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Influence of extreme heat or cold stresses on body pigmentation of *Drosophila melanogaster*

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ABSTRACT

Thoracic and abdominal pigmentation were measured in *Drosophila melanogaster* under a cold circadian stress (8–25 °C) and a heat one (18–33 °C) and compared to the phenotypes observed under similar but constant temperatures of 17 or 25 °C respectively. An isofemale line design permitted to submit each line (full sibs) to the four thermal regimes. Under cold stress, the pigmentation was similar to the value observed at constant 25 °C, suggesting a kind of functional dominance of the high temperature phase. In all cases, thermal stresses increased the individual environmental variance, i.e., increased the developmental instability. Genetic correlations between lines were not modified by the stresses but provided some unexpected and surprising results, which should be confirmed by further investigations: for example, negative correlations between pigmentation and body size or sternopleural bristle number. As a whole, the data do not confirm the hypothesis that under stressing conditions a hidden genetic variability could be unravelled, permitting a faster adaptation to environmental changes.

1. Introduction

Phenotypic traits are highly variable in wild living populations, and especially in plants and in ectotherm animals (Blum, 1988; Hoffmann and Parsons, 1997; Precht et al., 1973). This plasticity, generally considered as adaptive, was the main argument for Lamarck (1809) to propose as an evolutionary mechanism the heredity of acquired characters. Although the development of Mendelian genetics has shown that acquired characters are not inherited, plasticity by itself has received an increasing interest during the 21st century (DeWitt and Scheiner, 2004; Pigliucci, 2001; Pigliucci and Müller, 2010; Schlichting and Pigliucci, 1998; West-Eberhard, 2003). Phenotypic plasticity is now a significant component in the ecology of natural populations.

In the *Drosophila* evolutionary model, as well as in most insects, environmental temperature plays an important role in explaining the geographic distribution of species which are generally considered either tropical (i.e., cold sensitive) or temperate (i.e., cold tolerant) (Gibert et al., 2001, Kellermann et al., 2009). A few species, like *Drosophila melanogaster*, have been able to adapt to both temperate and tropical climates. In several species with a fairly broad latitudinal range, it is generally assumed that local thermal adaptation has produced latitudinal clines (e.g. Capy et al., 1993; van Heerwaarden and Sgrò, 2011; Hoffmann et al., 2002; Hoffmann and Week, 2007; Sgrò et al., 2010; Zwaan et al., 2000). The influence of temperature is not restricted to long range geographic variations. In a given place, temperature variability regulates the developmental conditions and modifies the phenotypes. Such variations which do not have a genetic basis are usually described as phenotypic plasticity. Temperature selective effects may involve two different processes: either a variation in the average temperature over successive seasons, or daily variations between day and night. Phenotypic plasticity in *Drosophila* according to average constant temperature is now well investigated and the response curves, or reaction norms, have been established for several quantitative traits (David et al., 2004; Gibert et al., 2004). More interestingly, it has been shown that the shapes of the reaction norms may change, in an adaptive way, between populations (Delpuech et al., 1995; Gibert et al., 1998a; Klepsatel et al., 2013; Morin et al., 1999; Pitchers et al., 2012; Mathur and Schmidt, 2016) or between species (Moreteau et al., 1997; Morin et al., 1999; Pétavy et al., 2001a, 2002; Overgaard et al., 2011). In other words, plasticity itself is a target for natural thermal selection. The

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effects of daily periodic variations are far less investigated (but see Colinet et al., 2016), although their amplitude may be important (Loeschcke et al., 1997; Feder et al., 1997).

In *D. melanogaster*, the development is possible between 11 and 31 °C. Outside this range a constant temperature does not permit the development and is considered as lethal. Lethal temperatures may, however, be tolerated if they are applied only during part of the day: the deleterious effects of the lethal phase being somehow 'cured' by a daily return to a benign temperature (Pétavy et al., 2001b).

In this paper, we asked the question: what are the specific effects of extreme daily thermal cycles upon phenotypic plasticity? We studied the effects of two extreme daily stresses, a cold one (8 °C during 12 h) and a heat one (33 °C during 12 h). Eight degree correspond to a strong stress: development is not possible below 11 °C and the developmental zero is 10 °C (Pétavy et al., 2001b). On the high temperature side, male sterility is at 30 °C and development not possible above 32 °C. The effects of thermoperiodic regimes on female's body pigmentation were investigated by comparison with flies reared at the same average constant temperature. In Drosophila, pigmentation can be investigated by considering two traits, the thorax with a darkly pigmented trident shape (David et al., 1985) and the abdomen (David et al., 1990; Pool and Aquadro, 2007). These both traits exhibit geographical clines relative to latitude (David et al., 1985; Munjal et al., 1997; Telonis-Scott et al., 2011) and several hypotheses have been proposed to explain these clines. The thermal budget hypothesis states that darker bodies will better absorb solar radiations favoring flight and general activity in cold environments. Another hypothesis invokes the role of melanin in UV protection (Bastide et al., 2014). Using a set of 33 isofemale lines (see David et al., 2005), we analyzed both phenotypic and genetic variability, revealing in most cases a clear increase of the phenotypic variability due to stress, but only little effect on genetic variability. This paper is the third of a series in which the same flies were simultaneously investigated for body size traits (Pétavy et al., 2004), thoracic sternopleural bristle number (Pétavy et al., 2006) and pigmentation (present paper). At the end of the paper, we compare the reactions of the three kinds of traits (size traits, bristle number and pigmentation) and also consider their genetic correlations. We show that the effects of stresses may vary according to the trait investigated.

2. Materials and methods

2.1. Populations and experiments

Wild living females were collected in two localities near Paris, Draveil (48°41′07″ N, 2°24′29″E) and Prunay (48°31′44″ N, 1°47′46″E), and isolated to establish a total of 33 isofemale lines (18 from Draveil and 15 from Prunay). Pairs (one female one male) were isolated in culture vials and reared at 21 °C under a 16: 8 light: dark photocycle, each pair initiating an isofemale line. From the first laboratory generation, ten pairs were randomly taken from each line as parents of the experimental flies. Three days later, parental groups oviposited directly for a few hours at 21 °C in a culture vial containing a high-nutrient, killed yeast food (David and Clavel, 1965, Blum, 1988; Hoffmann and Parsons, 1997, Lamarck, 1809, Capy et al., 1988). This operation was repeated four times for each line. Larval density was not precisely controlled but remained less than 150 adult flies per vial. After removal of the parents, the experimental vials were transferred to incubators regulated at ± 0.2 C with a continuous, of low intensity light. We used four regimes for each line: two constant mild temperatures, 17 and 25 C, and two alternating temperatures with a daily cycle of 12 h. We consider that one regime, 8–25 °C, imposed a cold stress, while the other, 18–33 °C, imposed a heat stress. In preliminary experiment, we checked that the effects of stronger stresses (6 or 35 °C induced an almost complete lethality, preventing any phenotypic analyses). For a constant temperature, the adult viability thresholds for a European population are 11 and 31 °C (Pétavy et al., 2001b). One day after emergence, adults were transferred to fresh food and maintained for a few days at 21 °C before measurements. We did not measure the viability in each line but, from other experiments with mass cultures of the same populations, viability was estimated to be higher than 90%.

2.2. Traits measured

Two kinds of pigmentation traits can be easily quantified in *Drosophila melanogaster*: the intensity of the trident pattern on the thorax mesonotum in both sexes, and the width of the black stripes on the abdomen terga 2–7 (A2-A7) in females (Capy et al., 1988; David et al., 1985, 1990). Discrete phenotypic classes are used: four for the trident pattern, from 0 (not visible) to 3 (very dark); eleven classes for the black area on abdomen terga from 0 (no pigment) to 10 (completely dark). For comparison with abdominal segments, trident pigmentation was adjusted to a maximum score of 10. In most cases we considered the six abdomen segments independently. In other cases it seemed however better to simplify the data, either by considering the sum of three successive segments (e.g. A2 + A3 + A4) or the total abdominal pigmentation (sum from A2 to A7). In all cases, the mean values were standardized to a maximum score of 10 in order to facilitate comparisons.

2.3. Data analyses

Data were treated using standard statistical methods included in the STATISTICA package (StatSoft 1996). For each sex, population and thermal regime, the variance within lines and between lines was calculated. A genetic variance was estimated from the between line variance, after a correction for family size (see Capy et al., 1994; David et al., 2005). The within line variance of full-sib families harbors both an environmental and a genetic component (Falconer and Mackay, 1996), but in most cases, the environmental component seems preponderant (David et al., 2005). Both variance components were used to calculate an intraclass correlation, which estimates broad sense genetic variability in the origin population and may be called isofemale line heritability (David et al., 2005) which is generally slightly higher than narrow sense heritability. We also considered the genetic coefficient of variation, sometimes called evolvability (Houle, 1992). Genetic correlations were estimated by calculating correlation coefficients between the means of isofemale lines without any correction for family size (see Gibert et al., 1998b). Coefficients of phenotypic correlation were also calculated within each isofemale line. In all cases, statistical comparisons were made on *z*-transformed values (Sokal and Rohlf, 1995).

3. Results

3.1. Mean phenotypic values

Mean thoracic and abdomen pigmentations for the pool of the two populations are given in Table 1 and a more detailed illustration is provided in Fig. 1. As expected (David et al., 1990), pigmentation scores were on average darker at lower temperatures. Moreover, an anteroposterior gradient was found for abdomen, the last segments being much darker. The data were submitted to ANOVA (not shown) and in all cases highly significant effects were due to line and thermal regime (*p values < 0001* for both).

A single significant population effect was found for the trident, i.e. a darker pigmentation in Draveil. Concerning the influence of the thermal regime, we considered separately the results for the thorax and the abdomen. The trident was much darker at low temperature, as expected from a well-known convex reaction norm (David et al., 1985) and the values were similar for CT 17 °C and AT 8–25 °C. This might be due to a compensation between the cold induced darkening and the warm induced lightening. If we compare, however, the 25 and 18–33 °C data, we found a much darker pigmentation under AT. This seems to be due