? Integr. Comp. Biol. 42, 415–423.

Johnson, M.D., Strong, A.M., 2000. Length-weight relationships of Jamaican arthropods. Entomol. News 111, 270–281.

Karan, D., Munjal, A.K., Gibert, P., Moreteau, B., Parkash, R., David, J.R., 1998. Latitudinal clines for morphometrical traits in *Drosophila kikkawai*: a study of natural populations from the Indian subcontinent. Genet. Res. 71, 31–38.

Leather, S.R., 1988. Size, reproductive potential and fecundity in insects: things aren't as simple as they seem. Oikos 51, 386–389.

Loria, R., Scharf, I., Subach, A., Ovadia, O., 2008. The interplay between foraging mode, habitat structure, and predator presence in antlions. Behav. Ecol. Sociobiol. 62, 1185–1192.

Manly, B.F.J., 2005. Multivariate Statistical Methods: A Primer, third ed. Chapman & Hall/CRC, Boca Raton, Florida.

Nakahira, K., Nakahara, R., Arakwa, R., 2005. Effect of temperature on development, survival, and adult body size of two green lacewings, *Mallada desjardinsi* and *Chrysoperla nipponensis* (Neuroptera: Chrysopidae). Appl. Entomol. Zool. 40, 615–620.

Niewiarowski, P.H., Roosenburg, W., 1993. Reciprocal transplant reveals sources of variation in growth rates of the lizard *Sceloporus undulatus*. Ecology 74, 1992–2002.

Nilssen, A.C., 1997. Factors affecting size, longevity and fecundity in the reindeer oestrid flies *Hypoderma tarandi* (L.) and *Cephenemyia trompe* (Modeer). Ecol. Entomol. 22, 294–304.

Petavy, G., Moreteau, B., Gibert, P., Morin, J.-P., David, J.R., 2001. Phenotypic plasticity of body size in *Drosophila*: effects of a daily periodicity of growth temperature in two sibling species. Physiol. Entomol. 26, 351–361.

Pigliucci, M., 2005. Evolution of phenotypic plasticitiy: where are we going now? Trends Ecol. Evol. 20, 481–486.

Renault, D., Hance, T., Vannier, G., Vernon, P., 2003. Is body size an influential parameter in determining the duration of survival at low temperatures in *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae)? J. Zool. 259, 381–388.

Renner, M., 2006. Repeatable measures of take-off flight performance in auklets. J. Zool. 268, 395–404.

Rogers, L.E., Buschbom, R.L., Watson, C.R., 1977. Length-weight relationships of shrub-steppe invertebrates. Ann. Entomol. Soc. Am. 70, 51–53.

Scharf, I., Ovadia, O., 2006. Factors influencing site abandonment and site selection in a sit-and-wait predator: a review of pit-building antlion larvae. J. Insect Behav. 19, 197–218.

Scharf, I., Filin, I., Golan, M., Buchshtav, M., Subach, A., Ovadia, O., 2008a. Phenotypic variation and plasticity in antlion populations: effect of population of origin and climate on morphology and life history development. J. Evol. Biol. 21, 162–172.

Scharf, I., Hollender, Y., Subach, A., Ovadia, O., 2008b. Effect of spatial pattern and microhabitat on pit construction and relocation in *Myrmeleon hyalinus* (Neuroptera: Myrmeleontidae) larvae. Ecol. Entomol. 33, 337–345.

Schoener, T.W., 1980. Length–weight regressions in tropical and temperate forest-understory insects. Ann. Entomol. Soc. Am. 73, 106–109.

Simon, D., 1988. Ant-lions (Neuroptera: Myrmeleontidae) of the coastal plain: systematical, ecological, and zoogeographical aspects with emphasis on the coexistence of a species guild of the unstable dunes. Ph.D. Thesis, Tel-Aviv University, Israel.

Speight, M.R., Hunter, M.D., Watt, A.D., 1999. Ecology of Insects: Concepts and Applications. Oxford University Press, Oxford.

Stalker, H.D., 1980. Chromosome studies in wild populations of *Drosophila melanogaster*. II. Relationship of inversion frequencies to latitude, season, wing-loading and flight activity. Genetics 95, 211–223.