

Interactive Effects of Rearing Temperature and Oxygen on the Development of *Drosophila melanogaster*

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ABSTRACT

Although higher temperatures strongly stimulate ectothermic metabolic rates, they only slightly increase oxygen diffusion rates and decrease oxygen solubility. Consequently, we predicted that insect gas exchange systems would have more difficulty meeting tissue oxygen demands at higher temperatures. In this study, *Drosophila melanogaster* were reared from egg to adult in hyperoxic (40%), hypoxic (10%), and normoxic (21%) conditions and in temperatures ranging from 15°–31.5°C to examine the interactive effect of temperature and oxygen on development. Hyperoxia generally increased mass and growth rate at higher rearing temperatures. At lower rearing temperatures, however, hyperoxia had a very small effect on mass, did not affect growth rate, and lengthened time to eclosion. Relative to normoxia, flies reared in hypoxic conditions were generally smaller (mass and thorax length), had longer eclosion times, slower growth rates, and reduced survival. At cooler temperatures, hypoxia had relatively modest or nonsignificant effects on development, while at higher temperatures, the effects of hypoxia were large. These results suggest that higher temperatures reduce oxygen delivery capacity relative to tissue oxygen needs, which may partially explain why ectotherms are smaller when development occurs at higher temperatures.

Introduction

Temperature has differential effects on ectothermic metabolic rate, oxygen diffusion rate, and oxygen solubility in fluids. For

instance, a 10°C rise in temperature within the biological range causes the metabolic oxygen demand of an ectotherm to increase 50%–300% (Hunter 1964; Withers 1992), while the diffusive rate of oxygen increases by only about 4% in air (Brown et al. 1991) and 40% in fluid (Seymour 1994). Furthermore, the solubility of oxygen in water declines by roughly 15% (Carpenter 1966). Consequently, increased temperatures may reduce oxygen delivery capacity relative to tissue oxygen requirements in ectothermic organisms (Sibly and Atkinson 1994; Atkinson 1996; Woods 1999).

Evidence from diverse taxa supports this idea. At warmer temperatures, spider crabs have reduced hemolymph PO_2 values (Frederich and Portner 2000), and both spider crabs and polychaete worms (Sommer et al. 1997) transition to anaerobic respiration, as evidenced by the presence of anaerobic end products. Furthermore, lizards (Hicks and Wood 1985), fish (Bryan et al. 1984; Schurmann and Steffensen 1992), alligators (Branco et al. 1993), and salamanders and crayfish (Dupre and Wood 1988) exposed to hypoxia prefer lower body temperatures than in normoxic conditions; insect pests succumb to anoxia more readily at higher temperatures (Donahaye et al. 1996); and trout eggs have higher mortality at high temperatures and low oxygen concentrations than at either high temperatures or low oxygen concentrations alone (Garside 1959).

The increased difficulty of tissue oxygen delivery at higher temperatures may help explain why almost all ectotherms are smaller when development occurs at higher temperatures (Ray 1960; Atkinson 1994). The effect of temperature on ectothermic size is well documented but poorly understood. However, it has been hypothesized for eggs (Bradford 1990; Woods 1999) and aquatic ectotherms (Atkinson 1994, 1996; Sibly and Atkinson 1994; Atkinson and Sibly 1997) that the smaller sizes associated with higher developmental temperatures are due to reduced oxygen availability.

We tested the effects of rearing temperature on the oxygen sensitivity of ectothermic development using *Drosophila melanogaster*. Fruit flies are ideal for this study because they have short generation times and large numbers are easily reared in small containers, facilitating experimental control of temperature and oxygen. We reared flies from egg to adult in hyperoxic (40%), normoxic (21%), and hypoxic (10%) conditions, in temperatures that ranged from 15°–31.5°C, and examined the effects on body size, time to eclosion, growth rate, and survival. If atmospheric oxygen levels limit development at higher temperatures, we predicted that, at warmer rearing temperatures, the flies in hyperoxia would have larger sizes, shorter eclosion

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times, and faster growth rates relative to flies reared in normoxia. In addition, we predicted that warmer temperatures would intensify the deleterious effects of hypoxia on development.

Material and Methods

Study Organism

Drosophila melanogaster were reared from egg to adult in a range of oxygen (10%, 21%, and 40%) and temperature (15°–31.5°C) treatments. In the hyperoxia experiment, an Ives strain of *D. melanogaster* was reared at 16°, 22°, 24°, 29.5°, and 31.5°C. In the hypoxia experiments, flies were reared in two separate experimental runs, designated “hypoxia test 1” (15°, 24°, and 30°C) and “hypoxia test 2” (18°, 24°, and 28°C). Hypoxia test 1 flies were an Oregon-R strain, and hypoxia test 2 flies were an Ives strain.

Egg Collection

Two or three days before eggs were collected for the experiments, we transferred several hundred mature flies to jars containing fresh food seeded with live yeast. On the day of egg collection, these flies were transferred to laying pots and given a grape-based nutritional substrate on which to lay their eggs (815 mL water, 683 mL Welch’s grape juice concentrate, 80 g dextrose, 50.5 g sucrose, 33 g agar, 27 g yeast, 33 mL 1.25 N NaOH, and 16.8 mL propionic acid). The flies were allowed a 2-h prelaying period to encourage females to lay any retained eggs, which were then discarded. We then collected all the eggs for each oxygen/temperature treatment in a 2–4-h period and transferred them into vials (9.5 cm long, 2.2 cm diameter) containing approximately 9 mL of dextrose diet. The dextrose diet was composed of 1.6 L water, 138 g dextrose, 9.9 g agar, 65 g cornmeal, and 34.4 g yeast, vigorously boiled for 20 min to kill the yeast. After the diet cooled to 55°C, 14.4 mL of Tegosept antifungal agent was added to further sterilize it so that oxygen consumption by diet microorganisms would be minimized.

Sample Sizes

Hyperoxia Experiment. In the hyperoxia experiment, we reared flies in vials placed in 0.95-L glass jars. We placed 12 eggs into each vial and five vials into each jar (60 eggs per jar). There were two jars for each oxygen/temperature treatment group (replicates). The total number of eggs in each treatment group was 120.

Hypoxia Experiments. In hypoxia test 1, there were no replicate treatment groups (i.e., one jar for each oxygen/temperature treatment), and each jar contained six vials with 10 eggs per vial. The total number of eggs in each treatment group was 60.

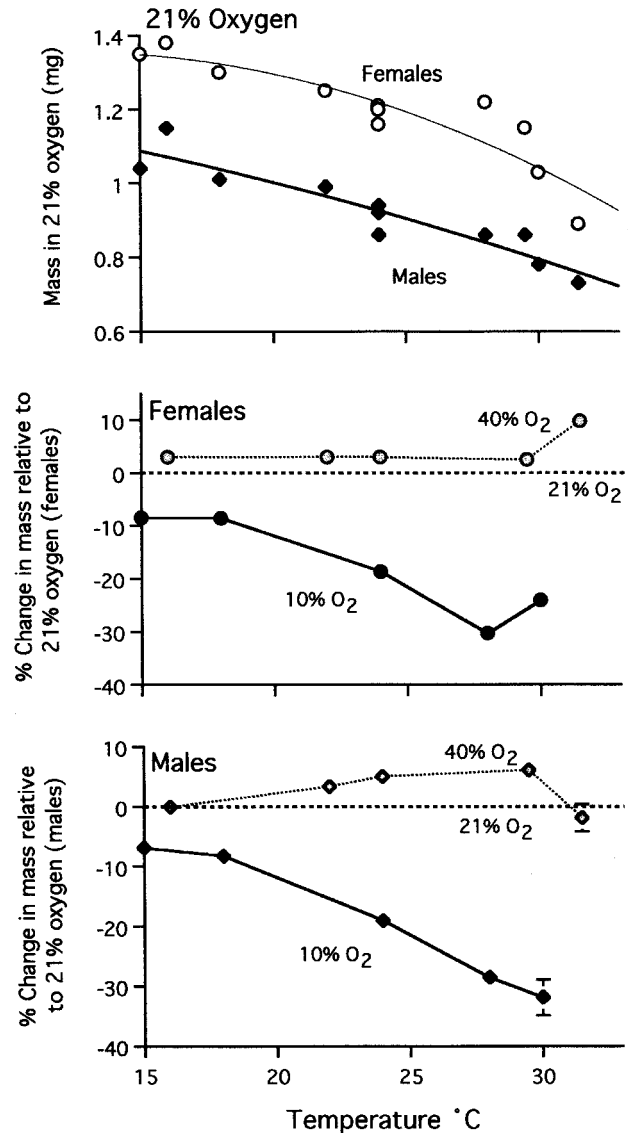


Figure 1. Effects of normoxia, hyperoxia, and hypoxia on mass. *Top panel*, as rearing temperature increased, the mass of *Drosophila melanogaster* reared in normoxia decreased for both females (open circles; $y = -0.001x^2 + 0.025x + 1.203$; $R^2 = 0.82$) and males (filled diamonds; $y = -0.000x^2 - 0.008x + 1.266$; $R^2 = 0.87$). *Middle, bottom panels*, relative effects of hyperoxia (shaded symbols) and hypoxia (filled symbols) on mass for females (*middle panel*) and males (*bottom panel*). In both the hyperoxia and hypoxia experiments, there was a significant effect of oxygen and a significant interaction between oxygen and temperature on mass. For this and subsequent figures, means \pm SEM are shown; SEMs not visible are not large enough to extend beyond symbol boundaries.

In hypoxia test 2, there were two jars for each oxygen/temperature treatment group (replicates). Each jar contained five vials with 15 eggs per vial (75 eggs per jar). The total number of eggs in each treatment group was 150.

Experimental Rearing Conditions

Flies were reared in temperature-controlled incubators (except 24°C flies, which were maintained at room temperature) under a 14L : 10D light cycle. We used premixed oxygen tanks for the 40% \pm 0.8% and 10% \pm 0.2% oxygen treatments and room air for the 21% oxygen treatment. Airflow was kept at 15 mL min^{-1} , as measured by a Cole-Parmer flowmeter (model N032-41; calibrated with a soap-bubble flowmeter). Rearing vials were covered with a fine mesh to encourage airflow and to prevent larval escape. Direct measures of oxygen levels in the air exiting the jars indicated that metabolic activity of the larvae and any microorganisms in the media reduced oxygen levels in the jars by <0.03% (Sable Systems Oxzilla oxygen analyzer). Additionally, measures of 100- μL air samples taken <1 mm above the diet had oxygen levels within 0.1% of the air flowing into the jar.

The relative humidity of the rearing chambers was kept near 100% by bubbling the air through a water column. This ensured that treatment effects were due to oxygen and not increased water loss due to higher temperatures or increased spiracular opening at decreased oxygen levels (Loudon 1989; Snyder et al. 1995). Measurements without vials indicated the air in the jars was 90%–94% relative humidity (measured with a Vaisala HMP 31UT humidity sensor, Helsinki, Finland, and a Vaisala HMI 32 meter calibrated with lithium chloride and potassium chloride saturated salt solutions; see Winston and Bates 1960). The humidity experienced by the flies, however, was probably closer to 100%, as water vapor often condensed in the vials during the experiment.

Hyperoxia Experiment. Flies were reared at temperatures of 16°, 22°, 24°, 29.5°, and 31.5°C. Temperatures were obtained by measuring the food temperature for each jar using copper-constantan thermocouples placed to a depth of 3–5 mm in the food after the flies had emerged. Jar temperatures were measured 10–25 times (day and night) over the course of several days with calibrated Physitemp BAT-10 thermocouple thermometers.

The measurements of food temperature indicated that temperatures varied due to incubator cycles and diurnal variations. The following range of temperatures was recorded for each temperature treatment: 16°C (16.1°–16.9°C), 22°C (21.7°–22.5°C), 24°C (23.4°–24.9°C), 29.5°C (28.4°–30.5°C), and 31.5°C (30.9°–31.4°C). The temperatures measured for the 21% and 40% oxygen treatment groups were not significantly different regardless of whether the entire experiment was analyzed (ANOVA, $F_{1,265} = 1.6$, $P = 0.21$) or whether each tem-

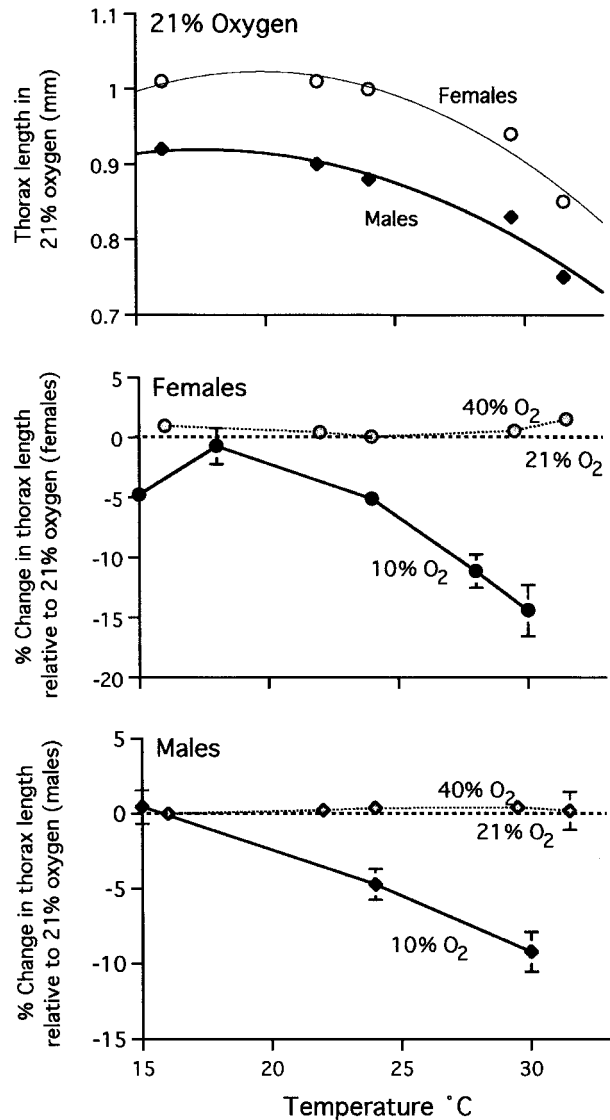


Figure 2. Effects of normoxia, hyperoxia, and hypoxia on thorax length. *Top panel*, as rearing temperature increased, the thorax length of *Drosophila melanogaster* reared in normoxia decreased for both females (open circles; $y = -0.001x^2 + 0.046x + 0.571$; $R^2 = 0.95$) and males (filled diamonds; $y = -0.001x^2 + 0.028x + 0.672$; $R^2 = 0.95$). *Middle, bottom panels*, relative effects of hyperoxia (shaded symbols) and hypoxia (filled symbols) on thorax length for females (*middle panel*) and males (*bottom panel*). In the hyperoxia experiment, there was a very small but significant effect of oxygen on thorax size in females but not males. There was no significant interaction between oxygen and temperature on thorax length regardless of gender. In the hypoxia experiments, there was a significant effect of oxygen and a significant interaction between oxygen and temperature on thorax length.

Table 1: 21% and 40% rearing oxygen

	R^2	Temperature	Oxygen	Temperature \times Oxygen
Log mass (Fig. 1):				
Females ($n = 446$)	.72	263.97**	31.76**	2.66*
Males ($n = 435$)	.73	267.96**	10.49**	2.56*
Log thorax length (Fig. 2):				
Females ($n = 446$)	.77	360.74**	4.54*	.60
Males ($n = 433$)	.79	384.00**	.26	.19
Log wing length:				
Females ($n = 443$)	.92	1,162.66**	6.94**	.89
Males ($n = 433$)	.93	1,394.54**	3.23	.49
Time to eclosion (Fig. 3):				
Females ($n = 447$)	.99	17,391.96**	54.85**	18.34**
Males ($n = 435$)	1.00	18,231.27**	94.67**	19.15**
Growth rate (Fig. 4):				
Females ($n = 446$)	.91	1,060.34**	2.30	6.04**
Males ($n = 435$)	.91	1,027.83**	.09	4.37**

Note. F ratios from ANOVA tests for log mass, log thorax length, log wing length, time to eclosion, and growth rate. Males and females were analyzed in separate ANOVA tests because they often had different responses to the temperature and oxygen treatments, causing significant three-way interactions between temperature \times oxygen \times sex. The following measurements had significant three-way interactions (with corresponding F ratio and P value): log wet weight, $F_{4,861} = 4.427$; time to eclosion, $F_{4,862} = 3.577$; growth rate, $F_{4,861} = 7.131$ ($P < 0.01$).

* $P < 0.05$.

** $P < 0.01$.

perature was analyzed separately (Tukey pairwise tests at each temperature, all $P > 0.35$). However, one of the duplicate jars in the 16°C treatment group was eliminated from the analysis because its temperature was higher than the other jars at that temperature (16.9°C).

Hypoxia Experiments. We reared flies in hypoxia test 1 at 15°, 24°, and 30°C and in hypoxia test 2 at temperatures of 18°, 24°, and 28°C.

Developmental Measures

As adult flies began to eclose, we collected them by aspiration every 1–9 h depending on emergence rate; flies were collected more frequently when emergence was rapid. Once collected, the flies were anesthetized (using ether in hypoxia experiments and carbon dioxide in hyperoxia experiments) and weighed using a Cahn C-33 microbalance ($\pm 2 \mu\text{g}$).

After collecting the flies, we measured thorax and wing lengths. In the hyperoxia experiment and hypoxia test 2 experiment, thorax length was measured from the posterior tip of the scutellum to the base of the most anterior humeral bristle (French et al. 1998) using a dissecting scope fitted with an ocular micrometer. In the hyperoxia experiment, all flies were measured, and in the hypoxia test 2 experiment, 20 female flies were randomly chosen from each jar for thorax measures (total of 40 thorax measures per temperature/oxygen treatment). In

hypoxia test 1, we measured thorax length from the posterior tip of the scutellum to the anteriormost portion of the scutellum. We obtained this measurement by transferring the image of the thorax from a dissecting scope to a television screen using a Hitachi 3CCD model HV-C20M video camera. We then measured the dimensions of the thorax and an adjacent black iodized insect pin of known dimension directly from the monitor screen using a Mitutoyo CD-6-inch BS micrometer (± 0.01 mm). Thorax measures were taken without knowledge of treatment group.

Wing lengths were measured only in the hyperoxia experiment. The wings were removed as close to the body as possible and adhered to paper with clear tape. To estimate wing size, the length of the third longitudinal vein was measured under a dissecting scope fitted with an ocular micrometer. The measurements for wing length were taken without knowledge of treatment group.

Time to eclosion (h) was calculated from the midpoint of the lay period to the time of emergence. Growth rate ($\mu\text{g}/\text{h}$) was calculated by dividing wet mass (mg) by the time to eclosion and then multiplying by 1,000.

Statistical Analysis and Data Presentation

Data were analyzed using SYSTAT (Wilkinson 1989), with the Type I error set at 0.05. To correct for unequal variances, analysis on all size data was performed on log-transformed data.

Table 2: 21% and 10% rearing oxygen

	R^2	Temperature	Oxygen	Temperature \times Oxygen
Log mass (Fig. 1):				
Females ($n = 480$)	.73	161.57**	391.17**	39.64**
Males ($n = 443$)	.77	219.31**	331.79**	34.28**
Log thorax length (Fig. 2):				
Hypoxia test 1:				
Females ($n = 128$)	.67	103.76**	91.97**	9.15**
Males ($n = 105$)	.57	62.99**	13.71**	5.75**
Hypoxia test 2:				
Females ($n = 222$)	.14	4.95**	14.98**	5.77**
Time to eclosion (Fig. 3):				
Females ($n = 480$)	.99	16,277.10**	284.32**	26.03**
Males ($n = 443$)	.99	12,813.02**	210.16**	15.84**
Growth rate (Fig. 4):				
Females ($n = 480$)	.91	732.15**	659.29**	112.34**
Males ($n = 443$)	.88	476.77**	471.23**	69.25**

Note. F ratios from ANOVA tests for log mass, log thorax length, time to eclosion, and growth rate. Males and females were analyzed in separate ANOVA tests because they often had different responses to the temperature and oxygen treatments, causing significant three-way interactions between temperature \times oxygen \times sex. Hypoxia test 1 and hypoxia test 2 were not grouped for log thorax length due to differences in measuring techniques between the two tests. The following measurement had a significant three-way interaction (with corresponding F ratio and P value): growth rate, $F_{4,903} = 9.53$ ($P < 0.01$).

** $P < 0.01$.

Data are presented as mean \pm SEM of the mean unless otherwise noted. Graphs showing the effects of temperature in normoxia include the data from the hyperoxia experiment and hypoxia experiments 1 and 2 (except for the thorax graph, which does not include hypoxia experiment 1 since a different protocol was used to obtain thorax measures in this experiment). For the graphs and data analysis, the results from hypoxia experiments 1 and 2 were combined to simplify presentation. However, the general trends were the same whether the two tests were analyzed individually or together.

Results

Effects of Temperature and Oxygen on Size

Regardless of oxygen treatment, flies had smaller masses, thorax lengths, and wing lengths when reared at higher temperatures (Figs. 1, 2; see top graphs for effects of temperature on size in normoxia). Flies reared in hyperoxia had significantly greater wet masses (but not thorax or wing lengths) than those reared in normoxia, whereas flies reared in hypoxia had significantly smaller wet masses, thorax lengths, and wing lengths than flies reared in normoxia. In addition, there was a significant interaction between temperature and oxygen on most measures of insect size (Table 1).

At the highest temperature of 31.5°C, female flies were about 10% heavier in 40% oxygen (Fig. 1, *middle panel*; Tukey pairwise comparison: mean log difference = 0.042; $P < 0.001$, $N = 60$). At 31.5°C, males were not significantly heavier in

hyperoxia; at 29.5°C, however, males were about 6% heavier in hyperoxia (Fig. 2, *bottom panel*; Tukey pairwise comparison: mean log difference = 0.026; $P = 0.003$, $N = 102$). At cooler temperatures, males and females were, on average, 3% larger in hyperoxia (ANOVA test of oxygen effect on female mass with 31.5°C removed from analysis: $F_{1,378} = 16.0$, $P < 0.001$; males with 31.5°C and 29.5°C removed: $F_{1,289} = 9.8$, $P = 0.002$). The effects of hyperoxia on thorax and wing lengths were very small, and there was no evidence of a temperature/oxygen interaction. Thorax size was about 1% larger in hyperoxia for female flies (Table 1; Fig. 2, *middle panel*) but not for male flies (Table 1; Fig. 2, *bottom panel*). Wing lengths were 1% smaller in 40% oxygen for female flies ($P = 0.009$; Table 1) but not significantly different for male flies ($P = 0.073$; Table 1).

Hypoxia depressed adult size more strongly at higher rearing temperatures (Table 2; Figs. 1, 2). For example, hypoxia reduced female mass 24% at 30°C and only 9% at 15°C (Fig. 1, *middle panel*). At 15°C, hypoxia did not significantly affect male thorax length (Tukey pairwise comparison: mean log difference = -0.002 ; $P = 0.999$, $N = 50$) and caused a 9% reduction in female thorax lengths (Tukey pairwise comparison: mean log difference = 0.021; $P = 0.005$, $N = 54$). At 30°C, however, hypoxia reduced thorax size by approximately 30%, for both males and females (Tukey pairwise comparison for females: mean log difference = 0.068; $P < 0.001$, $N = 29$; males: mean log difference = 0.0391; $P = 0.008$, $N = 22$).

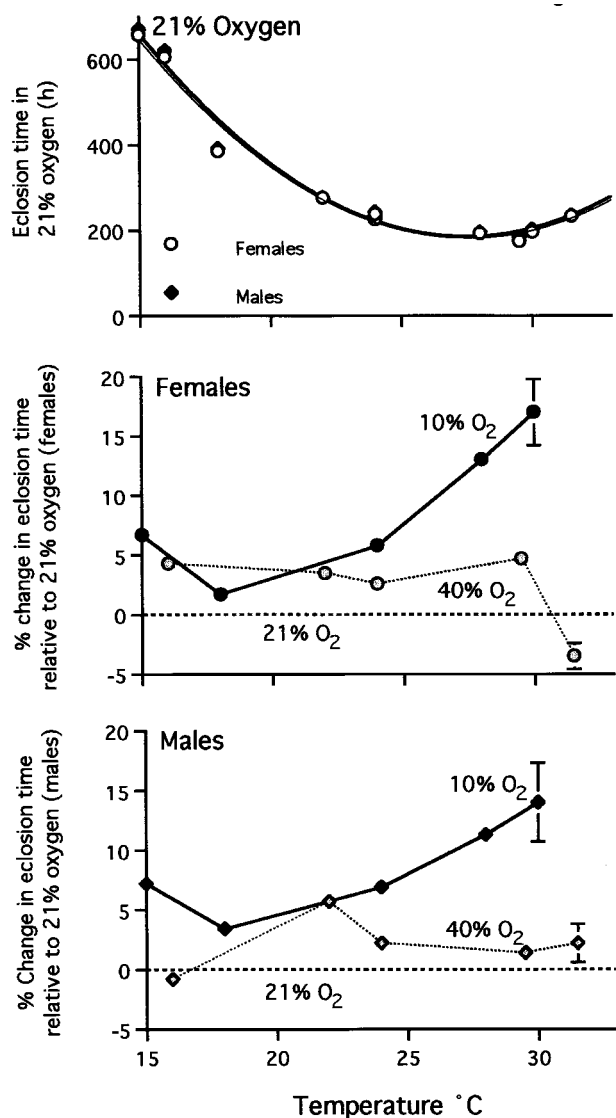


Figure 3. Effects of normoxia, hyperoxia, and hypoxia on eclosion time. *Top panel*, rearing temperatures lower than about 28°C reduced time to eclosion for both females (open circles; $y = 2.964x^2 - 163.144x + 2,427.220$; $R^2 = 0.98$) and males (filled diamonds; $y = 3.059x^2 - 168.082x + 2,494.424$; $R^2 = 0.98$). *Middle, bottom panels*, relative effects of hyperoxia (shaded symbols) and hypoxia (filled symbols) on time to eclosion for females (*middle panel*) and males (*bottom panel*). In the hyperoxia and hypoxia experiments, there was a significant effect of oxygen and a significant interaction between oxygen and temperature on time to eclosion.

Effects of Temperature and Oxygen on Time to Eclosion and Growth Rates

The effects of both hyperoxia and hypoxia on time to eclosion and growth rate were strongly temperature dependent (Table 1; Figs. 3, 4). At warmer temperatures, hyperoxia did not delay

eclosion time (Table 1; Fig. 3) and generally increased growth rate (Tukey pairwise for comparison females, 31.5°C: mean difference = 0.520; $P < 0.001$, $N = 60$; males, 29.5°C: mean difference = 0.210; $P = 0.025$, $N = 102$; Table 1; Fig. 4). In contrast, at cooler rearing temperatures, eclosion times were significantly delayed by hyperoxia ($\leq 22^\circ\text{C}$ for males and females; Tukey pairwise comparisons, $P < 0.001$) and growth rates were not affected by hyperoxia (Table 1; Fig. 4; $\leq 29.5^\circ\text{C}$ for females and 24°C for males: Tukey pairwise comparisons, $P > 0.75$). In hypoxia, eclosion was postponed and growth rate was reduced by 10% oxygen; higher temperatures exacerbated the effect (Table 2; Figs. 3, 4).

Effects of Temperature and Oxygen on Survival

In both the hyperoxia and hypoxia experiments, survival was reduced only at the highest rearing temperatures (log-linear analysis with all temperatures; hyperoxia experiment: $\chi^2_{0.05(8)} = 212.14$, $P < 0.001$; analysis with 31.5°C removed: $\chi^2_{0.05(5)} = 1.46$, $P = 0.9179$ [Fig. 5, *top panel*]; hypoxia experiment log-linear analysis: $\chi^2_{0.05(8)} = 79.34$, $P < 0.001$ [Fig. 5, *bottom panel*; Wilkinson 1989]). Hyperoxia had no effect on survival, suggesting that the high death rates at high temperatures were not due to oxygen limitation (log-linear analysis: $\chi^2_{0.05(8)} = 1.46$, $P = 0.9179$ [Fig. 5, *top panel*]). Hypoxia reduced survival at 30°C (G -test: $\chi^2_{0.05(1)} = 7.6008$, $P < 0.01$ [Fig. 5, *bottom panel*]), however, at 15°C, there was no significant difference in survival between 10% and 21% oxygen treatments (G -test: $\chi^2_{0.05(1)} = 0.14$, $P > 0.5$ [Sokal and Rohlf 1995]).

Discussion

In these experiments, oxygen concentration had pervasive effects on a variety of fitness-related traits, including adult size, time to eclosion, growth rate, and survival (Stearns 1992; Stearns and Kawecki 1994). The effects of oxygen were modulated in important ways by temperature, and, in general, the effects of both hyperoxia and hypoxia were most dramatic at higher rearing temperatures. The interaction between temperature and oxygen on fly development suggests that oxygen delivery becomes increasingly difficult at higher temperatures, and the metabolic demand for oxygen may outstrip the capacity for oxygen delivery in *Drosophila melanogaster*. The differential effects of temperature on metabolism, oxygen diffusion rates, and fluid solubility are relevant to most organisms. Consequently, the thermal dependence of oxygen demand relative to delivery capacity is likely to be quite general in ectotherms.

Effects of Temperature and Oxygen on Development

The developmental response of *D. melanogaster* to hyperoxic and hypoxic rearing conditions was strongly affected by temperature (Table 1). In general, hypoxia reduced size (mass, Fig.

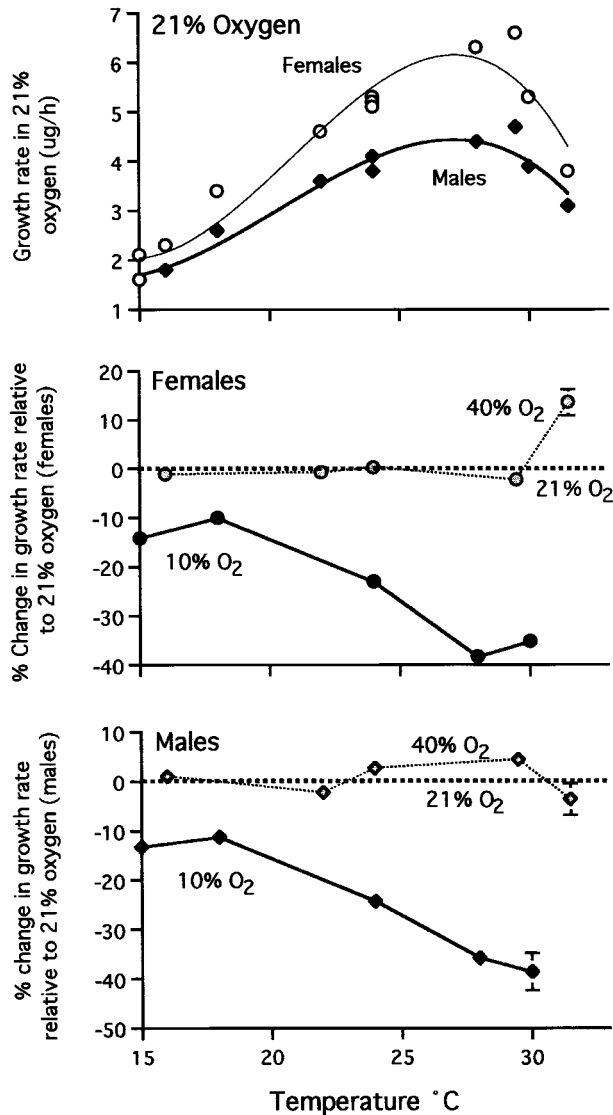


Figure 4. Effects of normoxia, hyperoxia, and hypoxia on growth rate. *Top panel*, rearing temperatures lower or higher than 28°C reduced growth rates for both females (open circles; $y = -0.004x^3 + 0.261x^2 - 4.939x + 31.499$; $R^2 = 0.93$) and males (filled diamonds; $y = -0.002x^3 + 0.132x^2 - 2.347x + 14.475$; $R^2 = 0.94$). *Middle, bottom panels*, relative effects of hyperoxia (shaded symbols) and hypoxia (filled symbols) on growth rates for females (middle panel) and males (bottom panel). In the hyperoxia experiment, growth rate was increased at higher rearing temperatures by 40% oxygen (31.5°C for females; 29.5°C for males). Hyperoxia did not increase growth rate at lower temperatures. In the hypoxia experiments, there was a significant effect of oxygen and a significant interaction between temperature and oxygen on growth rate.

1; thorax length, Fig. 2), growth rates (Fig. 4), and survival (Fig. 5) and increased time to eclosion (Fig. 3). The effects of hypoxia were most pronounced at higher rearing temperatures. The effects of hyperoxia on mass, time to eclosion, and growth rate differed between low and high rearing temperatures, suggesting that hyperoxia can affect development through different temperature-dependent mechanisms.

In general, at the highest rearing temperatures (31.5°C for females and 29.5°C for males), hyperoxia resulted in flies with larger masses (Fig. 1) and increased growth rates (Fig. 4). Interestingly, at 31.5°C, males did not have larger masses or faster growth rates in hyperoxia; this result may have been due to the males' high death rate at 31.5°C (78% death rate for males vs. 50% for females). The effects of hyperoxia at higher temperatures suggest that development was limited by oxygen availability in normal 21% oxygen.

The interaction between temperature and oxygen on fly development suggests that oxygen delivery becomes increasingly difficult at higher rearing temperatures. At higher temperatures, the inability of the tracheal system to meet oxygen demand may have reduced tissue oxygen levels enough to affect mitochondrial ATP production. To test this hypothesis, measurements of tissue metabolites and P_{O_2} at different temperatures and oxygen levels are necessary (Frederich and Portner 2000). Alternatively, demand for oxygen may have impeded feeding by limiting larvae to certain parts of the food—such as the surface—that contained adequate oxygen levels, or larvae and pupae may have devoted larger portions of their energy budgets to obtaining oxygen by ventilating or seeking oxygen-rich areas. An additional possibility is that smaller body sizes in low-oxygen or high-temperature environments may represent an acclimatory response to deal with reduced oxygen availability to the tissue, as smaller body sizes may reduce oxygen diffusion distances, thus increasing oxygen flux.

Interestingly, the thorax and wing length data do not support the hypothesis that 21% oxygen levels are limiting at higher temperatures. There was no significant interaction between oxygen and temperature on either wing or thorax length (Table 1; Fig. 2). Additionally, for reasons that are unclear, wing lengths were slightly smaller in all hyperoxic conditions.

At lower temperatures (16°–29.5°C for females and 16°–24°C for males), flies reared in hyperoxia were slightly larger than flies in normoxia, but these flies also took longer to eclose (Fig. 3); therefore, hyperoxia did not significantly affect their growth rates (Fig. 4). Because hyperoxia did not affect growth rates at lower rearing temperatures, it is unlikely that hyperoxia resulted in larger adult masses because 21% oxygen levels limited size. Rather, at cooler temperatures, flies were larger because they had a longer developmental period. A possible mechanistic explanation for the increased development time is that hyperoxia delayed molting, providing the flies more time to add body mass at each instar, ultimately resulting in larger adults. In support of this hypothesis, mealworm molting frequency varied

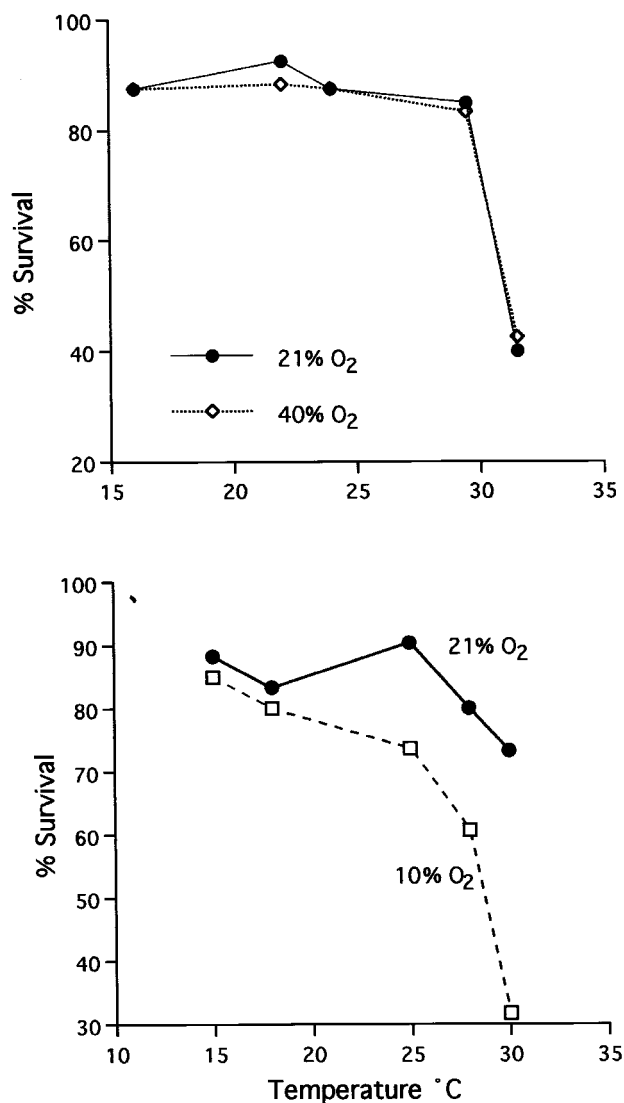


Figure 5. Survival in normoxia, hyperoxia, and hypoxia conditions. Percentage survival (males and females combined) to eclosion in the hyperoxia experiment (*top panel*) and the hypoxia experiment (*bottom panel*). Survival was significantly reduced at the higher temperatures in both experiments. There was no difference in survival between the 21% and 40% oxygen levels at any temperature. Survival was unaffected by hypoxia in the cooler temperatures but was significantly lower in hypoxia at the warmest temperature.

inversely with oxygen availability (Loudon 1988; Greenberg and Ar 1996).

Temperature and Insect Size

Approximately 80% of all ectotherms are smaller when development occurs at higher temperatures (Atkinson 1994; Berrigan and Charnov 1994; Atkinson and Sibly 1997). One proposed

explanation for this relationship is that increased temperatures result in oxygen limitations due to higher metabolic demands and decreased solubility of oxygen in fluids (Sibly and Atkinson 1994; Atkinson 1996; Woods 1999). In support of this idea, the maximum size of aquatic amphipods is better correlated with water oxygen content than with temperature (Chappelle and Peck 1999). In our direct tests of this hypothesis with *D. melanogaster*, increased oxygen partially compensated for the effects of rearing temperature on adult size. Our data suggest that oxygen delivery problems can account for about 15% of the thermal effects on size in this species.

Safety Margins for Oxygen Delivery and Insect Size

Insects are often believed to possess tremendously large safety margins for oxygen delivery because many insects can maintain normal resting metabolic rates and behavioral responses, such as walking, in atmospheres containing <5% oxygen (Galun 1960; Arieli and Lehrer 1988; Holter 1994; Holter and Spangenberg 1997; Greenlee and Harrison 1998). Short-term experiments on resting animals, however, may overestimate safety margins for oxygen delivery. In long-term rearing experiments with *Tenebrio molitor* beetles, 10% oxygen levels have reduced growth rates, survival (Loudon 1988; Greenberg and Ar 1996), and adult size (Greenberg and Ar 1996). Our experiments suggest that *D. melanogaster* have relatively small safety margins for oxygen delivery, as 10% oxygen reduced size and growth rate even at cooler rearing temperatures and 40% oxygen increased size and growth rate at the highest rearing temperatures.

If insects have small safety margins, then relatively small changes in oxygen availability from increased temperatures or reduced environmental oxygen could have a large effect on development and survival. Small oxygen safety margins may affect survival in natural environments since many insects reside in fairly hypoxic environments, such as underground burrows, organic wastes, grain stores, high elevations, and water environments (Anderson and Ultsch 1987). *Drosophila melanogaster* larvae are typically found in decaying plant matter, with their abdominal spiracles at the food-air interface (M. Frazier, personal observation). The boundary layer where larvae obtain oxygen may be hypoxic since rotting plants typically support large amounts of metabolically active bacteria and fungi. In addition, *Drosophila* larvae often reside in high-temperature environments (Feder et al. 1997), further compounding the problem of oxygen delivery.

Small safety margins for oxygen delivery also have implications for patterns of insect size on geological timescales. According to current geochemical models, atmospheric oxygen levels were as high as 35% during the Carboniferous period (Berner and Canfield 1989) and as low as 15% at the Permian-Triassic transition (Berner and Canfield 1989; Gruszczynski et al. 1989; Malkowski et al. 1989). The existence of giant insects during the late Paleozoic may have been a consequence of

increased oxygen levels (Graham et al. 1995; Dudley 1998), and the mass extinction event that occurred during the hypoxic Permian-Triassic transition (Raup 1979; Benton 1985; Labandeira and Sepkoski 1993; Erwin 1994) may have been partially attributable to decreased oxygen levels (Gruszczynski et al. 1989; Malkowski et al. 1989; but see Graham et al. 1995). If these patterns were truly driven by changes in atmospheric oxygen concentration, then current atmospheric oxygen levels must limit insect size. Our study supports this hypothesis by suggesting that larval *D. melanogaster* have relatively small safety margins for oxygen delivery.

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