Catchpole, Roger. Department of Biology, University of Leeds, Leeds, LS2 9JT. Email: r.d.j.catchpole@leeds.ac.uk The effect of substrate hardness on the oviposition behaviour in two species of *Drosophila*.

Drosophila feed on microorganisms that grow on a range of different substrates (Sang, 1950; Begon, 1982). The type of substrate is commonly used to identify the 'guild' to which a particular species might belong, *e.g.* fungal, fruit and sap. Rearing *Drosophila* under laboratory conditions can be done without the use of these substrates if the basic nutritional requirements of both the adult and larval life stages are met. Several authors have examined the exact nutritional requirements for growth in artificial culture (Begg and Robertson, 1950; Royes and Robertson, 1964; Sang 1978), although in practice a more pragmatic approach is usually taken (*e.g.* Ashburner and Thompson, 1978; Shorrocks, 1971). Most general food media usually contain varying proportions of sugar, agar, yeast, cereal and a mould inhibitor. Of particular interest to the current study are the effects that changes in the concentration of agar have on the oviposition behaviour of two species of *Drosophila*; *D. melanogaster* and *D. virilis*. Agar is an important element in a recipe because it provides a stable substrate in which the various ingredients can be evenly fixed and in which the larvae can burrow and feed. It also provides the adult with a stable oviposition substrate into which eggs can be inserted without sinking. This is important as the egg filaments must remain above the substrate surface to avoid anoxia.

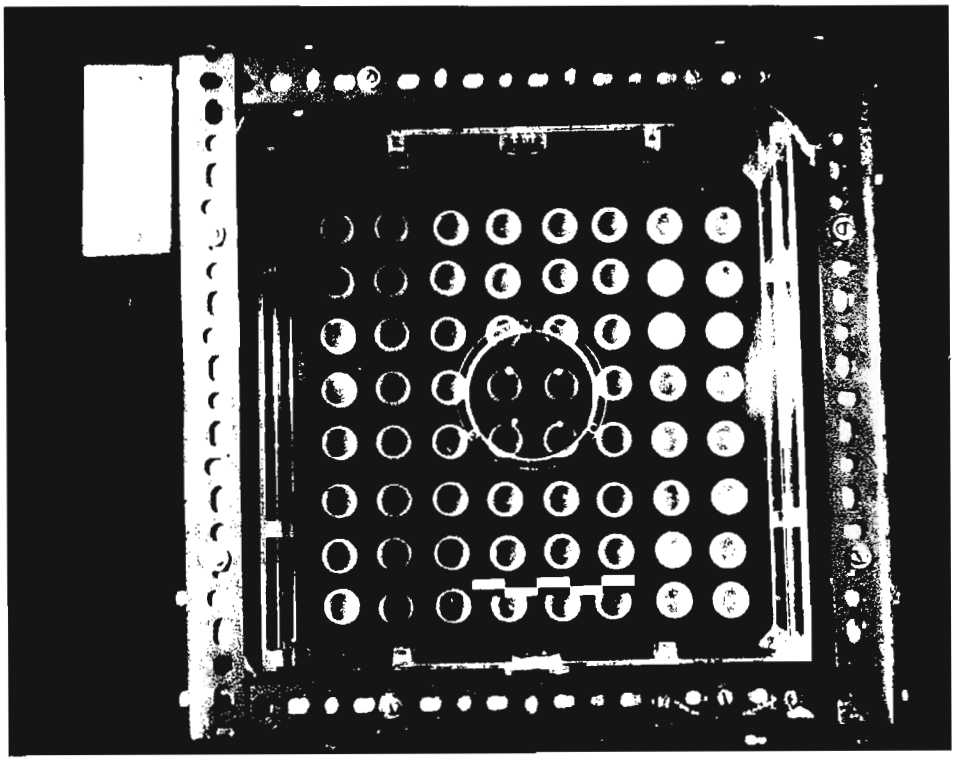

Figure 1. Perspex observation chamber with 200 mm scale bar and lighting unit.

In order to test the effect of different agar concentrations, a small amount of standard food medium (Shorrocks 1971) was placed in an equidistant array of nylon test tube caps, each of which contained 5ml of food. Six replicate patches of four different concentrations of agar (0.01g/ml, 0.02g/ml, 0.03g/ml and 0.04g/ml) were randomly arranged in a 4 × 6 array in the centre of the chamber as shown in Figure 1. Forty gravid females and 3 mature males were selected from stock populations of each species, sorted and placed in the observation chamber. When *D. melanogaster* was examined, individuals were only kept in the chamber for 24 hrs, whereas *D. virilis* needed a further 24 hrs before any appreciable oviposition had occurred. Each chamber was uniformly lit and kept in a constant temperature room for the duration of the experiment at a temperature of 20 ± 1°C. The light source was connected to a time switch which was set on a 12 hr light/dark cycle in order to avoid any interference with the flies' circadian oviposition rhythm. After exposure, the patches were removed and examined under a low power binocular microscope for the presence of eggs. This procedure was repeated three times to give three replicated experiments.

The results in Figure 2 clearly show that there were considerable differences in oviposition preference for food substrates of different hardness even when the concentration of agar changed by as little as 0.01g/ml. The greater number of eggs present on patches containing the lowest concentration of agar is of considerable interest as it may be indicating an active preference of oviposition site. An alternative interpretation could be that the females simply fed more often on

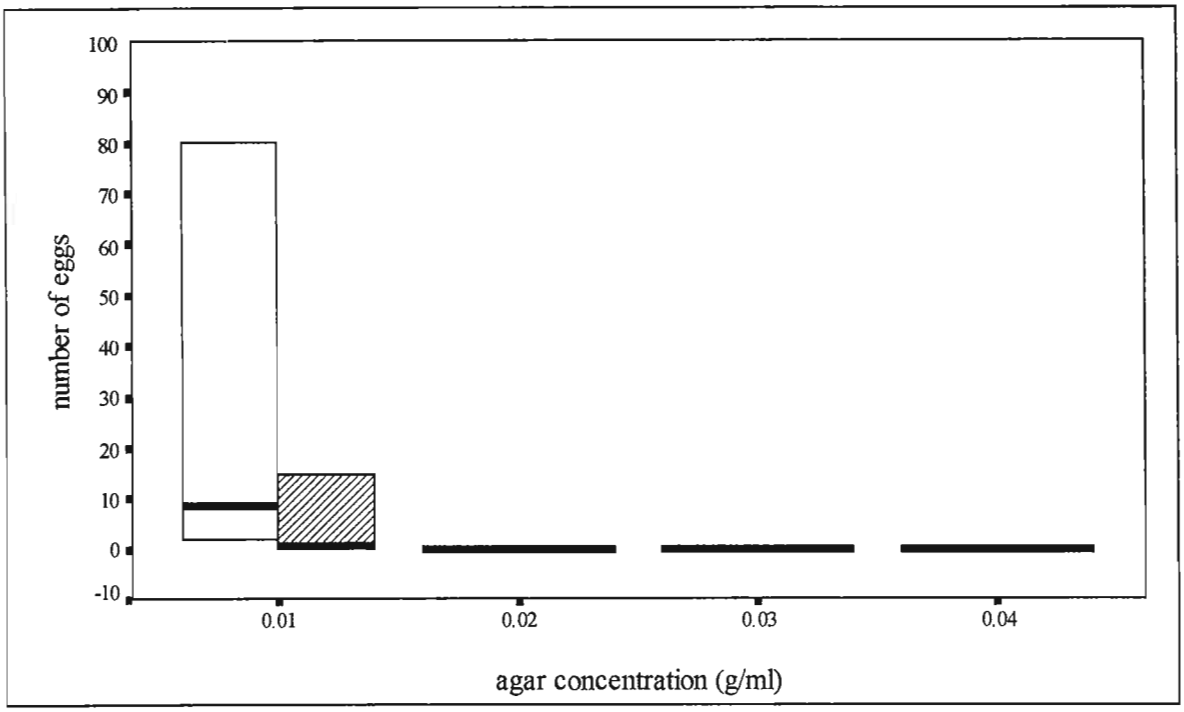

Figure 2. Oviposition preference in four different concentrations of agar. Graph shows median values and the interquartile range. Hatched bar shows data for *D. virilis* and the clear bar shows data for *D. melanogaster*.

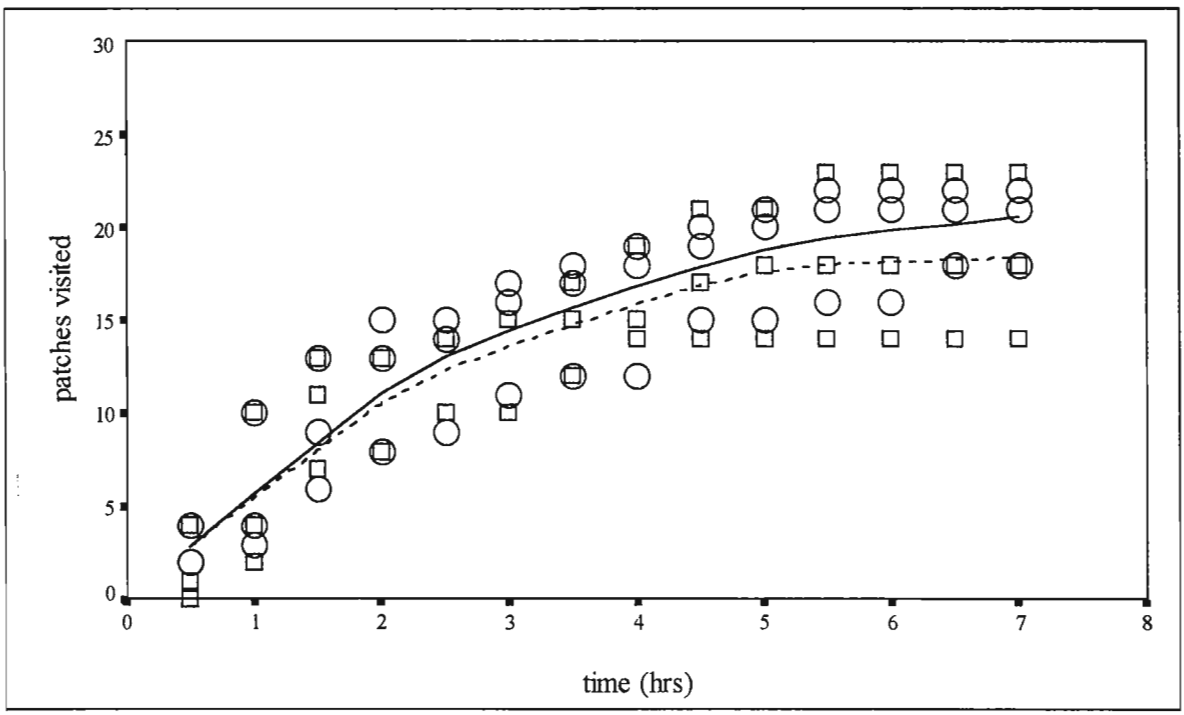

Figure 3. Cumulative number of patches visited during observation period. Dotted line and squares show *D. virilis* data while the solid line and circles show the *D. melanogaster* data.

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.

References: Ashburner, M., and J.N. Thompson, jr. 1978, The laboratory culture of *Drosophila*. In: *The Genetics and Biology of* Drosophila 2a (ed. M. Ashburner and T.R.F. Wright) pp. 1-81. Academic Press; Begg, M., and F.W. Robertson 1950, J. Exper. Biol. 26: 380-387; Begon, M., 1982, Yeasts and *Drosophila*. In: *The Genetics and Biology of* Drosophila 3b (ed. M. Ashburner, H.L. Carson and J.N. Thompson, jr.) p. 345-384. Academic Press; Kearney, J.N., 1979, The breeding site ecology of three species of woodland *Drosophila* (Drosophilidae). *PhD Thesis*, University of Leeds; Royes, W.V., and F.W. Robertson 1964, J. Exper. Biol. 156: 105-136; Sang, J.H., 1950, Biol. Rev. 25: 188-219; Sang, J.H., 1978, The nutritional requirements of *Drosophila*. In: *The Genetics and Biology of* Drosophila 2a (ed. M. Ashburner and T.R.F. Wright) pp. 159-190. Academic Press; Shorrocks, B., 1971, Dros. Inf. Serv. 46:149.

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: