Thermal capacity of different colour morphs in the pygmy grasshopper *Tetrix subulata*

Anders Forsman

Forsman, A., Department of Zoology, Uppsala University, Villavägen 9, S-752 36 Uppsala, Sweden

Received 15 January 1997, accepted 11 April 1997

I examined the relative importance of colour pattern, body size and shape for thermoregulation by experimentally subjecting adult female *Tetrix subulata* belonging to four different colour morphs (black, striped, brown, and white) to augmented irradiation levels. The results revealed significant variation in temperature excess (i.e., the difference between ambient and body temperature) among colour morphs, with black individuals having a mean temperature excess 49% greater than that of white individuals. Temperature excess was not significantly influenced by overall body size (measured as principal component 1 (PC1)) but increased with increasing values of PC2, suggesting that stout bodied individuals attained higher body temperatures than did individuals with low body mass relative to surface area. These results show that coloration may play an important role in determining body temperatures of grasshoppers exposed to direct irradiation, and suggest that reproductive females may be able to maintain higher body temperatures than non-reproductive females and males.

1. Introduction

Body temperature has profound effects on many aspects of the physiological and behavioural repertoire of ectothermic animals, with each bodily function apparently showing a thermal performance curve with a distinct maximum (e.g. Huey & Kingsolver 1989). In environments where animals cannot easily maintain optimum body temperatures, one may therefore expect individual differences in characters that affect thermal capacity (i.e., rates of heating and cooling of the body) to ultimately translate into differential fitness (Willmer 1991). For most ectotherms, body size is one of the most important determinants of body temperature (e.g. Willmer & Unwin 1981, Stevenson 1985). However, many fitness components may be correlated with body size for reasons other than differential body temperature. It may be difficult therefore to study the effect of thermal capacity on fitness in systems where differences in body temperature are due primarily to body size. Populations consisting of individuals differing in colour pattern, in contrast, are ideal for this purpose. Because colours differ in reflectance and absorbtion of incident radiation, the colour of an animal's surface may significantly influence the rate at which it converts solar radiation into body heat as well as its equilibrium body temperature (Digby 1955, Uvarov 1966, Forsman 1995a). Colour pattern is generally considered less important than body size in determining temperature excess in insects (e.g. Digby 1955, Willmer & Unwin 1981, Brakefield & Willmer 1985). However, studies of intraspecific variation have identified colour pattern as actually being more important than size (e.g. Watt 1968, Stewart & Dixon 1989), and even if the colour of an insect contributes no more than, say, 25% to its temperature excess (Digby 1955), this difference may have significant and predictable effects on animal capabilities. Insect populations polymorphic for colour pattern thus offer unique opportunities to study not only the relative importance of body size and coloration for temperature excess, but also the direct and indirect links between morphology, thermal capacity, physiological and behavioural performance and fitness.

Here I report on an experiment where adult female grasshoppers *Tetrix subulata* (L.) belonging to four different colour morphs were subjected to augmented irradiation levels. The chief purpose was to examine the relative importance of colour pattern, body size and shape for temperature excess.

2. Materials and methods

Tetrix subulata (L.) (Tetrigidae) is a small (approximately 10 mm), short-limbed ground-hopper that is widely distributed in Europe (Holst 1986). It is characterised by a long pronotum that extends beyond the apex of the abdomen and out over the middle of the hind tibia. It is polymorphic for colour pattern, a characteristic shared with many other species of grasshoppers (Rowell 1971). Within a single population one may at the same time find individuals varying from black, through yellowish-brown to white, with some individuals being monochrome and others having distinct patterns, such as a light narrow longitudinal stripe or two prominent black spots on the upper surface of the pronotum.

I collected adult female *Tetrix subulata* belonging to four different colour morphs (black, striped, yellowish-brown, and white) from a recently burnt forest clear-cutting outside Uppsala, Sweden, on 7 June 1995. Only the striped morph had an apparent pattern (black but with a yellowish narrow mid-longitudinal stripe on the pronotum); the black, brown and white morphs were uniformly coloured dorsally. The animals were brought to the laboratory where they were measured (pronotum length and maximum thorax width using digital callipers to the nearest 0.01 mm, and live body mass to the nearest 0.0001 g) and housed individually in plastic cages. Live grasshoppers were then individually attached to one of two thermocouples using a loop of thin thread so that the thermocouple was situated between the pronotum and the dorsal side of the abdomen. The two thermocouples (one naked and one affixed to a grasshopper) were attached to a quick-reading digital thermometer (FLUKE model 52 K/J) and placed side by side (2.5 cm apart) 10 cm below a lamp (LUMA model 3×longlife, 220 V, 40 W, opaque). Because movements may influence body temperature, each grasshopper was allowed 20 min to settle before measurements were taken. Ambient (naked thermocouple) and body temperatures of each grasshopper were recorded both before the lamp was switched on and after 15 min of irradiation. Individuals belonging to different colour morphs were measured in random order. The experiments were conducted at room temperature (20-22°C).

Temperature excess was calculated as the difference between ambient and body temperature (i.e., by subtracting the temperature reading of the naked thermocouple from the reading of the thermocouple with a grasshopper attached to it). To examine whether body temperature was affected by augmented illumination levels I tested for a difference in temperature readings between the two thermocouples using a paired comparisons t-test (Sokal & Rohlf 1981). This analysis was applied to data for each morph separately. An F_{max}-test (Sokal & Rohlf 1981) revealed significant heterogeneity of variances in temperature excess among colour morphs ($F_{9,9} = 7.71$, P < 0.01). I therefore tested for differences in temperature excess among morphs using the Kruskal-Wallis one-way analysis of variance (Siegel & Castellan 1988). To ensure that differences in temperature excess between morphs were not due to factors other than irradiation the sequence of tests was applied also to measurements taken before the light was switched on.

3. Results and discussion

Thirty-eight female grasshoppers were used in the experiment (pronotum length $\overline{X} = 12.1 \text{ mm}$, S.D. = 0.76 mm; thorax width $\overline{X} = 3.6 \text{ mm}$, S.D. = 0.16 mm; live body mass $\overline{X} = 0.066 \text{ g}$, S.D. = 0.0098 g). Significant positive associations were evident between all three pairs of size variables, with Pearson correlation coefficients ranging from 0.53 to 0.67 (all P < 0.001). A multivariate analysis of variance performed on pronotum length, thorax width and live body mass revealed no significant overall effect of colour morph on morphology (Wilk's Lambda = 0.64, $F_{9.78} = 1.73$, P = 0.10).

3.1. Relationship between colour pattern and temperature excess

Body temperatures did not differ from ambient temperature before the light was switched on (Table 1). Nor were there any differences in temperature excess among the four different colour morphs before the light was switched on (Kruskall-Wallis, $\chi^2 = 4.21$, *d.f.* = 3, *P* = 0.24) (Table 1). However, all four colour morphs showed a mean temperature excess significantly greater than 0 when exposed to augmented irradiation levels (Table 1). This shows that the radiation from the lamp was absorbed and translated into increased body temperatures. Kruskal-Wallis one-way analysis of variance further revealed highly significant variation in temperature excess among the four colour morphs ($\chi^2 = 19.77$, *d.f.* = 3, *P* = 0.0002). Multiple comparisons between treatments (Siegel & Castellan 1988: 213–215) revealed that black individuals had significantly greater temperature excess than did brown and white individuals, and that striped individuals had greater temperature excess than did white individuals. The three remaining comparisons were not statistically significant.

This greater thermal capacity of darker than lighter individuals when exposed to radiation (Table 1), conforms with expectations from theory and with most previous studies of other species of grasshoppers (reviewed by Uvarov 1966: 213– 215). The higher temperature excess of darker individuals may enable them to be active, grow, and lay eggs at lower ambient temperatures than lighter individuals (e.g. Huey & Kingsolver 1989, Hinks & Erlandson 1994). Other benefits possibly accruing to darker (and hence warmer) individuals are higher fighting ability and mating success (Willmer 1991). Such potential benefits of dark coloration may be particularly important in *Tetrix subulata* because these insects overwinter as adults and start to reproduce in early spring (Holst 1986), when ambient temperatures are low but solar radiation high. High body temperatures may also lower susceptibility to predation in these grasshoppers. Experimental evidence shows that warm individuals jump longer distances, and are more likely to escape from attacks by avian predators, compared with cold individuals (A. Forsman & S. Appelqvist unpubl.).

It should be emphasised, however, that the possible benefits accruing to dark individuals discussed above do not necessarily imply that the black colour morph has a selective advantage over lighter morphs. The fitness consequences of different colour patterns are influenced also by selection for crypsis mediated by visual predators. Indeed, experimental manipulation of phenotypes of free-ranging grasshoppers has shown that coloration significantly affects survival in this species (A. Forsman & S. Appelqvist, unpubl.). A superior thermal capacity of dark individuals therefore does not necessarily translate into higher body temperatures and superior performance in free-ranging individuals (e.g. Rowell 1971, Forsman 1995a), because selection imposed by visually oriented predators may favour different behaviours in individuals of different colour pattern (e.g. Schultz 1981, Gillis 1982, Forsman 1995b, A. Forsman & S. Appelqvist, unpubl.). Thus, in environments where animals are subjected to intense predation, black individuals may use their thermal advantage to reduce the time they expose themselves to predators rather than to increase body temperatures and activity levels (Forsman 1995a).

Table 1. Mean temperature excess (°C) for different colour morphs of *Tetrix subulata* under background and augmented radiation levels. *t* and *P* represent test results from paired comparison *t*-tests.

Colour morph						Light on		
eeleel melph	п	Mean ± S.E.	<i>t</i>	Р	Mean \pm <i>S.E.</i>	<i>t</i>	Р	
Black	10	0.02 ± 0.020	1.00	0.34	1.28 ± 0.059	21.60	0.0001	
Striped	10	0.05 ± 0.027	1.86	0.10	1.08 ± 0.053	20.25	0.0001	
Brown	10	-0.14 ± 0.092	1.52	0.16	0.93 ± 0.021	43.57	0.0001	
White	8	-0.04 ± 0.053	0.70	0.50	0.86 ± 0.026	32.78	0.0001	



Fig. 1. Relationship between temperature excess and body shape in *Tetrix subulata*. PC2 is a principal component measure of body mass relative to dorsal surface area.

3.2. Relationships between body size, shape and temperature excess

Previous studies suggest that temperature excess in insects is influenced not only by colour but also by morphology. To test whether temperature excess was affected by body size or shape I first applied a principal component analysis to a correlation matrix calculated from individual measures of pronotum length, thorax width and live body mass, using the procedure PRINCOMP (SAS Institute Inc. 1988). The principal component scores were not standardised to unit variance but have variances equal to their corresponding eigenvalue. Principal component 1 (PC1) explained 73% of the variance in morphology and is a good descriptor of overall body size, because the first eigenvector showed high positive loadings on all three variables (Bookstein 1989). Principal component 2 (PC2) explained 16% of the variance, and the second eigenvector had a high positive loading on wet body mass and moderate negative loadings on pronotum length and thorax width. PC2 may thus be thought of as a measure of relative body mass or stoutness, with high values indicating high body mass relative to dorsal surface area.

There was no significant association between temperature excess and body size (measured as PC1); this was true both for pooled data (r = -0.03, P = 0.87, n = 38), and when data for each colour morph were analysed separately (all P > 0.10).

This result is somewhat surprising considering the general consensus that size is more important than colour in determining temperature excess in insects (e.g. Digby 1955, Willmer & Unwin 1981, Brakefield & Willmer 1985). For instance, Digby (1955) argued that the coloration of an insect contributes no more than 25% to its temperature excess. However, I found that black individuals had a mean temperature excess almost 50% greater than that of white individuals. While the relative thermal excesses among colour morphs observed in this study (Table 1) are undoubtedly monotonically related to what would be seen under natural solar flux, the quantitative nature of the differences may differ. In fact, the magnitude of the difference may be even greater under natural conditions because absolute values of temperature excess are strongly influenced by the intensity and spectral composition of the radiation (Stewart & Dixon 1989). Most studies supporting the notion that size is the most important factor are based on interspecific comparisons, whereby variation in temperature among species may reflect differences not only in body size but also in shape (Willmer & Unwin 1981, Stewart & Dixon 1989). In contrast, studies that are based on intraspecific variation among individuals of the same stage typically identify colour pattern as being more important than size for thermal capacity in insects (e.g. Watt 1968, Stewart & Dixon 1989, this study).

There is little previous evidence for shape-related effects on thermal capacity in ectotherms (Willmer 1991). However, I found that temperature excess increased significantly with increasing values of PC2 (pooled data, r = 0.34, P < 0.05, n = 38) (Fig. 1), suggesting that stout bodied individuals attained higher equilibrium body temperatures than did individuals with low body mass relative to surface area. This result probably reflects a lower rate of heat loss by convection in stout bodied animals. The variation in shape, or stoutness, among the individuals used in the present experiment may reflect differences in reproductive status, but I have no data to test this possibility. The result nonetheless suggests that reproducing females that are full of eggs may be able to maintain higher body temperatures than non-reproducing females and males. This mechanism may favour females that increase clutch size at the expense of clutch frequency.

Finally, the variation in temperature excess among colour morphs remained highly significant also when I controlled statistically for the effect of PC2 (using analysis of covariance with PC2 as the covariate) (main effect of morph $F_{3,33} = 12.31$, P < 0.0001). This test result together with the apparent lack of variation in morphological variables among individuals of different colour morphs (see MANOVA results above), makes it very unlikely that the observed relationship between temperature excess and colour morph is simply a spurious correlation reflecting shape differences among morphs.

In summary, my experiment shows that the thermal capacity of these grasshoppers is significantly affected by coloration and body shape, but not by overall body size. Further studies are needed to unravel the ecological and evolutionary implications of these findings.

Acknowledgements. I thank D. Morse, B.G. Svensson, B.W. Svensson, and S. Ulfstrand for comments on the manuscript. Financial support was provided by The Swedish Natural Science Research Council and The Magnus Bergvall Foundation.

References

- Bookstein, F. L. 1989: "Size and shape": a comment on semantics. — System. Zool. 38: 173–180.
- Brakefield, P. M. & Willmer, P. G. 1985: The basis of thermal melanism in the ladybird beetle Adalia bipunctata. Differences in reflectance and thermal properties between morphs. — Heredity 54: 9–14.
- Digby, P. S. B. 1955: Factors affecting the temperature excess of insects in sunshine. — J. Exper. Biol. 32: 279–298.
- Forsman, A. 1995a: Heating rates and body temperature variation in melanistic and zigzag Vipera berus: does

colour make a difference? — Ann. Zool. Fennici 32: 365–374.

- Forsman, A. 1995b: Opposing fitness consequences of colour pattern in male and female snakes. — J. Evol. Biol. 8: 53–70.
- Gillis, J. E. 1982: Substrate colour-matching cues in the cryptic grasshopper Circotettix rabula rabula (Rehn & Hebard). — Anim. Behav. 30: 113–116.
- Hinks, C. F. & Erlandson, M. A. 1994: Rearing grasshoppers and locusts: review, rationale and update. — J. Orthopt. Res. 1994: 1–10.
- Holst, K. T. 1986: The Saltatoria of northern Europe. Fauna Entomol. Scand. 16: 1–127.
- Huey, R. B. & Kingsolver, J. G. 1989: Evolution of thermal sensitivity of ectotherm performance. — Trends Ecol. Evol. 4: 131–135.
- Rowell, C. H. F. 1971: The variable coloration of the Acridoid grasshoppers. — Adv. Insect Phys. 8: 145–198.
- SAS Institute Inc. 1988: SAS/STAT User's guide, Release 6.03 Edition. SAS Institute Inc., Cary, NC.
- Schultz, J. C. 1981: Adaptive changes in antipredator behavior of a grasshopper during development. — Evolution 35: 175–179.
- Seigel, S. & Castellan, N. J. Jr. 1988: Nonparametric Statistics For The Behavioral Sciences, 2nd ed. — McGraw-Hill, New York.
- Sokal, R. R. & Rohlf, F. J. 1981: Biometry, 2nd ed. W. H. Freeman and Company, New York.
- Stevenson, R. D. 1985: Body size and limits to the daily range of body temperatures in terrestrial ectotherms. — Am. Nat. 125: 102–117.
- Stewart, L. A. & Dixon, A. F. G. 1989: Why big species of ladybird beetles are not melanic. — Funct. Ecol. 3: 165–177.
- Uvarov, B. P. 1966: Grasshoppers and Locusts, vol. 1. Cambridge University Press, London.
- Watt, W. B. 1968: Adaptive significance of pigment polymorphism in Colias butterflies. I. Variation of melanin pigment in relation to thermoregulation. — Evolution 22: 437–458.
- Willmer, P. 1991: Thermal biology and mate acquisition in ectotherms. — Trends Ecol. Evol. 6: 396–399.
- Willmer, P. G. & Unwin, D. M. 1981: Field analyses of insect heat budgets: refelctance, size and heating rates. — Oecologia 50: 250–255.