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TRACHEAL HYPERTROPHY IN MEALWORMS: DESIGN AND PLASTICITY IN OXYGEN SUPPLY SYSTEMS

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Summary

Larval *Tenebrio molitor* L. (Insecta) were reared in three different levels of oxygen: 21 % (normal), 15 % and 10.5 %, all at 101.3 kPa (=1 atm) total pressure (remainder nitrogen). Some larvae were transferred from one oxygen level to another during development.

The main tracheae (branching off from the spiracular tracheae) were of greater cross-sectional area in lower ambient oxygen. Compared to larvae of the same body mass reared in 21 % oxygen, larvae reared in 15 % oxygen had main tracheae 40 % larger in cross-sectional area on average, and larvae in 10.5 % oxygen had main tracheae 120 % larger. This hypertrophy is not consistent with the widely accepted hypothesis that tracheae contribute an insignificant resistance to the net movement of oxygen in insect tracheal systems.

The magnitude of the hypertrophy is consistent with predictions from Fick's law of diffusion and with the hypothesis that diffusion is the primary mechanism for oxygen movement in the larval tracheal system of holometabolous insects.

Introduction

Using the mammalian respiratory system as an example, Taylor and Weibel (1981) articulated the principle of symmorphosis: that structural design should be matched to functional requirements. It follows from this principle that any part of an oxygen supply system should have just enough structure to support the maximal oxygen flow rate, that is that no part of the pathway will be overdesigned relative to the other parts, from the lungs to the mitochondria.

In contrast, one common conception about insect tracheal systems is that the capacity of the spiracles, tracheae and tracheoles is more than sufficient to supply the oxygen uptake of the tissues. This idea is based in part on short-term studies demonstrating impressive independence of behavior and rates of oxygen uptake in insects on lowered oxygen levels (reviewed in Loudon, 1988).

The insect respiratory system is different from the mammalian respiratory system in that, in insects, the main sources of oxygen at the tissues are air-filled

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Key words: hypertrophy, insect, oxygen, symmorphosis, tracheae.

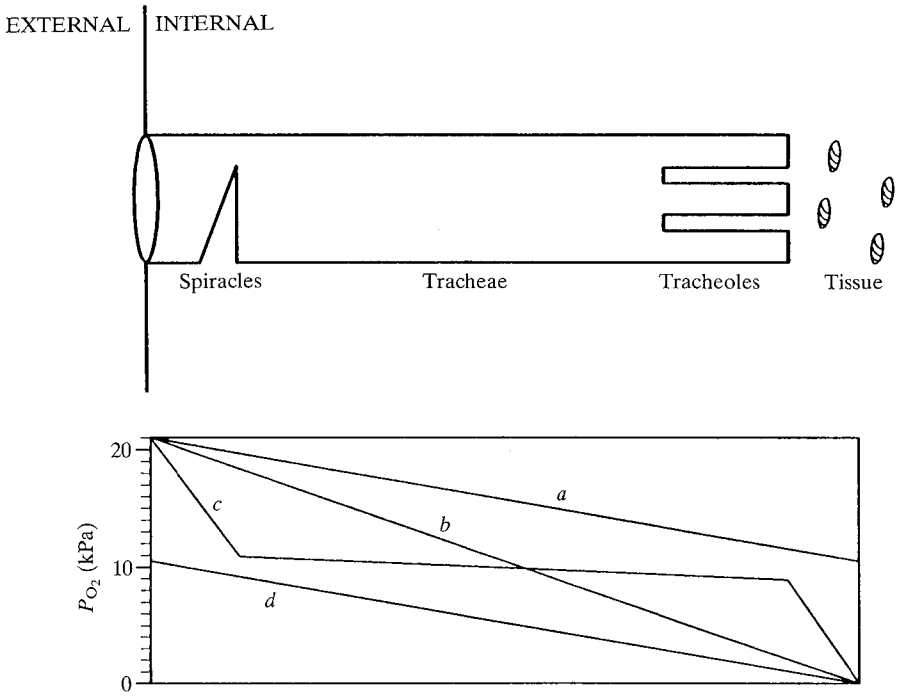

Fig. 1. Schematic diagram of the tracheal system of larval holometabolous insects. Below (labeled *a–d*) are some theoretical oxygen partial pressure gradients in the tracheal system for purposes of discussion (see text).

cuticular tubes called tracheoles, rather than blood equilibrated with air. Insect blood (hemolymph) only rarely contains respiratory pigments, and hence has a very low oxygen-carrying capacity. Tracheoles branch from larger cuticular tubes called tracheae and are in close contact with the tissues, sometimes indenting into the cells they supply. Tracheoles are the smallest branches ($<1\ \mu$m diameter), actually reaching the size below which diffusion itself becomes limited (Pickard, 1974). The tracheae connect to the outside air through paired, valved openings called spiracles (schematic diagram, Fig. 1). Tracheae are shed with the exoskeleton when the insect molts, and generally do not change in diameter between molts (Keister, 1948; Wigglesworth, 1954; Whitten, 1957) (insect tracheal systems have been reviewed in Whitten, 1972; Wigglesworth, 1972, 1983; Miller, 1974; Noirot and Noirot-Timothée, 1982).

Another generalization made about the design of the tracheal system is that the tracheae offer a trivial resistance to the movements of oxygen compared with the resistances offered by the spiracles and the cytoplasm (oxygen has to traverse the latter to reach the mitochondria). Another way of stating this is that the drop in partial pressure of oxygen along the length of the tracheae is thought to be small compared with the total drop in partial pressure of oxygen between the ambient air

and the mitochondria (e.g. line *c* in Fig. 1). Because oxygen diffuses five orders of magnitude faster in air than in water for the same gradient in oxygen partial pressure, this means that the air-filled tracheae will have a much lower resistance per unit length than the watery cytoplasm (Krogh, 1941; Miller, 1974; Kestler, 1984). Isolated mitochondria function independently of oxygen partial pressure until the partial pressure of oxygen is much less than 1 kPa (Weibel, 1984; Jones *et al.* 1985), and therefore the total drop in partial pressure of oxygen between air and mitochondria can theoretically be as large as 21 kPa (e.g. lines *b* and *c*, Fig. 1). Often-cited calculations by Krogh (1920) estimate the drop in oxygen partial pressure along the tracheae to be about 2 kPa in *Tenebrio molitor* larvae, *Cossus* larvae and *Lasiocampa* larvae, assuming oxygen transport by diffusion. Similar calculations by Thorpe and Crisp (1947) for *Aphelocheirus* adults estimate a drop of 0.3 kPa in oxygen between the spiracles and the tracheoles. These are very small decreases compared to a possible total decrease of 21 kPa.

A different result might be expected in flying insects because of their high rates of oxygen consumption. Supporting this, Weis-Fogh (1964) calculated that the decrease in partial pressure of oxygen between the ventilated primary tracheae and the terminal ends of the tracheoles could be between 5 and 13 kPa for the high oxygen consumption rates during flight in dragonflies. He concluded that diffusion was therefore sufficient to supply oxygen in this part of the oxygen pathway. One cannot make the generalization, however, that a large decrease in oxygen partial pressure is always found along tracheae in flying insects, as *Drosophila* flying in 6 % oxygen maintained 80–90 % of their corresponding oxygen consumption rates measured while flying in 21 % oxygen (Chadwick and Gilmour, 1940). This difference in results may relate to the differences in the sizes of the insects involved (Weis-Fogh, 1964) and, if so, the rate of oxygen consumption by flying dragonflies would be expected to decrease more rapidly with lowered ambient oxygen partial pressure than was found for *Drosophila*.

Spiracles in many insects are closed much of the time, only opening periodically (Buck, 1957; Miller, 1974; Kaars, 1981). Obviously, when the spiracles are closed they are a significant resistance to the movement of oxygen, and possibly even when they are open. Scheid *et al.* (1981) analyzed the rate of release of inert gases from the tracheal system of *Hyalophora cecropia* pupae. They concluded that the shape of the 'wash-out' curve was compatible with the open spiracle's being the major resistance to diffusion in the tracheal system. Tenney (1985) calculated that the spiracle was probably the site of almost the entire drop in partial pressure of oxygen in the respiratory system of *Carpocapsa saltitans* larvae (Mexican jumping beans). Buck and Keister (1958) calculated such low resistances for various parts of the tracheal system of *Agapema galbina* pupae that they concluded that only the spiracular valves, with their ability to close, could possibly afford any appreciable resistance to diffusion of gases. These cited calculations comparing the different parts of the insect tracheal system are the major reasons why the tracheae are thought to be of relatively minor resistance when the entire pathway is considered.

Earlier data reported by Locke (1958) contrast with both of these ideas, i.e.

(1) that the insect tracheal system is more than sufficient to supply oxygen (at least for nonflying insects) and (2) that the tracheae, in particular, offer trivial resistance to the movement of oxygen. He stated that the sizes of tracheae of *Tenebrio molitor* larvae were a function of ambient oxygen concentration; the increase in tracheal size at a molt was larger in larvae transferred into lower oxygen and smaller in larvae transferred into higher oxygen, as if they were being 'finely tuned' in response to the oxygen supply. As neither sizes of tracheae nor sizes of larvae were reported, it is difficult to make a complete functional interpretation of this information, but it does suggest that tracheae are a more significant resistance to the transport of oxygen than has been thought.

To pursue and extend Locke's original study (1958), *Tenebrio molitor* larvae were reared in different levels of oxygen: 21 % (normal), 15 % and 10.5 %, all at 101.3 kPa total pressure (remainder made up with nitrogen, percentages are for the dry gases). The diameters of the main tracheae branching from the spiracular tracheae were measured for larvae reared in the different oxygen treatments. This allowed evaluation of (1) whether tracheal size did respond to ambient oxygen level, and (2) the extent and pattern of hypertrophy. As tracheae are usually built around pre-existing tracheae at each molt (especially larval–larval molts) (Keister, 1948; Wigglesworth, 1954; Whitten, 1957), some larvae were transferred from one oxygen concentration to another to see if there is an effect of earlier tracheal size on future tracheal construction.

Quantification of tracheal hypertrophy may reveal information about the underlying mechanics of mass transport. The relative contribution of diffusion and convection in oxygen transport in tracheal systems of holometabolous larvae is still a matter of debate. Holometabolous larvae (larvae which will undergo complete metamorphosis such as *Tenebrio molitor* larvae) typically do not have air sacs (enlarged compressible tracheae) (Snodgrass, 1935) and, therefore, diffusion is usually assumed to be the primary mechanism for the movement of gases (Krogh, 1920; Dejours, 1981). Kestler (1984) has pointed out on theoretical grounds that gas movement by diffusion alone is practically an impossibility in the insect tracheal system. The net movement of oxygen molecules in the tracheal system is in one direction while the net movement of carbon dioxide and water vapor molecules is in the opposite direction. For the movement of the gases to be entirely by diffusion, the rates of net gas movement in opposite directions must exactly balance, or a pressure gradient will develop which will cause bulk movement of the gas mixture (convection). Oxygen, carbon dioxide and water vapor all differ in molecular weight, which means that each has a different diffusion coefficient. While it is possible to calculate a combination of rate of oxygen uptake, rate of carbon dioxide release and humidity conditions that will lead to an exact balance between the rates of gas movement in the opposite directions, rates of oxygen consumption are not affected by ambient humidity. This means that for any combination of rates of oxygen uptake and carbon dioxide release, there will be at most only one level of ambient humidity which will lead to an exact balance between the movements of gases by diffusion, and at all other levels of ambient

humidity there must be some degree of convection. It remains difficult, however, to deduce the relative importance of convection *versus* diffusion. In a rare empirical test, Tenney (1985 and personal communication) was able to demonstrate that the critical partial pressure of oxygen below which larval *Carpocapsa saltitans* (Mexican jumping beans) stopped jumping was lower if the total pressure was lowered, suggesting that diffusion, rather than convection, was the primary mechanism of mass transport of oxygen. The logic is that diffusion in the gas phase (i.e. diffusion along the spiracles, tracheae and tracheoles), but not convection, will specifically be enhanced at lower barometric pressure because diffusion coefficients increase as total pressure decreases (so oxygen movement by diffusion will be increased) (Frank-Kamenetskii, 1969). In the present study, measured tracheal sizes were compared quantitatively with predictions assuming each type of transport in turn. Because diffusion and convection scale differently with geometry (for a review see LaBarbera and Vogel, 1982), predicted morphology is a function of the assumed type of mass transport.

Materials and methods

Tenebrio molitor were purchased from Ward's Natural Science Establishment, Inc. Larvae were reared from the egg stage on flour in three different levels of oxygen in flow-through chambers as described in Loudon (1988). The partial pressures of oxygen in the three treatments were 21.3, 15.2 and 10.6 kPa (dry gas), with nitrogen making up the remainder to an ambient pressure of approximately 101 kPa (measured average 100.5 kPa). In the rearing chambers, the average humidity was 72 % relative humidity (±5 %, Vaisala transducer 6061 HM), the temperature was maintained between 26 and 28 °C, the light:dark cycle was 16 h:8 h, and the oxygen partial pressure was maintained within 1 kPa of the desired level (verified by analyzing gas samples from the chambers using a Lex-O_2-Con TL oxygen analyser, Hospex Co.).

Tracheal diameters were measured from whole mounts using a compound microscope. The use of whole mounts made it possible to identify specific tracheae. Whole mounts were made by cutting lateral strips from anaesthetized insects that included all the spiracles and main tracheae left attached to the integument and associated muscles (Fig. 2). Strips were fixed in modified Dietrich's (Kahle's) fixative: 1 part glacial acetic acid, 6 parts formalin, 15 parts 95 % ethanol, and 30 parts distilled water (Barbosa, 1974). After overnight fixation, the tissue was dehydrated through a series of ethanol dilutions, cleared in xylene for 1 h, and mounted on glass slides with Permount. After going through this procedure the tissue was rigid and transparent. Tracheae appeared as circular cylinders under the microscope.

For some whole mounts, visibility of the tracheae was enhanced by partial infiltration of the tracheal system with dye. Larvae were covered with chloroform and a vacuum was created (36 kPa absolute), held for 30 s, and released over 30 s. This was repeated once again. The larvae then lay in chloroform for 5 min.

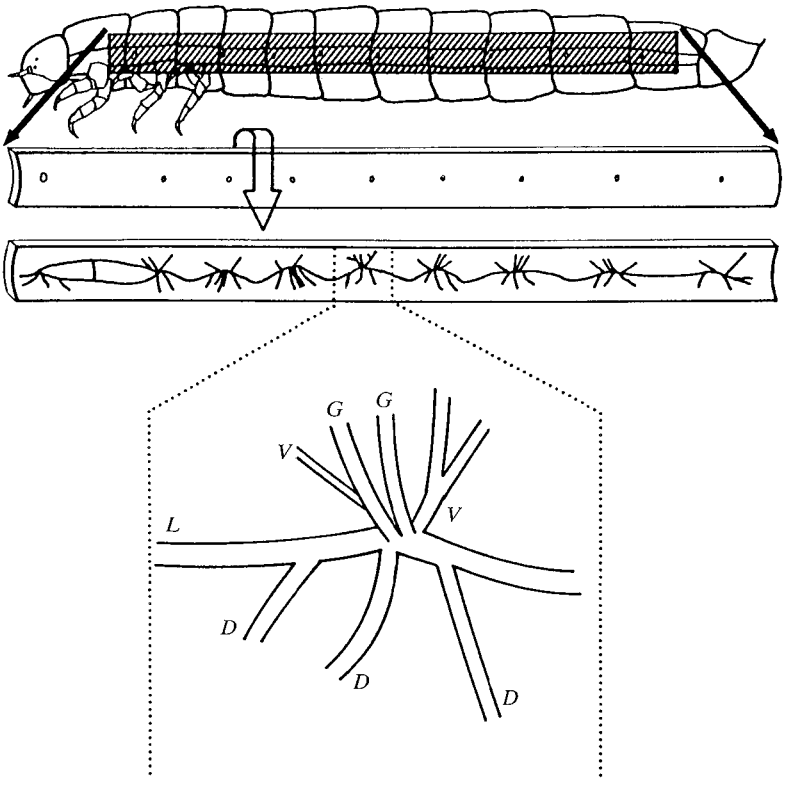

Fig. 2. A rectangular strip of body wall was removed for making whole mounts. The strip included all the spiracles and the main tracheae branching from the spiracular tracheae. The pattern of the main tracheae for the second to the seventh abdominal segments is shown in greater detail. *V*, ventral; *G*, visceral (gut); *L*, lateral longitudinal; *D*, dorsal.

This chloroform treatment sometimes improved the staining of the tracheae. After giving the larvae about 30 s for the chloroform to evaporate from the external surfaces, they were suspended over an aqueous mixture of Chlorazol Black (5 g in 50 ml water, plus 1 ml Triton X-100). A vacuum was created until the dye mixture started to boil (8 kPa absolute) and after 1 min, while maintaining the vacuum, the larvae were rolled into the dye. After another minute, the pressure was slowly increased to ambient (101.3 kPa) over 2 min. The vacuum was created again (8 kPa), held for 1 min, released over 2 min, and then the larvae remained in the dye mixture for 1 h before dissection. Fixation, clearing and mounting were as described above.

Only those large tracheae that branch close to the spiracular tracheae were measured. Diameters were measured to the nearest 5 μm. These measurements approximate the internal diameters because the width of the tracheal wall is much less than 5 μm. The tracheal system is bilaterally symmetrical, and the difference between measurements of left and right homologous branches was used as an

estimate of measurement error. Both members of 161 pairs of left–right homologues were measured, and members of 138 pairs (86 %) were within 5 μm of each other. Thereafter, only the left or the right member of a specific branch was measured. Although acetic acid can cause tissue swelling, and ethanol and xylene can cause tissue shrinkage (Humason, 1980), the tracheal diameters were not affected by this treatment or exposure to chloroform within this measurement error (comparing freshly dissected tracheae with the same tracheae after fixation, dehydration and clearing, or exposure to chloroform). Tracheal cross-sectional areas were calculated from the measured diameters assuming a circular cross-section.

Some larvae were transferred to a different oxygen treatment during development. There were four treatments: (1) larvae from 21 % oxygen that were transferred into 10.5 % oxygen, (2) larvae from 10.5 % oxygen that were transferred into 21 % oxygen, (3) larvae from 21 % oxygen that stayed in 21 % oxygen (control), and (4) larvae from 10.5 % oxygen that stayed in 10.5 % oxygen (control). Five larvae (size range before transfer 37–68 mg) were used for each of the four treatments, and the experiment was replicated twice. Tracheal sizes were measured after 32 days in the new conditions, using methods described above. The only mortality during these 32 day periods was in the 10.5 % oxygen control group, in which five of the 10 larvae died. Larvae were weighed to the nearest 0.1 mg on a Sartorius balance (repeated measurements were within ± 0.2 mg).

Statistical analyses were made using SAS software (SAS Institute, Cary, NC). Nonsignificant interaction terms were dropped from analyses. Partial sums of squares were used in significance tests (Type III SS in SAS). Analysis of variance will be referred to as ANOVA. In some instances where the assumptions of ANOVA were not met strictly, nonparametric tests were also performed. In no case were the results substantially different between the ANOVA and the comparable nonparametric test, and so the ANOVA results alone will be reported throughout.

Results

The number and position of major tracheae branching from the spiracular tracheae were not affected by oxygen level during larval development. The tracheal arrangement associated with the second to the seventh abdominal spiracles was the same for all larvae examined (Fig. 2), and thus these tracheae were used in statistical analyses because it was possible to make comparisons among serial homologues within individuals as well as among larvae. The three remaining pairs of spiracles (the thoracic, first abdominal and eighth abdominal) had different arrangements of tracheae, and will not be discussed further, as their associated tracheae showed a similar response in low oxygen to that of the other tracheae.

Tracheae branching from the spiracular tracheae (associated with the second to the seventh abdominal spiracles) were grouped into four categories (similar to

Snodgrass, 1935): dorsal tracheae that supply the dorsal musculature and the heart with oxygen (N=3 per spiracle), ventral tracheae that supply the ventral musculature and the nerve cord (N=2), visceral tracheae that supply the fat body, gut and gonads (N=2), and lateral longitudinal tracheae that connect adjacent spiracles (one to each adjacent spiracle – the anterior one was picked arbitrarily for measurement) (Fig. 2).

Size correlations between tracheae

Tracheal sizes within individual larvae were positively correlated after correcting for larval size, treating each oxygen treatment group separately. Since tracheal size increases with increasing larval size (see below), and tracheal size decreases with increasing oxygen partial pressure (see below), the influence of these two factors must be taken into account when looking at correlation patterns of tracheae within individual larvae. Otherwise, a positive correlation could be due to either of these two factors. The variation in tracheal size due to larval size was eliminated by multivariate regression of tracheal areas on larval head width (variates were all log-transformed). Correlation patterns among the residuals from the regression were examined. The residuals of every one of the 48 branches measured per larva (six spiracles each with eight branches) were compared in turn with every other (1128 different comparisons between pairs of branches). All three oxygen levels showed essentially the same correlation pattern. On average, the sizes of any two of these 48 branches were weakly but positively correlated (mean partial correlation coefficient r=0.53, N=1128 comparisons, 49 % of correlation coefficients were significantly different from zero at the $P \leq 0.05$ level). The correlation was not stronger within tracheae of a single spiracle or between serially homologous tracheae between spiracles. Therefore, in further analyses, the tracheae associated with different spiracles are averaged without loss of information.

Tracheal size as a function of oxygen concentration

The dorsal, ventral and visceral tracheae all hypertrophied significantly at lower oxygen levels (Table 1) (Fig. 3). A single graph is used to summarize the relationship between tracheal size and larval mass for these three tracheal groups (Fig. 3) because the individual graphs by tracheal group are virtually identical. In contrast, the lateral longitudinal tracheae were not significantly affected by oxygen level (Fig. 4) (Table 1). The dorsal, ventral and visceral tracheae all supply oxygen to tissues, while the lateral longitudinal tracheae primarily interconnect adjacent spiracles (sometimes tiny tracheae branch off the longitudinal tracheae and enter the body wall musculature). Thus, it is only the tracheae that primarily supply tissues that hypertrophy in lower oxygen. All measured tracheae increased significantly in size with increasing larval size (Figs 3 and 4) (Table 1).

Tracheal areas may be compared between oxygen treatments without adjusting for larval size, as larval size did not differ significantly between oxygen groups for the individuals used in morphological analysis (ANOVA, P=0.12, N=60).

Dorsal, ventral and visceral tracheae all increased by approximately the same amount in lower oxygen, although visceral tracheae consistently hypertrophied to a slightly larger extent (Table 2). Gross dissection did not reveal any differences in

Table 1. *ANOVA of tracheal cross-sectional area as a function of insect mass (live) and oxygen level* (N=*60 larvae*)

	Insect mass	Oxygen level
Tracheal group		
Dorsal	F_s=30.30***	F_s=40.54***
Ventral	F_s=28.86***	F_s=36.36***
Visceral	F_s=27.13***	F_s=55.08***
Lateral	F_s=29.37***	F_s=1.94 NS

The interaction terms were not significant and were dropped from the analyses.
Tracheal area and insect mass were log-transformed.
The null hypothesis is no effect of mass or oxygen level on tracheal cross-sectional area.
Notation: NS, not significant, ***=$P \leq 0.001$.

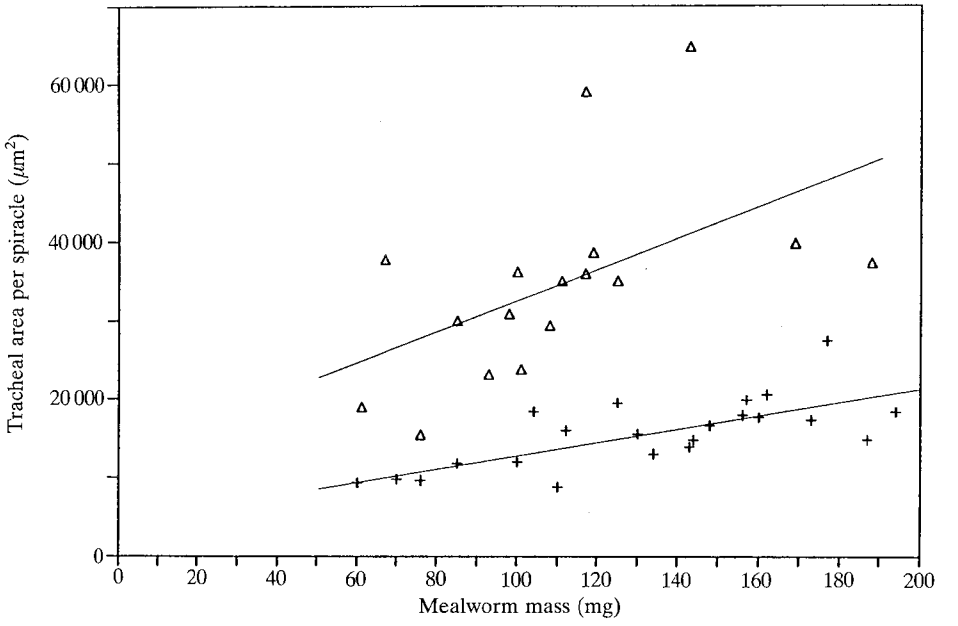

Fig. 3. Cross-sectional areas of dorsal, ventral and visceral tracheae were greater in lower partial pressures of oxygen. Larvae were reared in either 10.5 % (Δ) or 21 % (+) oxygen. Each point is for a single individual and is the average for the six pairs of abdominal spiracles under consideration, summing the areas of dorsal, ventral and visceral tracheae. The lines from linear regression are: larvae reared in 10.5 % oxygen, $y=196x+13\,110$ ($r^2=0.27$, $N=17$), and larvae reared in 21 % oxygen, $y=84.4x+4510$ ($r^2=0.51$, $N=23$), where y is average cross-sectional area per spiracle (in μm^2) and x is larval mass (in mg). Larvae reared in 15 % oxygen (not shown) had tracheal sizes intermediate between the two groups illustrated ($y=60.2x+15\,350$, $r^2=0.05$, $N=20$).

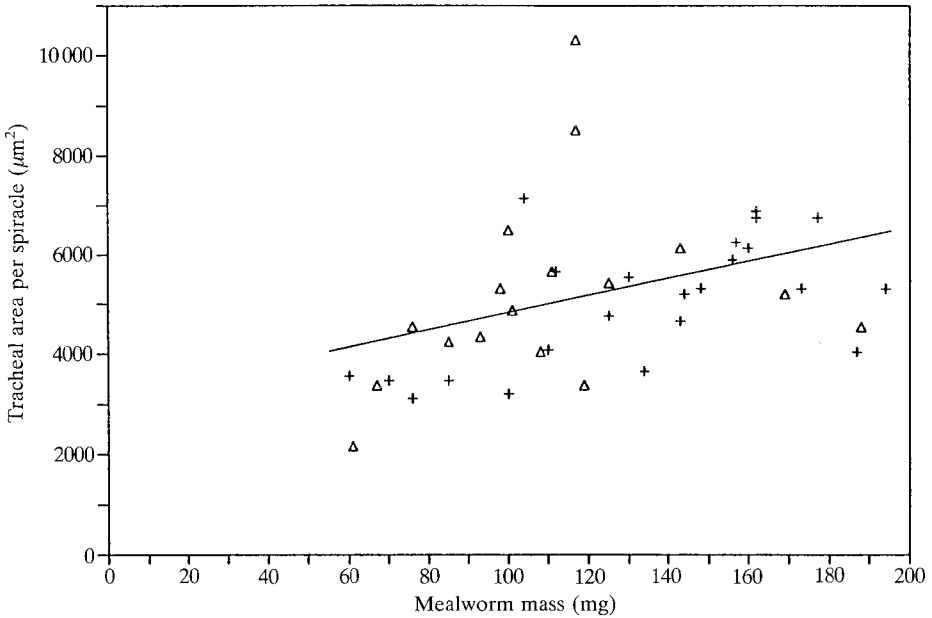

Fig. 4. Cross-sectional areas of the lateral longitudinal tracheae increased with larval mass but were not significantly affected by oxygen concentration. Larvae were reared in either 10.5 % oxygen (N=17) or 21 % oxygen (N=23). Each point is for a single individual and is the average for the six pairs of abdominal spiracles under consideration. The sizes of tracheae for larvae reared in 15 % oxygen (not shown) were not significantly different from those for the two groups illustrated. The line from linear regression is $y=17.3x+3130$ ($r^2=0.17$, $N=60$), where y is average cross-sectional area per spiracle (in μm^2) and x is larval mass (in mg).

anatomy or relative size of organs with oxygen concentration, so the meaning of the greater visceral tracheal hypertrophy is unknown. The cross-sectional areas of tracheae that supply tissues increased by about 40 % in 15 % oxygen and 120 % in 10.5 % oxygen.

Peritreme size

Spiracles of mealworms have a complex morphology, which makes it difficult to make a meaningful morphological measurement that will represent the functional aspects of the structure. The peritreme is the circular piece of cuticle that forms the border of the external opening of the spiracle. The anterior–posterior width of the elliptical peritreme was used as an indication of spiracular size because it is a straightforward measurement and is the size of the actual 'hole' that opens to the outside. The inside width of the peritreme was not affected by oxygen concentration (ANOVA of first abdominal spiracle peritreme width, including larval size as a covariate, P=0.83, N=35). Peritreme size was significantly but only loosely correlated with larval size (linear regression *versus* head width, r^2=0.34, P=0.0003, N=35). If peritreme size is reasonably representative of spiracular size,

Table 2. *Mean tracheal cross-sectional areas (μm^2) per spiracle by oxygen group and tracheal group (average for the second through to the seventh abdominal spiracles)*

Tracheae	21 % oxygen	15 % oxygen	10.5 % oxygen
	Cross-sectional area/spiracle (μm^2)		
Dorsal	5560±334	6980±697	11320±923
Ventral	3840±233	5170±424	7830±756
Visceral	6380±401	10330±888	15660±1424
D+V+V	15780±937	22480±1918	34810±3028
Lateral	5050±267	5470±314	5210±469
	Ratios relative to 21 % oxygen		
Dorsal	1.0	1.3	2.0
Ventral	1.0	1.3	2.0
Visceral	1.0	1.6	2.5
D+V+V	1.0	1.4	2.2
Lateral	1.0	1.1	1.0

D+V+V refers to the total cross-sectional area of tracheae supplying oxygen to the tissues (D+V+V=dorsal+ventral+visceral).

Mean sizes of larvae: 21 % oxygen, 133±38 mg (±1 S.D.); 15 % oxygen, 118±33 mg; 10.5 % oxygen, 110±33 mg.

Sample sizes: N=23 larvae for 21 % oxygen, N=20 for 15 % oxygen, N=17 for 10.5 % oxygen.

Values are means±1 S.E.

this means that although the tracheae that supply tissues change as a function of oxygen level, spiracular size does not.

Transfer results

What appears as tracheal hypertrophy could be due to the increased growth of the tracheae or the decreased growth of the rest of the body. Does tracheal hypertrophy actually exist, or are the larger tracheae seen in larvae in lower oxygen just a result of the greater total number of molts that larvae undergo in lower oxygen (Loudon, 1988)?

Tracheae do hypertrophy, as is evident comparing larvae from 21 % oxygen with larvae transferred from 21 % into 10.5 % oxygen (Fig. 5). Frequency of molting is not a function of oxygen concentration (Loudon, 1988), so these two groups of larvae molted the same number of times (approximately three times during this period). After starting with the same size of tracheae, larvae transferred into 10.5 % oxygen had larger tracheae than larvae that stayed in 21 % oxygen, after 32 days (ANOVA of dorsal, ventral and visceral tracheal area, P=0.0006, N=19).

Most tracheae for a larval instar are built around tracheae of the preceding instar (Keister, 1948; Wigglesworth, 1954; Whitten, 1957). Therefore, it is possible that tracheal hypertrophy, once developed, would persist into later stages. The results from the transfer experiment support this prediction. Larvae transferred from 10.5 % into 21 % oxygen started with the same size of tracheae as larvae that

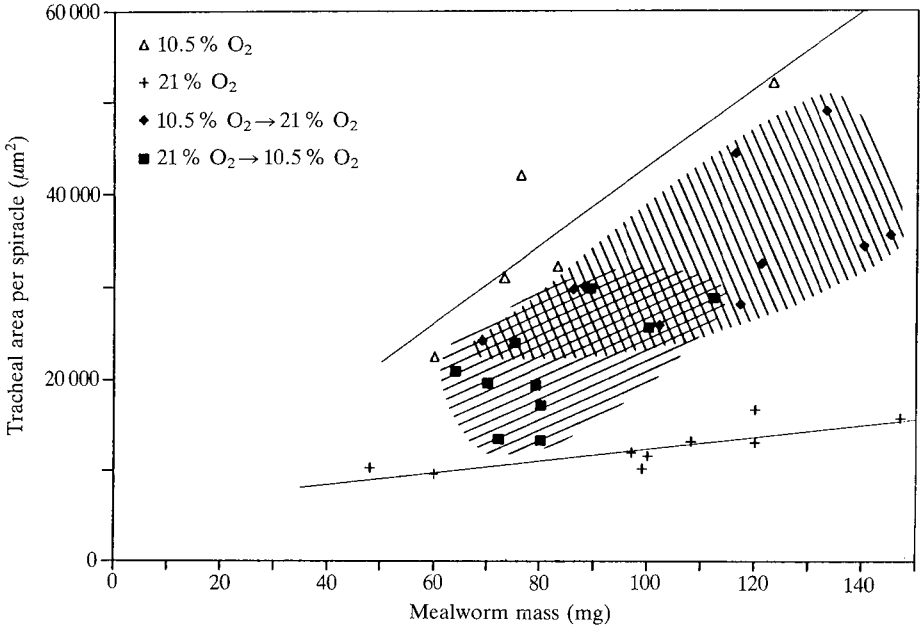

Fig. 5. Larvae were reared in either 10.5 % or 21 % oxygen, before transfer into new conditions for 32 days (controls were returned to their previous conditions). Dorsal, ventral and visceral tracheae increased in cross-sectional area in response to a lowered ambient oxygen partial pressure. Each point is for a single individual and is the average for the six pairs of abdominal spiracles under consideration, summing the areas of dorsal, ventral and visceral tracheae. The lines from linear regression are: larvae reared in 10.5 % oxygen, $y=422x+873$ ($r^2=0.79$, $N=5$), and larvae reared in 21 % oxygen, $y=65.3x+5910$ ($r^2=0.66$, $N=9$), where y is average cross-sectional area per spiracle (in μm^2) and x is larval mass (in mg).

stayed in 10.5 % oxygen, and underwent the same number of molts, but had the same size of tracheae after 32 days in different conditions (ANOVA of dorsal, ventral and visceral tracheal area, $P=0.61$, $N=15$). Therefore, earlier hypertrophy did persist despite a change in external conditions. Although their tracheae were the same size, their bodies were not: the larvae transferred into high oxygen were larger than the larvae that stayed in low oxygen, and therefore their bodies grew more with the same size of tracheae (shifted to the right in Fig. 5) (ANOVA of larval mass, $P=0.05$, $N=15$).

The sizes of lateral longitudinal tracheae in larvae transferred from one oxygen concentration to another were not affected by oxygen partial pressure (Fig. 6) (ANOVA of lateral tracheal area, $P=0.25$, $N=34$). This is not surprising, as the sizes of lateral longitudinal tracheae were independent of different, but constantly held, oxygen levels (see above).

Discussion

The main tracheae that supply oxygen to the tissues in larval *Tenebrio molitor*

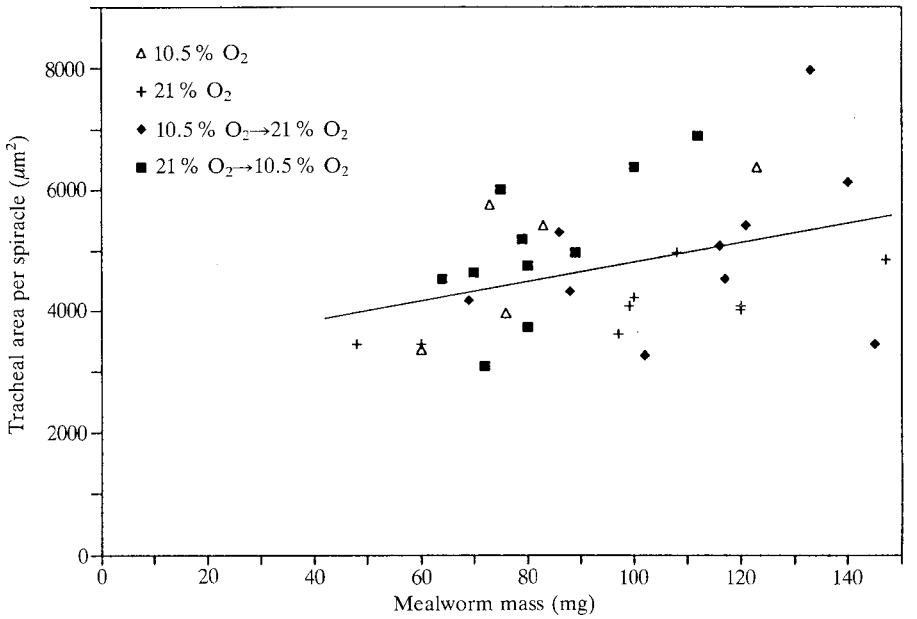

Fig. 6. In the transfer experiment, cross-sectional areas of the lateral longitudinal tracheae were not affected by ambient oxygen concentration, either constant or changing. Each point is for a single individual and is the average for the six pairs of abdominal spiracles under consideration. The line from linear regression is $y=16.2x+3190$ ($r^2=0.14$, $N=34$), where y is the average cross-sectional area per spiracle (in μm^2) and x is larval mass (in mg).

(dorsal, ventral and visceral) hypertrophy in lower oxygen levels, while the lateral longitudinal tracheae that interconnect adjacent spiracles do not. This does not mean that the longitudinal tracheae are incapable of hypertrophying, as it is possible that they may serve as a 'back-up' system in the event of spiracular blockage or damage. Locke (1958) did report hypertrophy of lateral longitudinal tracheae in *Rhodnius*, but this was only documented for *severed* lateral longitudinal tracheae (containing the lateral nodes) which were still connected to one adjacent spiracle, so this response seems to be related not to oxygen supply or demand but to wounding.

Fick's law, the Hagen–Poiseuille equation and hypertrophy

The extent of the tracheal hypertrophy is pronounced: larvae in 15 % oxygen have main tracheae on average 40 % larger in cross-sectional area than larvae in 21 % oxygen, and larvae in 10.5 % oxygen have tracheae on average 120 % larger than larvae in 21 % oxygen. This degree of hypertrophy can be compared with predictions based on Fick's law of diffusion and the Hagen–Poiseuille equation of convection to assess whether the observed morphological change is consistent with diffusion or convection as the primary mode of transport of oxygen.

Fick's law of diffusion describes the relationship between the rate of mass

transport by diffusion, the chemical potential gradient and the geometry of the system. For a gas, the chemical potential can be adequately described by the partial pressure. Treating the tracheae as a conglomerate circular cross-section cylinder (Fig. 1), Fick's law for diffusion along the tracheae can be written as:

$$\dot{M} = \Delta P_{O_2} \times \frac{A}{L} \times D \times \beta, \quad (1)$$

where $\dot{M}$ is net rate of transfer of oxygen molecules ($mol\,s^{-1}$), ΔP_{O_2} is the total difference in partial pressure of oxygen (Pa), A is the cross-sectional area of the cylinder (m^2), L is the length of the cylinder (m), D is the diffusion coefficient ($m^2\,s^{-1}$) and β is the solubility (=capacitance) coefficient ($mol\,m^{-3}\,Pa^{-1}$) (notation used is similar to that of Dejours, 1981).

The Hagen–Poiseuille equation describes the relationship between the volume flow rate, the difference in total pressure, and the geometry of the system. Written in comparable format to equation 1, it appears as:

$$\dot{V} = \Delta P \times \frac{A^2}{L} \times (8\mu\pi)^{-1}, \quad (2)$$

where $\dot{V}$ is the volume flow rate ($m^3\,s^{-1}$), ΔP is the total pressure difference along the cylinder (Pa), A and L are as defined above and μ is the dynamic viscosity ($Pa \cdot s$).

Note that the rate of diffusion scales linearly with cross-sectional area (equation 1), while the volume flow rate in convection scales with the square of cross-sectional area (equation 2). Both expressions assume steady-state conditions, no change in cross-sectional area along the length of the cylinder, no net movement across the walls of the cylinder, and neither absorption nor release along the length of the cylinder.

How does the relationship between the parameters in Fick's law compare with the extent of hypertrophy measured for the tracheae? Since the larvae being compared from the different oxygen treatments are on average the same size, the length of tracheae, L, will be assumed to be the same. The diffusion coefficient, D, and the solubility coefficient, β, can be treated as constants. It follows that the rate of diffusion, $\dot{M}$, will remain constant if ΔP_{O_2} and A change by reciprocal amounts (see equation 1).

If the partial pressure of oxygen at the mitochondria is taken to be zero (Jones *et al.* 1985), then

$$\Delta P_{O_2} = P_{O_2,\text{ambient}} - P_{O_2,\text{mitochondria}} \simeq P_{O_2,\text{ambient}}. \quad (3)$$

The drop in partial pressure along the tracheae is not known. If the drop in partial pressure along the tracheae is a constant proportion (C) of the total drop in partial pressure from ambient to mitochondria, then:

$$\text{in } 21\,\%\ O_2, \quad \Delta P_{O_2,\text{tracheae}} = 0.21C,$$

$$\text{in } 15\,\%\ O_2, \quad \Delta P_{O_2,\text{tracheae}} = 0.15C,$$

$$\text{in } 10.5\,\%\ O_2, \quad \Delta P_{O_2,\text{tracheae}} = 0.105C.$$

Therefore, the partial pressure gradients along the tracheae relative to the value for larvae reared in 21 % oxygen are:

$$\frac{\Delta P_{O_2,\text{ tracheae},\ 15\,\%}}{\Delta P_{O_2,\text{ tracheae},\ 21\,\%}} = \frac{0.15C}{0.21C} = 0.71,$$

$$\frac{\Delta P_{O_2,\text{ tracheae},\ 10.5\,\%}}{\Delta P_{O_2,\text{ tracheae},\ 21\,\%}} = \frac{0.105C}{0.21C} = 0.50,$$

The reciprocal of 0.71 is 1.4, and the reciprocal of 0.50 is 2.0. Therefore, increases in tracheal cross-sectional area of 1.4 and 2.0 times for larvae reared in 15 % oxygen and 10.5 % oxygen, respectively, will compensate for the assumed decrease in partial pressure gradient. These predicted increases lie within the 95 % confidence limits for measured tracheal hypertrophy in low oxygen (Table 3). (Note that these confidence intervals are conservative because they are based on a wide range of larval sizes.)

How does the extent of hypertrophy compare to predictions assuming convection as the primary mechanism of gas movement? The volume flow rate in the Hagen–Poiseuille equation (equation 2) refers to the gas mixture, not oxygen alone. Therefore, the rate of oxygen transport will remain unchanged if the cross-sectional area increases with the square root of the reciprocal of the decrease in oxygen content of the gas mixture. The observed increase in cross-sectional area is much greater than would be predicted from the Hagen–Poiseuille equation. Larvae reared in 15 % oxygen would be predicted to have an increase in tracheal cross-sectional area of 20 %, relative to larvae reared in 21 % oxygen $[(0.21/0.15)^{0.5}=1.2)]$, while larvae reared in 10.5 % oxygen would be expected to have a tracheal hypertrophy of 40 % in area relative to larvae reared in 21 % oxygen $[(0.21/0.105)^{0.5}=1.4]$ (Table 3).

Table 3. *Measured hypertrophy compared to predicted hypertrophy assuming (1) diffusion only and (2) convection only as the type of oxygen transport*

Oxygen treatment	Measured hypertrophy	Predicted hypertrophy assuming Diffusion	Convection
15 %	1.4(±0.29)	1.4	1.2
10.5 %	2.2(±0.46)	2.0	1.4

Measured hypertrophy is expressed as a ratio (±95 % confidence interval), the average tracheal cross-sectional area (dorsal+ventral+visceral) for the given oxygen treatment divided by tracheal cross-sectional area for larvae reared in 21 % oxygen.

95 % confidence limits for the empirically determined ratios were calculated using the standard analysis for propagation of errors through formulae, based on the statistics in Table 2; Mortimer, 1981, p. 280.

Therefore, the magnitude of the observed hypertrophy in the main tracheae is consistent with maintenance of a fixed rate of oxygen delivery by diffusion, but not by convection, with the stated assumptions. It is difficult to assess the magnitude of possible error introduced by some simplifying assumptions. For example, because spiracles open and close, it is clear that the system is not at steady state, but it is not clear if taking this into account in more complex calculations would qualitatively affect the above results. Furthermore, many changes may have taken place that were not measured (enzyme activities, numbers or positions of mitochondria, numbers of tracheoles, morphology of smaller tracheae, permeability of tracheal cuticle, etc.); calculations were performed assuming that the resistance of the tracheae changed by the same magnitude as the other (unknown) resistances, and that the rate of oxygen uptake was unchanged. If grossly different assumptions are made, the resulting calculations usually suggest that the magnitude of hypertrophy is greater than would be predicted assuming either diffusion or convection. I have been unable to construct a reasonable set of alternative assumptions that would make the observed magnitude of the hypertrophy consistent with convection but not diffusion.

Note that morphological compensation that maintains a constant diffusion *rate* of oxygen with changing ambient conditions will not hold the oxygen *partial pressure* constant at points along the tracheal system (e.g. compare lines *b* and *d* in Fig. 1). There is ample evidence that tracheal hypertrophy (and the other morphological and biochemical changes that presumably occur but were not measured) does not compensate fully for lowered ambient oxygen level, and this may be because of changes in the partial pressure of oxygen in the tissues. Although there are large changes in tracheal size, larvae reared in 10.5 % oxygen exhibited slower growth, greater mortality, a larger number of developmental abnormalities, and a significantly greater proportion of females than larvae reared in 21 % oxygen (the latter probably due to gender-related mortality) (Loudon, 1988). Growth of larvae in 15 % oxygen was not slowed relative to larvae in 21 % oxygen, but larvae in 15 % oxygen did exhibit other effects of low oxygen, such as a significantly greater proportion of females (Loudon, 1988). Jones *et al.* (1985) have shown that in mammalian cells, many metabolic changes occur at oxygen partial pressures *above* that critical partial pressure which maintains mitochondrial oxygen consumption. They interpret this to be because many enzymes, whether they use oxygen directly as a substrate or not, are differentially affected by low oxygen partial pressure.

Another point which should be made here is that although insect hemolymph rarely contains respiratory pigments, and hence has a very low oxygen-carrying capacity, it may play some role in delivering oxygen to the tissues it bathes (Wigglesworth, 1972). The whole tracheal system is bathed in hemolymph and, hence, if the oxygen partial pressure is lower in the tracheae because of a lower ambient oxygen level, the oxygen content of the hemolymph will also be lower. This may also contribute to the lowering of the oxygen concentration at the tissue level.

Spiracles

A single measurement made of the spiracles, the peritreme width, did not show a change with ambient oxygen partial pressure. If this measurement was representative of spiracular size, then the spiracular morphology was not affected by oxygen partial pressure. This is only consistent with the above argument if it is assumed that the spiracular resistance is a negligible part of the total resistance in the tracheal system of larval *Tenebrio molitor*, or if the resistance is changed because of differences in opening frequency. There is not enough evidence to evaluate this point for this species.

Transfer between oxygen concentrations

The results of the transfer experiments show that the tracheae themselves do hypertrophy, as opposed to the alternative hypothesis that smaller bodies are built around a predetermined size of tracheal system. This distinction may be immaterial to the insects themselves – if a larva has relatively large tracheae for its size, it may be of no consequence to the physiological state of the larva if it got there by adjusting body size or tracheal size to ambient conditions. This distinction is important, however, if the interest is in the mechanism of acclimation to low oxygen, or in predictions of morphological change.

Although the increase in tracheal size at a molt was greater when larvae were transferred into lower oxygen, the increase in tracheal size was not smaller when larvae were transferred into higher oxygen. This latter result is at odds with Locke's report (1958) that larvae transferred into higher oxygen (>21 % oxygen) showed a smaller increase in tracheal size at a molt relative to larvae in 21 % oxygen. I found that the tracheae increased by the same amount, and that the *relative* size of the tracheae decreased solely because of the increase in growth of the body in higher oxygen. Therefore, these data suggest that tracheal size is a function of previous tracheal size, as well as ambient conditions. This is probably because the tracheae are built around the tracheae of the previous instar, and so there may be a physical constraint on their development.

Morphological change as an indicator of process

Hypertrophy of respiratory exchange organs in low ambient oxygen has been documented for a variety of organisms (Dejours, 1981), but rarely are the morphological changes sufficiently quantified that inferences can be drawn about the process of mass transport itself, or the cues used by the tissues in forming new structures.

In this study, morphological change has been used to infer information about the underlying mechanics of mass transport. Another example where this approach would have been useful can be illustrated using data from Langille and O'Donnell (1986), who reported arterial diameter changes in response to experimentally altered blood flow in mammals. When flow was reduced to 30 % of its initial value, the diameter of the artery was reduced to 79 % of the control

artery (Langille and O'Donnell, 1986). It is interesting in itself that a change in the size of the artery was induced, but can we extract any additional information from the magnitude of the change? How does the flow reduction to 30 % relate to a diameter reduction to 79 %?

Referring back to the Hagen–Poiseuille equation (equation 2), it is clear that if the flow rate, $\dot{V}$, is decreased to 0.3 of its initial value, a compensating decrease in the diameter by the one-fourth power of 0.3 will keep the pressure drop along the artery the same (assuming that it stays the same length). It turns out that $(0.3)^{0.25}=0.74$, which is similar to the artery diameter after manipulation. Therefore, the magnitude of the change in arterial diameter is consistent with maintenance of a constant pressure drop along the artery.

There are obvious caveats to the use of morphological changes in providing information about the underlying fluid mechanics, one of which is that some resistances may be cheaper or easier to change than others. The change in tracheal size in *Tenebrio molitor* larvae does not prove that diffusion is a primary mechanism of mass transport but, because the rates of diffusion and convection scale differently with geometry, the change in tracheal size will differentially affect the two processes. Therefore, a morphological change that is consistent with Fick's law will have a disproportionate effect on convection in the system. Tracheal hypertrophy does not leave the larvae completely unaffected by ambient oxygen concentration, and this may be due to the changes in oxygen concentration in the tissues.

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