

## DIFFERENCES IN THERMAL BALANCE, BODY TEMPERATURE AND ACTIVITY BETWEEN NON-MELANIC AND MELANIC TWO-SPOT LADYBIRD BEETLES (*ADALIA BIPUNCTATA*) UNDER CONTROLLED CONDITIONS

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### Summary

The consequences of the elytral colour difference between non-melanic (red) and melanic (black) two-spot ladybirds for their thermal properties were studied by applying and testing a biophysical model. The expected differential effects of variation in transmission through the elytra, body size, width of the subelytral cavity, ambient temperature, radiation intensity and wind speed are described, assuming that the two colour patterns represent differences in elytral reflectance and transmittance. The model predicts a higher body temperature for melanic beetles under most conditions. Invasive temperature measurements on living beetles under ranges of specified conditions with respect to ambient temperature, radiative regime and wind speed were in qualitative agreement with

the model predictions and, considering the assumptions made, closely corresponded at the quantitative level. The consequences of the temperature differences for morph activity were studied by measuring walking speeds and the time needed to become active for each morph under the various conditions. The results are consistent with the differences in body temperature, assuming an optimum curve relating performance to body temperature. The colour difference between morphs appeared to be the principal factor influencing activity.

Key words: ladybird, *Adalia bipunctata*, thermal balance, colour morph, walking speed, physical model, melanism.

### Introduction

The two-spot ladybird, *Adalia bipunctata*, occurs as non-melanic morphs with black spots on red elytra and as melanic morphs which are predominantly black with red spots. This colour polymorphism, due to the expression of a supergene (Majerus, 1994), has been studied in the context of aposematism and mimicry, sexual selection and thermal melanism (for reviews, see Brakefield, 1985; Majerus, 1994). The theory of thermal melanism proposes that the melanic morphs are at an advantage under conditions of low temperature and a limited radiative regime, because a dark ectothermic insect will heat up faster and reach a higher equilibrium temperature when insulated, resulting in a higher level of activity and a reproductive advantage (Luis, 1961).

Although the role of colour in insect thermoregulation has been the subject of some controversy (see Digby, 1955; Heinrich, 1981, 1993; Willmer, 1982), a number of studies show an effect of melanization on thermoregulation (e.g. Watt, 1968, 1969; Kingsolver, 1985, 1988; Fields and McNeil, 1988; Kingsolver and Wiernasz, 1991; Goulson, 1994). Several observations and experiments support the operation of thermal

melanism in the two-spot ladybird. First, negative correlations were found between relative frequencies of melanic morphs and levels of sunshine (Benham *et al.* 1974; Muggleton *et al.* 1975; Brakefield, 1984a; see also Timofeeff-Ressovsky, 1940; Bengtson and Hagen, 1975, 1977; Scali and Creed, 1975). Second, differential reproductive activity between the colour morphs was observed in the field, in accordance with predictions from the thermal melanism hypothesis (Brakefield, 1984b,c). Furthermore, laboratory experiments showed a lower cuticular reflectance for melanic ladybirds (Brakefield and Willmer, 1985), resulting in higher body temperatures (Brakefield and Willmer, 1985; Stewart and Dixon, 1989) and higher activities (Benham *et al.* 1974) under a limited range of conditions. Similar results have been found for the seven-spot ladybird with varying degrees of melanism in India (Rhamhalinghan, 1985, 1987). Although most of the laboratory experiments yielded clear-cut results, their value in interpreting the field situation is limited, because the effects of variable environmental conditions, and their interactions, were not studied. A more detailed analysis of the thermal balance and its consequences for the activity of beetles of different size and

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colour under a range of experimental conditions is therefore clearly required.

This paper presents such an analysis and integrates the empirical data with a simple physical model of the heat exchanges occurring in ladybirds. The model is built on previous models, which were constructed in more general terms and successfully predicted body temperatures of various organisms (Porter and Gates, 1969; Gates, 1980; see also Bakken, 1976; Casey, 1988). Incorporating a number of reasonable assumptions and measurements of some basic properties of ladybirds, the model presented here predicts differences in body temperature between the colour morphs under different conditions. We test the predictions made by the model by measuring parameters related to activity under a range of environmental conditions. The effects of the key climatic parameters and their interactions can thus be estimated, and measurements of such parameters in the field can yield predictions about the importance of thermal effects on the fitness of the different colour morphs.

The objectives of the present study were as follows: (1) to use a simple physical model to compare the influence of a number of variables on body temperatures between the two main colour morphs of the two-spot ladybird; (2) to measure the internal body temperatures of freely moving, intact ladybirds under different conditions in the laboratory; (3) to measure the time necessary for the ladybirds to become active and to determine their walking speed under different combinations of ambient temperatures and intensities of insolation in the laboratory; and (4) to measure the effect of wind speed on the activity of the ladybirds.

## Materials and methods

### The model

#### Assumptions

The first assumption concerns the presence of temperature gradients within a ladybird. If such gradients exist, for example due to insulating layers, heat exchange must be considered both at the surface of the animal and across the insulating layer. For a number of insects, such gradients have been shown to exist (see Willmer, 1982; Heinrich, 1993), and it is reasonable to assume that the gap between the elytra and the body of the ladybird, when the wings are folded, represents an insulating layer (Willmer, 1982). It will be shown below that calculating body temperature in the situation with an insulating layer involves just one extra step compared with the simpler model without such a layer. The present model represents a system with a temperature gradient. A schematic representation of the possible avenues of heat exchange for a ladybird is given in Fig. 1. It will be assumed that heat exchange through conduction *via* the substratum is negligible, since the surface area in direct contact with the substratum (i.e. the tarsi) is very small. Furthermore, heat exchange *via* evaporation is generally assumed to be small for insects (Willmer, 1982) and will be ignored. Metabolic heat production is also ignored because it is likely to make only a small contribution to the total heat

balance. Finally, the dorsal surface of a ladybird is considered to be spherical, which enables standard formulae (Gates, 1980) to be used.

#### Steady-state balance of energy fluxes

The symbols used in the model largely correspond to those in Gates (1980) and are given in the Appendix. We consider the case that total incident radiation flux  $Q_s$  causes a thermal equilibrium with temperatures  $T_r$  and  $T_b$  of the elytra (i.e. the surface) and body, respectively, given an ambient temperature  $T_a$  and a wind speed  $v$ . The aim of the model is to predict  $T_b$  at equilibrium, taking into account different values for reflection from, and transmission through, elytra of different colours. At thermal equilibrium, energy gain across a particular surface equals energy loss. At the elytra, incident energy flux  $Q_s$  plus the energy flux through conduction from the body to the elytra  $Q_c$  equals the sum of the reflected energy flux  $Q_r$ , the energy flux transmitted through the elytra  $Q_t$ , the emission flux  $Q_e$  and the energy lost by convection  $C$  (Porter and Gates, 1969), with  $Q_e = \epsilon\sigma(T_r + 273)^4$  (in this paper, all temperatures are given in °C),  $C = h_c(T_r - T_a)$ ,  $Q_r = rQ_s$  (where  $r$  is reflectance),  $Q_t = tQ_s$  (where  $t$  is transmittance), all in  $\text{W m}^{-2}$ . The emissivity  $\epsilon$  is higher than 97% for most animal surfaces (Monteith, 1973; Gates, 1980), and therefore we assumed that  $\epsilon = 100\%$ ,

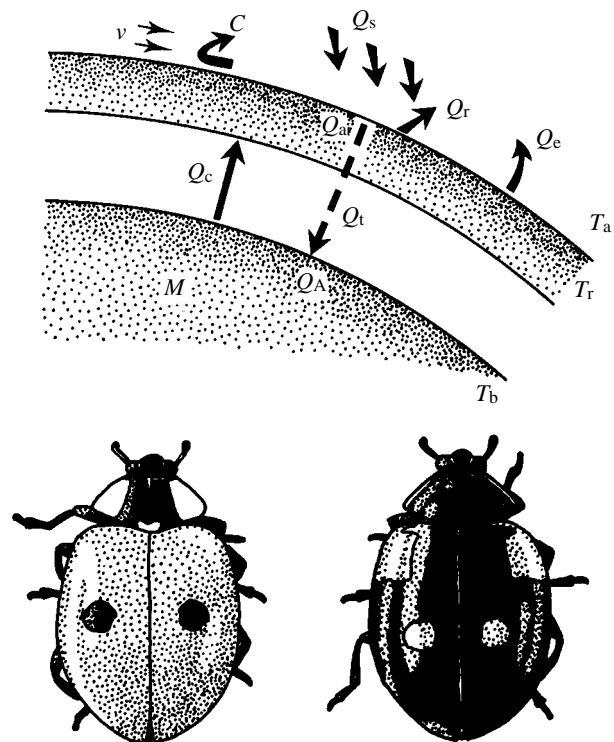


Fig. 1. Energy fluxes and other factors influencing heat exchange in a two-layer model.  $Q_s$ , incident radiation;  $Q_r$ , reflection;  $Q_a$ , absorption in elytra;  $Q_t$ , transmission;  $C$ , convection;  $Q_A$ , absorption in body;  $M$ , metabolic heat production;  $T_a$ , ambient temperature;  $T_r$ , surface temperature;  $T_b$ , body temperature;  $v$ , wind velocity;  $Q_e$ , emitted radiation;  $Q_c$ , conduction. The drawings at the bottom show a non-melanic (left) and a melanic (right) two-spot ladybird.

following Porter and Gates (1969) and Gates (1980). The Stefan-Boltzmann radiation constant  $\sigma=5.673 \times 10^{-8} \text{ W m}^{-2} \text{ K}^{-4}$ . The convection coefficient  $h_c$  (in  $\text{W m}^{-2} \text{ K}^{-1}$ ) is a complex function of the characteristics of the medium and the body (see Gates, 1980). It can be determined by combining the Nusselt number ( $Nu$ ) and the Reynolds number ( $Re$ ). The Nusselt number describes the ratio of the convective conductivity of the surface to the thermal conductivity per unit of dimension:  $Nu=h_c D/k$ , where  $k$  is the thermal conductivity of the medium,  $D$  is the characteristic dimension (in m) and  $k/h_c$  is the thickness of the boundary layer. The Reynolds number describes whether a flow, in this case over a surface, is laminar or turbulent:  $Re=vD/\nu$  where  $\nu$  is the velocity of the medium ( $\text{m s}^{-1}$ ) and  $\nu$  is the kinematic viscosity of the medium ( $\text{m}^2 \text{ s}^{-1}$ ). For air at  $20^\circ \text{C}$ ,  $k=25.7 \times 10^{-3} \text{ W m}^{-1} \text{ K}^{-1}$  and  $\nu=15.3 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ ; thus,  $Nu=38.9h_c D$  and  $Re=6.54 \times 10^4 \nu D$ . For a sphere under forced convection, the relationship between  $Nu$  and  $Re$  is  $Nu=0.37Re^{0.6}$  (Gates, 1980). At thermal equilibrium,  $Q_t$  equals  $Q_c$ , and hence:

$$Q_s = Q_r + Q_e + C. \quad (1)$$

Assuming that a ladybird is characterized by a spherical shape, the measurement of the characteristic dimension  $D$  establishes the relationship between  $h_c$  and  $\nu$ . If, for example,  $D=4 \text{ mm}$ , then the convection coefficient  $h_c=67.1\nu^{0.6}$ . Substituting this expression for  $h_c$  into equation 1, gives:

$$Q_s(1-r) = \epsilon\sigma(T_r + 273)^4 + 67.1\nu^{0.6}(T_r - T_a). \quad (2)$$

Equation 2 is the equation for the energy balance at the surface of a two-spot ladybird (see Porter and Gates, 1969), and if  $Q_s$ ,  $r$ ,  $\nu$  and  $T_a$  are measured, it yields the temperature of the elytra,  $T_r$ . If there are no temperature gradients within a ladybird, the surface (=elytral) temperature  $T_r$  equals the body temperature  $T_b$ .

In the presence of an insulating layer,  $T_b$  need not be equal to  $T_r$ . At equilibrium, heat gain within the body, which is equal to the amount of radiation that is transmitted through the elytra  $Q_t$ , equals heat loss across the insulating layer. The heat loss depends on the conductivity of the insulating layer, its thickness and the temperature difference across the layer. For a sphere with radius  $R$  (in m), the equation for the energy balance across the insulating layer is:

$$Q_t = \frac{k(T_b - T_r)}{R_r - R_b} \times \frac{R_b R_r}{R_b^2}, \quad (3)$$

where  $R_b$  is the radius of the body and  $R_r$  is the radius of the elytra;  $R_b < R_r$  (see Gates, 1980, p.276). Rewriting equation 3 gives:

$$T_b = T_r + \frac{tQ_s R_b (R_r - R_b)}{kR_r}. \quad (4)$$

$T_r$  can be calculated using equation 2 and, if the transmittance  $t$  of the elytra and the radius of the body and elytra are measured,  $T_b$  can be calculated. By subtracting the ambient temperature  $T_a$  from  $T_b$ , the temperature excess  $\Delta T$  is found.

Equations 2 and 4 were used to predict the body temperature  $T_b$  and hence the temperature excess  $\Delta T$  at thermal equilibrium of two-spot ladybirds with black or red elytra under various combinations of incident radiation, ambient temperature and wind speed. It was assumed that the colour morphs differ only in reflectance and transmittance of the elytra. These parameters were determined empirically.

#### Experimental animals

For the experiment investigating ladybird activity (see below), two-spot ladybirds *Adalia bipunctata* L. were collected in September 1992 in the 'de Hoge Veluwe' National Park in The Netherlands. They were sexed using characters described in Majerus and Kearns (1989), de Jong *et al.* (1991) and Randall *et al.* (1992). Twenty-four melanic and 64 non-melanic pairs were kept in Petri dishes 8.5 cm in diameter, containing a filter paper 7 cm in diameter, at  $20^\circ \text{C}$  and with a photoperiod of 18 h:6 h L:D. They were fed daily with an ample supply of laboratory-bred aphids (*Acyrtosiphon pisum* Harris) and they were transferred to clean Petri dishes at least once every week to promote egg laying and to reduce transmission of diseases. Petri dishes containing egg batches were collected and hatched larvae were fed as described above. After their second moult, larvae were kept in pairs to reduce mortality due to cannibalism. If one larva pupated, the other was transferred to a new Petri dish for the same reason. Newly emerged ladybirds were fed for 5 days, until the adult colour pattern had developed, and subsequently stored in the dark at  $3^\circ \text{C}$  until they were used for experiments.

The ladybirds used for body temperature measurements and for the determination of the influence of wind speed were collected in the Utrecht University 'de Uithof' campus, The Netherlands (close to 'de Hoge Veluwe'), in 1993 and reared as described above.

#### Measurement of transmittance of the elytra

The transmittance ( $t$ ) of the red and black areas of ladybird elytra was estimated using a Beckman DU-50 spectrophotometer. Wavelengths in the range 200–900 nm were scanned at a speed of  $750 \text{ nm min}^{-1}$ ; measurements beyond this range were not possible with our equipment (see Discussion). Elytra of freshly killed ladybirds (frozen at  $-20^\circ \text{C}$ ) were removed and mounted on a small strip of black plastic (4 cm × 1 cm) over a small round hole (diameter 1 mm) using a small ring of Plasticine. Elytra were mounted in such a way that either a red or a black area covered the hole. The plastic strip was placed in a quartz cuvette and the transmittance spectrum measured against background readings from the same set-up without an elytron mounted. The spectrum was compared with the energy spectrum of the light used in the experiment, i.e. the amount of energy emitted by the lamps as a function of wavelength, so that the proportion of energy transmitted could be estimated. To establish a value for the average transmittance through melanic and non-melanic elytra, the areas of black and red coloration were estimated using an image analysis system (see Windig, 1991). Elytra were removed from a random sample of field-collected two-

spot ladybirds (collected in Prinsenbeek, Tilburg and Etten-Leur, The Netherlands) and the black area and the total surface area were measured for 67 melanic and 67 non-melanic elytra. The elytra were left intact and were not flattened in order to obtain a value representing the amount of radiation intercepted by a ladybird illuminated from above.

#### *Body temperature and activity measurements*

All observations were made in a cool room, in which the temperature was controlled with a Danfoss RT3 thermostat. A frame was constructed, at the top of which 13 halogen lamps (Philips type 13117, 17 V, 150 W) were positioned in a grid, facing downwards, with 11.5 cm separating neighbouring lamps. The lamps were connected in series; thus, the total voltage was 221 V. They were operated using a variable power switch to prevent damage to the lamps. When operated at full power, the spectrum of light produced by the lamps resembles that of bright sunshine (Philips specification). A glass plate was constructed underneath the lamps, which could be moved up and down to control the intensity of the light falling on it. The ladybirds were placed on this glass plate during experiments. Glass was used to avoid heating of the working plane (the maximum temperature increase above ambient, measured immediately after switching off the lights, was approximately 2 °C), and reflection from below was prevented by placing a dull black container filled with water underneath the glass plate. The lamps were positioned in such a way that a homogeneous beam of light reached the glass plate. A transparent plastic sheet (30 cm × 40 cm) bearing a grid of hexagons (1.4 cm between opposite sides of each hexagon) was laid on top of the glass for making measurements of the walking speeds of ladybirds. Incident radiation over the whole spectral range of the lamps (300–2400 nm) was measured using a pyranometer (Kipp & Zonen, CM6) and recorded using a datalog system developed in the Department of Biology, University of Leiden. Temperatures were measured on the glass plate with a sensor shielded from direct irradiation. Measurements made in various places in the climate room indicated that there was minimal temperature variation within the room.

#### *Experimental design*

##### *Body temperature*

Body temperatures were measured in 26 living melanic and 25 living non-melanic ladybirds, at 3 °C and 675 W m<sup>-2</sup> (conditions relevant for the field). A small hole was made in the ladybird by piercing the rostral part of the right elytron with a sharpened needle normally used for mounting insects. The needle was inserted in such a way that the point was situated in the thorax. The needle was removed and replaced by a type K Chromega-alomega thermocouple (diameter 25.4 μm, length 0.31 m, coating Nobecutan) which was glued with Cyanolit into the tip of a micropipette. Body temperatures were recorded using a digital multimeter (Keithley, type 871). A second thermocouple was used to measure the ambient temperature. After insertion of the thermocouple, the ladybird was allowed to settle down and the body temperature to stabilize. The ladybird

was then transferred to the experimental arena by carrying it on a small piece of glass. The initial body temperature ( $T_{b,initial}$ ), which was close to  $T_a$ , was measured. The lights were switched on and the temperature was recorded every 10 s. To establish warming-up curves for individual ladybirds, the initial body temperature was subtracted from each reading (thus giving temperature excesses corrected for deviations from the ambient temperature due to the handling of the ladybirds and their metabolism). The resulting curves can be described by:

$$\Delta T(t) = \Delta T_{max}(1 - e^{-t/\tau}), \quad (5)$$

in which  $\Delta T_{max}$  is the maximum attained temperature excess and  $\tau$  is the time constant of the temperature increase. Least-squares regression estimates were determined for the two parameters ( $\Delta T_{max}$  and  $\tau$ ) for each ladybird and these were compared between the non-melanic and melanic beetles. After 5 min, a fan, which was placed on the glass plate 30 cm from the ladybird and directed towards it, was switched on, and the effect of a wind speed of 1.9 m s<sup>-1</sup> on body temperature was studied.

##### *Ladybird activity*

Nine series of experiments were performed with all possible combinations of three temperatures (3, 7 and 13 °C) and three light intensities (420, 675 and 1175 W m<sup>-2</sup>) relevant to field conditions (1175 W m<sup>-2</sup> is equivalent to bright sunshine and, in early spring, when the ladybirds come out of hibernation, ambient temperatures are in the range used in the experiments). Ladybirds were re-used in the different series. They were kept individually in the dark in 8.5 cm diameter Petri dishes and they were kept at an experimental temperature for at least 48 h before the experiment. During the experiments, there was no other light source but the experimental one, and ladybirds that were not being tested were kept in the dark. Each series of experiments was started with 50 individuals per colour morph, chosen at random from a total stock of approximately 200 non-melanic and 200 melanic ladybirds. The only exception was series I (3 °C, 675 W m<sup>-2</sup>), which was started with 100 ladybirds.

Four ladybirds at a time, two non-melanic and two melanic, were placed on the grid under the lamps in their Petri dishes. Only those ladybirds that were immobile on the bottom of the Petri dish with their elytra facing upwards were used. The lids of the Petri dishes were removed immediately, and the time until each ladybird started moving was recorded. When all four ladybirds were active, the walking speed was recorded for each by counting the number of grid-lines crossed per unit time. After the experiments, all ladybirds were weighed using a top-pan balance (Sartorius RC210D, MC1) and pronotum width and maximum ladybird width were measured with a stereo microscope fitted with an ocular micrometer (×25). At our experimental temperatures, none of the ladybirds flew during the experiment.

Two control experiments were also carried out. The first examined whether ladybird age could have influenced the results. Following completion of the nine experimental series (after 22 days), the first series was repeated using the same ladybirds. A second control experiment was performed to test whether any differences observed between melanic and non-melanic ladybirds

were due to their elytral colour. Elytra and wings were removed from 10 non-melanic and 10 melanic individuals and, after a few hours, time to activity and walking speed were recorded as described above at 3 °C and 675 W m<sup>-2</sup>. This control experiment was carried out twice with different groups of beetles. No loss of haemolymph after excision of the elytra and wings was observed. Note that the abdomens of melanic and non-melanic ladybirds are similarly coloured (dark brown or black).

The effect of wind was investigated using ladybirds from the Utrecht stock. Time to activity and walking speed were measured as described above for 50 melanic and 50 non-melanic ladybirds at 3 °C and 675 W m<sup>-2</sup>, for three wind speed regimes. Wind was produced by the fan described above. Wind speeds were measured at the position of the beetles using a thermo-anemometer (Elbanton, DGT 100). They were estimated to be 1.9 or 4.6 m s<sup>-1</sup> with the fan on, and 0.4 m s<sup>-1</sup> with the fan off, due to the cooling fan in the climate room. Ladybirds were tested in pairs of one melanic and one non-melanic individual.

## Results

### Reflectance

Values of the elytral reflectance  $r$  were taken from Brakefield and Willmer (1985), who estimated an average  $r$  of 19% for non-melanic and 6% for melanic *A. bipunctata* morphs.

### Transmittance

Mean transmittances for the range of wavelengths used (200–900 nm) were 0.5% (range 0.09–1.1%,  $N=12$ ) for the black part of melanic elytra and 4.6% (range 1.0–8.4%,  $N=14$ ) for the red part of non-melanic elytra. Average proportions of black cuticle were 77.7±0.9% (S.E.M.,  $N=67$ ) for melanic and 4.7±0.2% (S.E.M.,  $N=67$ ) for non-melanic beetles. Hence, the average transmittance through a melanic elytron is 1.4% and that through a non-melanic elytron is 4.4%, assuming that transmittance beyond a wavelength of 900 nm is similar to that in the range where measurements were possible.

### Predicted temperature excess ( $\Delta T$ )

The body temperature  $T_b$ , and hence  $\Delta T$ , was predicted by solving the steady-state energy balance equations 2 and 4 using the values of reflectance and transmittance given above. Examples for specified conditions of the effects of variation in ambient temperature  $T_a$ , incident radiation  $Q_s$ , wind speed  $v$ , the width of the subelytral cavity  $R_r-R_b$ , and the characteristic dimension  $D$  on the temperature excess are shown in Fig. 2. Each of these factors was varied while keeping all others constant at a particular value. The melanic morphs have a higher temperature excess than the non-melanic beetles for the parameter values used in Fig. 2A because the effect of reflection (equation 2) dominates that of transmission (equation 4). The influence of ambient temperature on the temperature excess is the same for melanic and non-melanic beetles. Temperature excess decreases with ambient temperature because, in order to balance equation 2, when  $T_a$  increases,  $T_r$  increases at a slower rate because it appears in two terms in the equation. The decrease

in temperature excess with ambient temperature approaches linearity over the range of ambient temperatures considered in Fig. 2A (the theoretical relationship is slightly curvilinear owing

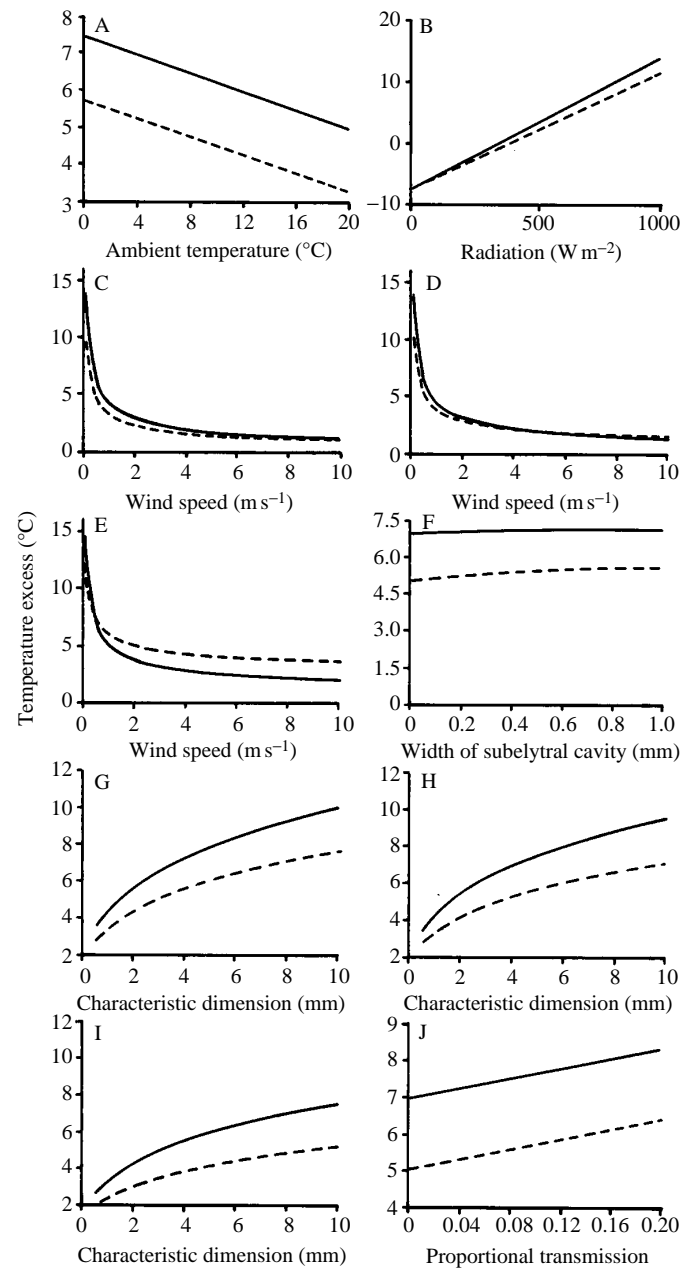


Fig. 2. Predictions of the energy flux model showing the effects of a number of factors on the temperature excess of melanic (solid line) and non-melanic (dashed line) ladybirds. Default parameter values are (unless otherwise stated):  $D=4$  mm,  $R_r=2.0$  mm,  $R_b=1.7$  mm,  $v=0.4$  m s<sup>-1</sup>,  $T_a=3$  °C,  $Q_s=675$  W m<sup>-2</sup>,  $r_{\text{non-melanic}}=0.185$ ,  $r_{\text{melanic}}=0.06$ ,  $t_{\text{non-melanic}}=0.044$ ,  $t_{\text{melanic}}=0.014$ . See Materials and methods for details of the model; the abbreviations are explained in the Appendix. (A) Ambient temperature, (B) radiation intensity, (C) wind speed with default transmission  $Q_t (=tQ_s)$ , (D) wind speed with a 2.5-fold increase in transmission, (E) wind speed with a 10-fold increase in transmission, (F) width of subelytral cavity, (G) body size (characteristic dimension) at  $T_a=0$  °C, (H) body size at  $T_a=3$  °C, (I) body size at  $T_a=15$  °C, (J) transmission.

to the emission term in equation 2, where  $T_r$  is a fourth-power function of  $T_a$ ). The slopes are the same for both morphs (Fig. 2A). Fig. 2B shows that predicted temperature excess increases with the level of irradiation for both non-melanic and melanic ladybirds. Again, the increase approaches linearity over the range of ambient temperatures considered ( $T_r$  is a fourth-power function of  $Q_s$ ). The effect is stronger for melanic morphs since a smaller proportion of radiation is reflected. Fig. 2C shows that the temperature excess drops with increasing wind speed (if  $v$  increases in equation 2,  $T_r$  decreases exponentially to balance the equation), and that the effect is stronger for melanic than for non-melanic ladybirds (see also Fig. 2D,E). This is because the proportion of radiation transmitted through the elytra is then 'shielded' from convection by the insulating layer. Because transmittance of melanic elytra is lower than that of non-melanic elytra, a higher proportion of radiation is affected by wind speed in melanic morphs. The effect of the variation in the thickness of the sub-elytral cavity is shown in Fig. 2F; there is a slow increase in temperature excess with increasing thickness of the sub-elytral cavity. This can be understood by examining equation 4; it is intuitively obvious that increasing the insulating layer will lead to an increase in temperature excess. The effect of this variable is slightly stronger for non-melanic beetles than for the melanic morphs because the amount of radiation transmitted through their elytra is larger, but the effect is marginal relative to those of the other variables considered here. Fig. 2G-I shows that temperature excess is positively related to body size and that the effect of size is greater for the melanic morphs. In equation 2, the convection coefficient  $h_c$  decreases exponentially with  $D$ ; thus, the temperature excess needs to be larger to balance the equation. For higher ambient temperatures (Fig. 2H,I), the effect is smaller for the reasons given above for Fig. 2A. Fig. 2J shows a linear increase in temperature excess with proportional transmission (see equation

4). The parameters presented in Fig. 2 are, of course, interrelated, and variation in one of them may have substantial consequences for the relationships between other parameters (see also Porter and Gates, 1969). If, for example, the transmission through the elytra is actually 2.5 times higher than that shown in Fig. 2C, the curves for the effect of wind speed on  $\Delta T$  for melanic and non-melanic ladybirds cross, giving melanic morphs that are cooler than non-melanic morphs at high wind speeds (Fig. 2D). This effect is even stronger at higher levels of transmission (Fig. 2E) because, at higher levels of transmission, a relatively larger part of the radiation in the non-melanic morphs is absorbed in the body and not in the elytra, so that the effect of wind becomes relatively smaller in non-melanic beetles. However, the general forms of the relationships for a range of parameter values relevant to field conditions are accurately described above and can be qualitatively compared with actual observations on living ladybirds.

#### Body temperature

Initial body temperatures, measured in the dark directly before putting the beetles under the light ( $T_{b,initial}$ ), were not significantly different between melanic and non-melanic ladybirds (Mann-Whitney  $U$ -test,  $N_1=26$ ,  $N_2=25$ ,  $z=0.25$ ,  $P=0.80$ ). The average warming-up curves under irradiation with  $675 \text{ W m}^{-2}$  at  $3^\circ\text{C}$  are given in Fig. 3. Although a comparison between Fig. 3A and Fig. 3B shows that the melanic ladybirds tend to warm up slightly faster than the non-melanic ones, the difference in average  $\tau$  was not significant (non-melanic:  $\tau=81.7 \text{ s}^{-1}$ , range=33.6–268.7  $\text{s}^{-1}$ ,  $N=25$ ; melanic:  $\tau=79.2 \text{ s}^{-1}$ , range=32.6–471.4  $\text{s}^{-1}$ ,  $N=26$ , Mann-Whitney  $U$ -test,  $z=0.82$ ,  $P=0.41$ ). The maximum temperature excesses ( $\Delta T_{max}$ ), however, were significantly higher for melanic ladybirds (non-melanic:  $\Delta T_{max}=6.4^\circ\text{C}$ , range=4.1–8.3  $^\circ\text{C}$ ,  $N=25$ ; melanic:  $\Delta T_{max}=7.8^\circ\text{C}$ , range=5.2–14.5  $^\circ\text{C}$ ,  $N=26$ ; Mann-Whitney  $U$ -

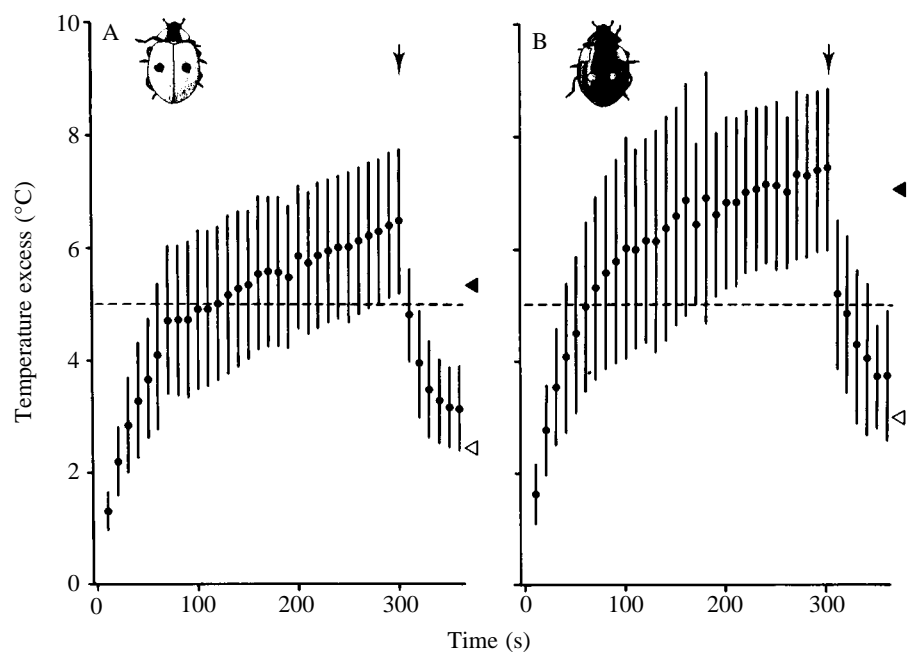


Fig. 3. Average warming-up curve at  $3^\circ\text{C}$  for (A) non-melanic ( $N=25$ ) and (B) melanic ( $N=26$ ) ladybirds. The bars show standard deviations. Values are  $T_b - T_{b,initial}$ . At time zero, beetles were put under the lights ( $675 \text{ W m}^{-2}$ ). The vertical arrow indicates the point at which the fan was switched on. The predicted temperature excesses are indicated by arrowheads on the right ( $\blacktriangle$ , without wind;  $\triangleleft$ , with wind). The dashed line at  $5^\circ\text{C}$  is included as a reference line to facilitate comparison between A and B.

Table 1. Results from principal components analysis used to determine a general size index for the ladybirds

Variable	PC1	PC2	PC3	PC4
Body mass	-0.48	-0.81	-0.33	0.02
Elytra length	-0.50	-0.04	0.82	-0.29
Maximum body width	-0.52	0.34	-0.02	0.79
Pronotum width	-0.50	0.48	-0.48	-0.54
Proportion of variance explained	0.76	0.10	0.08	0.06

test,  $z=2.69$ ,  $P<0.05$ ). Spearman rank correlations were calculated between fresh body mass and both  $\Delta T_{max}$  and  $\tau$ . Applying a sequential Bonferroni test (Dunn-Šidák method, Sokal and Rohlf, 1995; see Rice, 1989) revealed that none of these correlations was significant at an experimentwise error rate of 5%. The influence of wind is also shown in Fig. 3. In both melanic and non-melanic ladybirds, the body temperature dropped substantially when the fan was turned on. The difference in  $\Delta T_{max}$  between melanic and non-melanic beetles was no longer significant at a wind speed of  $1.9\text{ ms}^{-1}$  (Mann-Whitney  $U$ -test,  $z=1.24$ ,  $N_1=18$ ,  $N_2=17$ ,  $P=0.22$ ).

Ladybird activity

Influence of radiation and ambient temperature on time to activity and walking speed

The mean ( $\pm$  S.E.M.) ambient temperatures across the nine series of experiments were  $3.3\pm 0.7^\circ\text{C}$  ( $N=20$ ),  $7.1\pm 0.4^\circ\text{C}$  ( $N=17$ ) and  $12.9\pm 0.5^\circ\text{C}$  ( $N=18$ ), respectively. The information on several size variables (body mass, length of elytra, maximum width of ladybird, width of pronotum) was combined in a principal components analysis. The first principal component (PC1; Table 1) provides an overall index of size. General linear modelling (GLM, Minitab-package; see Minitab reference manual, release 7, p. 8/27-8/40) was performed to investigate the contribution of the three intensities of radiation, the three ambient temperatures, the colour of the elytra (melanic or non-melanic) and body size (PC1; Table 1) on time to activity and walking speed (Table 2); PC1 was entered as a covariate and a fixed-effect model was used in the tests carried out. Table 2 shows that body size did not contribute significantly to variation in time to activity, whereas the three other factors all had strongly significant effects (see below). Only one interaction,

that between temperature and radiation intensity, was significant. Walking speeds were significantly influenced by every variable including body size (PC1) (Table 2, and see below). Significant interactions occurred between temperature and both light intensity and morph.

Fig. 4A presents a plot of time to activity against ambient temperature for the three experimental light intensities for melanic and non-melanic ladybirds. The significance of the differences between the morphs is given in Table 3. Fig. 4A clearly shows that increasing temperature and light intensity result in a shorter time to activity for both non-melanic and melanic beetles and that, for all conditions, the non-melanic morphs take longer to become active (see Table 3). Fig. 4B shows a similar plot for the walking speeds after the ladybirds had become active. Spearman rank correlations were calculated between walking speed and both the period for which the ladybirds had been under the lamps until the end of the speed measurement and the period for which the ladybird had been active until the end of the speed measurement. Applying a sequential Bonferroni test (Dunn-Šidák method) to each of these sets of 18 correlations, none was significant at an experimentwise error rate of 5%. It is therefore unlikely that the period during which the beetles had been illuminated or active before the walking speed was determined had any strong influence on the results. Both light intensity and ambient temperature had a positive influence on walking speed, and walking speeds were generally higher for melanic than for non-melanic beetles (Fig. 4B; Table 3).

Influence of body size

The body sizes of the ladybirds used in the different series of experiments were not significantly different (ANOVA,  $F=0.856$ ,  $d.f.=17$ ,  $P=0.63$ ); most ladybirds were re-used for all the series of experiments. To examine the nature of the effect of body size on the walking speed in the GLM analysis (see Table 2), a Spearman rank correlation was calculated for each series of experiments (Table 4). Although applying a Bonferroni test (Dunn-Šidák method) showed that none of the correlations was significant, five out of six tests carried out at  $3^\circ\text{C}$  yielded  $P<0.05$  (for all the other conditions  $P$ -values were much higher). Thus, at low ambient temperature, larger beetles of each morph probably tend to walk faster than smaller individuals.

Table 2. Results from general linear model analysis used to analyse the influence of ambient temperature, light intensity, colour morph and PC1 (size index; see Table 1) on time to activity and walking speed of ladybirds

Factor	Time to activity			Walking speed		
	F	d.f.	P	F	d.f.	P
PC1	2.92	1	0.09	10.83	1	<0.01
Temperature	175.80	2	<0.001	523.0	2	<0.001
Light	33.34	2	<0.001	51.37	2	<0.001
Morph	106.40	1	<0.001	75.98	1	<0.001
Temperature $\times$ light	6.56	4	<0.001	4.38	4	<0.01
Temperature $\times$ morph	1.85	2	0.16	7.81	2	<0.001
Light $\times$ morph	2.06	2	0.13	2.55	2	0.08
Temperature $\times$ light $\times$ morph	0.86	4	0.49	2.32	4	0.06

Table 3. Significant differences between melanic and non-melanic ladybirds in time to activity and walking speeds for the various experimental conditions

Temperature (°C)	Light intensity (W m <sup>-2</sup> )	Time to activity		Walking speed	
		<i>z</i>	<i>P</i>	<i>z</i>	<i>P</i>
3	420	3.48	<0.001*	2.24	0.02*
7	420	4.95	<0.001*	0.97	0.33
13	420	2.89	<0.01*	2.18	0.03*
3	675	3.58	<0.001*	5.21	<0.001*
7	675	4.11	<0.001*	3.67	<0.001*
13	675	1.69	0.09	2.32	0.02*
3	1175	3.91	<0.001*	4.65	<0.001*
7	1175	2.76	<0.001*	3.00	<0.01*
13	1175	3.30	<0.001*	0.98	0.33

Mann–Whitney *U*-test; sequential Bonferroni test applied for each experimental condition (Dunn–Šidák method, experimentwise error rate=0.05).

An asterisk indicates a significant difference between the two colour morphs.

#### Control series

To test whether age, previous treatment or variation in experimental conditions played a role in the experiments described above, the first series of experiments (3 °C, 675 W m<sup>-2</sup>) was repeated at the end of the series of experiments, using the same ladybirds. For both melanic and non-melanic beetles, time to activity was not significantly different from the value for the first replicate (Mann–Whitney *U*-test, melanic: *z*=0.33, *N*<sub>1</sub>=83, *N*<sub>2</sub>=19, *P*=0.74; non-melanic: *z*=1.09, *N*<sub>1</sub>=78, *N*<sub>2</sub>=19, *P*=0.28). As in the first replicate, there was a significantly shorter time to activity for melanic than for non-melanic beetles (Mann–Whitney *U*-test, *z*=2.93, *N*<sub>1</sub>=19, *N*<sub>2</sub>=19, *P*=0.003). Similar results were found for the walking speed: there was no significant difference between replicates (melanic: *z*=0.14, *N*<sub>1</sub>=81, *N*<sub>2</sub>=19, *P*=0.89; non-melanic: *z*=1.36, *N*<sub>1</sub>=77, *N*<sub>2</sub>=18, *P*=0.17), and the walking speed for melanic beetles was significantly higher than that for non-melanic beetles (*z*=2.69, *N*<sub>1</sub>=19, *N*<sub>2</sub>=18, *P*=0.007).

Two series of experiments were performed in which the elytra and wings of melanic and non-melanic ladybirds were surgically removed prior to testing. Both series were at a light intensity of 675 W m<sup>-2</sup>, series I at 3.95 °C and series II at 4.9 °C. Time to activity and walking speeds were measured. The two series were combined, because there were no significant differences between them for both parameters and colour morphs (Mann–Whitney *U*-test, warming-up times, melanic: *U*=20, *N*<sub>1</sub>=10, range=23–147 s, *N*<sub>2</sub>=9, range=25–210 s; non-melanic: *U*=12, *N*<sub>1</sub>=10, range=10–124 s, *N*<sub>2</sub>=8, range=37–221 s; walking speed, melanic: *U*=20, *N*<sub>1</sub>=10, range=0.1–0.4 lines s<sup>-1</sup>, *N*<sub>2</sub>=9, range=0.1–0.3 lines s<sup>-1</sup>; non-melanic: *U*=11, *N*<sub>1</sub>=10, range=0.1–0.5 lines s<sup>-1</sup>, *N*<sub>2</sub>=8, range=0.1–0.3 lines s<sup>-1</sup>). A sequential Bonferroni test (Dunn–Šidák method) with an experimentwise error rate of 5% was applied. For the combined series, times to activity were not significantly different between

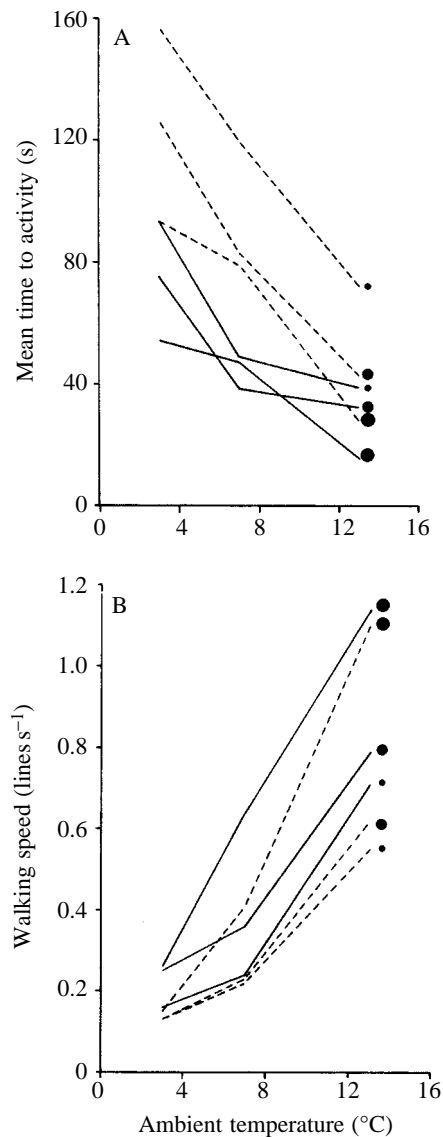


Fig. 4. Mean time to activity (A) and walking speed (B) versus ambient temperature for the three light intensities for melanic ladybirds (solid lines) and non-melanic ladybirds (dashed lines). The light intensities are indicated by the size of the dots on the right-hand side: small dots, 420 W m<sup>-2</sup>; intermediate dots, 675 W m<sup>-2</sup>; large dots, 1175 W m<sup>-2</sup>.

non-melanic and melanic ladybirds (*z*=0.73, *N*<sub>1</sub>=19, *N*<sub>2</sub>=18, range=23–210 s and 10–221 s, respectively, *P*=0.47) nor were walking speeds (*z*=0, *N*<sub>1</sub>=19, *N*<sub>2</sub>=18, range=0.1–0.4 lines s<sup>-1</sup> and 0.1–0.5 lines s<sup>-1</sup>, respectively, *P*=1.0), using a sequential Bonferroni test (Dunn–Šidák method) with an experimentwise error rate of 5%.

#### The influence of wind

The series of experiments at a wind speed of 0.4 m s<sup>-1</sup> (without the extra fan switched on) yielded results for time to activity that were not significantly different from the corresponding series in the experiments described above, as expected (Mann–Whitney *U*-test, warming-up time: melanic:



Table 4. Spearman rank correlations between walking speed and body mass for the nine experimental series

Temperature (°C)	Light intensity (W m <sup>-2</sup> )	Melanic			Non-melanic		
		<i>r</i>	<i>N</i>	<i>P</i>	<i>r</i>	<i>N</i>	<i>P</i>
3	420	0.34	44	0.03	0.11	39	0.49
7	420	0.09	49	0.52	-0.02	42	0.91
13	420	-0.09	45	0.57	-0.04	43	0.82
3	675	0.27	79	0.02	0.36	77	0.002
7	675	0.15	45	0.32	0.25	41	0.12
13	675	-0.03	46	0.84	-0.03	43	0.85
3	1175	0.36	48	0.01	0.31	46	0.04
7	1175	-0.17	48	0.24	0.18	44	0.24
13	1175	-0.18	47	0.22	-0.06	41	0.72

$z=0.78$ ,  $N_1=83$ ,  $N_2=21$ ,  $P=0.43$ ; non-melanic:  $z=1.41$ ,  $N_1=78$ ,  $N_2=24$ ,  $P=0.16$ ). Mean values for time to activity in the different wind regimes are shown in Fig. 5A. The time to activity was significantly shorter for non-melanic ladybirds at a wind speed of  $1.9 \text{ m s}^{-1}$  than at a wind speed of  $0.4 \text{ m s}^{-1}$  (Mann–Whitney *U*-test,  $z=3.49$ ,  $N_1=24$ ,  $N_2=43$ ,  $P=0.0005$ ). There was no difference between values at wind speeds of  $1.9$  and  $4.6 \text{ m s}^{-1}$  for the non-melanic beetles (Mann–Whitney *U*-test,  $z=0.66$ ,  $N_1=43$ ,  $N_2=40$ ,  $P=0.51$ ). For the melanic ladybirds, there was no evidence for an influence of wind speed on time to activity. The significant difference between non-melanic and melanic time to activity at a wind speed of  $0.4 \text{ m s}^{-1}$  (see above,  $z=3.58$ ,  $N_1=83$ ,  $N_2=78$ ,  $P=0.0003$ ) had disappeared at  $1.9 \text{ m s}^{-1}$  ( $z=1.55$ ,  $N_1=46$ ,  $N_2=43$ ,  $P=0.12$ ) and at  $4.6 \text{ m s}^{-1}$  ( $z=2.09$ ,  $N_1=43$ ,  $N_2=40$ ,  $P=0.04$ ), applying sequential Bonferroni tests (Dunn–Šidák method) for the above comparisons.

The influence of wind speed on walking speed is shown in Fig. 5B. A comparison between melanic walking speeds at wind speeds of  $0.4 \text{ m s}^{-1}$  and  $1.9 \text{ m s}^{-1}$  shows a significant decrease (Mann–Whitney *U*-test,  $z=6.52$ ,  $N_1=81$ ,  $N_2=46$ ,  $P \leq 0.001$ ). For non-melanic beetles, there was no significant difference between walking speeds at these wind speeds ( $z=0.37$ ,  $N_1=77$ ,  $N_2=43$ ,  $P=0.71$ ). The comparison between values at wind speeds of  $1.9$  and  $4.6 \text{ m s}^{-1}$  yields a significant decrease in walking speed for both melanic ( $z=2.24$ ,  $N_1=46$ ,  $N_2=43$ ,  $P=0.02$ ) and non-melanic beetles ( $z=2.30$ ,  $N_1=43$ ,  $N_2=40$ ,  $P=0.02$ ). Differences between melanic and non-melanic beetles were significant at  $0.4 \text{ m s}^{-1}$  (see above,  $z=5.21$ ,  $N_1=81$ ,  $N_2=77$ ,  $P \leq 0.001$ ), but at  $1.9$  and  $4.6 \text{ m s}^{-1}$  they were not ( $z=0.42$ ,  $N_1=46$ ,  $N_2=43$ ,  $P=0.68$  and  $z=0.07$ ,  $N_1=43$ ,  $N_2=40$ ,  $P=0.95$ , respectively), applying sequential Bonferroni tests (Dunn–Šidák method) for the comparisons.

### Discussion

These new empirical data, together with the application of a physical model to ladybird morphs characterized by different elytral reflectances and transmittances, provide a sound basis for thermal melanism and the influence of the major climatic factors on thermal budgets and beetle activity. Calculations from our generalized model show that both the properties of the ladybirds

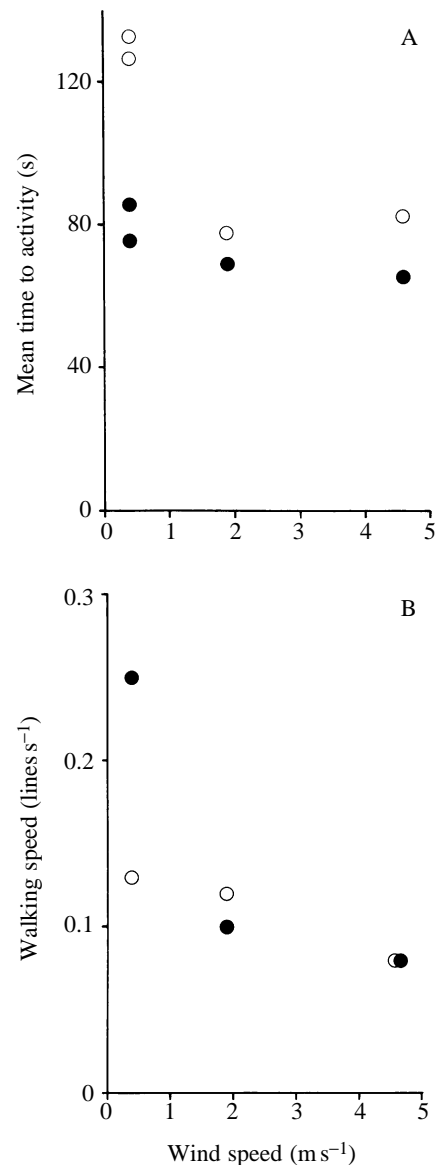


Fig. 5. Mean time to activity (A) and walking speed (B) at different wind speeds for melanic (●) and non-melanic (○) ladybirds at  $3^\circ\text{C}$  and  $675 \text{ W m}^{-2}$ .

(reflection, transmission, body size, width of subelytral cavity) and climatic factors (radiation intensity, ambient temperature, wind speed) are expected to influence equilibrium body temperature. All these factors are interrelated and may vary between individual ladybirds of each colour morph, both within and across environments. Examples of variable properties of ladybirds are body size (including sexual dimorphism), the amount of black on the elytra (both between and within morphs) and the intensity of colour of the elytra (which is partly age-linked). Each of these can affect reflectance or transmittance. Future, more complete tests of the predictive value of our model will need to take such variation into account. However, these data on the mean body temperatures of melanic and non-melanic ladybirds over a range of specified environmental conditions provide a first examination.

Our measurements show that, at 3°C and 675 W m<sup>-2</sup>, the average equilibrium temperature of living melanic ladybirds was significantly higher than that of non-melanic ladybirds: the mean temperature excess for melanic morphs was 7.8±2.2°C (*N*=26) and for non-melanic morphs was 6.4±1.2°C (*N*=25). For these conditions, the model would predict, for a ladybird with a characteristic dimension of 4 mm, a temperature excess of 7.07°C for melanic morphs and 5.34°C for non-melanic morphs. The small discrepancies between the measured and predicted values may have been influenced by a slightly larger average body size and some warming-up of the air under the lights, thus slightly reducing temperature excesses (see Fig. 2A; the gradual increases in  $\Delta T$  occurring after the initial warming phase shown in Fig. 3 may be a consequence of such warming-up effects). Furthermore, estimates of the mean transmission of radiation through the elytra may deviate from the actual values for the beetles used in the experiments. The measurements of transmittance exclude all wavelengths higher than 900 nm and will probably be underestimates since the assumption was made that the transmittance at longer wavelengths was, on average, the same as that in the range 200–900 nm. A larger transmittance would yield a slightly higher temperature excess for both melanic and non-melanic beetles. However, even if all radiation of longer wavelengths is transmitted, the difference in temperature excess between morphs would be largely unaffected, and the maximum absolute increase in  $\Delta T$  would be 2.7°C. In reality, however, not all radiation of long wavelengths is expected to be transmitted (see Porter, 1967), so this effect on  $\Delta T$  will be smaller. Porter (1967) gives an example of a black carabid beetle, where transmission was mostly in the infrared spectrum and was approximately 2.4% of the incident radiation. Potential problems associated with the invasive techniques used for measuring body temperature could be avoided in the future by employing thermal imaging technology (see e.g. Stavenga *et al.* 1993).

Our model predicts that an increase in wind speed reduces the temperature excess of melanic beetles at a faster rate than that of non-melanic beetles (Fig. 2C–E). For beetles of 4 mm diameter, the predicted temperature excesses at a wind speed of 1.9 m s<sup>-1</sup> (representing a slight breeze) are 3.0°C and 2.4°C for melanic and non-melanic morphs, respectively. The measured effect is qualitatively as expected: a drop in

temperature excess for both non-melanic and melanic morphs and a reduction in the difference between morphs. Again, the actual temperatures measured are quite close to those predicted by the model (3.7°C and 3.0°C; see Fig. 3). The results of these experiments clearly show the importance of wind speed in understanding differences in equilibrium body temperatures between the morphs.

We also investigated the warming-up rate of each morph. The considerably more complex models that have been devised to describe this parameter (Gates, 1980) predict that dark insects will warm up faster than lighter ones (Brakefield and Willmer, 1985). We found no significant difference between non-melanic and melanic ladybirds in the rate of increase in body temperature, but the variation between individual beetles was very large. This may have been due to variations in the activity of the beetles. We found no clear influence of body mass on either equilibrium body temperature or warming-up rate, although body mass is expected to be positively correlated with the equilibrium temperature (Fig. 2G–I) and negatively with warming-up rate (Digby, 1955; Willmer and Unwin, 1981; Brakefield and Willmer, 1985; Stevenson, 1985). Perhaps the size range of the ladybirds used in these experiments was not sufficiently large to detect a significant size effect.

We also measured the walking speed and time to activity of each morph to examine the consequences of the differences in body temperatures and warming-up rates. Most behavioural processes can be related to body temperature by means of a performance curve (see e.g. Huey and Kingsolver, 1989). Fig. 4B shows that both ambient temperature and radiation intensity positively influence walking speed. Under almost all circumstances, walking speeds of melanic beetles are significantly higher than those of non-melanic morphs (Fig. 4B; Table 3). The results of the control experiment in which the wings and elytra were removed show that such differences are due to the different properties of the pigmented elytra. The effect is strongest at intermediate conditions of temperature and radiation (Fig. 4B; Table 3). The model predicts that, at low radiation intensities, the difference in temperature excess between melanic and non-melanic ladybirds is relatively small (Fig. 2B). If the ambient temperature is also low, actual body temperatures of both morphs are expected to be low and in the lower tail of the curve linking walking speed to body temperature. If this curve is similar for both morphs, the small temperature difference is then expected to lead to relatively small differences in performance. For the highest radiation intensities and temperatures used in this study, the predicted body temperature for both morphs will be approximately 20°C, which is presumably close to the optimal body temperature for walking (see Heinrich, 1993, p. 520). Close to this optimum, even relatively large differences in temperature excess are expected to lead to only minor differences in walking speed, because of the levelling off of the performance curve.

The body mass of the ladybirds only had an effect on walking speed at the lowest temperature (3°C). Fig. 2G–I predicts that the effect of body size on temperature excess will be strongest at low ambient temperatures. Depending on the precise shape

of the curve linking body temperature to walking speed, this could lead to a measurable effect of body size on walking speed. Close to the optimum body temperature, at higher ambient temperatures, the effect of body size is smaller and, because the optimum curve levels off, the small effect on body temperature is likely to lead to differences in walking speed that are no longer measurable. A similar argument applies to the difference between melanic and non-melanic beetles in the accumulation of enough heat to become active; this is expected to be most pronounced at low, limiting light intensities and particularly at low ambient temperatures. Fig. 4A shows that our measurements are consistent with this prediction.

The effects of wind speed on walking speeds are also consistent with the predictions from the model (Figs 2C–E, 5B). Body temperatures are expected to drop with increasing wind speed and to a greater extent for melanic than for non-melanic beetles. This is because heat loss through convection is proportional to the temperature difference between the body and the surrounding medium, which is larger for melanic beetles. This effect is expected to decline with increasing wind speed. Measured walking speeds were significantly higher for melanic morphs than for non-melanic beetles at  $0.4 \text{ m s}^{-1}$ , but not at higher wind speeds, while the walking speeds of both melanic and non-melanic morphs decreased with increasing wind speeds (Fig. 5B). The effect of wind on time to activity showed a negative trend for each morph, which was unexpected assuming that time to activity is a measure of the time needed to reach a threshold temperature for walking. It is possible that the presence of wind encourages the ladybird to start moving, perhaps to avoid cooling effects or desiccation or the danger of becoming dislodged. Although none of the ladybirds walked at the start of the measurements in any series of experiments, suggesting that the time to activity does represent the time necessary to reach some minimum threshold walking temperature, time to activity may also be a measure of behavioural willingness to walk after being transferred from the dark to the light. This willingness may also be influenced by wind speed, as described above.

Our measurements and model predictions lead to a number of conclusions relevant to thermal melanism in ladybirds. First, the thermal advantage to melanic morphs is expected to be more pronounced at low ambient temperatures, because the contribution of radiation to the time to activity is then relatively large. Second, higher wind speeds will rapidly reduce any potential thermal advantage to melanic morphs, because convective cooling is proportional to the surface temperature of the insect. Third, variable levels of sunshine, especially at low ambient temperatures, are expected to favour melanic morphs since they respond faster to changes in radiation intensity. Fourth, a high intensity of radiation can lead to a larger thermal advantage for melanic morphs. This study provides a basis for exploring the relevance of these effects in natural populations.

One factor influencing body temperature which has, until now, received surprisingly little attention in the ladybird literature is heat loss through convection (see Digby, 1955; Church, 1960; Stevenson, 1985; Heinrich, 1990). Our results emphasize that convection, especially in small insects

(Stevenson, 1985), is one of the major parameters determining body temperature. In general, our development of the model shows that, at low ambient temperatures, even very small insects (up to 10 mg) are expected to exhibit a temperature excess in still air. This is consistent with measurements on other insects reported by Willmer and Unwin (1981) and Stevenson (1985). Therefore, in our opinion, Stevenson's conclusion that small insects cannot elevate their body temperature above that of the ambient air is incorrect.

## Appendix

$C$ ( $\text{W m}^{-2}$ )	convection
$D$ (m)	characteristic dimension
$h_c$ ( $\text{W m}^{-2} \text{K}^{-1}$ )	convection coefficient
$k$ ( $\text{W m}^{-1} \text{K}^{-1}$ )	thermal conductivity of medium
$M$	metabolic heat production
$Nu$	Nusselt number
$Q_a$ ( $\text{W m}^{-2}$ )	energy flux absorbed by the elytra
$Q_A$ ( $\text{W m}^{-2}$ )	energy flux absorbed by the body
$Q_c$ ( $\text{W m}^{-2}$ )	conduction
$Q_e$ ( $\text{W m}^{-2}$ )	emission
$Q_r$ ( $\text{W m}^{-2}$ )	reflection
$Q_s$ ( $\text{W m}^{-2}$ )	incident radiation
$Q_t$ ( $\text{W m}^{-2}$ )	transmission
$r$	reflectance
$R$ (m)	radius of a sphere
$R_b$ (m)	radius of the body
$R_r$ (m)	radius of the elytra
$Re$	Reynolds number
$t$	transmittance
$T_a$ ( $^{\circ}\text{C}$ )	ambient temperature
$T_b$ ( $^{\circ}\text{C}$ )	body temperature
$T_r$ ( $^{\circ}\text{C}$ )	surface temperature
$v$ ( $\text{m s}^{-1}$ )	velocity of medium (wind)
$\Delta T$ ( $^{\circ}\text{C}$ )	temperature excess
$\varepsilon$	emissivity
$\nu$ ( $\text{m}^2 \text{s}^{-1}$ )	kinematic viscosity of medium
$\sigma$ ( $=5.673 \times 10^{-8} \text{ W m}^{-2} \text{K}^{-4}$ )	Stefan–Boltzmann radiation constant
$\tau$ ( $\text{s}^{-1}$ )	time constant

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