Collins, M.F., J.K. Hewitt and J.F. Gogarty.
University of Birmingham, Birmingham,
England. Time-sampling courtship behavior
in **Drosophila melanogaster.**

Biometrical genetic analysis requires scores on many individuals to provide adequate data for statistical analysis and parameter estimation. To measure the courtship behavior of individual files continuously over long periods is therefore impractical, and so procedures are needed which allow behavior to be time-sampled while yielding scores that reflect reliably the behavior under observation.

A plastic mating wheel of diameter 10cm with ten separate observation chambers (Hotta & Benzer 1976) each of area 0.64^2 and volume $0.38cm^3$ was used to observe courtship behavior. The flies were first lightly anaesthetised with CO_2 to facilitate transfer to the wheel using a soft paint brush. The wheel itself is such that it consists of a plastic disc for the females with ten holes which are initially out of register with those for the males. Two further outer discs with one hole each allow flies to be placed in either male or female chambers. The flies are allowed to accommodate to the wheel for five minutes. The two inner discs are then rotated to bring the male and female chambers into register and then the courtship of flies is noted. After each test the wheels are dismantled and washed thoroughly.

For all courtship tests the male behavior was categorised into four easily identifiable components: orientation, wing vibration, licking and attempted copulation, and copulation itself. Licking and attempted copulation were scored together as one category as licking is a behavior of short duration which would not be detected reliably by a time-sampling procedure and moreover it is known that licking is usually followed by attempted copulation. A score was given to a pair of flies every 30 seconds. The male courtship behavior was recorded over a ten minute period giving each male 20 courtship scores.

A courtship index (CI) is defined for a given male as the proportion of observation periods that showed any courtship behavior. Four further courtship indexes are defined for each behavioral element as the proportion of observation periods that showed any orientation (OI), wing vibration (WVI), licking and attempted copulation (LACI), and copulation itself (COPI). These four indices sum to the total courtship index. The copulation index should of course be a good indicator of mating speed.

An experiment was performed to test the validity of the time-sampling procedure described above. The courtship behavior of single pairs of flies in one chamber of a wheel was video-recorded for 10 minutes. The behavior was decoded using a computer as an event recorder. The continuous observation (CO) and
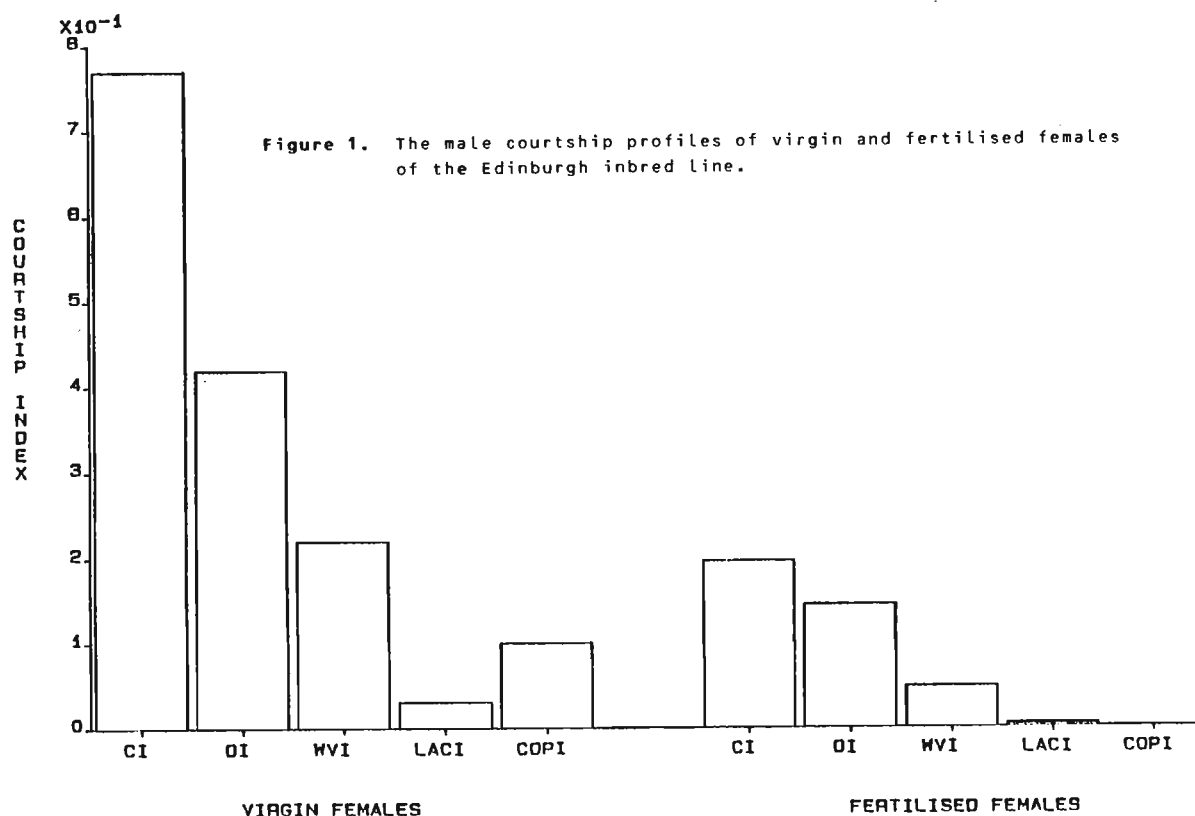

Figure 1. The male courtship profiles of virgin and fertilised females of the Edinburgh inbred line.

Table 1. Group means and between individual correlations for continously observed and time-sampled courtship behavior.

Behavior	Mean proportions of observations in each category of behavior (N=80)				Correlations across individuals[*]
	Continuous		Time-sampled		
	Mean	S.E.	Mean	S.E.	
Orientation (OI)	0.392	0.027	0.381	0.024	0.94
Wing Vibration (WVI)	0.113	0.013	0.114	0.015	0.90
Licking & attempted copulation (LACI)	0.019	0.002	0.028	0.004	0.51
Copulation (COPI)	0.248	0.023	0.250	0.022	0.99
Overall courtship (CI)	0.772	0.028	0.773	0.028	0.96

[*]Correlations were computed within each inbred line (N=20 per genotype) and pooled across inbred lines. All are significant at the 1% level.

the derived time-sampling (TS) scores were thus obtained from viewing the videotape once. The courtship behavior of twenty pairs of three day old flies of each of four inbred lines of **D.melanogaster** was recorded. Analysis of this data is presented in Table 1.

Highly significant correlations were found between time-sampled and continuously observed courtship. As would be expected, the behavior which is the least reliably time-sampled is licking and attempted copulation, the behavior which has the least total duration and occurs in the shortest bouts. It should also be noted that the very close agreement between the mean time-sampled proportion of observations spent in a particular courtship element and the mean proportion of time spent in a particular courtship phase for the continuously observed data would, of course, be expected for a reliable measure averaged over 80 pairs of flies. Nevertheless it is strong evidence for the reliability and validity of the time-sampling technique. We can conclude that time-sampling gives good agreement with continuous observation for proportional time spent in particular categories of behavior, except where they are infrequent or of short duration. The courtship index scores have been subjected to tests for skewness and kurtosis and no significant departures from a normal distribution were found and hence the data require no transformation.

A further experiment was designed to test this time-sampling procedure. Could the time-sampling technique reliably detect the often cited effect that virgin males court fertilised females less vigourously than virgin females (Connolly & Cook 1973)? The male courtship behavior of fifty virgin and fifty fertilised females of one inbred strain was time-sampled for ten minutes. Figure 1 shows the male courtship profiles of these virgin and fertilised females. The results clearly show a marked reduction in the male courtship of fertilised females for each courtship element. Not surprisingly no males copulated with a fertilised female within the ten minute observation period and furthermore well over 75% of the overall courtship of the fertilised females was spent in orientation with little progression to the more important courtship elements. The method is therefore sensitive to the differences in courtship intensity caused by exposure to virgin or mated females.

The economy of the method makes courtship amenable to the detailed analyses of biometrical genetics (see Collins & Hewitt 1984). Further application of the method should permit analysis of the role of genotype-environment interaction in mating behavior. It is also hoped that the method should be useful in the screening for further mutants of courtship behavior and possibly in the study of the effects of drugs on courtship behavior. A more detailed presentation of this work has been submitted to Behavior Genetics.

References: Collins, M.F. & J.K. Hewitt 1984, Heredity 53:321-337; Connolly, K. & R. Cook 1973, Behav. 64: 142-166; Hotta, Y. & S. Benzer 1976, P.N.A.S. 73: 4154-4158.

Cook, J.L. and D.T. Kuhn. University of Central Florida, Orlando, Florida USNA. Spatial distribution of tuh abnormalities.

Transformations of the eye-antenna to posterior abdominal tergites and genitalia appear in the tumorous-head strain (tuh-lh; tuh-3). A casual analysis would lead one to assume that the distribution patterns are random. The objective of this communication is to show that the defects seem to respect at least some of the developmental restrictions shown for the eye-antenna (Baker 1978; Campos-Ortega & Waitz 1978).