

Effects of phenotypic variation onto body temperature and flight activity in a polymorphic insect

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Abstract. According to biophysical principles, colour and size are important phenotypic factors that may influence body temperature and activity in ectothermic insects. In taxa showing female-limited polymorphism, males and female morphs differ in body colour, size and activity pattern. However, no previous study has evaluated whether such phenotypic and behavioural variation relates to differences between males and female morphs in thermal properties. In the present study, the relationships between body colour, size, activity and body temperature are examined under laboratory and field conditions, for the polymorphic damselfly *Enallagma cyathigerum* (Charpentier, 1840) (Odonata: Zygoptera). Contrary to expectation, males and female colour morphs of this species do not differ in thermal properties (i.e. heating characteristics or field body temperatures). When questioning phenotype and activity, temperature does not appear to be relevant for understanding the maintenance of female-limited polymorphism.

Key words. Ambient and body temperature, colour, damselflies, female-limited polymorphism, size, thermal properties, thermoregulation.

Introduction

The behavioural and ecological performance of ectotherms depends largely on their body temperature (Huey & Kingsolver, 1989). Body temperature, in turn, is primarily a function of ambient conditions. Nonetheless, flying ectothermic insects in temperate and arctic regions often require body temperatures exceeding those of the surrounding environment for flight (Dreisig, 1995; Van Dyck & Matthysen, 1998). The ability of an individual to attain suitable body temperatures for activity may rely on its phenotypic properties. Phenotypic factors known to influence body temperature are colour and size (Digby, 1955; Willmer & Unwin, 1981; Heinrich, 1996). Dark-coloured individuals heat up faster and attain higher steady-state body temperatures than light-coloured phenotypes (Watt, 1968; De Jong *et al.*, 1996; Forsman *et al.*, 2002). Therefore, dark-coloured individuals can be active under

colder ambient conditions, allowing them to accumulate more resources or increase mating success (Willmer, 1991) when environmental temperatures are relatively low. However, this comes at the cost of an increased risk of overheating at high ambient temperatures. Heating capacity also is affected by body size, with larger individuals warming up more slowly, but achieving greater temperature excess, than smaller ones (Digby, 1955; Stevenson, 1985; Heinrich, 1996).

In natural populations of many insect species, different female colour morphs coexist with one conspecific male phenotype (butterflies: Cook *et al.*, 1994; damselflies: Fincke *et al.*, 2005). Female-limited polymorphism is controlled genetically by variation at a single locus with sex-limited expression (Andrés & Cordero, 1999; Sánchez-Guillén *et al.*, 2005) and selection appears to contribute to its maintenance (Andrés *et al.*, 2000; Wong *et al.*, 2003; Van Gossum *et al.*, 2005). Current adaptive explanations assume mostly that multiple female morphs have evolved primarily as a mechanism to avoid costly sexual harassment (Cordero *et al.*, 1998; Miller & Fincke, 1999; Sherratt, 2001; Van Gossum *et al.*, 2001a). In addition, it is suggested that differences in thermal properties between female morphs may contribute to the

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maintenance of female-limited polymorphism (Svensson & Abbott, 2005; Bots *et al.*, 2007; Van Gossum *et al.*, 2007). Based on phenotypic differences, it can be expected that males and different female morphs will vary in body temperature. First, whereas female morphs usually do not appear to differ in size (Lajeunesse & Forbes, 2003; Robb *et al.*, 2006; but see also Cordero *et al.*, 1998), females are often larger than males, which is the result of natural selection promoting large clutch sizes (Fincke *et al.*, 1997). Second, female morphs show variation in body colouration, with one female morph resembling the conspecific male in body colouration (andromorph), whereas the other does not (heteromorph). These phenotypic differences between males and female morphs may affect heating rates, body temperature and, ultimately, aerial activity. Interestingly, males and different female morphs show different proportions of time spent perching and flying (Forbes *et al.*, 1997; Van Gossum *et al.*, 2001b). For example, heteromorphs fly more regularly and cover larger distances than andromorphs. Despite the reported differences in phenotype and activity of species showing female-limited polymorphism, to our knowledge, no studies exist evaluating differences in thermal properties.

The present study examines the hypothesis that phenotypic differences between males and female morphs influence heating rate, body temperature and flight activity for the damselfly *Enallagma cyathigerum* (Charpentier, 1840) (Odonata: Zygoptera). Males of this species show bright blue body colouration with limited black patterning, whereas female body colouration is either brown to greenish brown (heteromorph) or blue as the conspecific male (andromorph). Both female morphs have a higher amount of black abdominal patterning than the male (Askew, 2004). Blue is considered to be a lighter colour than brown as it is known to function as an 'overheating protector' in odonates (Sternberg, 1996). Therefore, heteromorphs are expected to heat up faster, reach higher equilibrium temperatures and initiate flight at lower ambient temperatures than males, whereas trait values are expected to be intermediate for andromorphs. In addition, it is anticipated that larger animals will heat-up more slowly. These predictions are tested by determining heating characteristics and the threshold for flight in a laboratory heating experiment and by assessing body temperatures and activity pattern at a wide range of ambient conditions in the field. If the hypothesis that morphs do differ in thermal properties gains support, future studies may explore further its relevance in maintaining female-limited polymorphism.

Materials and methods

Study species

The polymorphic damselfly *E. cyathigerum* is widespread throughout Europe and is found near a variety of water bodies ranging from acidic fens to slightly eutrophic ponds (Askew, 2004). For the location employed in the present study, the species is univoltine and the flight period lasts from May until August.

Heating experiment

Heating rates of males and female morphs were assessed under controlled laboratory conditions in June 2005. To this end, mature damselflies were caught in the same heathland area where field observations were performed. After emergence from the water, damselflies require several days to weeks (depending on the species) to develop their mature body colouration (Corbet, 1999). To avoid the confounding effects of immaturely coloured individuals, only individuals engaged in mating were collected. All collected individuals were inspected visually to have a mature body colouration and, for females, a swollen abdomen (indicating the presence of developed eggs and confirming mature status). After capture, the damselflies were transported to the laboratory in small plastic vials inside a cool box to minimize handling stress.

At the start of a heating experiment, an insect temperature probe (a copper-constantan thermocouple Type MT-29/1B with time constant 0.015 s mounted in a 29-gauge hypodermic needle; Physitemp Instruments, Clifton, NJ) was inserted ventrally into the thorax of the damselfly. This position was chosen because flight activity depends on the temperature of the flight muscles, which are located in the thorax (Dudley, 2000). The probe and the damselfly were mounted on a rod mimicking a natural roosting posture. To prevent damselflies escaping or dislodging from the insect probe, their abdomen was attached to the rod with double-sided tape. The entire set-up was then placed in a refrigerator and was cooled for approximately 120 s until the temperature of the individual measured 9–10 °C, which is well below the normal lower activity temperature (± 19 °C) of *E. cyathigerum* (Hilfert-Rüppell, 1998). Although, in some Odonates, cooling may temporarily change body colouration (Sternberg, 1987), this was not the case for *E. cyathigerum* during the present study. Next, the set-up was placed under a light source in a blacked out room. The light source was a mercury vapour lamp (Hönle, SOL 500, Hönle UV Technologie, Gräfelfing, Germany) radiating a spectrum that corresponds to that of natural sunlight (Van Dyck & Matthysen, 1998). Preliminary heating experiments showed that a distance of 0.55 m between the damselfly and the lamp resulted in suitable rates of heating. The insect probe was connected to a data logger (Delta T logger type DL-2, Delta T devices, Cambridge, U.K.; accuracy 0.01 °C) and body temperature was recorded with 1-s intervals within seconds of removal from the fridge (T_{initial}) until termination of the experiment. Temperature registration was stopped when the damselfly started flapping its wings (i.e. opened its wings towards a horizontal position and showed wing beat movements). The body temperature recorded at this point is further referred to as T_{flight} . The threshold for flight is of major importance in damselflies given that all their activities rely highly on a quick and efficient ability to fly: from catching prey, to mating and escaping from predators. After completion of the heating experiment, the damselfly was measured for fresh body mass (MT5 balance, mettler Toledo, Zaventem, Belgium; ± 1 μg) and body length (from the tip of head to the end of abdomen;

Absolute digimatic caliper, Mitutoyo, Veenendaal, The Netherlands; ± 0.01 mm).

Only measurements from individuals ($n = 43$) that opened completely and moved left and right wings within 120 s of the start of the experiment were retained for further analysis. The described procedure resulted in data on the changes in body temperature of male and female morphs during the time they were heated. To obtain individual estimates of T_{\max} (i.e. equilibrium temperature that would be attained if the damselfly would not start flying) and τ (i.e. time constant of temperature increase), heating curves of individual damselflies were fitted with the model (all $r^2 > 0.96$):

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

in which t is time and $T(t)$ is the body temperature at time t (De Jong *et al.*, 1996).

Field measurements

Fieldwork was conducted on seven different days between 30 June and 19 July 2003, at a heathland nature reserve (north of Antwerp, Belgium). The weather conditions varied considerably over this period; thus, the body temperatures obtained are characteristic for the wide range of thermal circumstances under which this species can be active. No measurements were made on rainy days, when *E. cyathigerum* remains inactive. Body temperature was measured in individuals found both near and away from the reproductive area (i.e. the water's edge).

On days with suitable weather, a constant procedure was used to assess body temperatures and weather conditions. Upon encountering an adult damselfly, its sex, morph, activity (perching, flying, or mating) and height in the vegetation (to the nearest 0.1 m) was noted. The damselfly was then caught in an insect net. An insect thermal probe (technical description above; see also Shelly, 1982; Watanabe, 1991) was inserted ventrally into the thorax, directly through the net, avoiding any direct contact between the researcher and the animal. Body temperature (T_b) was read, within 5 s of capture, from a portable electronic thermometer (Bailey-Bat 12, Physitemp Instruments, Clifton, NJ; precision 0.1 °C) connected to the insect probe. Immediately thereafter, air temperature (T_a) was determined at the site of capture, at a height of 1 m, with the insect probe shielded from direct sunlight (Christian & Tracy, 1985). Wind (Beaufort scale: http://www.zetnet.co.uk/sigs/weather/Met_Codes/beaufort.htm) and solar radiation conditions (cloud coverage, on a scale from one to eight with one a clear sky and eight completely overcast) were also noted. Body length was measured (from the tip of head to the end of abdomen) using a digital calliper (Digimax, York Survey Supply Centre, York, U.K.; ± 0.1 mm). Finally, the damselfly was released at the site of capture.

Statistical analysis

The variation in T_{initial} , τ , T_{\max} , T_{flight} , body length and mass among males and female colour morphs were analyzed using

analysis of covariance (ANCOVA). Explanatory variables (fixed effects) were body mass, type (male, andromorph female, heteromorph female) and their interactions. In addition, T_{initial} was included as an extra explanatory variable into the analyses of τ , T_{\max} and T_{flight} . In all analyses, type was treated as a class variable. Post-hoc paired comparisons between morph means were carried out with Tukey–Kramer adjustment.

Differences in body length, T_a and T_b among males and female colour morphs were assessed with the ANCOVA procedure. In the analysis of T_a , explanatory variables were activity (perching, flying, mating), type, and their interactions. In the analysis of T_b , also T_a , T_a^2 , height in the vegetation and body length were included as additional explanatory variables. In all analyses, activity and type were treated as class variables. Post-hoc paired comparisons between morph means were carried out with Tukey–Kramer adjustment.

Analyses were conducted in SAS 9.1 (SAS Institute Inc., 2005). Model selection started with the full model, including all interactions and preceded with the removal of non-significant terms until only significant terms remained in the model (Verbeke & Molenberghs, 1997). Descriptive statistics are reported as the mean \pm SE.

Results

Heating experiment

Males and female colour morphs used in the heating experiment differed in body mass ($F_{2,40} = 26.72$, $P < 0.01$). On average, males (37.62 ± 1.44 mg) weighed considerably less than heteromorphs (Tukey– $P < 0.01$; 47.77 ± 1.48 mg) and andromorphs (Tukey– $P = 0.01$; 44.98 ± 1.59 mg). Female morphs, however, did not differ in mean body mass (Tukey– $P = 0.41$). The relationship between body length and mass did not differ among males, andro- and heteromorphs ($F_{2,38} = 0.146$, $P = 0.87$). Because body mass was highly correlated with body length within males and within both female morphs ($F_{1,40} = 36.33$, $P < 0.01$, $r^2 = 0.69$), body mass was used as the sole index of size in further analyses.

Positioning the set-up below the vapour lamp took a few seconds, during which the animal's T_b increased slightly. However, T_{initial} was independent of type ($F_{2,40} = 1.06$, $P = 0.36$) and body mass ($F_{1,40} = 0.26$, $P = 0.62$), indicating that this increase in T_b did not differ consistently among groups. We found no evidence for differences between males, andromorph and heteromorph females in the three studied characteristics of the heating curves (τ , T_{flight} , T_{\max}) (all $P > 0.05$; Table 1). Thermal time constants (τ), T_{\max} and T_{flight} were also independent of mass (τ : $F_{1,37} = 0.06$, $P = 0.81$; T_{\max} : $F_{1,39} = 0.40$, $P = 0.53$; T_{flight} : $F_{1,40} = 1.19$, $P = 0.28$) and T_{initial} (τ : $F_{1,40} = 1.88$, $P = 0.18$; T_{\max} : $F_{1,38} = 0.07$, $P = 0.79$; T_{flight} : $F_{1,35} = 0.43$, $P = 0.52$).

Field measurements

Body temperatures (T_b) were obtained of 280 male and female damselflies active at a variety of ambient conditions.

Table 1. Thermal time constants (τ), body temperatures at the onset of flight (T_{flight}) and equilibrium body temperatures (T_{max}) for males and two female colour morphs of the damselfly *Enallagma cyathigerum*, as derived from the heating experiment.

	τ (s)		T_{flight} (°C)		T_{max} (°C)	
	Mean	Range	Mean	Range	Mean	Range
Males ($n = 16$)	90.7 \pm 6.9	43.3–144.6	22.2 \pm 0.9	15.6–27.5	32.5 \pm 2.7	15.8–61.7
Andromorph females ($n = 13$)	108.1 \pm 8.5	70.2–170.4	23.0 \pm 0.9	18.3–31.2	34.1 \pm 2.3	24.2–50.1
Heteromorph females ($n = 14$)	104.5 \pm 6.4	73.1–154.6	22.3 \pm 0.9	15.9–29.2	33.7 \pm 1.6	25.4–44.4

Data are the mean \pm SE.

With one exception (T_b 1.2 °C below T_a), T_b was always higher than T_a (0.3–8.7 °C). Air temperatures (T_a) were in the range 15.9–31.2 °C, cloud coverage varied from a bright open sky to completely overcast (0–8) and wind conditions varied between 0 and 4 Beaufort. Weather parameters were inter-correlated (T_a and cloud coverage, $n = 280$, $r = -0.72$, $P < 0.01$; T_a and wind velocity, $n = 280$, $r = -0.26$, $P < 0.01$). To simplify comparisons among groups, we therefore chose to keep T_a as the sole measure of ambient conditions in further analyses.

Males and female colour morphs measured for field body temperatures differed significantly in body length ($F_{2,277} = 12.72$, $P < 0.01$). On average, males (32.1 \pm 0.1 mm) were significantly larger than both andromorph females (Tukey- $P = 0.03$, 31.6 \pm 0.2 mm) and heteromorph females (Tukey- $P < 0.01$, 31.4 \pm 0.1 mm). Female morphs, however, did not differ in mean body length (Tukey- $P = 0.27$).

In the statistical model for T_a , the interaction activity \times type was significant ($F_{3,269} = 7.39$, $P < 0.01$), indicating that males and female colour morphs exhibited different types of behaviour at different air temperatures. Males and both female morphs engage in mating at relatively high T_a , but females (of both morphs) tended to perch and fly at lower T_a than males (Fig. 1).

Both T_a ($F_{1,275} = 19.41$, $P < 0.01$) and T_b^2 ($F_{1,275} = 62.29$, $P < 0.01$) contributed significantly to the statistical model of T_b . Figure 2 shows that the quadratic nature of the relationship between T_b and T_a results from a relatively smaller difference between these two measures at high T_a . Body length also tended to affect T_b , with larger animals attaining lower T_b ($F_{1,272} = 3.78$, $P = 0.05$). Controlling for T_a and body length, males and female colour morphs tended to differ in T_b ($F_{2,272} = 3.14$, $P = 0.05$). Under all environmental conditions, males had slightly higher T_b than females, with female colour morphs showing comparable T_b . However, Figure 2 indicates that this difference between the sexes was only minor (approximately 0.5 °C), which may suggest that this effect is biologically insignificant. Indeed, when body length was excluded from the model, differences in T_b among males and female colour morphs were no longer statistically significant ($F_{2,273} = 2.14$, $P = 0.12$). The effects of activity ($F_{2,270} = 1.13$, $P = 0.32$) and height in the vegetation where the damselfly was detected ($F_{2,263} = 1.31$, $P = 0.25$) on T_b were also non-significant.

Discussion

Studies on a variety of species, with differentially coloured morphs (including insects similar in size to damselflies), lend empirical support to the concept that body colouration and body size (Honěk, 1993; Forsman, 1997; Van Dyck & Matthysen, 1998; Bittner *et al.*, 2002; Pereboom & Biesmeijer, 2003; Kemp & Krockenberger, 2004; Tanaka, 2005) can have a profound effect on the heating rate and body temperature of ectotherms. Nevertheless, no effects of colour and size are observed in the polymorphic damselfly *E. cyathigerum*, either under experimental or under natural conditions. Differences in activity pattern between males and females (both andro- and heteromorphs) are observed; however this appears to be caused by the dependence of behaviour on ambient conditions.

The heating experiment suggests that the differences in colour and black patterning among males and female morphs do not affect heating rates and equilibrium temperatures. The experiments also fail to find any effect of body size. Because the same equipment (light source and thermocouple probe) is used as in a previous experiment, where differences in heating rate are present between differently coloured individuals

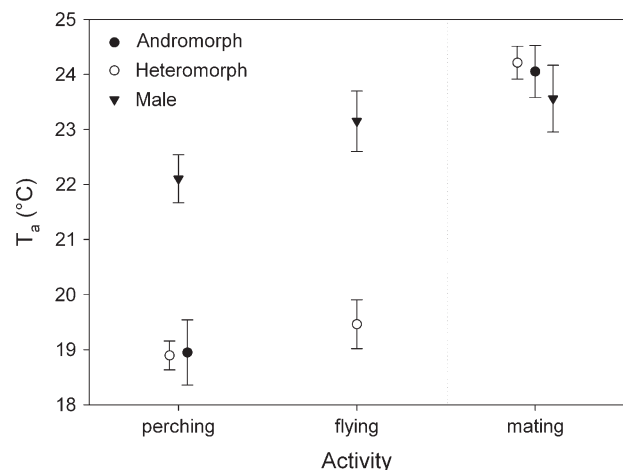


Fig. 1. The activity pattern of males and female morphs of the damselfly *Enallagma cyathigerum* as a function of air temperature (T_a). Both female morphs are found perching and flying at lower T_a than males, but no difference exists for mating individuals.

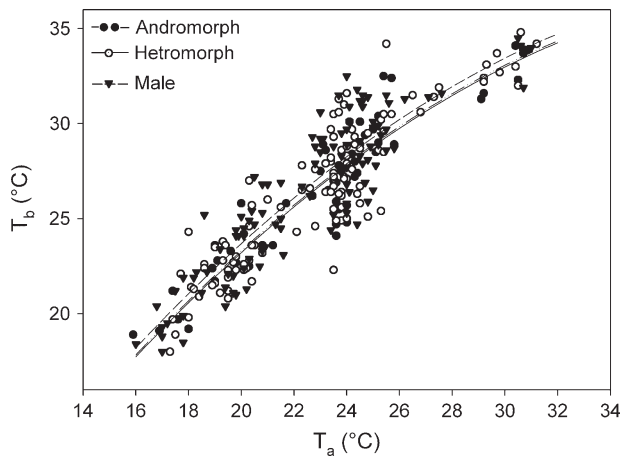


Fig. 2. Relation between air temperature (T_a) and body temperature (T_b) for males, andromorph and heteromorph females of the damselfly *Enallagma cyathigerum*. At all environmental condition, the T_b of males appears slightly higher than T_b of both female morphs. However, this effect is no longer significant when body size is removed from the statistical model.

of a butterfly (Van Dyck & Matthysen, 1998), the lack of effect in the present study is not due to imprecise measurements. More likely, the differences among individuals in colour and size are too small to have a measurable effect. It can be argued that differences in heating properties that may have remained unnoticed are too small to have any ecological significance, certainly when considering the relative importance of the other elements in the energy budget equation that do not depend on colour and/or size (e.g. convective heat exchange, posturing; Gates, 1980; Campbell & Norman, 1998).

Field measurements show that *E. cyathigerum* can be seen at a wide range of air temperatures ($16\text{ °C} < T_a < 32\text{ °C}$). Because there are no available data on the temperature sensitivity of ecologically relevant physiological functions, and we also have insufficient information on the distribution of operative temperatures in the habitat (Bakken, 1992), the thermoregulatory effectiveness (sensu Hertz *et al.*, 1993) cannot be comprehensively assessed. The present data suggest little or no thermoregulation occurs (linear regression of T_b on $T_a \approx 1$), which is in line with the view that damselflies have limited behavioural or physiological control over their body temperatures (Shelly, 1982; Watanabe, 1991). Despite this, the analysis picked up a significant quadratic effect in the relationship between T_b and T_a , indicating some thermoregulatory control. The temperature excess ($T_b - T_a$) in field-active *E. cyathigerum* decreases slightly at higher air (and body) temperatures, suggesting that animals are then actively avoiding overheating. Possibly, *E. cyathigerum* regulates its body temperatures behaviourally at very high air temperatures by operating in more shaded or denser vegetation (J. Bots, personal observation).

Interestingly, female (both morphs) *E. cyathigerum* are seen perching and flying at lower air temperatures (and thus

body temperatures) than males. One hypothesis could be that males and females have different thermal preferences or physiological optima, or that females become active more readily at suboptimal temperatures. However, this appears to be in disagreement with the lack of variation in T_{flight} in the heating experiment of the present study. An alternative explanation involves the thermal dependence of mating behaviour and the typically male-biased operational sex ratio in damselflies (Parr & Palmer, 1971; Stoks, 2001a, b). Mating occurs only at relatively high temperatures. Due to the skewed operational sex ratio, all females flying or perching at high air temperatures will immediately be grasped by a sexually interested male. That is, solitary perching or flying females can only be caught (by the experimenter) under relatively poor ambient conditions. The situation for males is different, with solitary males being observed under all weather conditions. Those males seen solitary despite favourable weather conditions most likely are unable to acquire a partner due to the surplus of males.

In conclusion, contrary to expectations, no effects are found with respect to body colouration or size on heating rates, body temperatures and the activity pattern of males and female morphs. Therefore, it is considered unlikely that temperature-mediated effects contribute to the maintenance of female-limited polymorphism. However, it cannot be ruled out that temperature plays a role. Physiological functions other than flight activity, such as egg production, may also be temperature sensitive, and physiological optima may differ between female morphs (Svensson & Abbott, 2005).

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