Pupation-temperature range in 12 *Drosophila* species from different ecological backgrounds

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Summary. A comparison of pupation-temperature range was made in the laboratory on a temperature gradient (3–38 °C) using 12 species of *Drosophila* representing four species groups and four different ecological backgrounds (temperate-montane forest: *virilis* group; desert: *repleta* group; cosmopolitan: *melanogaster* group; tropical forest: *willistoni* group). Within groups, differences are found which usually reflect species' distributions. Comparisons of species' mating-, oviposition- and pupation-temperature ranges reveal that pupation most often occurs at temperatures beyond those for mating and oviposition. Each species reflects a different combination of temperature effects. Individual species have different temperature-limits for mating, oviposition and pupation. Temperatures permissive for one response are not predictive of limits on other responses. Among species, temperature can affect a particular response differently. Within groups, species differences can be at high and/or low temperatures for any response, and temperature effects among closely related species can manifest themselves in one, or any combination of responses. One cannot predict which responses will be most and least limited, or at which end of the temperature scale a response will be most limited. Among groups, *common,* but not *absolute* temperature ranges generally correspond to the geographic distributions and ecological backgrounds of the species triads. The evaluation of temperature effects on species, based on a single activity, may not be adequate for predicting adaptive strategies.
Key words. Drosophila; temperature-effects; pupation; mating; oviposition; adaptive strategies.

Introduction

Species' geographic and microhabitat distributions are determined, in part, by environmental conditions permissive for survival and reproduction in the adult, as well as the successful maintenance of preadult stages. One vital aspect of the *Drosophila* life cycle is the ability of third instar larvae to pupate, since puparia remain immobile and exposed to potentially harmful environmental factors[13]. An environmental variable known to influence pupation in *Drosophila* is temperature (see Grossfield[10], for review).

Sokal et al.[24] observed pupation in *D. melanogaster* between 19–31 °C, with maximum height occurring at 22–25 °C. Mensua[16] also found that height increased from 13 °C to 17 °C and 25 °C, but then decreased at 29 °C. Neither paper reported temperature limits for pupation in the laboratory.

Other pupation-temperature data include the field observations of Basden[2] that *D. deflexa* larvae can overwinter and then pupate above 10 °C. Ives[11] reported that *D. melanogaster* can also overwinter as larvae. However, in a wine cellar, McKenzie[14] found no *D. melanogaster* puparia below 14 °C. At high temperature, McKenzie and McKechnie[15] found *D. melanogaster* puparia, but none for *D. simulans,* in grape residues measured at 29 ± 4.1 °C.

In the laboratory, Fogleman and Markow[8] detected a small but significant difference in the pupation site preferences (PSP) of *D. mettleri* and *D. nigrospiracula* (24.4 ± 0.2 °C and 23.1 ± 0.2 °C, respectively). Although interspecific competition has been shown to be influenced by temperature effects on the larval stage in *Drosophila*[3,9,20], it is not known how extensive species differences in pupation-temperature range are, nor is it known what the potential importance of such differences may be in determining the distributions and adaptive strategies of species.

We have shown considerable interspecific variation in both mating- and oviposition-temperature ranges[22,23], while for individual species, not only are these temperature ranges different, but temperature limits cannot be predicted from one response to the other. Kaneshiro et al.[12] stated that *Drosophila* species usually show less specificity in their adaptations for larval sites than for oviposition sites. Yet, Coyne et al.[6] report that adaptive differentiation in response to temperature stress among populations of *D. pseudoobscura* is more pronounced for puparia than for adults. Here, we report how species' pupation-temperature ranges compare with their temperature ranges for oviposition and mating[22,23]. Such information is not only useful for better understanding the ecological ramifications of temperature effects on species, but also for the husbandry and maintenance of *Drosophila* in the laboratory[1].

Materials and methods

Animals, housing and experimental apparatus. Four *Drosophila* species triads representing four species groups from different ecological backgrounds were chosen as follows:

1) Temperate-montane forest: *virilis* group (*D. virilis, D. americana, D. montana**).
2) Desert: *repleta* group (*D. arizonensis, D. mojavensis, D. mulleri**).
3) Cosmopolitan: *melanogaster* group (*D. melanogaster, D. simulans, D. ananassae**).
4) Tropical forest: *willistoni* group (*D. paulistorum*-Amazonian, Interior, Transitional*).

The *virilis, repleta* and *melanogaster* triads each consist of two sibling species and a third more distantly related species (*), while the *willistoni* triad is represented by three semispecies. The close genetic relatedness of Amazonian and Interior[21] was used as the 'sibling' criterion

(see Schnebel and Grossfield[22], for histories of each population, details of culture, and a description of the experimental apparatus).
Briefly, all species were reared on a raisin-based culture medium at 19–20 °C, 67–70% RH, and constant light. An aluminium bar with 90 holes for shell vials was used to establish a 3–38 °C temperature gradient, which was produced using a laboratory hot plate under one end of the bar and a water-circulating cold plate under the other end. Relative humidity was maintained at 100%.

Experimental procedures. Five third instar larvae, grown in population bottles in the rearing chamber, were removed with a microspoon and placed on the surface of the food medium in a fresh vial. Vials were then placed in the apparatus at one of the experimental temperatures for the appropriate pupation period (four replicates/species/temperature). The number of puparia formed after this time was recorded for all species. Pupation periods: *melanogaster* and *willistoni* groups: 48–72 h; *virilis* and *repleta* groups: 96–120 h.

Analysis of data. Species comparisons at each temperature are based on analyses of variance comparing the percentage of larvae pupating in all species tested at that temperature (four replicates/species). Pupation percentages were first transformed using the angular transformation[25]. Significant species differences were determined using the Student-Neuman-Keuls test ($p = 0.05$).

Results and discussion

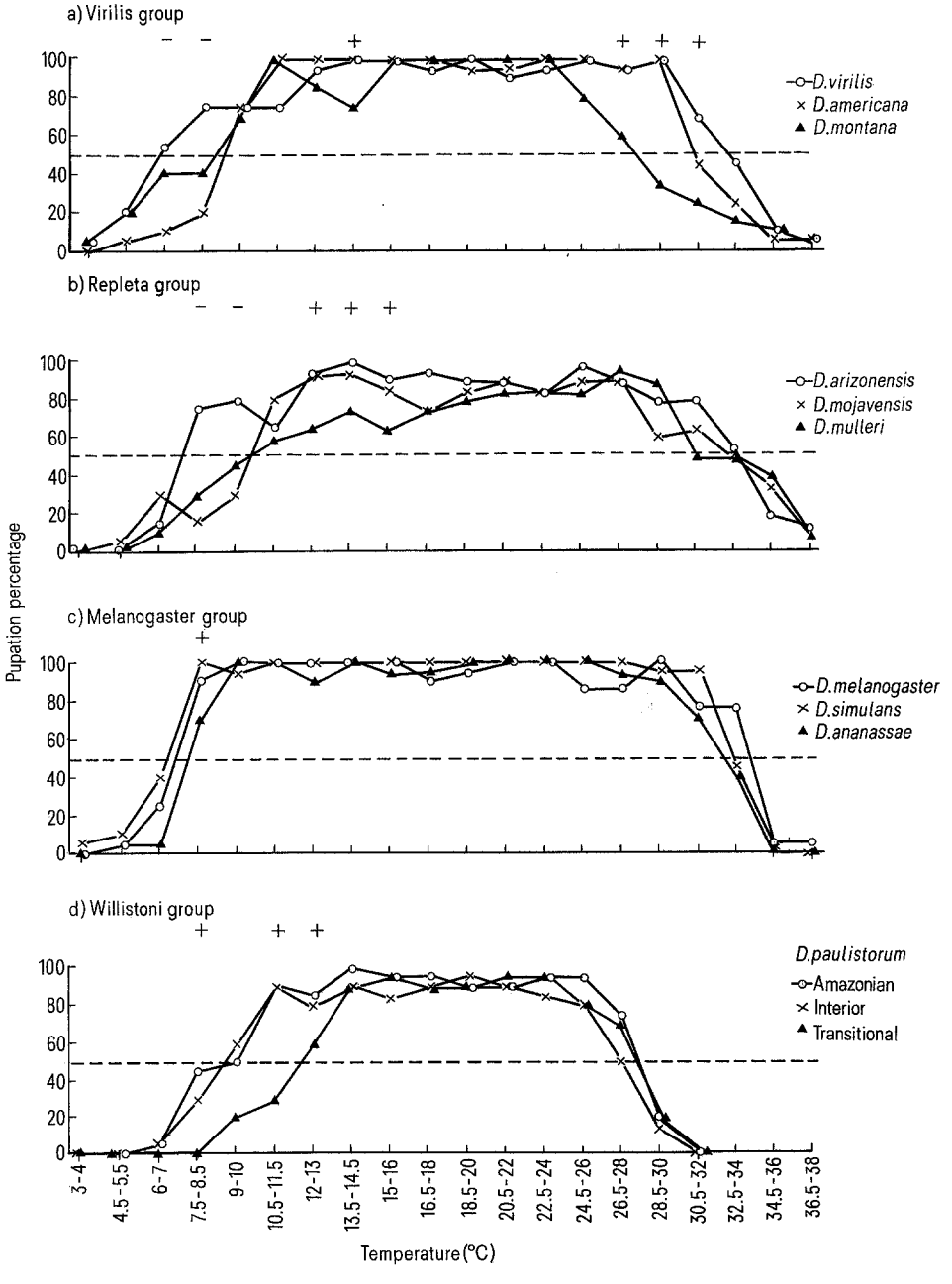
Within group comparisons. The data in figure 1 show species differences in pupation-temperature range within triads. *Virilis* group. The distribution of the two sibling species, *D. virilis* and *D. americana,* include warmer areas than that of *D. montana*[26]. As was found for mating[22], the pupation percentages of both siblings are significantly higher than those of *D. montana* at warm temperatures (fig. 1A: 26.5–30 °C; only *D. virilis* is significantly higher than *D. montana* at 30.5–32 °C). This is also observed at 13.5–14.5 °C. However, the latter difference disappears at immediately higher and lower intervals and does not appear meaningful.
The greater cold-temperature pupation percentages of *D. virilis* over its sibling, *D. americana* (fig. 1A: 6–8.5 °C), correspond to those previously found for mating and oviposition[22,23]. The more cosmopolitan distribution of *D. virilis*[26] may be due, in part, to such low-temperature adaptations. The absence of warm-temperature differences between these two siblings, however, contrasts with earlier findings of greater mating and oviposition ability of *D. virilis* over *D. americana,* demonstrating that temperature constraints can vary for pupation, mating and oviposition and that interspecific differences need not be consistent among temperature-dependent responses.
Repleta group. The significantly lower pupation percentages of *D. mulleri* as temperatures become colder (fig. 1B: 12–16 °C), may be related to habitat differences among the three species. The Sonoran Desert can reflect extreme environmental fluctuations. Mosiño Aleman and Garcia[17] report that the mean annual range of temperatures in the area encompassing Navojoa, Sonora (collection site of *D. arizonensis* and *D. mojavensis*) can be as high as 21 °C, whereas in the Monterrey region (collection site of *D. mulleri*), it is only 7–14 °C. The larvae of *D. arizonensis* and *D. mojavensis* are likely to encounter greater temperature variations than the larvae of *D. mulleri.* The increased ability of the siblings to pupate at lower temperatures than *D. mulleri* may, therefore, be adaptive. Interestingly, no cold-temperature differences were observed for mating or oviposition among these species[22,23]. Furthermore, *D. mulleri* showed significantly less warm-temperature mating and oviposition than both *D. arizonensis* and *D. mojavensis,* yet no pupation differences are found at high temperatures.
Comparisons of *D. arizonensis* and *D. mojavensis* reveal significantly higher cold-temperature pupation percentages for *D. arizonensis* (fig. 1B: 7.5–10 °C). Since cold temperatures are common in desert regions[5,17], these differences in pupation ability could provide opportunities for reducing competition between this pair of closely related species. Whether pupation occurs at these temperatures under natural conditions is not known.
Melanogaster group. The higher pupation percentages of *D. melanogaster* and *D. simulans* over *D. ananassae* at cold temperatures (fig. 1C: 7.5–8.5 °C) are consistent with our findings for mating and oviposition. These differences have been related to the greater tolerance for cold in *D. melanogaster* and *D. simulans,* and their ability to expand into the temperate zone, as compared to the circumtropical *D. ananassae*[4,19,22,23]. The lack of interspecific differences at high temperatures suggests that warm-temperature constraints on pupation are less stringent than those observed for mating and oviposition, in that both *D. melanogaster* and *D. simulans* had higher mating percentages[22], and *D. melanogaster* showed significantly greater oviposition[23] under identical experimental conditions.
The absence of *D. melanogaster* puparia below 14 °C in a wine cellar[14] appears to be due to reasons other than an inability to pupate at these temperatures. Here, *D. melanogaster* shows close to maximal pupation at temperatures as low as 7.5 °C, and a small percentage of puparia is still present at 4.5 °C (fig. 1C). McKenzie and McKechnie[15] state that differences between *D. melanogaster* and *D. simulans* in the number of puparia found in grape residues at high temperatures can be explained by their relative tolerances to ethanol and acetic acid. They raise the question, however, of whether temperature alone or the interaction of these variables may be influential. The present data, demonstrating NS temperature differences in pupation ability between these species, indicate that the independent effect of temperature does not contribute to the different distributions of puparia in these species. The only result suggesting that *D. melanogaster* may have a greater potential for pupating at warm temperatures is the observation of a small percentage of puparia between 34.5–38 °C, while for *D. simulans,* pupation does not occur above 34 °C (fig. 1C). *Willistoni* group. The distributions of *D. paulistorum* semispecies[7] have been related to different climatic temperatures[23]. The broader range of temperatures associated with the regions in which Amazonian and Interior are found, are

reflected in their ability to mate and oviposit at both lower and higher temperatures than Transitional[22,23]. For pupation, similar differences are found only at low temperatures (fig. 1D: 7.5–13 °C, differences at 9–10 °C are not significant), confirming that temperature-limits are response-specific. As was found for oviposition[23], no pupation-temperature differences are observed between the more closely related Amazonian and Interior semispecies.

Among group comparisons. The table reveals that *common* pupation-temperature ranges (temperatures at which all three species can pupate) generally reflect the geographic distributions of each triad, i.e., the temperate-montane *virilis* group has the lowest temperature limits (the *melanogaster* group has equally low limits), the desert *repleta* group has the highest limits, and the cosmopolitan *melanogaster* group has a broader temperature range than the endemic tropical *willistoni* group. This correspondence of *common* temperature range with the ecological background of each group is also seen for mating and oviposition.

Differences in *absolute* ranges (temperatures beyond the *common* range at which one or two species can pupate) are less distinct. Although the *willistoni* group is clearly the most temperature-restricted triad for pupation, the *absolute* ranges of the other three groups are almost identical. This demonstrates that among species from different ecological backgrounds, there is considerable overlap in the temperatures permissive for pupation. Additionally, when the *common* and *absolute* ranges for pupa-

Figure 1. Pupation percentages at different temperatures in 12 *Drosophila* species from four species groups. +, sibling species not significantly different, one or both siblings significantly different from the third species. –, sibling species significantly different. ——, dashed line indicates a 50% level of success.



tion, mating and oviposition are compared in each triad (table), 13 out of 16 comparisons show that pupation can occur at temperatures beyond those for mating and oviposition. The only exceptions are, a) the *common* range for mating in the *virilis* triad extends to 3 °C, compared to 4.5 °C for pupation, and b) the *absolute* range for pupation in the *willistoni* triad is more limited at warm temperatures than are mating and oviposition (mating: 9–32 °C, oviposition: 7.5–34 °C, pupation: 6–30 °C).

Implications. Data from one population do not necessarily represent the temperature responses of the species[27]. However, they do represent a step toward describing the physiological limits of those responses. Even if population variability is found, interspecific analyses are important in helping define phenotypes that can then be examined at the intraspecific level[18].

It should be emphasized that these results derive from mature larvae in the test system. It is not clear whether similar results would stem from placing earlier stages of the life cycle in this system.

The generally broader temperature ranges observed for pupation in the four triads support the conclusions of Kaneshiro et al.[12] that *Drosophila* species usually show less specificity for larval sites. One explanation for this phenomenon relates to the potential for movement by larvae of many species beyond their hatching sites to adjacent areas subjected to a greater range of temperatures. However, caution is needed before generalizing among all species. Figure 2 summarizes the mating-, oviposition- and pupation-temperature ranges in the 12 *Drosophila* species used in our experiments. In most species, pupation is the least restricted response. This is not the case, however, in *D. americana* at cold temperatures, or in *D. paulistorum* Amazonian and Interior at warm temperatures.

Other than the broader temperature ranges seen for pupation, no consistent patterns emerge with respect to how temperature effects manifest themselves in these three responses. For example, oviposition is the most limited response at low temperatures in the *virilis* and *repleta* groups, yet mating is more low-temperature-limited in the *willistoni* group. The only consistent theme appears to be inconsistency. Each species reflects a different combination of temperature effects (fig. 2).

From the data in figure 2, the following points emerge with respect to temperature effects on mating, oviposition and pupation:

1) For individual species, temperature-limits on mating, oviposition and pupation are different, and temperatures permissive for one activity are not predictive of limits on other responses.

2) Among species, temperature can affect a particular response differently.

3) Within groups, a) species differences in temperature-limits may contribute to microniche differences; b) species differences can be at high and/or low temperatures for any response; c) if temperature is an important factor influencing species' adaptive strategies, it can manifest itself through differential effects on one, or any combination of responses, i.e., closely related species for which competition may be greatest show different temperature-limits in one response (Amazonian/Interior), two responses *(arizonensis/mojavensis, melanogaster/simulans)* and three responses *(virilis/americana)*; d) one cannot predict which responses will be most and least limited, or at which end of the temperature scale a response will be most limited.

4. Among groups, *common*, but not *absolute* temperature ranges generally correspond to the geographic distributions and ecological backgrounds of the triads.

These points strongly suggest that the evaluation of temperature effects on species, based on the examination of a single activity, may not be adequate for predicting adaptive strategies.

Common and *absolute* temperature ranges for mating, oviposition and pupation in four *Drosophila* species group triads

Species groups	*Common* ranges/*absolute* ranges (°C) Mating	Oviposition	Pupation
virilis	3–30/3–34	9–32/6–34	4.5–36/3–38
repleta	9–34/9–36	12–36/10.5–38	6–38/4.5–38
melanogaster	10.5–32/6–34	10.5–34/7.5–34	4.5–34/3–38
willistoni	13.5–30/9–32	10.5–30/7.5–34	9–30/6–30

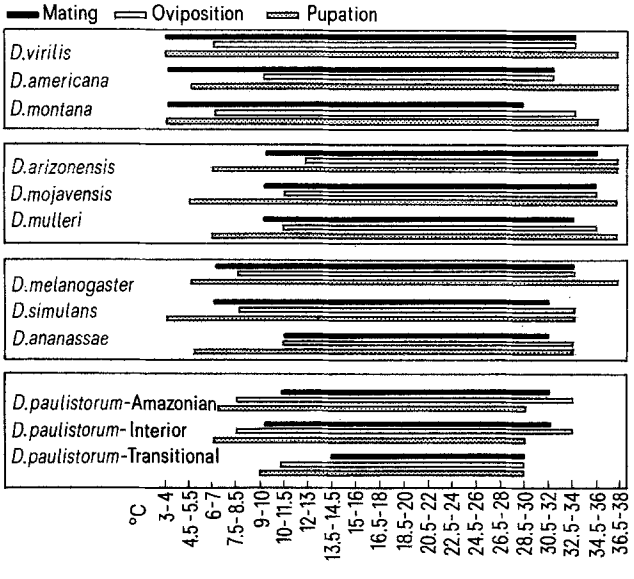


Figure 2. Mating-, oviposition- and pupation-temperature ranges in 12 *Drosophila* species.

Acknowledgments. Supported in part by USPHS Grant 2 S07 RRO7132 and by awards from CUNY-FRAP. We wish to thank G. Fisher for her work on the illustrations.

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0014-4754/86/060600-05$1.50 + 0.20/0

Short Communications

Influence of brown adipose tissue on deep cervical temperature during sleep in the young rabbit[1]

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Summary. In young rabbits the slope of the temperature in the deep cervical region close to brown adipose tissue increased during desynchronized sleep at low ambient temperature. No increase occurred at neutral ambient temperature. In control rabbits (after disappearance of brown adipose tissue), the slope of deep cervical temperature did not increase during desynchronized sleep at low or neutral ambient temperatures.
Key words. Sleep; brown adipose tissue; rabbit.

Central control of body temperature is markedly altered during desynchronized sleep (DS), i.e. rapid eye movement or paradoxical sleep, as compared with synchronized sleep (SS), i.e. slow wave sleep. In particular, it has been shown that the sympathetic control of vasomotion in cutaneous heat exchangers ceases during DS (see ref. 4 for a review of temperature regulation during sleep). Sympathetic efferent activity also controls brown adipose tissue (BAT), a main effector of nonshivering thermogenesis. The present study was undertaken for the following reasons:
a) a repatterning of sympathetic outflow occurs during DS[5];
b) the control of a metabolic thermoregulatory effector (BAT) might differ from that of a motor thermoregulatory effector, e.g. cutaneous vasculature;
c) the BAT thermogenic role is especially well established at a very young age, when
d) the sleep cycle exhibits a high DS content[6].

The experiments were performed on young New Zealand white rabbits implanted, under general anesthesia (sodium pentobarbitone 40 mg/kg s.c. 45 min after premedication with Flunitrazepam 0.5 mg/kg i.m.), with electrodes for EEG recordings. Thermistors were also placed in the hypothalamus, and deep in the cervical region, located bilaterally between lateral and posterior BAT cervical lobes[7]. Since white fat replaces the BAT deposits in the adult rabbit, ontogenetic timing of the recording sessions was critical. The rabbits were implanted just after weaning at 4 weeks of age, and the recording sessions carried out during the 5th to 6th week of age (weight 600–700 g), depending on the speed of post-operative recovery. At this age the cervical lobes consist mainly of brown fat. Preliminary recordings of nuchal EMG were also carried out. As previously shown in the adult[8], EMG activity was attenuated in the young rabbit during SS and strongly depressed during DS at the ambient temperatures (T_a's) considered. Therefore, EMG recording was discontinued in sub-