ON THE RELATIVE IMPORTANCE OF BODY WEIGHT AND SURFACE AREA MEASUREMENTS FOR THE PREDICTION OF THE LEVEL OF OXYGEN CONSUMPTION OF *LIGIA OCEANICA* L. AND PREPUPAE OF *DROSOPHILA MELANOGASTER* MEIG.

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INTRODUCTION

Although there is general agreement about the variation in metabolic rate with increasing body size, the reason for this variation is obscure. In general it is assumed that oxygen consumption is proportional to some function of body weight, and that the relationship of the former to surface area is fortuitous and due to the relationship of surface area itself to body weight. But measurements of the surface area of the individuals used in the oxygen determinations are almost invariably lacking for poikilotherms, and rare for other animals. In recent papers (Ellenby, 1951, 1953) the relationship of oxygen consumption to surface area and to body weight has been investigated for prepupae of *Drosophila melanogaster* and for the littoral isopod *Ligia oceanica*. For forty-seven *Drosophila* prepupae and eighty male *Ligia* information was available on body weight, and the surface area (or a linear dimension related to it) of the actual animals used in the oxygen consumption determination. The oxygen consumption per unit surface varied with weight for *Drosophila* but remained approximately constant for *Ligia*. For both animals, the material suggested that the level of oxygen consumption might be predicted more precisely from a measurement of surface area than from one of body weight, and this in spite of the greater accuracy of the weight determinations. In other words, the level of oxygen consumption might be more closely related to surface area than to body weight. In the present paper, the material is examined in greater detail in order to see how far the suggestion can be substantiated.

DROSOPHILA

The relationship of oxygen consumption to body weight appears to be approximately exponential, and it is therefore conveniently evaluated by a linear regression analysis based on the logarithms of the observations. The usual normal law assumptions appear to be valid for the transformed observations. Preliminary calculations (Ellenby, 1953) showed that the residual mean square about the regression line for log oxygen consumption on log surface area was some three-quarters of that for a regression line for log oxygen consumption on log body weight. A less valid test for *Ligia* (Ellenby, 1951) also suggested that oxygen consumption might be more closely related to surface area than to body weight. But the technique of linear regression can consider only two variables at the same time, either oxygen and body weight, or oxygen and surface area. In the present treatment the material is examined by employing the technique of multiple regression; this can consider any number of independent variables in relation to a single dependent variable and assess their relative importance. In the present case the technique is used to assess the relative importance of body weight and surface area in predicting the value of the dependent variate, oxygen consumption. As before, the logarithms of the observations are used in the analysis.

The following abbreviated notation is used throughout this section:

Log (O.C.) = $\log_{10}$ (oxygen consumption in mm.^{3} per hour at S.T.P.);
Log (B.Wt.) = $\log_{10}$ (body weight in mg.);
Log (S.A.) = $\log_{10}$ (surface area in mm.^{2}).

The values of these three variables were known for each of forty-seven *Drosophila* prepupae: thirty-three sets of values for diploid females, five for diploid males, and nine for triploid females.

Separate multiple regression equations of the form

$$\log(\text{O.C.}) = a_0 + a_1 \log(\text{B.Wt.}) + a_2 \log(\text{S.A.})$$

were fitted by least squares for each of these three homogeneous groups.

No significant differences were found between the diploid males and females, but the results for the triploid females were of a different character; the latter are therefore treated separately.

Diploid male and female Drosophila

The analysis of variance for the combined diploid male and female data is given in Table 1. The total sum of squares for log (O.C.) is 0·169 with 37 D.F. This sum of squares is partitioned into two components; the 2 D.F. sum of squares removed by fitting a multiple regression equation of the type mentioned above, and the residual sum of squares for the fitted regression equation. The table shows that $F_{2,35} = 156$, so that, as expected, there is a significant multiple regression of log (O.C.) on log (B.Wt.) and log (S.A.).

The initial part of the table is concerned with testing whether it is an advantage to have both the dependent variates log (B.Wt.) and log (S.A.) in the regression equation, or whether practically as good a prediction of oxygen consumption could be obtained by using body weight or surface area alone. The first line shows that the sum of squares removed by a linear regression of log (O.C.) on log (S.A.) is significant since $F_{1,35} = 313$. The second line shows that the extra sum of squares removed by the multiple regression of log (O.C.) on log (S.A.) and log (B.Wt.) is negligibly small since $F_{1,35} = 1/108$. (This variance ratio is not significantly small, the lower 5% point being 1/251.)

A similar procedure for log (O.C.) and log (B.Wt.) is repeated in the third and fourth lines of the table. The linear regression of log (O.C.) on log (B.Wt.) removes a significant sum of squares ($F_{1,38}=283$); however, in this case, the extra sum of squares removed by the multiple regression is also significant ($F_{1,35}=29$).

The conclusions to be drawn from this analysis are that the multiple regression equation does not give any significant reduction in the error mean square when compared with the simple linear regression of log (O.C.) on log (S.A.). The multiple regression is, however, significantly better than the linear regression of log (O.C.) on log (B.Wt.).

Table 1. *Analysis of variance for multiple regression*; Drosophila *diploid male and female data combined*

Source of variation	Sum of squares	D.F.	Mean square	F
Linear regression on log (S.A.)	0·152	1	0·152	313
Regression on the component of log (B.Wt.) orthogonal to log (S.A.)	0·000	1	0·000	0
Linear regression on log (B.Wt.)	0·138	1	0·138	283
Regression on the component of log (S.A.) orthogonal to log (B.Wt.)	0·014	1	0·014	29
Multiple regression on log (B.Wt.) and log (S.A.)	0·152	2	0·076	156
Residual	0·017	35	0·000	—
Total	0·169	37	—	—

In other words, assuming a regression of log (O.C.) on log (S.A.), a negligible increase in the precision with which log (O.C.) may be predicted is obtained by taking into account log (B.Wt.). But, on the other hand, after fitting a linear regression of log (O.C.) on log (B.Wt.), a significant improvement in the precision is obtained by taking log (S.A.) into account also. Clearly, although both log (B.Wt.) and log (S.A.) give significant information about log (O.C.), the log (S.A.) variable is the more important of the two; and this in spite of its relatively inaccurate measurement.

The simple regression line linking log (O.C.) and log (S.A.) has a slope of +1·19, with an estimated standard error of 0·066. The slope differs significantly from unity ($P < 1\%$), confirming, as already deduced indirectly (Ellenby, 1953), that oxygen consumption is not quite proportional to surface area.

Triploid female Drosophila

The individual residual mean squares for the three groups of data showed no evidence of heterogeneity; the three residual sums of squares left after fitting the three separate multiple regressions were therefore pooled. The error variance of Table 2 has been estimated from this pooled information instead of from the residual error for the triploid group alone; the effect is to give a residual mean square of 0·000480 with 38 D.F. rather than a mean square of 0·000280 with 6 D.F.

The arrangement of Table 2 is then fundamentally the same as that of Table 1. From the last two lines it is seen that $F_{2,38}=38$ so that there is a significant multiple regression of log (O.C.) on log (B.Wt.) and log (S.A.). The first and second lines of the table show that the linear regression of log (O.C.) on log (S.A.) is significant ($F_{1,38}=69$), and that the extra sum of squares removed by the multiple regression of log (O.C.) on both log (S.A.) and log (B.Wt.) is also significant ($F_{1,38}=16$), that is, log (B.Wt.) contributes significantly.

Table 2. *Analysis of variance for multiple regression; triploid female data*

Source of variation	Sum of squares	D.F.	Mean square	F
Linear regression on log (S.A.)	0·029	1	0·029	60
Regression on the component of log (B.Wt.) orthogonal to log (S.A.)	0·008	1	0·008	16
Linear regression on log (B.Wt.)	0·036	1	0·036	76
Regression on the component of log (S.A.) orthogonal to log (B.Wt.)	0·000	1	0· 00	0
Multiple regression on log (B.Wt.) and log (S.A.)	0·036	2	0·018	38
Pooled residual	0·018	38	0·000	—

The middle section of the table shows that the linear regression on log (B.Wt.) is significant ($F_{1,38}=76$); however, the extra sum of squares removed by the multiple regression is now negligibly small ($F_{1,38}=1/15$), showing that log (S.A.) contributes practically no additional information about log (O.C.) beyond that already provided by log (B.Wt.).

The position, then, is almost the exact opposite of that found for the diploid males and females. A prediction of oxygen consumption for diploid males and females from surface area measurements is not improved significantly by the additional information from body weight; in the case of the triploids, however, a prediction based on body weight is not improved significantly by the additional information from surface area. For the diploids, therefore, surface area is the more important; but for the triploids it is body weight.

As already described (Ellenby, 1945), the surface area of the puparium is measured after the imago has emerged, the empty case being flattened between microscope slides. A certain number are excluded if they are badly broken in the process. In view of the scarcity of triploids, it seemed possible that they may have been judged less rigorously as one could hardly afford to lose a single one. However, comparison of the variances of the surface-area measurements for diploids and for triploids showed that they do not differ significantly ($P>30\%$). Whatever the reason, therefore, for the difference between the triploid and diploid results it is not due to any systematic difference between the accuracy of the surface-area measurements in the two groups.

LIGIA

A plot of log length against log breadth for male *Ligia* (Ellenby, 1951) gave a straight line with a slope which did not differ significantly from unity, showing that the animals remained more or less the same shape with increasing body size; and this conclusion was also supported by an examination of the growth of the pleopods relative to body length. Since, for bodies of similar shape, surface area is proportional to the square of a linear dimension, $(\text{length})^2$ was used for estimating surface area in the work already cited; and 'log length' is used in the present treatment. Multiple regression analyses were also carried out including 'breadth' but, presumably because of the larger percentage error of these measurements, this dimension was found to be very inferior to both length and weight. Consequently these analyses have not been presented here (even though the simple regression of log oxygen consumption on log breadth was highly significant), and the multiple regression analysis given below is based only on the oxygen consumption, body weight, and length (excluding the antennae and uropods) of the eighty male *L. oceanica* used in the previous work.

The following abbreviated notation is adopted:

Log (O.C.) = $\log_{10}$ (oxygen consumption in $mm.^3$ per hour, at S.T.P.);
Log (B.Wt.) = $\log_{10}$ (body weight in mg.);
Log (L.) = $\log_{10}$ (length in cm.).

The analysis shows that log (B.Wt.) is the most important single variate, and that the increase in accuracy obtained by including log (L.) in a multiple regression equation is not statistically significant. That is, log (L.) is found to be relatively unimportant statistically compared with log (B.Wt.) for the prediction of log (O.C.) for male *Ligia*.

The results of the analysis are presented in Table 3. The last two lines of the table show that there is a significant multiple regression of log (O.C.) on log (B.Wt.) and log (L.). The first and second lines of the table show that the linear regression of log (O.C.) on log (B.Wt.) is significant, and that the extra sum of squares removed by the multiple regression including log (L.) is negligible ($F_{1,77} = 0.2$); very little additional information is therefore provided by log (L.).

Table 3. *Analysis of variance for multiple regression*; Ligia oceanica *data*

Source of variation	Sum of squares	D.F.	Mean square	F
Linear regression log (B.Wt.)	5·44	1	5·44	699
Regression on the component of log (L.) orthogonal to log (B.Wt.)	0·00	1	0·00	0
Linear regression on log (L.)	5·38	1	5·38	692
Regression on the component of log (B.Wt.) orthogonal to log (L.)	0·06	1	0·06	8
Multiple regression on log (B.Wt.) and log (L.)	5·44	2	2·72	350
Residual	0·60	77	0·01	—
Total	6·04	79	—	—

Linear regression of log (O.C.) on log (L.) is significant, as shown in the third line of the table, with $F_{1,77} = 692$; and as shown in the next line, including log (B.Wt.) gives a significant improvement in accuracy ($F_{1,77} = 7.8$).

Although log (B.Wt.) is the most important single variate, from our present point of view it is interesting to note that, in spite of the relative inaccuracy of the length measurements, log (L.) does not show up too badly; the residual mean square for simple linear regression of log (O.C.) on log (B.Wt.) is 0·0077 compared with 0·0085 for the residual mean square for linear regression of log (O.C.) on log (L.).

DISCUSSION

The results show, then, that for *Ligia* and triploid prepupae of *Drosophila*, body weight is more important for predicting the level of oxygen consumption than surface area: on the other hand, surface area gives a better prediction for diploid male and female prepupae.

It would ill become us to belittle the *Ligia* data which, in fact, compare favourably with that from other animals; but the conclusions drawn from the diploid *Drosophila* material and the comparison of triploids and diploids are of most interest, and these are based on material of exceptional reliability. In any case, it would be unwise to compare information based on a series of isopods differing in age and body weight with that derived from a group of insect prepupae differing in body weight but of the same age, and undergoing the cataclysm of insect metamorphosis. Compared with the *Ligia* individuals, the prepupae were genetically uniform; the measurements of oxygen consumption are based on animals of the same age, and would not have been influenced by bodily movements or digestive and related processes; body weight would also be more reliable, for the prepupae were reared under controlled conditions and weighed after many hours at constant humidity; and surface area was actually measured rather than estimated from a linear dimension. Accurate measurement of surface area is, however, extraordinarily difficult, and even a polished metal plate may have a true surface ten times its apparent surface (Bowden & Rideal, 1928); but, although the method by which puparial surface was measured is as accurate as most, it is of greater importance that there are grounds for maintaining that the measured surface bears a constant relation to the true surface over the whole size range (Ellenby, 1953). Nevertheless, as *determinations*, the surface area measurements are less accurate than those of body weight, and the finding that surface gives the better prediction of level of oxygen consumption for diploid prepupae is therefore of added significance.

The success of a prediction of the level of oxygen consumption from a measurement of body weight, or of surface area, will depend on the accuracy with which the latter are measured and on the closeness of their relationship to factors governing the level of metabolism. Clearly, even if physical surface were directly connected with the level of metabolism, it may still be possible to predict oxygen consumption with greater accuracy from body weight; for it may be possible to estimate the surface area of an individual animal more accurately from its weight than it can be measured directly. A demonstration that oxygen consumption may be predicted more accurately from body weight may only be due, therefore, to the fact that body weight can be measured more accurately.

On the other hand, although an animal may be weighed at any instant with very great accuracy, the body weight may be unreliable from the standpoint of metabolism; the chemical balance may be very trustworthy, but not the use to which it is put. For example, the water content of a particular animal may vary considerably from instant to instant, so that a prediction of oxygen consumption from wet weight is likely to be inaccurate for this reason alone. Surface area may then give a better prediction of oxygen consumption merely because it is to a large extent independent of water content, and not because it is causally related to metabolism. In other words, under these circumstances, surface area may be a better predictor than body weight because it is a more reliable measure of 'body size'.

A more accurate prediction of the level of oxygen consumption from body weight, therefore, or from surface area, does not necessarily imply a closer relation of either of these physical properties to the real factors governing the level of metabolism.

The evidence from the *Drosophila* material is therefore of particular importance in relation to this impasse: for the triploid females and the diploid males and females give different answers. It is unlikely that these different prepupae, covering the same range of body size, would show class differences in their composition of the minor sort which might operate among a collection of animals raised under a variety of nutritive conditions. Their body weights would therefore be of the same reliability as *determinations*, and the accuracy with which surface area was measured would be the same for the different classes. Yet the results show, nevertheless, that while surface area gives the better prediction for diploids, weight is better for triploid females. It would be strange indeed if this difference were not entirely because the comparison was, in fact, between diploid and triploid organisms.

Differences in the body weights of organisms may be accompanied by relative differences in their composition or their structure or in both. The superiority of body weight, therefore, may be due to a variety of causes. On the other hand, minor factors can be excluded in the case of the diploid/triploid comparison. The superiority of surface area as a standard for predicting the level of oxygen consumption of diploid male and female prepupae of *Drosophila* strongly suggests that, in this case at least, factors related to the surface itself may have a determining influence on the level of metabolism.

SUMMARY

1. Previous investigations with *Ligia oceanica* and prepupae of *Drosophila melanogaster* (Ellenby, 1951, 1953) have suggested that it may be possible to predict the level of oxygen consumption more precisely from a measurement of surface area (*Drosophila*) or body length (*Ligia*) than from body weight, in spite of the greater accuracy of the latter measurement. The point is now examined more closely by applying the technique of multiple regression to the original data.

2. For *Ligia*, it is shown that the suggestion cannot be upheld, for the level of oxygen consumption can be predicted with greater accuracy from body weight than from a function of body length.

3. On the other hand, for diploid male and female prepupae of *Drosophila*, it is shown that surface area does, in fact, give a better prediction than body weight. In the case of triploid female prepupae, however, body weight is superior.

4. It is shown that there are no grounds for believing that the measurements of surface area were less accurate in the case of the triploids; for this and other reasons, it is suggested that the difference between diploids and triploids may be due to a fundamental difference between the two sorts of prepupae.

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