Larval competition in *Drosophila melanogaster*. I. Estimation of larval growth parameters

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Despite extensive research into the competitive interactions between the larvae of *Drosophila* for food, there have been few studies of the biological characteristics of the larvae which might underly competition. Here we present a sensitive method for estimating the larval feeding rate, larval gut capacity, larval conversion efficiency and larva–adult conversion efficiency, using radioactively labelled yeast. Two developmental stages, defined by the time since oviposition, were investigated in eight genetically distinct strains of *Drosophila melanogaster*. Significant genetic variation was recovered for all parameters at the second instar but only for conversion efficiency at the third instar. Feeding rate and the gut capacity had large and heterogenous error variances, especially in the third instar, while conversion efficiencies were relatively more stable.

INTRODUCTION

Competition has long been a subject of investigation in plants and animals (de Wit, 1960; Dawson, 1966; Sokal and Bryant 1967; Ayala *et al.* 1972; Shorrocks and Begon, 1975; Ford and Diggle 1981; Mather and Caligari, 1981, 1983; Spitters 1983; Spitters and Aerts, 1983). The many conveniences of *Drosophila* have made it a popular organism with which to investigate competitive interactions. However, despite the generally acknowledged presence of competition between the larvae in both natural (Grimaldi and Jaenike, 1984; Atkinson, 1985) and laboratory conditions (Sang, 1950; Ayala *et al.*, 1972; Mather and Caligari, 1981; Bakker, 1983), there has been little investigation of the biological characteristics of larvae that might determine competitive success. The studies of Bakker, (1961) de Jong, (1976) and Nunney, (1983) make inferences about larval processes through examination of the emerging adults. The studies by Robertson (1960a; 1960b; 1963) also infer the nature of the larval period through examination of the adults. Studies of the larvae themselves either concern changes in their biochemical constitution (Robertson, 1978) of their behaviour whilst feeding (Burnet *et al.*, 1977; Sewell *et al.*, 1975; Bauer and Sokolowski, 1984). Feeding variables such as cephalopharyngeal retraction and locomotory

behaviour may be related to food intake, but do not provide an estimate of the rate at which it is ingested.

Here we use a new method for estimating the rates of food acquisition and utilisation, characters important in determining competitive success (Bakker, 1961), which involve a time course assay using radioactively labelled yeast. In an accompanying paper (de Miranda and Eggleston, 1987) we attempt to correlate these parameters with statistical parameters derived from the analysis of competitive ability (Mather and Caligari, 1981, 1983).

Measurement of the feeding rates of *Drosophila* larvae involves two phases, the first concerning the rate at which food is ingested and the second the rate at which it is processed and incorporated into body tissue. A time course assay of the incorporation of radioactively labelled yeast will therefore produce a biphasic linear graph. The first phase depicts the rate at which the label is taken into the larval gut, and provides an estimate of the capacity to ingest food. After a period of feeding the larval gut will be filled with radioactive yeast and the larva will then start to excrete radioactive products. Any increase in the amount of label present in the larval body beyond this point depends on the efficiency with which food is processed into body tissue, as well as the rate at which it is taken in.

This constitutes the second phase of the graph (fig. 1). If we make the reasonable assumption that the rate of ingestion is the same for phase 1 and phase 2, then the ratio of the slopes for phases 1 and 2 will provide an estimate of conversion efficiency. The point of intersection of the two lines provides an estimate of the capacity of the larval gut and hence of the size of the larva. If radioactively labelled larvae complete their development on non-radioactive yeast we can measure, through analysis of the adults, any sexual differences in larval feeding behaviour and an estimate of the efficiency with which the radioactive label is retained through larval development and into adulthood.

Figure 1 Representation of the progress of larval feeding as described in the text. Phase 1 concerns solely the rate of ingestion (b_1), while phase 2 concerns the rate at which the food is absorbed into the larval tissue (b_2). The point of intersection provides an estimate of the capacity of the larval gut (g).

EXPERIMENTAL DETAILS

Radioactive yeast was cultured using the low sulphate medium (LSM) described by Douglas *et al.*, (1979) which maximises both the yield of yeast cells and the incorporation of radioactive sulphate ($S^{35}O_4$). The yeast used as a starter for the culture (Sigma YSC-2) was the same as that used in the accompanying analysis of competitive behaviour (de Miranda and Eggleston, 1987). For the second instar assays 200 μCi S^{35} /100 ml LSM was used whereas the third instar assays used 100 μCi S^{35} /100 ml LSM. The yield of yeast cells was between 1.2 and 1.5 g/100 ml LSM which was resuspended to 600 mg/ml (wet weight) in a 1 per cent solution of glucose in LSM and about 90 per

cent of the label could be centrifuged with the cells. The radioactive yeast solution was stored at 0°C until required.

Eight strains of *Drosophila melanogaster*, denoted 25A, 25B, 25C, 25D and 27A, 27B, 27C, 27D, were investigated. These strains are a subset of two larger sets of chromosome substitution lines constructed 6 months earlier involving the T19 and T25, and T27 and T5 inbred lines of *Drosophila melanogaster* (Linney *et al.*, 1971), and they were maintained by sibmating. Their chromosomal constitution is described in the accompanying paper (de Miranda and Eggleston, 1987). For each strain 4 replicate time course assays were conducted for both second and third instar larvae (53 and 77 hours after oviposition respectively, at 25°C). The larvae were allowed to develop on 9.0 cm petridishes containing 25.0 ml 2 per cent bactoagar with 15 ml/l 10 per cent nipagin in ethanol as an antifungal agent to which 4.0 ml of 100 mg/ml YSC-2 yeast suspension was added as a source of food. The yeast was allowed to dry overnight at 25°C and about 320 eggs were transferred to each petridish. An excess of food was provided to minimise competition and to ensure that estimates of the feeding rate would not be influenced by varying levels of starvation. The dishes were incubated at 25°C $\pm$ 0.5°C at constant humidity levels for 53 or 77 hours, producing either second or third instar larvae respectively.

For each assay 3.0 ml radioactive yeast solution (200 μCi yeast for 53-hour larvae, 100 μCi for 77-hour larvae) was deposited in a 5 cm petri dish and dried to a uniformly solid consistency at 25°C. Prior to each assay 2 small samples (approximately 30 mg) of the thickened yeast were weighed and dissolved in 5.0 ml of water to determine the radioactivity per μg yeast which was used to convert all radioactivity estimates to estimates of the amount of yeast consumed. The larvae were washed from the petridishes, rinsed three times on filter paper under light vacuum to remove all traces of non-radioactive yeast, dried and deposited *en masse* on the radioactive yeast. At 0, 5, 10, 20, 30, 45, 60, 90, 120, 150 and 180 minute intervals approximately 50 larvae were collected from the radioactive yeast, rinsed with water, dried on tissue paper, counted and homogenised. The homogenates were transferred to scintillation vials with three changes of 0.25 ml Ringers solution and 4.0 ml of Beckman's readymix scintillation fluid was added to each vial. At the 180-minute interval two extra samples of 50 larvae were removed from the radioactive yeast, rinsed and distributed over two plastic vials containing standard *Drosophila* medium to

complete their development at 25°C. Upon emergence these flies were counted, sexed, weighed and homogenised. The homogenates were transferred to scintillation vials using three changes of Ringers solution containing 5 per cent lipsol (a detergent supplied by LIP Ltd., West Yorks., England) to facilitate the transfer of hydrophobic components. Radioactivity was determined as before.

There exists a vast literature concerning the problem of biphasic regressions and discontinuous changes in normally distributed variables (Shaban, 1980; Sprent, 1961; Hinckley, 1969 and Jones and Molitoris, 1984). Here the plots in each assay were divided into two groups (Phase 1 and Phase 2) to minimise the combined residual regression variation when two simultaneous linear regressions were fitted to the data. The last plot to be included in phase 1 and the first plot in phase 2 were used as the centres for their respective regressions; as near as possible to each other and to the putative breakpoint. After determining the best possible allocation of plots to phases 1 and 2, the point of intersection (X_g) was calculated. The data were then reanalysed using X_g as the single centre of regression for both slopes, *i.e.*,

$$S(X_{d1})^2 = \sum_{i=1}^m (X_i - X_g)^2$$

$$S(X_{d2})^2 = \sum_{i=m+1}^n (X_i - X_g)^2$$

where m = number of plots in phase 1, n = total number of plots, $S(X_{d1})^2$ and $S(X_{d2})^2$ are the sums of squares in X for phases 1 and 2 respectively. The basic residual regression variance (RRV) was calculated as

$$RRV = RSS / (n - 4)$$

where RSS is the residual sums of squares $\sum^n (O - E)^2$ and $(n - 4)$ is the number of degrees of freedom, 3 df having been lost for the three linear parameters estimated and 1 df for estimating X_g (Sprent, 1961; Jones and Molitoris, 1984). The approximate error for g (the Y value at the breakpoint) becomes

$$VAR(g) = RRV / n$$

and the approximate error variances for the two slopes are

$$VAR(b_1) = RRV / S(X_{d1})^2$$

$$VAR(b_2) = RRV / S(X_{d2})^2.$$

Since this is essentially a non-linear regression using linear techniques all variance estimates are approximate rather than exact. The fiducial limits for X_g can be calculated using Fieller's theorem (Sprent, 1961), while Hinckley (1969) describes a method to calculate the exact limits for X_g using maximum likelihood procedures.

An approximate test of the null hypothesis that the biphasic regression provides no better fit to the data than a single linear regression was described by Jones and Molitoris (1984). For the experiments described here the biphasic regression consistently gave a significantly better fit to the data and in only 6 out of 64 such tests was the simple linear regression found to be as good.

The efficiency of the conversion of food to body tissue is estimated as

$$c = b_2 / b_1 \quad (\text{see fig. 1}).$$

Fieller (1940, 1944) derived the fiducial limits for this ratio based on the t -distribution. From these limits for c the variance can be calculated. Davies (1955) also gives an approximate formula for calculating the variance around c which generally is in close agreement with the variance derived from Fieller's limits.

The larva-adult conversion efficiency (n) estimates the proportion of the radioactivity present in the larvae after 180 minutes of feeding (L_{180}) that is retained through to the adult stage (A).

$$n = A / L_{180}$$

Since for each larval assay 2 sets of adults were raised, we can obtain a duplicate variance of the adult radioactive content for each replicate assay. The radioactive content of the larvae at 180 minutes is estimated using the regression equations. The residual regression variance in this case provides the basis for the variance estimate appropriate to the estimated value of the larval radioactive content at 180 minutes. These two variance estimates can then be used to obtain the variance of the estimated larva-adult conversion efficiency.

RESULTS

Fig. 2 depicts the four replicate biphasic linear regressions for the second instar larval assays of a representative genotype. There is good concurrence of the observed data with the predicted biphasic linear model and the linear fit is generally very good, testifying to the inherent accuracy of the assay procedures and conditions. There is also considerable variation between the four replicate

Figure 2 Four replicate time course assays of larval feeding behaviour at 53 hours of age for a representative genotype. The rate of food uptake by the larvae is represented by the slope of the first phase, while the rate of food absorption into the larval tissue is represented by the slope of the second phase. The point of intersection of the two slopes estimates the larval gut capacity.

sets of larvae in all aspects of feeding behaviour as measured by this assay which is probably due to divergence during the pre-assay incubation periods. It is not surprising, therefore, that this replication error is far greater for the 77-hour larvae than for the 53-hour larvae, both in absolute terms and relative to the assay related errors, which are based on the residual regression variances. For such primary regression parameters as the slopes and the point of intersection the variation between the replicate assays is from 20 to 500 times the size of the residual regression based errors. For derived parameters such as the conversion efficiencies the replicate error variance is only about 2 to 6 times as large as the assay derived error. In all cases the replicate error variances were used.

Table 1 shows the mean feeding rates of the larvae at 53 hours and 77 hours after oviposition (b_{53} and b_{77} respectively), together with their replicate error variances. There was significant variation between genotypes in their feeding rates at 53 hours ($\chi^2_{(7)} = 54.345$) but not at 77 hours ($\chi^2_{(7)} = 7.542$), a result that is primarily due to the large replication errors for the third instar feeding rates. The third instar feeding rate is on average about 4 times as large as the second instar feeding rate.

Table 2 shows the mean larval gut capacity at the second and third instar stages (g_{53} and g_{77} respectively). As was the case for the feeding rates,

significant genotypic variation was found for second instar larvae ($\chi^2_{(7)} = 36.867$) but not for third instars ($\chi^2_{(7)} = 7.005$). The average third instar gut capacity is about 5 times as great as that of the second instar.

Table 3 contains the estimates of mean larval conversion efficiency (c_{53} and c_{77} for the second and third instar stages respectively), recording the proportion of radioactive label taken into the gut that is retained in the body. For the 53-hour-old larvae this figure averages at 36.5 per cent, while

Table 1 Estimates of the mean larval feeding rates (b) in μg yeast/minute for second and third instar larvae (53 and 77 hours respectively). $\text{VAR}(b)$ is the variation between the estimates from the 4 replicate assays, corrected to apply to the mean values

Genotype	Feeding rates			
	53 hours		77 hours	
	b	$\text{VAR}(b)$ ($\times 10^{-4}$)	b	$\text{VAR}(b)$ ($\times 10^{-3}$)
27A	0.06808	0.51771	0.30588	0.38278
27B	0.11904	1.27190	0.36000	6.62040
27C	0.03447	0.17536	0.33492	4.28570
27D	0.11506	8.11730	0.41309	3.32180
25A	0.12425	16.19800	0.56404	26.61180
25B	0.12451	3.47120	0.64114	41.19520
25C	0.10399	2.05190	0.39208	17.38030
25D	0.24269	5.41800	0.53230	9.37980

Table 2 Estimates of mean larval gut capacity (*g*) in μg yeast as determined by the assay for second and third instar larvae (53 and 77 hours respectively). VAR (*g*) is the variation between the estimates for the 4 replicate assay, corrected to apply to the mean values

Genotype	Larval gut capacity			
	53 hours	77 hours		
<i>g</i>	VAR (<i>g</i>)	<i>g</i>	VAR (<i>g</i>)	
27A	5.61917	0.39056	20.7330	1.77087
27B	4.01248	0.41220	31.1088	106.26800
27C	2.03675	0.20464	19.2560	7.49810
27D	8.40254	2.88157	36.6112	32.61550
25A	6.80606	3.84934	33.8251	102.60300
25B	7.98160	0.92027	32.7572	106.43000
25C	9.33518	1.55809	33.4554	85.82810
25D	10.10460	1.31976	43.35760	63.4053

Table 3 Estimates of the mean larval conversion efficiency (*c*) as determined by the assay for second and third instar larvae (53 and 77 hours respectively). Each value is the proportion of the radioactive label taken into the gut that is retained by the larval tissue. VAR (*c*) is the variation between the estimates for the 4 replicate assays, corrected to apply to the mean value

Genotype	Larval conversion efficiency			
	53 hours	77 hours		
<i>c</i>	VAR (<i>c</i>)	<i>c</i>	VAR (<i>c</i>)	
	($\times 10^{-3}$)		($\times 10^{-3}$)	
27A	0.41161	6.13576	0.51527	5.0909
27B	0.29273	3.14391	0.23235	7.58160
27C	0.42997	2.84850	0.29503	0.31522
27D	0.22547	1.18940	0.24547	5.43110
25A	0.22147	1.16149	0.26335	1.72950
25B	0.39211	4.79714	0.19008	1.69350
25C	0.46503	7.95424	0.27397	4.53750
25D	0.48052	2.57713	0.18680	2.98870

for the 77-hour-old larvae this is only 27.5 per cent. For both instars there is significant variation among the eight genotypes in conversion efficiency ($\chi^2_{(7)} = 20.320$ and $\chi^2_{(7)} = 20.689$ for c_{53} and c_{77} respectively).

Table 4 shows the proportion of the total radioactive label present in the larvae after 180 minutes of feeding that is retained through to the adult state (n_{53} for second instar larvae, n_{77} for third instar larvae). There is significant variation among genotypes in both the second and third instar stages in the ability to retain radioactive label ($\chi^2_{(7)} = 15.653$ and $\chi^2_{(7)} = 19.535$ for n_{53} and n_{77} respectively). This significant variation was obtained from a very narrow range and a replicate error variance 3 to 4 times smaller than for larval conversion efficiencies, making this parameter the least variable and most consistent of the four presented

Table 4 Estimates of the mean larva to adult conversion efficiency (*n*) as determined by the assay for second and third instar larvae (53 and 77 hours respectively). Each value is the proportion of radioactive label present in the larval tissue after 180 minutes of feeding which is retained through further development into the adult tissue. VAR (*n*) is the variation between the estimates for the 4 replicate assays, corrected to apply to the mean values

Genotype	Larva to adult conversion efficiency			
	53 hours	77 hours		
<i>n</i>	VAR (<i>n</i>)	<i>n</i>	VAR (<i>n</i>)	
	($\times 10^{-4}$)		($\times 10^{-4}$)	
27A	0.36449	4.78320	0.50987	2.93377
27B	0.34912	8.27236	0.48603	5.66938
27C	0.44906	40.31050	0.49878	15.34020
27D	0.36150	24.34770	0.43240	9.26612
25A	0.33895	0.41744	0.42690	6.62570
25B	0.27736	11.16740	0.42282	6.85790
25C	0.29102	1.95702	0.38767	16.43870
25D	0.34679	5.77018	0.37665	9.25327

here. On average, about 34.7 per cent of the radioactivity ingested and absorbed by the second instar larvae can be recovered from the adults, while the corresponding figure for the third instar larvae is 44.3 per cent. This difference is readily explained. Most loss is incurred during the post-assay developmental period, which is shorter for the third instar larvae than for the second instar larvae.

The data suggest a relationship between the rate of feeding and larval gut capacity for both instars. The rate of feeding is a function of mouthpart size and retraction rate, neither of which is necessarily dependent on larval size so that a large gut capacity or larval size is probably a consequence of a high feeding rate, rather than the reverse. There also appears to be a negative relationship between the larval feeding rates and gut capacity, and the larva-adult conversion efficiency.

Correlation coefficients between second and third instar larval parameters are given in table 5(i). There is a positive correlation between the instar stages for all parameters, although only one of the coefficients is significant (that for larval gut capacity (*g*)). This implies that the second instar body size is instrumental in determining the third instar body size. Several factors may be involved in this process, including the timing of the second to third instar larval moult and the rates of larval growth in the two instar stages. These suggestions are tested with a further series of correlation coefficients (table 5(ii)), documenting the relationship between the larval feeding rates and gut capacity (or body size) at the two instar stages. Again, all correlations are positive, with the most

Table 5 Correlation coefficients for 6 df, showing some of the relationships between the estimated biological parameters. The correlation between the two larval instar stages for feeding rate (b), gut capacity (g), larval conversion efficiency (c) and larva to adult conversion efficiency (n) is shown in (i). The correlations between feeding rate (b) and gut capacity (g) for each larval stage are shown in (ii) and the correlations between larva-to-adult conversion efficiency (n) and gut capacity (g) for the same larval stages are shown in (iii). The significance of the correlation coefficients is given as $p > 0.05$ (NS); $p < 0.05$ (*); $p < 0.01$ (**). See text for details

	(i)			(ii)		(iii)	
	$r_{(53,77)}$	P		b_{53}	b_{77}	n_{53}	n_{77}
b	0.584	NS	g_{53}	0.718*	0.537NS	-0.711*	-0.545NS
g	0.820	*	g_{77}	0.907**	0.616NS	-0.892**	-0.869**
c	0.116	NS					
n	0.625	NS					

significant correlation between the second instar feeding rate and the third instar gut capacity. Those correlations which concern the relationship between larval gut capacity (g) and larva-adult conversion efficiency (n) are shown in table 5(iii). All are negative, implying that bigger larvae are likely to lose a larger percentage of their food intake during further development.

DISCUSSION

Although competitive interactions in *Drosophila* have been widely investigated very little effort has been directed towards identifying and measuring larval growth parameters in spite of their probable importance in determining the outcome of competition. Only Burnet *et al.* (1975) have attempted to obtain direct estimates of larval feeding rates with a view to explaining their competition results. The logistic models developed to predict the result of competition (de Jong, 1976; Nunney, 1983) place strong emphasis on basic parameters such as feeding rate, conversion efficiency and minimum pupation weight, without taking into account the most obvious feature of larval growth, namely the existence of three distinct instar stages (although Nunney, (1983) does include an age dependent increase in feeding rate). Here we have attempted to estimate most of the parameters required to define any growth equation and to distinguish between the instar stages. The most promising parameters for further study are feeding rates and the fraction of the food consumed that is retained to the adult stage. However, the general principles of this assay technique can be applied to any type of input-output system where both the rate and the efficiency of production must be estimated.

Significant genetic variation was found for all second instar parameters, but for only two of the

third instar parameters. It may be that these methods are not sensitive enough to detect third instar genetic variation in feeding rate and gut capacity. Previous experience (Sewell *et al.*, 1975) suggests that enough genetic variation is expressed in the third instar for selection on the rate of mouthpart retraction to be effective. Other reports stress the interplay between larval growth, medium conditioning and other aspects of environmental variation, which would obscure the genetic control of larval growth as the larvae grow older (Sang, 1950; Bakker, 1961; Robertson 1960a, 1960b, 1963). Part of the lack of significant genotypic variation in the third instar larval feeding rate and body size arises from the large and heterogeneous error variances associated with these estimates. Altapov (1929) also found large differences between two otherwise identical experiments in the timing of the change over from the second to the third instar state. Any differences between replicates in peak instar moulting times would produce variable proportions of second instar larvae after 77 hours of growth, or differences in the time already spent as third instars. The first of these would affect the replicate error variance of third instar feeding rates whereas the second would affect the replicate error variance of third instar larval body size. Third instar larvae may also depart from the central assumption that larval rates of feeding were the same for phases 1 and 2. To minimise this possibility larvae were grown on an excess of yeast to prevent them from becoming hungry. However, third instar larvae burrow extensively in the non-nutritive agar (unlike the second instar larvae which mainly inhabited the surface layer), and consequently may have been hungry at the start of the assay. Differences between replicates in the extent to which this occurred would increase the replicate error variance for third instar feeding rates. In addition, initial feeding rates

during the assay may then have been greater than the final feeding rates, causing the third instar larval conversion efficiencies to be underestimated. It does appear that characters concerned with the utilisation of resources are far less affected by random environmental fluctuations than larval feeding rates and larval body size. While the third instar feeding rate on average exceeds the second instar feeding rate by a factor of about 4, their body sizes differ on average by a factor of about 5. The second instar feeding rate is hence *greater* in relation to its body size than that of the third instar. Feeding rates are determined to some extent by the size of the mouthparts and second instar mouthparts are larger in relation to the body length than the third instar mouthparts (Altapov, 1929). These findings support the assumption of Nunney (1983) that the larval feeding rate is some exponential function of the larval body size, and that the exponent is less than one.

Although the sex ratio in *Drosophila melanogaster* generally remains uniform even under the competitive conditions of laboratory culture, suggesting an equality in competitive ability, the sexes may nonetheless differ in the individual parameters that comprise competitive ability (Bakker, 1961; Nunney, 1983). Differences may be found in the length of the larval period, mean adult weight at eclosion and minimum weight required for pupation. However, we found no evidence for any systematic difference between the sexes in their larval feeding behaviour.

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