softer patches and that the presence of eggs was coincidental rather than deliberate. This is not supported from data on wild populations which suggest that there is an effective separation between oviposition and breeding sites in at least some species (Kearney, 1979). Another explanation could have been that only the softest patches were 'discovered' during the oviposition period. This was not supported by the data shown in Figure 3, however, which were obtained from direct observation during the first eight hours of the experiment. The graph clearly shows that during this period, both species discovered most of the patches that were present. Interestingly no eggs were laid on the standard concentration of 0.02g/ml which was used to rear the stocks during the preceding year. This suggests that even though the stocks had been in the laboratory for some time, variation was still present for oviposition preferences. Whether this was linked to higher survival during the preadult stages within the softer substrate was unknown. There appears to be a need for further research on the response of wild caught stocks to general food substrates as this data suggests that there may be some room for improvement, especially from the perspective of the individual *Drosophila*. In summary, patch hardness appeared to have a profound affect on the choice of oviposition site. Both species chose a patch that was 'softer' than the standard substrate on which they were reared. This may be closely linked to fitness within wild populations or could be an artefact of adult feeding behaviour.

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Catchpole, Roger, and Bryan Shorrocks. Department of Biology, University of Leeds, Leeds, LS2 9JT. Email: r.d.j.catchpole@leeds.ac.uk An alternative method for population estimation in large scale laboratory populations of *Drosophila*.

When large laboratory populations of *Drosophila* are being studied, more traditional estimates of population size such as mark-release-recapture may not be practical, because of the sampling effort required. This is especially the case if a large number of replicates are being studied. As an alternative, the number of individuals present on a standard area can be counted to give an estimate of the total population size. This note describes a method for determining population density through the use of curvilinear functions fitted to observations that were taken at known population densities for two species of *Drosophila*: *D. melanogaster* and *D. virilis*.

Drosophila populations are commonly kept in a number of different containers in laboratories which range from half pint milk bottles, that support several hundred individuals, to large scale population microcosms that are capable of supporting many thousands of individuals. Generally, larger populations are usually kept in purpose built 'cages' such as the one shown in Figure 1. When such containers are used it is easily possible to define an area in which the number of individuals can be counted at regular intervals. The container shown in Figure 1 has two transparent inspection windows which are ideally suited to this activity.

Before any estimates of population size could be produced it was necessary to calibrate the windows using known densities of flies. Adults were removed from established cage populations of each species and placed in empty cages at densities of 10, 30, 50, 300, 600 and 1200 individuals. A sex ratio of 50:50 was maintained in order to simulate the age structure that would be present under 'normal' sampling conditions. After sorting, the flies were allowed to recover from anaesthetisation for 24 hrs before any observations were made. The numbers of individuals on the windows were subsequently recorded at 15 minute intervals, over an 8 hour period. Immediately after each observation the cages were tapped to dislodge any individuals that had settled to ensure independent counts. The cages were placed in an incubator at 20±1°C and illuminated throughout the observation period.

Initially a number of different functions were fitted to the data for each species. These were derived from the straight line, quadratic, cubic, power, exponential and logistic equations. In each species the models that provided the three best fits were the straight line ($y = b_0 + b_1x$), quadratic ($y = b_0 + b_1x + b_2x^2$) and cubic ($y = b_0 + b_1x + b_2x^2 + b_3x^3$). The adequacy of the fit was determined by comparing the adjusted coefficients of determination (R^2_{adj}) for each model which were calculated in the following manner:

$$R^2_{adj} = 1 - \frac{(n-1)(1-r^2)}{n-p}$$

where n = number of observations, p = number of parameters and r = correlation coefficient (SAS, 1990). Although both R^2 and R^2_{adj} are commonly used to describe the goodness of fit of particular models, R^2_{adj} was chosen in this case as it generally provides a better estimate of a models adequacy by adjusting for the number of parameters that are present (SAS, 1990). Using R^2 as a selection criterion can be misleading because as the number of parameters increases, so the fit of the model will improve. Unless a perfect straight line is present, a simple model will always have a lower value in comparison to a higher order model such as a quadratic or cubic. The values for the models with the three highest R^2_{adj} coefficients are shown in the legend of Figures 2 and 3. The best linear estimate of population density was chosen by applying a stepwise multiple regression to successive powers of the independent variable. This procedure sequentially adds and removes additional variables to determine whether changes in the model significantly reduce the residual variance that is present (Sokal and Rohlf, 1995). The significance level for retaining a variable was adjusted to 0.017 from an initial probability of 0.05 using the following formula where p = number of parameters and a = probability level (Sokal and Rohlf, 1995):

Table 1. Model determination for *D. melanogaster*. Stepwise multiple regression of successive polynomial terms.

variables in the equation			
variable	b	t	p
b_1 x (straight line)	0.161	74.017	<0.001
b_0 (constant)	0.492	12.209	<0.001
variables not in the equation			
b_2x^2 (quadratic)	n/a	1.905	0.057
b_3x^3 (cubic)	n/a	1.551	0.122

Table 2. Model determination for *D. virilis*. Stepwise multiple regression of successive polynomial terms.

variables in the equation			
variable	b	t	p
b_2x^2 (quadratic)	0.006	52.030	<0.001
b_0 (constant)	1.171	18.904	<0.001
variables not in the equation			
b_1 x (straight line)	n/a	-0.007	0.879
b_3x^3 (cubic)	n/a	-0.114	0.019

$$1-(1-\alpha)^{\frac{1}{p}}$$

The results for this analysis are shown in Tables 1 and 2 which indicate that the best fit for *D. melanogaster* was the straight line while the quadratic provided a better fit for *D. virilis*. The models gave the following transformed values for the different parameters:

D. melanogaster (straight line fit $y = b_0 + b_1x$)
$y = 0.492 + 0.161\,x$

D. virilis (quadratic fit $y = b_0 + b_1x + b_2x^2$)
$y = 1.189 + -0.003\,x + 0.006\,x^2$

In order to estimate the number of individuals that were present, the equations for each model had to be re-arranged and solved for x. Simply swapping the variables in the analysis would have violated one of the basic assumptions of model I regression; that the independent variable is measured without error and is a 'fixed' variable (Sokal and Rohlf, 1995). In consequence the equations were re-arranged in the following way so that the actual numbers that were present in the cages could be estimated from the observed values:

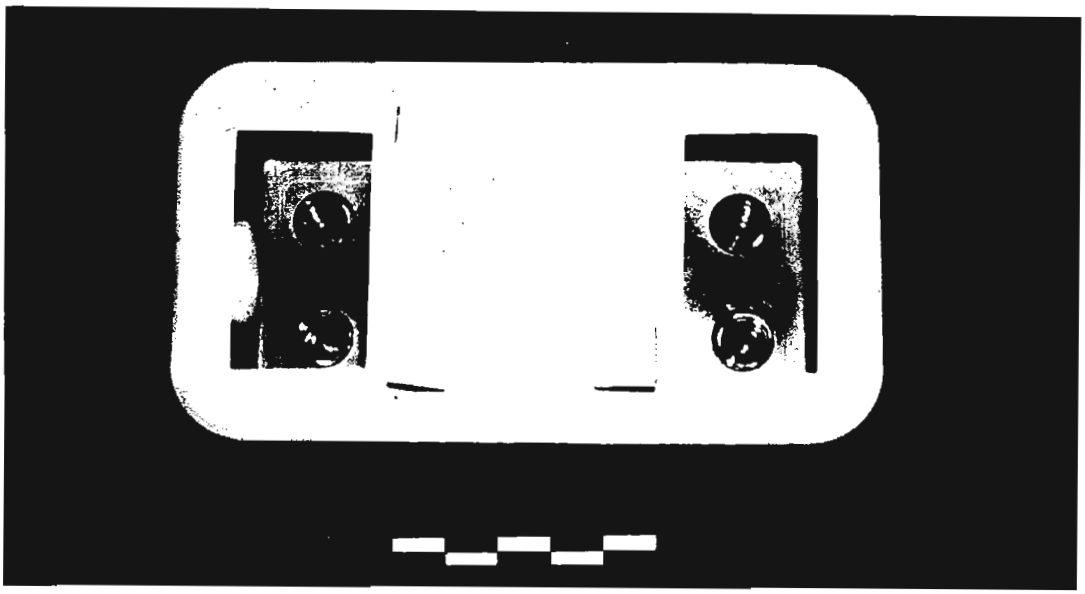

Figure 1. Polythene population cage with 200 mm scale bar.

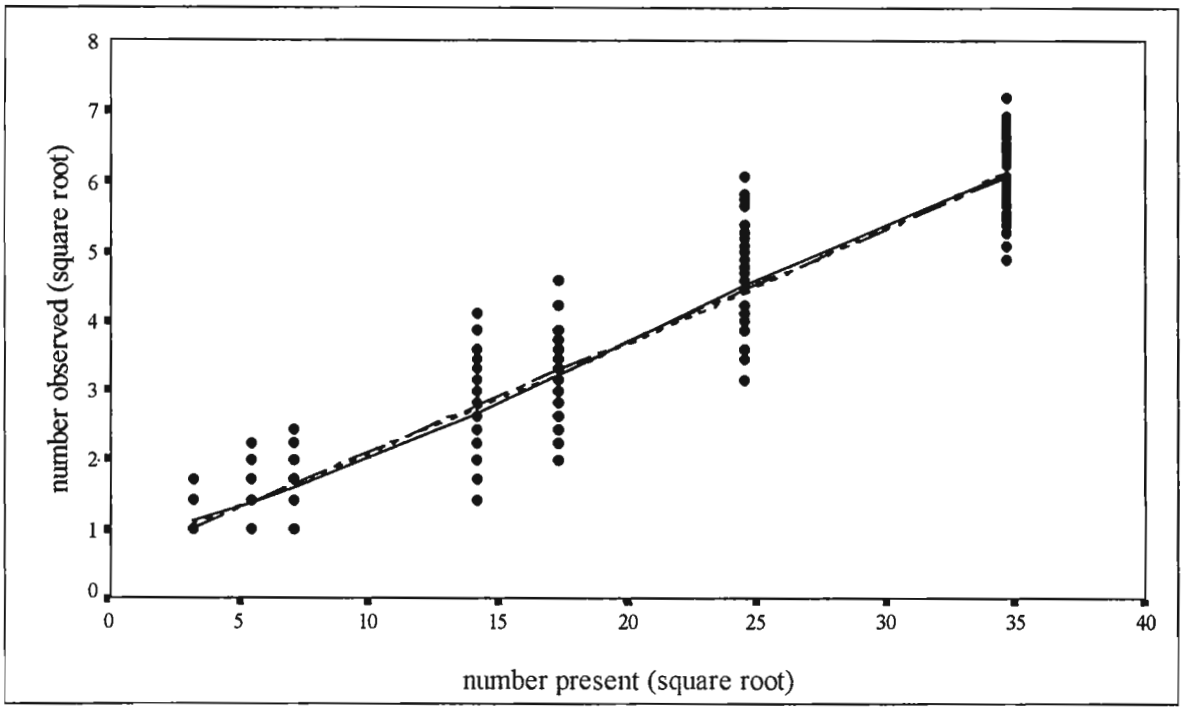

Figure 2. Curve estimation for *D. melanogaster* populations. Solid line represents the cubic fit ($R^2 = 0.931$), dotted line the quadratic fit ($R^2 = 0.929$) and the long dotted line the linear fit ($R^2 = 0.929$).

straight line ($y = b_0 + b_1x$) $$x = \frac{y - b_1}{b_0}$$

quadratic ($y = b_0 + b_1x + b_2x^2$) $$x = \frac{-b_1 \pm \sqrt{b_1^2 - 4(b_0 - y)b_2}}{2(b_0 - y)}$$

There were very few qualitative differences between the three models that were fitted to the data for *D. melanogaster*. The analysis clearly indicated that the straight line was the most suitable model and that the addition of extra terms had no effect on the amount of residual variance that was present. The data for *D. virilis*, however, was more complex and a higher order model was more suitable. Although the cubic model was also significant, the quadratic model had the highest level of significance and, qualitatively, also gave the best fit, see Table 2 for details. The data for *D. virilis* showed that at low densities the window counts failed to consistently detect flies up to a density of about 200 individuals. This meant that for some of the counts, no individuals were observed when they were actually present in the cage. Bearing this in mind, the straight line gave a qualitatively better estimate for populations at lower densities but was less adequate at moderate densities, see Figure 3 for details.

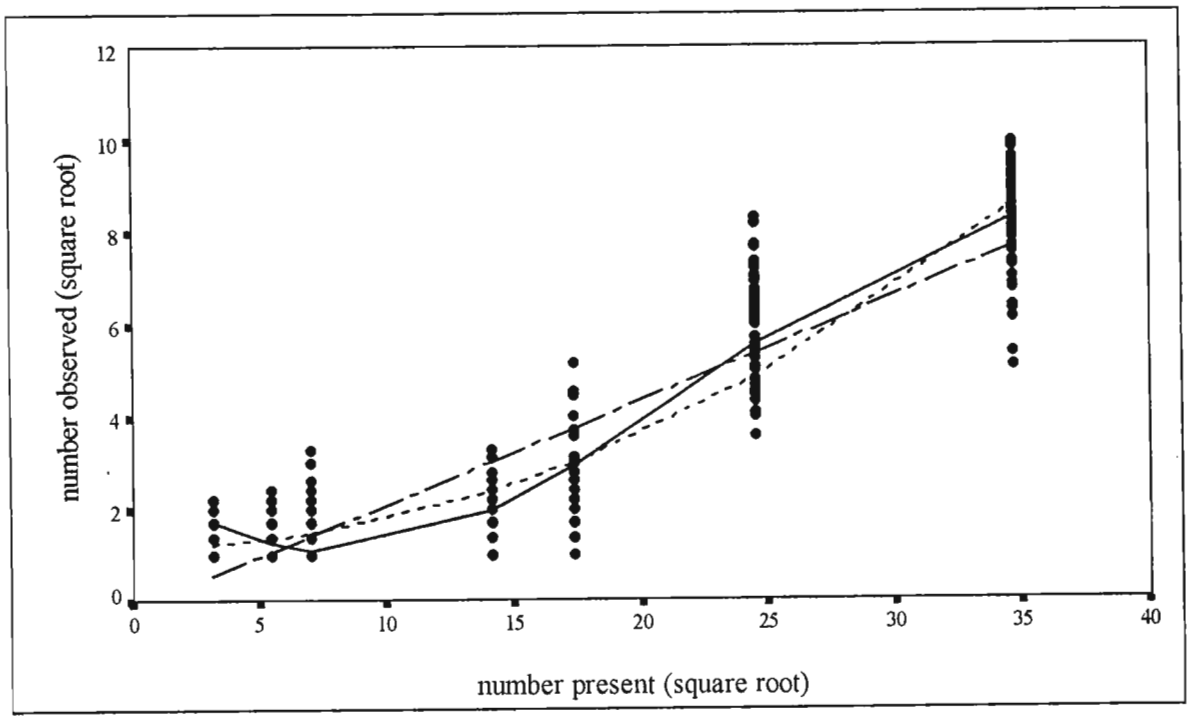

Figure 3. Curve estimation for *D. virilis* populations Solid line represents the cubic fit ($R^2 = 0.889$), dotted line the quadratic fit ($R^2 = 0.866$) and the long dotted line the linear fit ($R^2 = 0.813$).

After the most suitable models were identified, they were then used to estimate changes in population density for 40 replicate populations during the course of a long term study. Sampling all these populations took approximately one hour each week in contrast to the considerably greater length of time that would have been required even for the most basic mark-release-recapture estimate. The method provides a quick and economic way of measuring changes in population density within large cage environments.

References: SAS, 1990, *SAS/STAT User's Guide: Volume 2 (Version 6, Fourth Edition)*. SAS Institute Inc.; Sokal, R.R., and F.J. Rohlf 1995, *Biometry*. W.H. Freeman.