Geographical Variation in the Acclimation Responses of Drosophila to Temperature Extremes
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GEOGRAPHICAL VARIATION IN THE ACCLIMATION RESPONSES OF *DROSOPHILA* TO TEMPERATURE EXTREMES

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Abstract.—Populations may adapt to climatic stresses by nonplastic or plastic changes in stress resistance. Plastic changes include a range of different acclimation mechanisms. A few previous studies with *Drosophila* suggest interspecific variation in plastic responses to climatic stresses, but there is not much evidence for variation within species. Tropical and temperate populations of *Drosophila melanogaster* and *Drosophila simulans* were compared for plastic responses to cold and heat stress. Tropical populations tended to be less resistant to cold stress than temperate populations. In contrast, *D. melanogaster* populations showed similar acclimation responses to cold stress following different acclimation treatments, which included keeping adults at low temperatures for a few hours or several days and culturing larvae at a low temperature. Populations of *D. simulans* also showed similar plastic responses to cold stress after adults were acclimated at a low temperature for several days. In the heat resistance experiments, there was no evidence for population differences in acclimation response in either *D. melanogaster* or *D. simulans* when adults were exposed to high and low temperatures for a few days. Genetic variation for acclimation response may therefore be mainly restricted to the interspecific level, although larger experiments are required to detect small quantitative differences between populations.

The continued survival and reproduction of insects in a changing environment will often depend on evolutionary changes that counter environmental stresses, a process that may involve two types of changes (Hofmann and Parsons 1991). Insects may adapt by altering their ability to counter a climatic stress, irrespective of the environments they experience prior to the onset of the stress. Insects may also counter a stress by changing the way they respond to conditions that precede a stress.

This second type of evolutionary change involves the selection of genotypes with altered levels of plasticity. Plastic changes that are used by insects to counter a stress include the process of acclimation, in which case stress resistance is increased following exposure to nonlethal conditions. They also include responses to environmental cues that result in an insect avoiding a stress or in an insect entering a dormant stage of its life cycle (Levins 1968).

To determine whether insect populations adapt via such plastic changes requires information about genetic variation for plasticity in evasion responses and acclimation within and between populations. A few insect studies have examined the genetic basis of variation in plastic responses involving stress evasion, and these have shown that responses to conditions inducing dormant phases may be

under genetic control. For example, in the milkweed bug, Dingle (1978) found that a high proportion of the variance for the critical period inducing diapause was genetic. Selection of nondiapausing strains under a particular light regimen was achieved in a few generations. Similarly, larvae of the pitcher-plant mosquito could be readily selected for an altered incidence of diapause after a long photoperiod (Istock 1978). Geographical variation in cues inducing stress evasion responses has also been shown to be under genetic control in a few instances (Tauber et al. 1986). For example, Tauber and Tauber (1982) found that diapause and reproduction in an Alaskan population of a lacewing were controlled by photoperiod, but this factor did not influence diapause and reproduction in a California population.

In contrast, very little genetic research has been carried out on plastic responses involving acclimation for increased stress resistance. Apart from the few *Drosophila* studies reviewed below, almost nothing is known about genetic variation for acclimation ability within and between insect populations. Acclimation responses would seem to present a good opportunity for investigating plastic adaptation, because much is already known about the types of acclimation responses insects use to counter different stresses.

Genetic studies on acclimation ability need to consider the following questions in assessing the likelihood of plastic adaptation:

1. How readily can evolutionary changes in acclimation responses occur? This question requires an evaluation of genetic variation for acclimation ability within populations. While many experiments have shown that increased stress resistance can be readily selected in insects (Huey and Kingsolver 1989; Hoffmann and Parsons 1991), conditions likely to result in acclimation have only been incorporated in a few selection regimes (see, e.g., Tucić 1979); even in these cases plastic and nonplastic responses have not been distinguished after selection. It is therefore not known if insect populations can readily undergo evolutionary changes in acclimation response.

2. Have populations exposed to different climates diverged for acclimation responses, or are population differences in stress resistance mostly nonplastic? There is ample evidence that insect populations can differ in their ability to counter environmental stresses (Hoffmann and Parsons 1991) and in physiological mechanisms likely to underlie differences in stress resistance (Gibbs et al. 1991). However, not much is known about population differences in acclimation ability. This also applies to organisms other than insects, with a few notable exceptions (see, e.g., Brown and Feldmeth 1971; Tsuji 1988).

3. Are costs involved in an increased ability to acclimate? Costs have been postulated as a way of accounting for the absence of genotypes with a very high degree of plasticity (Heslop-Harrison 1964; Bradshaw 1965), but there is not much relevant data. Genotypes with a high acclimation ability may have reduced fitness under benign conditions because of structural/energetic constraints. Such constraints may be imposed by mechanisms needed to detect cues preceding a stress and respond to the stress once it has been detected.

4. Are theoretical predictions about environmental conditions favoring plastic and nonplastic changes met? While some environmental changes have been pos-

tulated to favor plastic over nonplastic changes (Levins 1968; Lynch and Gabriel 1987; Hoffmann and Parsons 1991), there have been only a few attempts at empirical evaluation. For example, Bradley's (1978) research on copepods suggested that rapid temperature changes selected for nonplastic responses, whereas individuals could largely counter slow changes in temperature by acclimation.

5. Are genes controlling plastic and nonplastic responses to stress independent? The question of whether the means of traits and their plasticity are likely to evolve independently has been investigated in studies on morphological traits. For example, Scheiner and Lyman (1990) generated plastic variation in thorax size by exposing *Drosophila melanogaster* to different temperatures. Using family selection, they were successful in selecting for altered levels of plasticity without much change in mean thorax size, which suggests that genes influencing mean thorax length and its plasticity were partly independent. These findings may not apply to acclimation responses. The same physiological mechanisms could underlie a plastic response and nonplastic variation in stress resistance, in which case genotypes with a high level of stress resistance might show a decreased acclimation response (Hoffmann 1990).

PLASTICITY AND STRESS RESISTANCE IN *DROSOPHILA*

We are attempting to answer some of these questions by investigating resistance to climatic stress in *Drosophila*. Much of the previous research in this area has involved exposing individuals to a stress without the possibility of acclimation. For example, genetic variation in heat resistance within populations has usually been examined by exposing *Drosophila* directly to heat stress (see, e.g., Hosgood and Parsons 1968; Morrison and Milkman 1978; Stephanou et al. 1983). Only some studies have considered genetic variation in plastic responses, and most of this work has involved comparisons of populations from different environments.

One of the few studies examining acclimation for heat resistance is Maynard Smith's (1957a) comparison of acclimation in inbred and outbred strains of *Drosophila subobscura*. Individuals were acclimated by keeping them as adults or larvae at 15° or 25°C. While inbred and outbred strains did not differ consistently in adult acclimation responses, outbred strains showed an increased larval acclimation ability. This difference suggests genetic variation in plastic responses within populations, but the relevance of these results to variation between strains without a history of inbreeding is not known. The only evidence for such variation was obtained by Oudman et al. (1992) using lines of *Drosophila melanogaster* differing at the ADH and aGPDH enzyme loci. They found that differences in the heat resistance of genotypes at these loci depended on the rearing temperature, which suggests that the loci contributed to variation in larval acclimation.

The effects of acclimation on heat resistance in populations of several *Drosophila* species were examined by Levins (1969). Adults were acclimated by being exposed to constant temperatures (13°–29°C) for a few days. By comparing the heat resistance of flies acclimated at the temperature extremes, Levins concluded that there were species and population differences in acclimation ability. For

example, none of the *Drosophila willistoni* populations showed an acclimation response, whereas exposure to a high temperature did not appear to increase resistance in some *Drosophila simulans* populations but increased resistance by as much as 50% in other *D. simulans* populations. However, interpretation of these experiments is difficult because they were carried out with different assistants in different times and places. Moreover, population differences in acclimation response were inferred indirectly rather than by a statistical analysis of population by acclimation treatment interactions.

The heat and cold resistance of Japanese populations of two widespread *Drosophila* species, *Drosophila virilis* and *Drosophila immigrans,* was examined by Yamamoto and Ohba (1982). Adult *D. virilis* were more resistant to both temperature extremes than adult *D. immigrans,* and there were smaller resistance differences between populations. Flies were also acclimated by holding them at 14° or 25°C for 2 d before testing heat resistance. The 25°C treatment increased heat resistance to a much greater extent in *D. immigrans* than in *D. virilis,* but no statistical analysis of population differences in acclimation ability was presented.

Acclimation for cold resistance has also been examined in Japanese populations of the *melanogaster* species group (Kimura 1988). There were large species differences in cold resistance after acclimation to 3°C, but comparable data for flies without prior exposure to cold conditions were not collected. Species restricted to cold environments were more resistant than those from warm environments, but populations did not differ in resistance. Kimura suggests that the development of cold hardiness following acclimation evolves ''in accordance with the progress of speciation'' (p. 1295), which accounts for the lack of variation between populations, although some of the populations appeared to have been maintained in the laboratory at a constant temperature for a long time.

Apart from research on temperature acclimation, one *Drosophila* study (Hoffmann 1991) has considered geographical variation in acclimation responses for desiccation stress. Populations of *D. melanogaster* and *D. simulans* from tropical and temperate sites were compared. Previous experiments (Hoffmann 1990) had shown that resistance in these species can be increased by prior exposure to a nonlethal desiccation stress. Populations from the tropical site were more sensitive to desiccation than temperate populations, but there were no differences in acclimation responses. Acclimation was not detected in *Drosophila birchii,* a species restricted to the tropics, but did occur in its sibling species *Drosophila serrata,* which is more widespread. This observation suggested variation in acclimation at the interspecific level but not at the intraspecific level. *Drosophila melanogaster* lines selected for increased desiccation resistance were also scored for acclimation responses. Selected lines showed less acclimation than unselected lines (Hoffmann 1990), even when selected lines were exposed to a longer prior stress period than the control lines to compensate for their greater level of resistance. This result indicates that genes controlling acclimation and nonplastic desiccation resistance were not independent.

In summary, the few studies carried out to date suggest that acclimation responses can vary between related *Drosophila* species, but there is only limited evidence for genetic variation for acclimation responses within and between pop-

ulations. Many of the population comparisons have failed to compare acclimation and nonacclimation treatments directly. Only some types of acclimation responses have been examined, and there has been little attempt to relate acclimation treatments and stresses to conditions likely to be experienced in the field.

POPULATION COMPARISONS FOR HEAT AND COLD ACCLIMATION

We have examined the acclimation responses of Australian *Drosophila melanogaster* and *Drosophila simulans* populations for cold and heat resistance. We have focused on the resistance of adults rather than larvae. While larval heat resistance may be an important ecological trait because of extreme summer temperatures in the breeding sites of *Drosophila,* adults are more likely to experience extreme cold temperatures. This is because *D. melanogaster* and *D. simulans* overwinter in temperate regions at the adult stage when low temperature extremes are correlated with winter survival (Izquierdo 1991).

The cold resistance of adults may be influenced by several forms of acclimation. Resistance is affected by the temperature *Drosophila* have previously experienced at the larval stage, and it may also be influenced by temperatures experienced by parents of flies if there are maternal/paternal factors carried into the next generation. In addition, two types of adult acclimation responses for cold resistance have been identified. First, the resistance of *Drosophila* and other insects can be rapidly increased by short exposure to a low temperature (Lee 1989). This acclimation effect, often called a hardening response, is likely to be important in countering daily temperature fluctuations or short cold spells. Second, the resistance of *Drosophila* can be increased to an even greater extent by exposing adults to low temperatures for a few days (Kimura 1988). This response is likely to be important in countering long periods of cold stress such as seasonal cold spells, which are usually preceded by a gradual decrease in temperature. Acclimation for cold resistance therefore encompasses a number of responses likely to be controlled by different physiological mechanisms. Genetic variation could occur for each of these responses, and any description of plasticity for stress resistance must consider all the responses as well as genetic interactions between them and nonplastic stress resistance.

In the experiments below, we consider responses to temperature extremes (cold and heat) in two populations of *D. melanogaster* and *D. simulans* from the east coast of Australia. One population (Melbourne) is from southern Australia (38°S), while the other (Cairns) is from the tropics (17°S). Melbourne has a temperate climate, and temperature extremes vary from about 0°C in winter to over 40°C in summer. The mean daily minimum temperature in July is 5°C compared to a daily maximum of 26°C in January. In contrast, temperatures are less variable and much warmer in Cairns. Extremes vary from about 10°C in winter to 35°–40°C in summer, and the mean daily July minimum is 17°C compared to a January maximum of 31°C.

These diverse climates are likely to select for different genotypes, and there is ample evidence for genetic divergence between tropical and temperate Australian populations of *D. melanogaster.* Quantitative traits that show genetic divergence

include body size and resistance to desiccation, ethanol, and cold (see Parsons 1982). There are also electrophoretic differences between these populations (Oakeshott et al. 1982). Population differences in stress resistance can often be interpreted from an adaptive perspective. For example, Melbourne strains are relatively more resistant than tropical strains to desiccation and a $-1°C$ cold stress (Stanley and Parsons 1981). These populations therefore provide an opportunity to investigate the role of acclimation in adaptation to different climates. In contrast, *D. simulans* may show less divergence for quantitative traits between these populations, as in the case of ethanol resistance (Anderson and Oakeshott 1986).

In the cold resistance experiments, we consider the response of these populations to the short- and long-term adult acclimation responses described above, and we also consider plastic changes resulting from larval and parental culture conditions. We have stressed flies at two temperatures ($-2°$ and $-5°C$) in these experiments. Two stress levels were used to encompass the range of stresses used by other workers (Tucić 1979; Stanley and Parsons 1981; Czajka and Lee 1990; Davidson 1990) and to use stresses that give very different mortality curves. The $-2°C$ temperature is close to the minimum winter temperature experienced in Melbourne, although it should, of course, be emphasized that behavioral responses may modify the temperatures actually experienced by the flies. Temperatures just below $0°C$ are low enough to cause mortality in *D. melanogaster* because this species cannot survive temperatures well above its supercooling point of $-20°C$ (Tucić 1979; Czajka and Lee 1990). Flies were acclimated only by exposure to nonlethal low temperatures. Although photoperiod can also influence cold resistance in some insects, this factor does not affect the adult diapause or resistance of *D. melanogaster* (Kimura 1988).

Increased resistance to a heat stress can be induced in *Drosophila* by a short or long exposure to prior stress periods (Maynard Smith 1957b; Levins 1969; Lindquist 1986) as well as larval culture temperature (Maynard Smith 1957b), but we have only considered long-term adult exposure. We have followed the protocol of Levins (1969) to test the reproducibility of his findings using the temperate and tropical populations of *D. melanogaster* and *D. simulans*. Both the acclimation temperatures ($13°–29°C$) and stress temperature ($39°C$) are experienced at the sites where the populations originated, although these temperatures may not necessarily be experienced by flies because of behavioral evasion. Mean summer and winter temperatures are much higher in Cairns than in Melbourne, but high temperature extremes are similar in the two populations. Melbourne flies might be expected to show a different acclimation response because of the greater daily and seasonal temperature variability experienced by flies from this population.

<div align="center">MATERIALS AND METHODS</div>

<div align="center">*Stocks and Measurement of Cold Resistance*</div>

Each laboratory stock was initiated with the progeny of 35–50 inseminated females collected from the field. Melbourne flies were collected from an apple orchard, and Cairns flies were obtained from a banana plantation. Flies were

cultured on a sucrose-dead yeast medium under continuous light at $25° \pm 0.5°C$ unless stated otherwise. Stocks were maintained by mass transfer of a few hundred individuals each generation.

Experiments were carried out 5–10 generations after flies were brought in from the field. Laboratory culture does not seem to have much effect on acclimation ability. In preliminary experiments, we have found that the acclimation responses of stocks tested one to two generations after they are brought into the laboratory are similar to those of stocks from the same location that have been under laboratory culture for a year (about 15 generations).

Flies for the cold resistance experiments were cultured at a low density in 600-mL bottles. Adults were collected from bottles when they were 0–2 d old. After being aged under different conditions as described below, flies were sexed under carbon dioxide anesthesia. To measure cold resistance, vials were set up with 10 males and 10 females. Flies were left to recover from anesthesia for a day before they were transferred to plastic vials. These were placed in a 24-vial rack and submerged in a Braun refrigerated bath. Exposure temperatures in the vials were recorded with a calibrated thermometer and varied 0.2°–0.6°C in an experiment. Flies were left to recover at 25°C. Following Czajka and Lee (1990), we scored flies that could stand, walk, or fly as alive. The cold resistance of males and females was scored separately, and a sex factor was not examined in the analysis because male and female data were not independent.

Cold resistance of Drosophila melanogaster after Adult Acclimation

Rapid hardening, $-2°C$ stress.—This experiment measured the effects of rapid cold hardening on resistance to a $-2°C$ stress. Czajka and Lee (1990) found that a 2-h exposure to 5°C markedly increased the subsequent survival of *Drosophila melanogaster* when they were stressed at $-5°C$ for 2 h. Our preliminary experiments indicated a similar effect when adults were exposed to temperatures in the 4°–6°C range. Flies were therefore exposed to 4°C for 2 h before being transferred to $-2°C$ for 23 h (the actual stress varied between $-1.5°$ and $-2.0°C$). This stress period provided intermediate mortality levels. Flies had been cultured at 19°C rather than 25°C and were 3–5 d old at the time of testing, an age when rapid hardening ability is at a maximum (Czajka and Lee 1990). The experimental design is given by

$$Y_{ijklm} = a + b_i + c_j + d_{ij} + e_{k(ij)}, \tag{1}$$

where a is the grand mean, b_i the acclimation treatment effect, c_j the population effect, d_{ij} the interaction between population and acclimation treatment, and $e_{k(ij)}$ the error term. Six replicates were set up for each population and treatment combination.

Rapid hardening, $-5°C$ stress.—This experiment tested the effects of rapid hardening on resistance to an extreme stress. Flies were exposed to 4°C for 2 h and stressed at $-5°C$ for around 50 min. This stress period ensured some survivors in both the control and acclimated treatments. Recorded temperatures in the experiments varied between $-4.9°$ and $-5.5°C$. The 50-min exposure time is shorter than the 2-h period used by Czajka and Lee (1990) because we found that a 2-hour period resulted in 100% mortality of both acclimated and nonacclimated

flies. Three repeats of this experiment were carried out, so that 360 flies were tested overall for each treatment. Flies were 2–4 d old at the time of testing. The experimental design follows equation (1) except that a main effect due to the repeat experiments was also included. We expected mean mortalities to differ between repeats because of temperature variation in the refrigerated bath.

Long-term cold hardening.—To measure cold hardening, we initially undertook experiments in which adults were exposed to 15°C for 7 d. These experiments were carried out with both *D. melanogaster* and *Drosophila simulans* and are described below. In addition, we examined the effect of a second exposure period at 6°C, because Kimura (1988) suggested that this treatment further increased the cold hardiness of *Drosophila*. Flies were acclimated by exposing them to 15°C for 14 d or by exposing them to 15°C for 7 d followed by 7 d at 6°C. The experimental design follows equation (1) because only one experiment was carried out. Resistance was increased markedly by both treatments, and flies had to be stressed for 11 h at −5°C (actual recorded temperatures were −4.8° to −5.3°C) to obtain intermediate mortality levels. The resistance of 14-d-old flies not acclimated at the low temperature could not be tested at the same time because these had all died after an exposure period of less than 2 h.

Effect of Larval and Parental Temperature on Cold Resistance
of Drosophila melanogaster

These experiments were carried out only with *D. melanogaster*. Populations were initially cultured in bottles at 18° or 25°C, and populations from each of these temperatures were cultured for a second generation at both 18° and 25°C, to give four parental/larval culture treatments. By setting up cultures for the second generation at different times, flies from each treatment emerged simultaneously. Flies were collected within 16 h after emergence and aged for 2–3 d at 25°C before being stressed. Because larval culture temperature had a smaller effect on cold resistance than long-term adult acclimation, treatments could be compared by exposing adults for the same length of time.

Flies were stressed at two temperatures, −5°C (45 min) and −2°C (135 min). There were four repeat experiments at −5°C (recorded temperatures varied between −4.9° and −5.5°C) and six repeat experiments at −2°C (recorded temperatures varied between −2.0° and −2.3°C). The 135-min exposure time used for the −2°C stress is much shorter than the 23-h exposure period we used in the experiment described above. We suspect that a shorter time was required to achieve intermediate mortalities because of the small increase in the level of cold stress in this experiment.

The experimental design is given by

$$Y_{ijklm} = a + b_j + c_k + d_l + f_m + cd_{kl} + cf_{km} + df_{lm} + cdf_{klm} + e_{n(ijklm)}, \quad (2)$$

where b_j is the block (experiment) term, c_k the parental temperature term, d_l the culture temperature term, f_m the population term, and $e_{n(ijklm)}$ the error term. The remaining terms are the interactions, and the df_{lm} and cf_{km} terms are of particular interest because they represent the interactions between population and larval/parental acclimation. In each experiment, three replicates were set up for a treatment-population combination.

Cold Resistance of Drosophila melanogaster and Drosophila simulans Populations

Flies of both species were bred at 25°C. After emergence, flies were aged for 7 d at 13° or 25°C. Preliminary experiments showed that acclimation at 13°C markedly increased cold resistance in both species. Almost all of the *D. melanogaster* and *D. simulans* flies held at 25°C were dead after exposure to −2°C for 4 h or more, while flies acclimated at the lower temperature did not start to die until after 12 h at −2°C. The two treatments could therefore not be compared in the same experiment.

Flies were stressed at −2°C (range −1.9° to −2.4°C) for 17.5 h (18°C flies) or 110 min (25°C flies). Three repeat experiments were set up for the 18°C treatment, and four repeats for the 25°C treatment. The experimental design is given by

$$Y_{ijklm} = a + b_i + c_j + d_k + cd_{jk} + e_{l(ijk)},\qquad(3)$$

where b_i is the block (repeat experiment) term, c_j the species term, d_k the origin term, cd_{jk} the origin by species interaction, and $e_{l(ijk)}$ the error term. There were six replicates for each population-species combination in an experiment.

Heat Resistance of Drosophila melanogaster and Drosophila simulans Populations

The experiments follow the procedures in Levins (1969). Flies were reared at 19°C and aged (sexes mixed) after emergence for 2 d at 19°C. Flies were then sexed, and females were placed at 13°, 25°, or 29°C for 4 d at a density of 10 females per vial. Females were transferred to empty vials prior to testing for heat resistance. The vials were placed in a 39°C incubator and held at high (>80%) humidity. Flies were initially scored for knockdown. Females on their sides or back at the bottom of a vial were considered to be knocked down. Vials were removed after a time, and females were transferred to a different set of vials with food that were kept at 19°C. The number of females that recovered (i.e., were mobile) in these vials was scored after 24 h.

Species were tested separately because *D. melanogaster* was more resistant to heat than *D. simulans*. Each experiment was set up as a series of blocks. A block consisted of only six vials (one vial for each population and acclimation treatment combination) to facilitate rapid scoring of knockdown numbers. For *D. melanogaster,* knockdown resistance was scored after 22 min, and vials were removed from heat after 28 min to test recovery. Knockdown in *D. simulans* was scored after 10 min, and vials were removed from heat after only 14 min. Five blocks were set up for each species. The experimental design follows equation (1) except that there was a main effect due to block.

RESULTS

Cold Resistance of Drosophila melanogaster after Adult Acclimation

Rapid hardening, −2°C stress.—Acclimation increased cold resistance significantly in the males, and the female results ($P < .10$) suggest a similar trend (table 1). Melbourne flies of both sexes were more resistant than Cairns flies (fig.

THE AMERICAN NATURALIST

TABLE 1

ANALYSES OF VARIANCE FOR COLD RESISTANCE OF *DROSOPHILA MELANOGASTER* AFTER ACCLIMATION

Effect	df	Females	Males
Short-term acclimation, −2°C stress:			
Population	1	41.03***	95.41***
Acclimation	1	14.42*	49.87**
Population × acclimation	1	1.58	.33
Error	20	3.58	6.75
Short-term acclimation, −5° stress:			
Repeat	2	77.93***	90.50***
Population	1	6.98	8.56
Acclimation	1	108.48***	294.38***
Population × acclimation	1	.32	13.99
Error	60	2.22	6.12
Long-term acclimation, −5°C stress:			
Population	1	63.21**	24.59**
Treatment	1	153.55***	.13
Population × treatment	1	10.87	.64
Error	20	10.13	3.82

NOTE.–Numbers represent mean squares; ANOVAs were carried out on 10 × arcsine-transformed proportions. For an explanation of the acclimation treatments, see text.
* $P < .10$.
** $P < .05$.
*** $P < .001$.

1), and the two populations showed a similar acclimation response as indicated by the absence of a significant interaction term (table 1).

Rapid hardening, −5°C stress.—The 2-h exposure to 4°C significantly increased resistance in both sexes (table 1), in agreement with Czajka and Lee (1990), although the acclimation response was not as marked as in their study. Populations did not differ overall in resistance and showed similar acclimation responses (fig. 1). The absence of a significant population difference contrasts with the −2°C stress results. This finding suggests that the detection of population differences may depend on the stress level that is applied, although population differences were detected in a subsequent experiment involving a −5°C stress (see below).

Long-term cold hardening.—The 14 d that flies spent at cool temperatures resulted in a high degree of cold resistance. Females held for 7 d at 15°C and 7 d at 6°C were more resistant than those held at 15°C for 14 d (fig. 1). This difference may reflect the effects of cold hardening or differences in the aging rate at the two temperatures. However, the 6°C treatment did not influence the resistance of the males (table 1). Melbourne flies of both sexes were more resistant to cold than Cairns flies regardless of the acclimation treatment, and the absence of interactions indicates that populations did not differ in the extent to which the 6°C exposure further enhanced resistance.
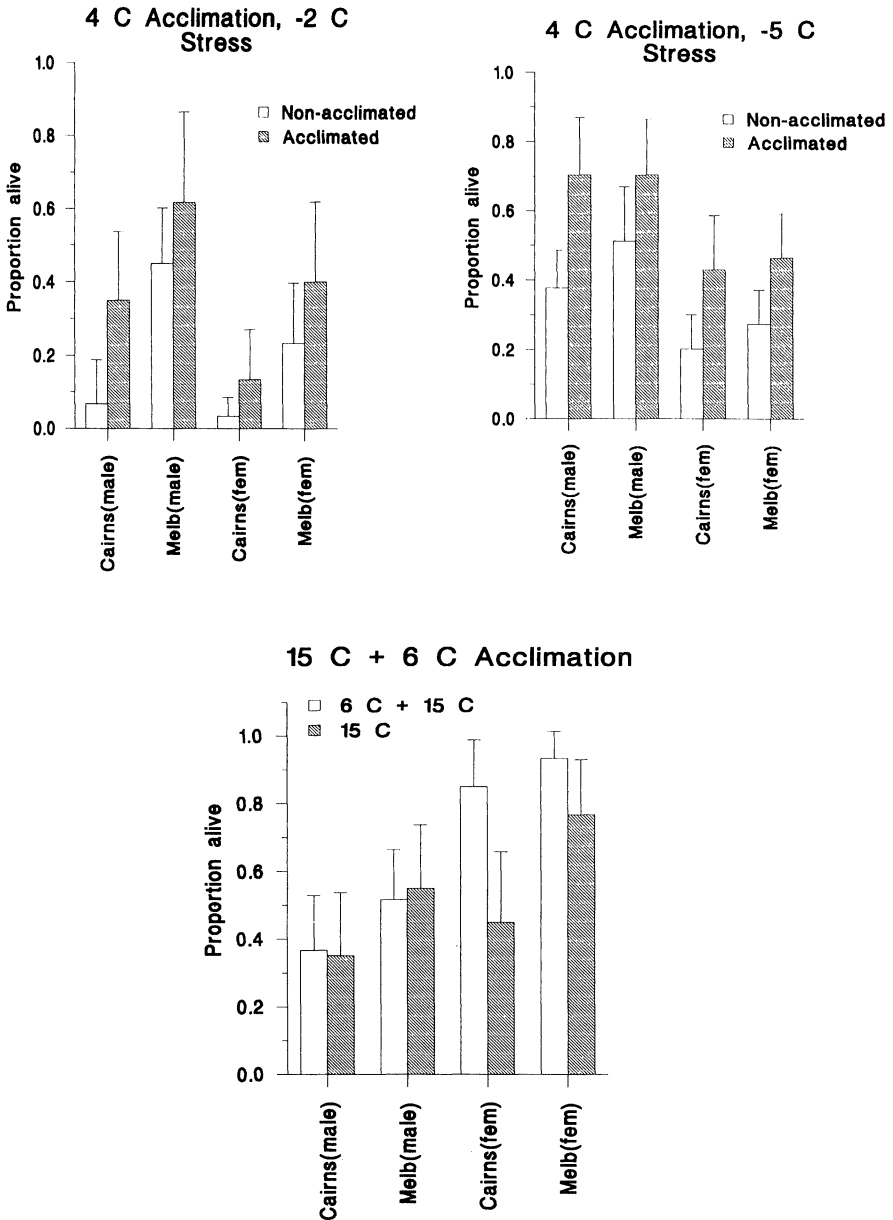
FIG. 1.—Short- and long-term acclimation responses of *Drosophila melanogaster* populations. Flies were acclimated for 2 h at 4°C in the short-term acclimation treatments and stressed at either −2° or −5°C. For the long-term acclimation experiment, flies were acclimated at 15°C for 14 d or at 15°C for 7 d followed by 7 d at 6°C. *Error bars* for the short-term (−2°C stress) experiment and the long-term experiment are SDs based on six replicates. *Error bars* for the −5°C short-term acclimation test are the mean SDs of the three repeat experiments.

TABLE 2

ANALYSES OF VARIANCE FOR CULTURE AND PARENTAL TEMPERATURE EFFECTS ON COLD RESISTANCE OF
DROSOPHILA MELANOGASTER

Effect	df	Females	Males
Flies stressed at − 2°C:			
Repeat	5	78.85***	115.04***
Population	1	24.49**	1.97
Culture temperature	1	835.79***	982.80***
Parental temperature	1	9.58	1.83
Culture temperature × parental			
temperature	1	6.20	.89
Population × culture			
temperature	1	2.77	6.19
Population × parental			
temperature	1	4.61	1.26
Population × culture temperature			
× parental temperature	1	3.79	.02
Error	96	3.40	4.04
Flies stressed at − 5°C:			
Repeat	3	50.26***	27.09***
Population	1	23.06*	63.94***
Culture temperature	1	197.71***	403.89***
Parental temperature	1	6.60	.30
Culture temperature × parental			
temperature	1	.05	.82
Population × culture			
temperature	1	3.79	3.67
Population × parental			
temperature	1	14.68*	3.03
Population × culture temperature			
× parental temperature	1	6.48	4.95
Error	63	3.44	3.23

NOTE.—Numbers represent mean squares; ANOVAs were carried out on 10 × arcsine-transformed proportions of flies that were alive 24 h after the cold stress. Parents and progeny were cultured at 18° or 25°C.
  * $P < .05$.
  ** $P < .01$.
  *** $P < .001$.

Effect of Larval and Parental Temperature on Cold Resistance of Drosophila melanogaster

For the − 2°C cold stress, the ANOVAs (table 2) indicate a significant effect of larval culture temperature on the cold resistance of both sexes, but parental temperature did not significantly influence resistance. As might be expected, larvae cultured at 18°C were more resistant than those cultured at 25°C (fig. 2). This difference was evident in both populations, and the absence of a significant interaction between population and culture temperature indicates that the acclimation effect was of a similar magnitude in Melbourne and Cairns flies. Melbourne females were more resistant than Cairns females, but this population difference was not evident in the males.

Similar results were obtained for the − 5°C stress experiments (table 2). Larvae cultured at 18°C were more resistant than those cultured at 25°C (fig. 2), and the
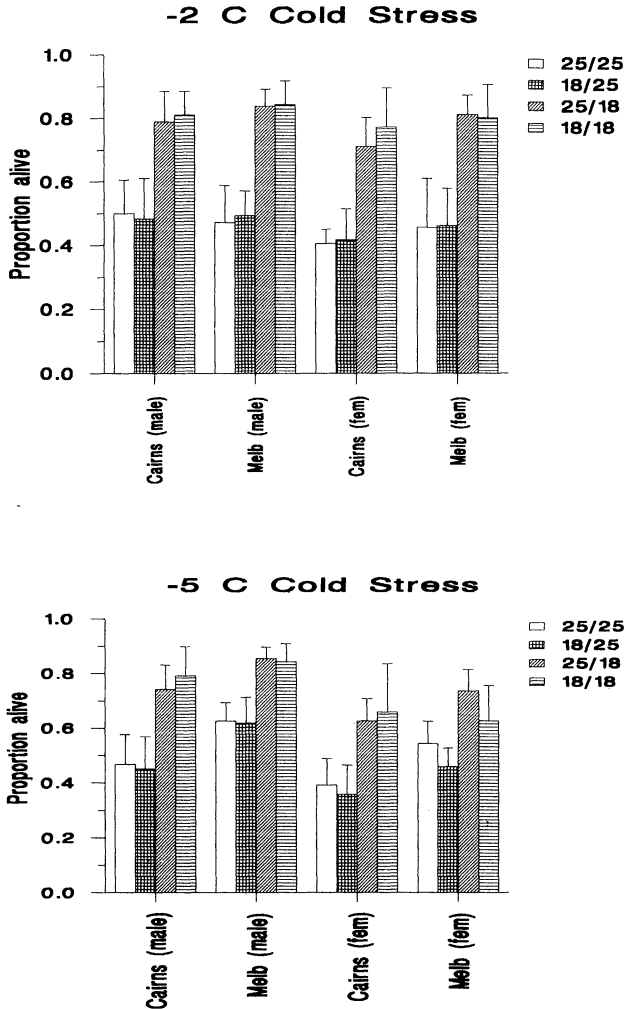
FIG. 2.—Effect of larval and parental culture temperature on the cold resistance of *Drosophila melanogaster* populations. Flies were reared at 25° or 18°C (parental temperature), and progeny from each type of parent were also reared at 25° or 18°C (culture temperature). The *first number* in the legend refers to the parental temperature, and the *second number* refers to the culture temperature. *Error bars* are the mean SDs of the six (−2°C stress) or four (−5° stress) repeat experiments.

absence of a significant interaction indicates that this acclimation response was similar in the two populations. In both sexes, there were significant differences between populations because Melbourne flies were more resistant than Cairns flies. For the female data, there was a significant ($P < .05$) interaction between parental temperature and population, because of the fact that Melbourne females from parents cultured at 25°C were more resistant than those from parents cultured at 18°C, whereas no such difference was evident in the Cairns population.

TABLE 3

ANALYSES OF VARIANCE FOR COLD RESISTANCE OF *DROSOPHILA MELANOGASTER* AND
DROSOPHILA SIMULANS

Effect	df	Females	Males
Acclimated at 13°C:			
Experiment	2	8.29	9.01**
Species	1	40.49***	66.98****
Population	1	13.18*	22.86***
Population × species	1	3.39	.31
Error	60	3.50	2.06
Nonacclimated (25°C):			
Experiment	3	104.49****	44.24****
Species	1	38.02***	90.54****
Population	1	2.85	1.26
Population × species	1	1.71	1.13
Error	80	4.62	3.62

NOTE.—Numbers represent mean squares; ANOVAs were carried out on 10 × arcsine-transformed proportions of flies alive after 24 h. Flies were aged for 7 d at 25°C or acclimated by holding them at 13°C for 7 d.
* $P < .10$.
** $P < .05$.
*** $P < .01$.
**** $P < .001$.

This parental effect is the opposite of what might be expected. The significance of the "repeat" term probably reflects small differences in the cold stress temperature.

Cold Resistance of Drosophila melanogaster *and* Drosophila simulans *Populations*

The cold resistance of both species was increased markedly by the 13°C treatment, as reflected by the vastly different times flies held at 13° and 25°C had to be exposed to −2°C to obtain intermediate mortality levels. There were significant differences in cold resistance between the species for flies that had been held at 13° and at 25°C (table 3). After acclimation at 13°C, *Drosophila simulans* was less resistant to cold stress than *Drosophila melanogaster,* but *D. simulans* was significantly more resistant than *D. melanogaster* after flies were held at 25°C. The extent to which cold resistance was increased by the 13°C treatment was therefore greater in *D. melanogaster* than in *D. simulans*.

Population differences were not evident when flies were held at 25°C. However, Melbourne males of both species were significantly more resistant than Cairns males after acclimation at 13°C (fig. 3). A similar difference was evident in the females although the population effect was only marginally significant ($P < .10$). The absence of significant interactions between the species and population effects indicates that differences between the populations were of a similar magnitude in both species.
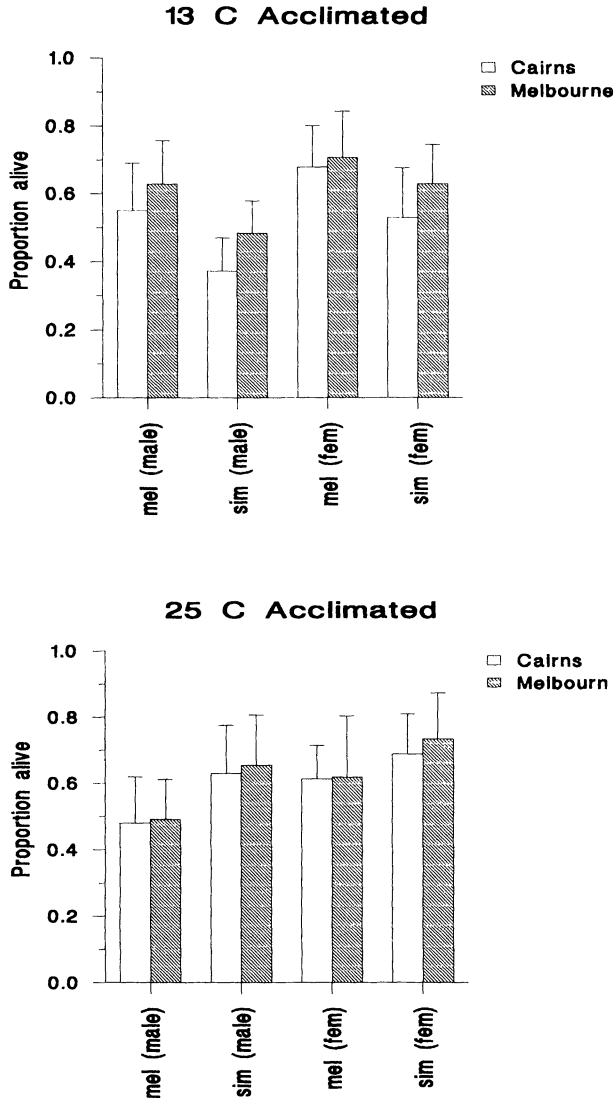
13 C Acclimated

25 C Acclimated

FIG. 3.—Cold resistance of *Drosophila melanogaster* and *Drosophila simulans* populations after adults were aged for 7 d at 13° or 25°C. *Error bars* are mean SDs of the three 14°C repeat experiments or four 25°C repeat experiments.

Heat Resistance of Drosophila melanogaster *and* Drosophila simulans *Populations*

Table 4 presents ANOVAs for knockdown and recovery, and means for the recovery data are plotted in figure 4. Acclimation influenced the resistance of both species, regardless of whether resistance was scored as knockdown or recovery. Females acclimated at 29°C were the most resistant, followed by the 25° and 13°C treatments. The only apparent exception is the Cairns *D. simulans* females,

THE AMERICAN NATURALIST

TABLE 4

ANALYSES OF VARIANCE FOR HEAT RESISTANCE OF *DROSOPHILA MELANOGASTER* AND
DROSOPHILA SIMULANS

Effect	df	Knockdown	Recovery
Drosophila melanogaster:			
Population	1	9.42	42.30**
Acclimation	2	162.13***	117.75***
Block	4	11.77	21.33**
Population × acclimation	2	1.07	7.01
Error	20	5.34	3.90
Drosophila simulans:			
Population	1	2.35	79.58**
Acclimation	2	92.43**	25.83*
Block	4	44.55*	89.45***
Population × acclimation	2	11.37	7.46
Error	20	12.59	7.24

NOTE.—Numbers represent mean squares; ANOVAs were carried out on 10 × arcsine-transformed proportions of flies that recovered or were not knocked down. Females were acclimated by holding them at 13°, 25°, or 19°C for 4 d.
* $P < .05$.
** $P < .01$.
*** $P < .001$.

which seemed to have a similar level of resistance when they were acclimated at 29° and 13°C. When the 29° and 13°C data from the *D. simulans* populations are compared by a one-way ANOVA, the difference between acclimation treatments is significant in Melbourne ($P < .05$) but not in Cairns. However, the interaction effects are not significant in the ANOVAs, which indicates that the acclimation responses of the populations cannot be distinguished when they are compared directly. Interaction terms are also nonsignificant when only the data for the 29° and 13°C treatments are compared. There were overall population differences for the recovery measure, which reflects greater resistance of Melbourne females to knockdown in both species.

DISCUSSION

The experiments provide little evidence for intraspecific variation in the different types of acclimation responses for cold resistance. The populations of *Drosophila melanogaster* from Cairns and Melbourne showed the same degree of acclimation when adults were exposed to sublethal cold stresses for a few hours or for several days. Moreover, larval culture temperature had a similar effect on resistance in both populations. Similarly, the Cairns and Melbourne *Drosophila simulans* populations showed the same degree of acclimation when adults were aged at different temperatures. These conclusions applied regardless of whether flies were stressed at −5° or −2°C. The results are therefore consistent with other *Drosophila* studies on cold resistance (Yamamoto and Ohba 1982; Kimura 1988), which indicates little population differentiation in acclimation responses for cold resistance.
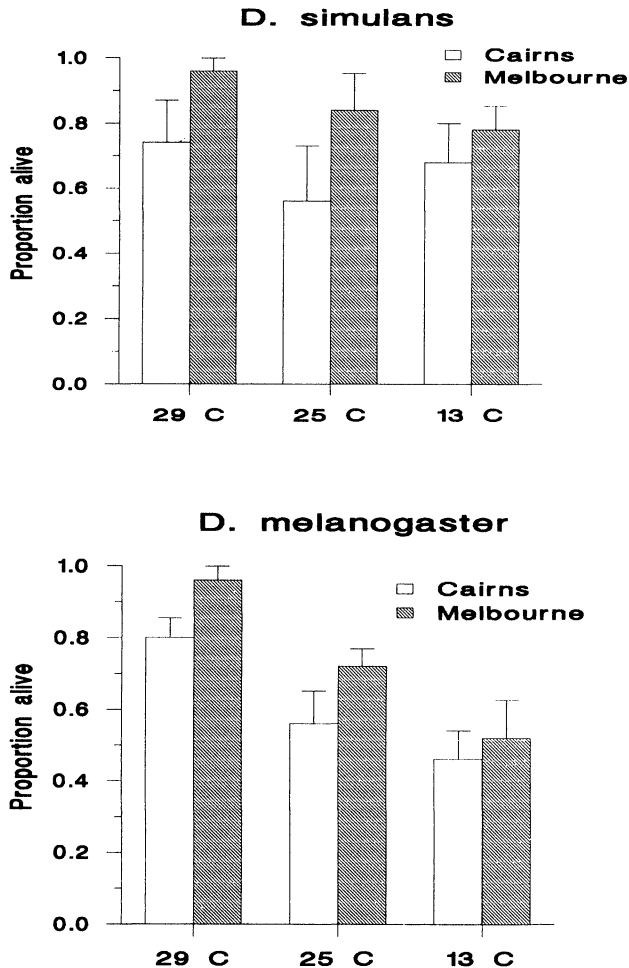
D. simulans

D. melanogaster

Fig. 4.—Heat resistance of *Drosophila melanogaster* and *Drosophila simulans* popula-tions. Adults were aged for 4 d at 13°, 25°, or 29°C. Flies were stressed at 39°C and scored for recovery after exposures of 28 min (*D. melanogaster*) and 14 min (*D. simulans*). Means are based on five replicates, and *error bars* represent SEs based on five replicate blocks.

We have also failed to repeat the findings of Levins (1969) on acclimation for heat resistance. Levins found that both *D. simulans* and *D. melanogaster* populations varied markedly in their acclimation responses. We were unable to find such differences despite using acclimation regimens and a heat stress that were similar to those used by Levins. It is possible that we may have detected population differences in acclimation ability if a larger number of populations had been examined. However, the Cairns and Melbourne populations were deliber-ately chosen to represent very different climatic regimes.

A limitation of the experiments described by Levins (1969) is that the acclima-tion-by-population interactions were not tested directly but were inferred by a

comparison of the 29° and 13°C treatments carried out separately for each experiment. It is worth noting that our *D. simulans* results indicate a significant difference between the 29° and 13°C treatments for the Melbourne population but not for the Cairns population when these populations are compared separately. However, the absence of a significant population-by-acclimation interaction in the ANOVA indicates that populations did not differ in acclimation responses. Conclusions based only on separate comparisons of the means should therefore be treated cautiously when differences between treatments are small and there is a high degree of interreplicate variation. To detect a significant difference in the heat acclimation response of two populations would require a very large and well-designed experiment.

A lack of power to detect differences in acclimation response is evident in some of our experiments (and those of other *Drosophila* researchers) on cold resistance. In the *D. melanogaster* experiments on rapid acclimation, simulations show that the population-by-acclimation term in the ANOVAs would not have been significant even if Cairns had shown an acclimation response as little as 30% of that of the Melbourne population. This observation is true even for the $-5°C$ stress treatment, which involved several repeat experiments and 18 replicates for each population-acclimation combination. We can therefore only conclude from our results that both *D. melanogaster* populations were acclimated after a short exposure period, although the means for the treatments (fig. 1) suggest that the acclimation response was of a similar magnitude in the populations.

In contrast, there is more power to detect variation in acclimation response in the larval acclimation experiments, because of the size of the difference between the acclimated and nonacclimated treatments. Simulations indicate that the population-by-acclimation interaction would have been significant if Cairns males had shown an acclimation response that was 70% of the response shown by Melbourne males. Even so, smaller differences in acclimation ability would have remained undetected in these experiments. Such small differences were likely to have been apparent only in the experiments in which adults were exposed to low temperatures for 1 or 2 wk. Because this acclimation treatment had such a drastic influence on resistance, small differences in acclimation ability would have led to large population differences in resistance following acclimation in *D. melanogaster* and *D. simulans,* which was clearly not the case (fig. 3).

It should be emphasized that the detection of population-by-acclimation treatment interactions can depend on the scale of measurement when populations differ markedly in their overall levels of resistance. In the ANOVAs carried out in this study, interactions were tested on survival data after arcsine transformation. This means that acclimation effects are assumed to be independent of overall population means. An increase in survival following acclimation from 20% to 30% in one population is therefore considered roughly the same as an increase from 70% to 80% in another population. However, it may be more appropriate to assume that acclimation effects and the overall resistance of populations are not independent. In this case, an increase in survival from 20% to 30% may represent a larger acclimation response than an increase from 70% to 80%, because the former situation represents a larger proportional increase in resistance. Log trans-

formations can be used to test for interactions if acclimation responses are as-sumed to be proportional to overall population means. We have therefore reana-lyzed the results of some of our experiments using log-transformed data. In no case did this reanalysis provide evidence for significant population-by-acclimation interactions.

Despite the absence of population differences in acclimation ability, we have confirmed earlier results (Stanley and Parsons 1981) on nonplastic differences in cold resistance between tropical and temperate Australian populations. Mel-bourne *D. melanogaster* were relatively more resistant regardless of how cold resistance was measured. This difference was evident when larvae were cultured at different temperatures or when adults were aged at low temperatures. Adult *D. simulans* from Melbourne were also more resistant than those from Cairns after adults were acclimated at a low temperature, and the population difference was of a similar magnitude in the two species. Nevertheless, nonplastic popula-tion differences were not detected in three experiments, which suggests that cau-tion is required when concluding that populations do not show differences in stress resistance on the basis of a single test of resistance. For example, Davidson (1990) concluded that the cold resistance of *D. simulans* from Melbourne did not differ from those originating from Townsville, a tropical site near Cairns, and this result may reflect the fact that only one measure of cold resistance was used.

The absence of population differences in acclimation response in contrast to nonplastic differences may indicate that climatic adaptation at the intraspecific level involves nonplastic changes acting independently of acclimation. The rea-sons for an absence of plastic adaptive changes are not known. A number of factors may favor nonplastic adaptation to an environmental stress (Hoffmann and Parsons 1991). These include low levels of genetic variance for acclimation ability within populations or costs associated with increased acclimation re-sponses. We are presently investigating genetic variance for acclimation re-sponses within populations using selection experiments and strain comparisons.

The acclimation responses of the sibling species *D. melanogaster* and *D. sim-ulans* were similar. Both species could be cold hardened by short exposure to a low temperature (*D. simulans* data are not presented), and both species showed large increases in resistance following adult exposures of a few days. Neverthe-less, there was evidence for species differences in acclimation ability following adult exposure to 13°C because *D. simulans* adults were more cold-resistant after being held at 25°C, whereas *D. melanogaster* flies were more resistant after being held at 13°C, which indicates that *D. melanogaster* showed a greater acclimation response to the low temperature. This difference was small compared to the overall acclimation effect; the resistance of flies of both species was increased considerably when flies were aged at 13°C as indicated by the short time (1.8 h) required to score resistance of the 25°C flies compared to the long time (17.5 h) for the 13°C flies. Hence, while there is evidence for some interspecific variation in acclimation response in agreement with other *Drosophila* species comparisons (Kimura 1988), these small differences do not suggest that *D. melanogaster* and *D. simulans* follow different strategies in countering climatic stresses.

In conclusion, we have failed to demonstrate significant differences in the accli-

mation responses of *D. melanogaster* and *D. simulans* populations from different climates. Our findings suggest that, at least in these two species of *Drosophila*, plastic responses to temperature stress do not readily evolve. These findings parallel those of Brown and Feldmeth (1971), who failed to detect differences in the thermal acclimation responses of populations of desert pupfish exposed to environments with different temperatures.

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