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DEVELOPMENT OF *TENEBRIO MOLITOR* IN LOW OXYGEN LEVELS

CATHERINE LOUDON*
Department of Zoology, Duke University, Durham, NC 27706, U.S.A.

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Abstract—*Tenebrio molitor* L. (Coleoptera) were reared from eggs to adults in 21, 15 and 10.5% oxygen at one atmosphere total pressure (remainder nitrogen). Larvae reared in 10.5% oxygen grew more slowly than larvae reared in higher levels of oxygen (size per unit time). Timing of moulting was independent of oxygen level. Larvae reared in 10.5% oxygen underwent a greater total number of moults than larvae in higher levels of oxygen, but all larvae reached the same final larval size. The sex ratio of surviving pupae was 1:1 in 21% oxygen, but a significantly greater proportion of females were found in lower oxygen levels. Mortality was higher and developmental abnormalities were more common in lower oxygen levels. The experimental oxygen levels were not low enough to influence the rate of oxygen uptake, yet oxygen level affected the growth rate, sex ratio and mortality of these larvae. This paper suggests that maintenance of a "normal" rate of oxygen uptake is not a sufficient indication of unchanged physiological state; oxygen-independence of the rate of oxygen uptake is not equivalent to oxygen-independence of the animal.

Key Word Index: Growth rate, hypoxia, oxygen, stored products, *Tenebrio molitor*

INTRODUCTION

Many insects develop in environments in which the partial pressure of oxygen departs significantly from standard conditions. Insects that live in stagnant ponds, in logs, in soil, in rotting carcasses, in dung, within other animals as parasites, in stored grain, or at high altitude can all be exposed to low partial pressures of oxygen. Decaying logs may contain as little as 0.5% oxygen, compared with 21% oxygen in normal dry air (Paim and Beckel, 1964). A horse's stomach (where larvae of the horse bot fly *Gasterophilus intestinalis* develop) contains very little oxygen (0.1% oxygen cited in Levenbook, 1950). The oxygen content of the soil depends heavily on the rate of oxygen uptake of soil flora and fauna (roots and microorganisms), the soil compactness, and soil water content: the anoxic zone can begin at a depth of only 1.5 mm for waterlogged soil with a high oxygen uptake rate, or 8 m for loose dry soil with a low oxygen uptake rate (for a variety of profiles see Glinski and Stepniewski, 1985). In stored grain, the oxygen level drops and the carbon dioxide level rises from the respiration of the grain and associated microorganisms as well as the insects. In airtight underground grain storage in Argentina, the carbon dioxide level increased to 9.5% in 70 days, and the oxygen decreased to 1.8% in 644 days (Cotton, 1963). Some insects may live most of their lives in normal air but spend part of their lives at lower oxygen levels. For example, some lepidopteran larvae live on the surfaces of plants but pupate underground.

The effects of oxygen partial pressure on development are relatively unexplored for insects, probably because insects are infamous for their independence of ambient oxygen level down to very low oxygen partial pressures. For example, Mani (1968) was not surprised that large numbers of insects were found high in the Himalayas above the elevation where mountain sheep, ibex, and yak were found (barometric pressure less than half that at sea level). He stated that most insects are known to be "extraordinarily resistant" to the dearth of oxygen found at extremely low atmospheric pressures. To support this generalization he cited a number of references in which insects were subjected to very low pressures without appreciable (visible) effect.

In contrast to the large number of studies demonstrating relative independence to ambient oxygen level, there are a small number of studies suggesting that insects are in fact very sensitive to the oxygen level in their environment. This discrepancy is not due to differences between species or stages but is due to whether the effects are evaluated on a short-term or long-term basis. Here "short-term" and "long-term" refer only indirectly to the length of the study—what is important is the time between exposure to low oxygen levels and measurement of effect. In general, long-term evaluation was much more sensitive in detecting effects of oxygen level than short-term evaluation. For example, in a short-term study (effects assessed at the same time as exposure), Knipling *et al.* (1961) found that a very long time was required to kill half of the adults in 100% nitrogen; for 7 species from 3 orders the range was from 1.5 h (*Aedes egypti*, yellow-fever mosquito) to 36 h (*Tribolium confusum*, confused flour beetle). In a long-term study, Brooks (1965) found that exposing cockroach nymphs (*Blattella germanica*) to 100% nitrogen for only 2 min once a week increased the percentage of aborted egg capsultes in the adults. In another long-term study, a daily 5-min exposure to 100% nitrogen resulted in 30–40% mortality in 8 days for last-instar crickets (*Acheta domesticus*) (Woodring *et al.*, 1978).

*Current address: Department of Ecology and Behavioral Biology, University of Minnesota, Minneapolis, MN 55455, U.S.A.

Since it can take hours of oxygen deprivation to kill many insects, it is surprising that a few minutes without oxygen once a day or once a week can cause such high mortality, unless short-term and long-term effects are only indirectly related.

In addition to mortality, the immediate change in the rate of oxygen uptake, or the immediate change in behaviour are also measured in short-term evaluation of the effect of low ambient oxygen level. The rationale for measuring rate of oxygen uptake is that there is a "critical" level of oxygen below which the rate of oxygen uptake decreases and above which the rate of oxygen uptake is constant. This critical oxygen level (which is naturally a function of temperature, activity, developmental stage, size, etc.) is interpreted as the point below which the "normal" oxygen consumption rate can no longer be maintained, or the point at which the oxygen delivery system has reached its functional limitations. It is usually assumed that the insect is unaffected by oxygen concentration above this critical level, since its rate of oxygen uptake is unchanging in this range. Adult insects (resting) generally maintain a constant rate of oxygen uptake until the molar fraction of oxygen is lowered to 5% or below (compared to 21% oxygen in air) (*Termopsis nevadensis* termites, Cook, 1932; *Aedes aegypti* mosquitoes, Galun, 1960; *Phormia regina* flies, Keister and Buck, 1961). Some larvae and non-diapausing pupae have a slightly higher threshold, but a decline in rate of oxygen uptake is still usually not seen until the molar fraction of oxygen falls to 10% or below (*Calliphora erythrocephala* flies, Fraenkel and Herford, 1938; *Phormia regina* flies, Keister and Buck, 1961; *Tenebrio molitor* larvae, Loudon, 1986, and unpublished; for review see Keister and Buck, 1974).

When behavioural assays are used to determine oxygen sensitivity, the critical oxygen levels are reported to be even lower. Tenney (1985) reported that Mexican jumping beans (seed pods inhabited by larvae of the moth *Carpocapsa saltitans*) cease normal jumping at around 3% oxygen. Larvae of *Orthosoma brunneum* (Cerambycidae) continue to feed normally until the oxygen falls below 1% (Paim and Beckel, 1964). Different *Drosophila* species have different thresholds producing immobility, which vary from 1.6 to 2.8% oxygen (Csik, 1939). Adult mosquitoes (*Aedes egypti*) can still walk, climb and fly when the total pressure is lowered to 0.20 atmospheres (Galun and Fraenkel, 1961). Thus, in short-term studies, the oxygen level must be lowered to a fifth or less of standard conditions (21% of 1 atm) before a change in behaviour is seen.

The discrepancies between long-term and short-term studies of oxygen sensitivity suggest that short-term measurements do not adequately represent the physiological state of an insect. In particular, the rate of oxygen uptake is a commonly used short-term measurement to assess physiological state in an aerobic organism. To demonstrate experimentally that the rate of oxygen uptake is an inadequate indication of physiological state, it would be necessary to demonstrate long-term effects of low oxygen in the absence of short-term change in the rate of oxygen uptake. The studies cited above do not test this, since either the oxygen treatments affected the rate of oxygen uptake, or because long-term measurements were not made.

This study evaluates the long-term effects of low oxygen levels on development in levels of oxygen that do not affect the rate of oxygen uptake (Loudon, 1986; Loudon, unpublished). *Tenebrio molitor* larvae were raised from eggs to adults in three different levels of oxygen: 21% (the normal percentage found in dry air), 15% and 10.5%, with the remainder nitrogen. *Tenebrio molitor* larvae live in stored grain, where the oxygen levels can be lower than in normal air (Cotton, 1963). Growth rates and timing of moulting were measured. Some larvae were transferred from one oxygen level to another during their larval development to assess the effect of previous conditions on growth rates.

MATERIALS AND METHODS

Tenebrio molitor were obtained from Ward's Natural Science Establishment, Inc. A stock culture was maintained on bran (Poultry and Livestock Feed, Stevens Milling Co., Broadway, NC) in plastic boxes (25 cm × 31 cm, 11 cm high) in a Tyler box (walk-in, environmentally controlled chamber). The Tyler box held the temperature between 26 and 28°C under 16 h light–8 h darkness.

There were three experimental oxygen treatments: 0.21, 0.15, and 0.105 atm oxygen (remainder nitrogen; partial pressures are for dry air). A value of ±0.01 atm was arbitrarily chosen as an acceptable degree of variation in the experimental oxygen treatments.

Experimental larvae were raised in controlled oxygen levels using flow-through rearing chambers located in the Tyler box. Only first-generation insects, raised from eggs collected from the stock cultures, were used in the rearing chambers. Larvae were reared in small plastic cups (diameter 43 mm) within the rearing chambers. This made it possible to keep track of groups or individuals.

The four rearing chambers for the experimental oxygen treatments (one 21%, one 15% and two 10.5% oxygen) were constructed of $\frac{1}{4}''$ plexiglas, and were 24 cm × 24 cm (base) × 10 cm (height). Gases of controlled composition entered continually through a glass tube sealed into one side of the chamber, and exited through another glass tube into the Tyler box air. Access to the insects was through a hole in the top of the rearing chamber. The hole was sealed with an O-ring pressed between two layers of plexiglas.

Pre-mixed oxygen-nitrogen mixtures (21, 15 and 10.5% oxygen) were purchased from National Welders Supply Co. (Raleigh, NC) and the oxygen content of each mixture verified using an oxygen analyzer (Lex-O_2-Con TL, Hospex Co., Chestnut Hills, MA). After calibration, repeated measurements of a standard using the oxygen analyzer were within 0.001 atm of the known partial pressure of oxygen. Pre-mixed gases were always within 0.01 atm of the desired partial pressure of oxygen.

Before entering the rearing chambers the humidity in the gas was increased to 76% r.h. by bubbling it through saturated sodium chloride solution (Winston and Bates, 1960). Since the tubing leading into the

salt solution clogged with salt crystals unless the gas was first humidified by bubbling through pure water, the gas passed through three Erlenmeyer flasks before reaching the rearing chamber: pure water, saturated aqueous sodium chloride, and then an empty flask (which served as a trap). The humidity in the rearing chambers was approx 72% (±5%) r.h., measured with a Vaisala transducer (6061 HM, Vaisala Co., Woburn, MA). This increase in humidity lowered the oxygen partial pressure from the gas cylinders by 0.005 atm (21% oxygen chamber) or less. One 10.5% oxygen chamber was kept at higher humidity (>98% r.h.) by bubbling the gas through water only.

Gas entered the rearing chambers at a rate between 8 and 14 ml/min. This slow flow rate was sufficient to keep the oxygen within 0.01 atm of the desired value, verified by measurement of air samples taken from the chambers using the Lex-O_2-Con oxygen analyzer. Each time the chamber was opened to remove or insert insects, the flow was increased to 1500–2000 ml/min (fast flow) for several minutes until at least 3 times the total volume of the chamber had flushed through. This was done to return the chamber to its steady-state oxygen level as quickly as possible.

In a flow-through rearing system such as this, the barometric pressure will affect the partial pressure of oxygen in the rearing chambers. The mean barometric pressure recorded was 0.992 atm (standard deviation 0.005 atm, barometric measurements corrected for temperature). Therefore, barometric pressure did not significantly affect the oxygen partial pressures in the rearing chambers.

Volume flow rates of gases entering the rearing chamber were measured to the nearest 100 ml/min with a calibrated rotameter (fast flows) or to the nearest ml/min by timing bubble release in the saturated salt solution (calibrated by collection of bubbles in an inverted graduated cylinder) [slow flows].

The larvae in the rearing chambers were reared on white, unbleached flour containing 10% torula yeast. Each dish contained 1.5 g of flour (a depth of 2 mm). This shallow depth ensured that the larvae were exposed to the oxygen concentration in the rearing chamber. (Hughes, 1943) has cautioned that the external atmosphere over flour may be no guide to conditions within the flour, as 100% nitrogen produced immediate anaesthesia in exposed mites, but mites in flour over alkaline pyrogallol (oxygen scavenger) were alive and feeding for 36 h).

Disturbance of larvae was kept to a minimum by censusing as infrequently as possible (average once a week). Shed cuticles and changes in head width were used to keep track of instar number when ecdyses of individual insects were followed.

Larvae were weighed to the nearest tenth of a milligram on a Sartorius balance (repeated measurements were within ±0.2 mg). Larval head widths were measured under a dissecting microscope with an ocular micrometer, using the maximal width of the head when viewed dorsally. Repeated measurements of a single head were within ±0.05 mm. Pupae were sexed following Doyen (1966).

For the transfer experiments, in which larvae were transferred from one oxygen level to another, larvae in the size range of 37–68 mg (before transfer) were used. They were specifically assigned to treatment groups so that each treatment group had a similar initial size distribution. Larvae were either "transferred" to the same oxygen level in which they had developed (controls) or were transferred to a different oxygen level. Only the extreme oxygen levels, 21 and 10.5%, were used for this experiment. The experiment was performed twice, each time using 5 larvae for each of the four treatments: from 21 to 21%, from 21 to 10.5%, from 10.5 to 10.5% and from 10.5 to 21%. Growth was measured after 32 days.

Statistical analyses were done using SAS software (SAS Institute, Cary, NC). Growth rate of larvae varied between batches of eggs, and therefore date of egg collection was entered into analyses as a main effect where appropriate. Nonsignificant interaction terms were dropped from analyses. Variates were tested for significant deviations from a normal distribution. If variates were not normally distributed, nonparametric methods were used in lieu of analysis of variance (ANOVA).

RESULTS

Timing of moulting

Larvae reared in lower oxygen moulted at the same rate as larvae reared in higher oxygen (Fig. 1). Due to high mortality and early termination of some measurements, the sample sizes for the later instars (after instar 14) are too small for a satisfactory analysis of timing. Therefore, the second through fourteenth instars were analyzed (hatching to the first instar by definition was day 1). The number of days required to reach an instar was not significantly different between the three oxygen treatments for any instar (ANOVA used for instars with normally distributed variables; Kruskal–Wallis used for early instars which were not normally distributed; $P > 0.05$

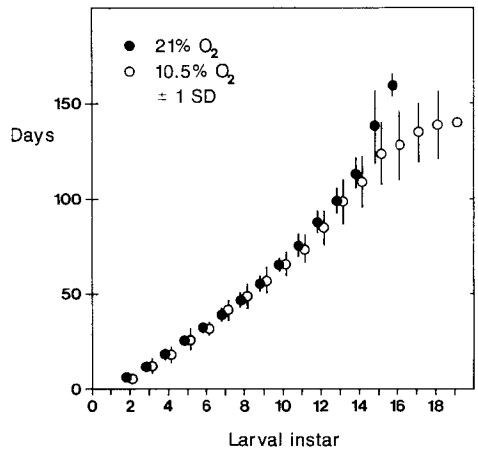

Fig. 1. The number of days to reach different larval instars. Larvae reared in 15% oxygen (not shown) were statistically indistinguishable from either oxygen group. Points are offset for clarity. Total sample sizes: $n = 23$ for 21% oxygen, $n = 20$ for 10.5% oxygen. Sample sizes decreased through time due to mortality. Sample sizes were ≥15 to the 10th instar in 10.5% oxygen, and the 14th instar in 21% oxygen. Sample sizes were ≤5 starting at the 16th instar for both groups.

Table 1. Frequencies of final instar number

	% oxygen		
Final larval instar	10.5	15	21
12		1	1
13		2	1
14		1	8
15		1	5
16	1		2
17	2		
18	d		
19	d		

Individual mealworms followed from egg to pupation.
d = a larva had reached this instar but died before pupating.

for all 15 analyses, total $n = 57$, sample sizes decline with increasing instar number). As no instar showed a significant difference between treatments, no adjustment was made for multiple comparisons.

Larvae in 10.5% oxygen underwent a greater total number of larval moults than larvae in 15 or 21% oxygen (Table 1) [Kruskal–Wallis, $P < 0.001$ including values for larvae in 10.5% oxygen that died before pupating, $P < 0.025$ not including those values]. As larvae in different oxygen treatments were moulting at the same time, and larvae in 10.5% oxygen had a greater number of larval moults, it follows that larvae in 10.5% oxygen would have a longer total larval development (time from hatching to pupation). There was a trend towards longer larval period in 10.5% oxygen (average 159 days) than in 15 or 21% oxygen (averages 130 days and 137 days respectively). However, this trend is not significant for this small sample size (ANOVA for time to pupation, $P = 0.30$, $n = 2$ for 10.5% oxygen, $n = 5$ for 15% oxygen, $n = 16$ for 21% oxygen; total $n = 23$).

Size

Although larvae moulted at the same time in different oxygen treatments, larvae in lower oxygen increased in size by smaller increments at each moult (Fig. 2). Each larva had a straight growth trajectory following Dyar's law (Wigglesworth, 1972); that is, a straight line was obtained when the logarithm of head width was plotted against number of instars. The correlations between head width (log-transformed) and instar number were very high: for single individuals the range of r^2 was 0.96–1.00, with an average $r^2 = 0.99$. Therefore, the slope from the regression of head width (log-transformed) on instar number was used as a measure of growth rate for a single individual. Growth rate was not significantly different between individuals reared in 21% oxygen and 15% oxygen (ANOVA of slopes, $P = 0.37$ for oxygen treatment, $n = 33$), but slopes were significantly lower for individuals reared in 10.5% oxygen compared with 21 or 15% oxygen (ANOVA of slopes, $P = 0.0052$ contrasting 10.5 and 15% oxygen, $n = 31$; $P = 0.0001$ contrasting 10.5% oxygen and 21% oxygen, $n = 40$).

As larvae in the different oxygen treatments started out at the same size (i.e. subsamples of eggs), these results mean that larvae in 21 and 15% oxygen grew at the same rate, while larvae in 10.5% oxygen grew at a significantly lower rate. The average slope for individuals reared in 21% oxygen was 0.15 ± 0.01 (1 SD), compared with 0.11 ± 0.02 in 10.5% oxygen. This corresponds to an average increase in head width of 16% at each instar for larvae in 21% oxygen and an increase of 12% at each instar for larvae in 10.5% oxygen.

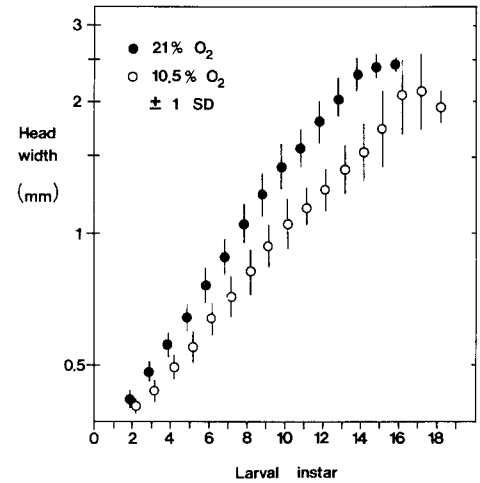

Fig. 2. Head width (mm) as a function of larval instar. Larvae reared in 15% oxygen (not shown) were statistically indistinguishable from the 21% oxygen group. Points are offset for clarity. Total sample sizes: $n = 23$ for 21% oxygen, $n = 20$ for 10.5% oxygen. Sample sizes decreased through time due to mortality. Sample sizes were ≥ 15 to the 10th instar in 10.5% oxygen, and the 14th instar in 21% oxygen. Sample sizes were ≤ 5 starting at the 16th instar for both groups.

Two different measures of larval size were used throughout this study: head width and total body mass. Either one can be used interchangeably as the relationship between mass and head width was not a function of the level of oxygen during development. There was complete overlap in the relationship between mass and head width in the different oxygen treatments (Fig. 3). A straight line was obtained when the log-transformed variables were plotted against each other (Fig. 3). Linear regression of the log-transformed variables, combining all three oxygen treatments ($n = 150$), resulted in the expression,

$$M = 10.7\,HW^{2.8} \quad (r^2 = 0.95)$$

where mass (M) is in mg and head width (HW) is in mm.

Final larval size

Although larvae reared in lower oxygen increased in size by smaller increments at each moult, they underwent a greater total number of moults to reach the same final larval size. Due to mortality, the sample size of final larval instars was not high enough to make this assertion on the basis of final larval size alone. However, in most insects, final larval size should be highly correlated with pupal size and adult size. There is a high degree of correlation in *Tenebrio molitor*; adult weight (starved, less than a week since ecdysis) is highly correlated with pupal weight ($r^2 = 0.85$, $n = 50$), and pupal weight is highly correlated with final larval weight ($r^2 = 0.91$, $n = 20$). As the sample size was largest for the pupal stage, it was

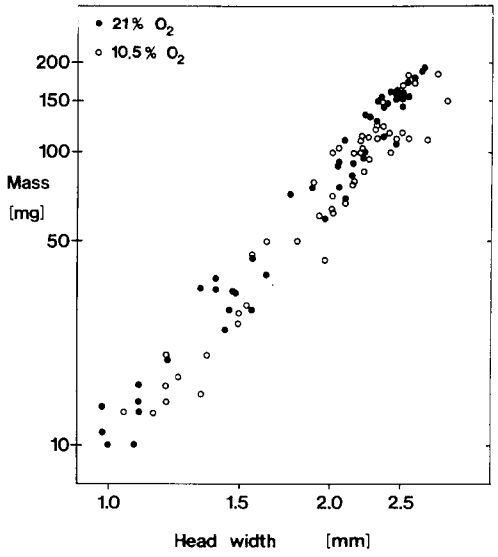

Fig. 3. The relationship between head width and mass. Only one head width and mass measurement was plotted for each individual larva. Sample sizes: $n = 50$ for 21% oxygen, $n = 50$ for 10.5% oxygen.

used for comparison. Pupal weight was not significantly different in the three oxygen treatments (Table 2) [ANOVA, $P = 0.84$, $n = 189$]. As pupal size was not a function of oxygen concentration, this means that final larval size and adult size were independent of oxygen concentration.

Mortality

Mortality was greater in lower oxygen. For individuals raised in isolation (which excludes cannibalism as a source of mortality), 64% of the larvae survived from egg to the tenth instar in 21% oxygen, 61% in 15% oxygen, and 49% in 10.5% oxygen (sample sizes 36, 23, and 35 respectively). Table 3 shows the sample sizes of all pupae (raised individually or in groups) collected over a 7-month period from the three different oxygen treatments, which gives a rough estimate of differential mortality to the pupal stage. Larvae usually die while moulting; dead bodies were encased in two cuticles. There was no particular size or age at which mortality was especially high, other than hatching from the egg (24% of eggs in individual dishes did not hatch, $n = 78$).

Transfers

Growth rates of mealworms transferred from one oxygen level to another were independent of prior conditions (Table 4). Mealworms transferred into 21% oxygen had the same average weight gain whether they were originally in 21% oxygen or 10.5% oxygen (ANOVA, $P = 0.36$). Similarly, mealworms transferred into 10.5% oxygen had the same average weight gain whether they were originally from 10.5% oxygen or 21% oxygen (ANOVA, $P = 0.93$). These results mean that acclimation to an oxygen level does not result in faster growth in that oxygen level, relative to larvae first acclimated to a different oxygen level. The only mortality during the transfer period was in the low oxygen group that remained in low oxygen; 50% mortality occurred both times that the experiment was performed. Thus, larvae acclimated to low oxygen do not grow faster or have lower mortality in low oxygen than larvae acclimated to higher oxygen.

Table 2. ANOVA of pupal size (mg)

Source	*df*	SS	*E*	*P*	
Gender	1	26.6	1.10	0.34	ns
Oxygen	2	1,468.2	0.04	0.84	ns
Gender × Oxy	2	2,090.7	1.57	0.21	ns
Error	183	122,198.2			

Neither of the main effects (oxygen concentration and gender) nor the interaction term were significant ($n = 189$).

Table 3. Percentage of female pupae

Oxygen level	% female	*N*	Difference from 50%
21	49	121	ns
15	63	57	$P < 0.05$
10.5	82	11	$P < 0.05$

A greater proportion of female pupae were found in the lower oxygen treatments. G tests were done on each oxygen level to test for differences from 50% female using the Williams correction for G.

Humidity

If larvae in lower oxygen open their spiracles more frequently to obtain a sufficient supply of oxygen (Buck, 1957), it is conceivable that the reduction in growth rate is a reflection of greater tracheal water loss rather than the effects of lower oxygen, even though mealworms were in >70% r.h. To test for the effects of humidity, one 10.5% oxygen chamber was kept saturated with water vapour (>98% r.h.). The flour in this chamber became mouldy very quickly, and was replaced weekly. Larvae in the humid 10.5% oxygen chamber were larger than larvae in the less humid 10.5% oxygen chamber (ANOVA of slopes, $P = 0.005$, $n = 28$), but were still not as large as larvae in 21% oxygen (ANOVA, $P = 0.01$, $n = 29$). As the larvae in the humid 10.5% oxygen chamber were still distinguishable from the larvae in the 21% oxygen chamber, it is clear that the differences in growth rate were not solely due to differences in dehydration levels.

Sex ratios

The sex ratio was a function of oxygen partial pressure. Morphological features distinguishing males from females in *Tenebrio molitor* have been

Table 4. Size of mealworms after transfer into 10.5 or 21% oxygen

	Transferred from	
Transferred into	10.5	21
10.5	83 ± 24	82 ± 15
21	112 ± 25	100 ± 29

Size is in mg (±1 SD), measured 32 days after transfer. Initial sizes of four larval groups before transfer: 50–51 ± 8–10 mg. Sample sizes are $n = 10$ except for the group transferred from 10.5% oxygen to 10.5% oxygen, in which half died during the 32 days ($n = 5$).

described for the pupal and adult stages but not the larval stage (Doyen, 1966). Therefore, gender was determined at the pupal stage. The proportion of female pupae was greater in lower oxygen (Table 3). As the eggs in each rearing chamber were from the same source, i.e. subsamples from eggs laid by adults from the stock culture, this implies either (1) differential mortality of female and male larvae as a function of oxygen level or (2) induction of female characteristics in genetically male insects in low oxygen.

Differential mortality could result from gender-specific growth in different oxygen treatments. The oxygen by gender interaction for pupal size is not significant, however (Table 2), so if differential growth response is the cause of the differential mortality, it was not seen in the larvae that survived to the pupal stage.

Female morphological characteristics were induced in genetically male mosquitoes (*Aedes stimulans*) by elevated temperature during larval development (Anderson and Horsfall, 1963). In the mosquito study, elevated temperature resulted in an entire range of male–female intermediates (Anderson and Horsfall, 1963), whereas in the present study no male–female intermediates were seen.

As chromosomal studies were not conducted, it is impossible to completely differentiate between the alternative explanations. However, the fact that no male–female intermediates were seen favours differential mortality as an explanation for the skewed sex ratios.

Pupal–adult intermediates

The incidence of abnormal adults was an order of magnitude higher in lower oxygen: approx 40% of the adults in 10.5% oxygen ($n = 16$) versus approx 3% of the adults in 15% ($n = 39$) or 21% oxygen ($n = 101$) resembled hormonally-induced pupal–adult intermediates (Bowers, 1971), with small warped elytra and an abdomen wrapped in pupal cuticle. However, in most cases the pupal cuticle was overlying normal adult cuticle, and so they were not true pupal–adult intermediates. The only true pupal–adult intermediates were seen in 10.5% oxygen, where two out of the six abnormal adults retained pupal abdomens.

DISCUSSION

Tenebrio molitor larvae in 21 or 15% oxygen grew at the same rate, while larvae in 10.5% oxygen grew more slowly. Slowed growth was a direct result of low oxygen and was not due to increased tracheal water loss. A comparable low level of oxygen has been shown to decrease the speed of development in embryonic lake trout (0.08 atm) [Garside, 1959], and young rats (0.12 atm) [Bartlett and Remmers, 1971]. This is not a general phenomenon, however, as young guinea pigs develop at the same rate in 0.11 atm and 0.18 atm oxygen (Banchero *et al.*, 1985).

Little information is available for comparison with other insects. Puparium formation is delayed in fly larvae (*Calliphora vomitoria*) in 10 or 5% oxygen, and adult emergence is slowed by exposure of the puparium to 1% oxygen (body sizes not reported, Houlihan, 1974). Houlihan's study and the other long-term studies cited in the introduction differ from the present one in the intermittent nature of the exposure to low oxygen. In this study, the developing insects were exposed to constant levels of low oxygen starting at the egg stage (except for the larvae in the transfer experiment, which were transferred from one constant oxygen level to another). Curiously, acclimation to low oxygen does not lead to faster growth rate or lower mortality in low oxygen, compared to larvae acclimated to higher oxygen and then transferred to low oxygen. Exposure to low oxygen appeared to have cumulative detrimental effects; not only was mortality much higher in lower oxygen, but also the only treatment group in the transfer experiment that had mortality during the 32-day period was the group that was reared entirely in 10.5% oxygen.

The *timing* of the moulting cycle, however, was not influenced by low oxygen, and larvae in 10.5% oxygen moulted at the same times as their cohorts in higher oxygen. Interestingly, temperature affects the timing of moulting in many insects, including *Tenebrio molitor* (Ludwig, 1956). One might expect that higher temperature and lower oxygen concentration would perturb the moulting cycle in a similar way because higher temperature leads to a greater oxygen demand. In mammals, the physiological changes (long-term) that takes place in response to greater oxygen demand (due to cold) are similar to those that take place in response to a decreased partial pressure of oxygen (Banchero *et al.*, 1985). Apparently these two different environmental variables, temperature and oxygen level, have completely different effects on the moulting cycle in *Tenebrio molitor* larvae.

Although larvae in 10.5% oxygen grew more slowly than larvae in higher oxygen, larvae in 10.5% oxygen underwent a greater total number of moults to pupate at the same size. This regulation of size at metamorphosis is analogous to the threshold size for metamorphosis that has been documented in another insect (*Manduca sexta*, Nijhout, 1975). *Tenebrio molitor* differs from *Manduca sexta*, however, in that the threshold size for metamorphosis in *M. sexta* is fairly constant between individuals (Nijhout, 1975), whereas in *T. molitor*, there is no threshold size above which a larva always pupates and below which a larva always undergoes another larval moult. This result is consistent with that of Stellwaag-Kittler (1954), who reported that neither head width nor number of instars was an absolute indication for which larval instar was final in *T. molitor*.

An increasing percentage of females was seen in lower oxygen, even in 15% oxygen where growth was not slowed. Of the two alternative explanations, (1) differential mortality of the genders and (2) induction of female characteristics in genetically male beetles, differential mortality is more likely as no male–female intermediates were seen. Alstad and Edmunds (1983) have suggested that a sex ratio favouring the homogametic gender can result from viability selection. In *Tenebrio molitor*, the female is the homogametic gender, so a preponderance of females would be expected in the more extreme oxygen levels, as was seen.

This study documented the effects of different

constant oxygen levels on the entire development of an insect. The importance of continuous exposure for the whole life of the insect is that the complete potential for acclimation to these conditions can be realized. These experimental oxygen levels were insufficiently low to affect the short-term rates of oxygen uptake (Loudon, 1986; Loudon, unpublished). This means that a level of oxygen that was insufficient to affect the rate of oxygen uptake nevertheless had a number of effects on the growth and mortality of these larvae. Growth was not slowed until the oxygen was lowered to 10.5%, but the mortality and sex ratio data demonstrated that even in 15% oxygen there were physiological effects of the lowered oxygen. These results are an experimental demonstration that an unchanged rate of oxygen uptake is not equivalent to an unchanged physiological state. A parallel to this result at the whole organism level can be found at the level of the cell; a number of metabolic changes have been documented in cells in suspension in oxygen concentrations much higher than those which affect their rate of oxygen consumption (Jones *et al.*, 1985).

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