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

Any examination of the literature associated with the measurement of sexual isolation, as well as the general courtship behaviour of *Drosophila,* shows that individuals of a range of ages are commonly used in different studies on the same species (*e.g.,* Stalker, 1942; Spieth, 1951). Both the receptivity of the female (Manning, 1959) and the willingness of the male to initiate courtship (Spieth, 1974) has been shown to be age dependent. In view of this, it is important to determine when maturity has been reached before any study of courtship behaviour is undertaken. Differences in temperature, diet and genotype will all lead to different rates of development that make any definitive estimate of when a particular species reaches maturity problematic. In spite of this fact many studies show no evidence of any direct measurement (*e.g.,* Spieth, 1951; Bastock and Manning, 1955; Bastock, 1956; Cowling and Burnet, 1981; Cobb *et al.,* 1986; Crossley *et al.,* 1995).

The differences that can be present, both within and between species, are well illustrated in the following data that were collected as part of a larger study on the effects of founder events and selection on isolation within two species; *D. melanogaster* and *D. virilis*. As a large number of assortative mating tests had to be carried out, it was important to determine the age at which each species became sexually mature and therefore receptive to courtship. Newly emerged individuals of each species were collected at six hour intervals over a number of days and stored individually in glass vials on standard food medium (Shorrocks, 1971). These virgins were then used in single pair mating tests to determine the maturity threshold for each species. The latency to key stages in the mating sequence were recorded in order to determine the relationship between age and sexual maturity in both males and females. The measurements consisted of latency to tarsal contact where the male first makes deliberate physical contact with the female, latency to the first wing vibration where the male first begins actively to court the female and latency to copulation where the male mounts the female and intromission occurs. All observations were carried out at a constant temperature ($21 \pm 2^{\circ}C$) and in uniform lighting conditions. Five pairs were observed for each time interval.

Analysis of the effect of age on the courtship behaviour of *D. melanogaster* showed that there was no significant relationship for any of the key behaviours that were measured between 2-15 days, as may be seen from Table 1 and Figures 1-3. Only data for adults aged between 2-15 days were used, as sample sizes were not constant after 15 days and

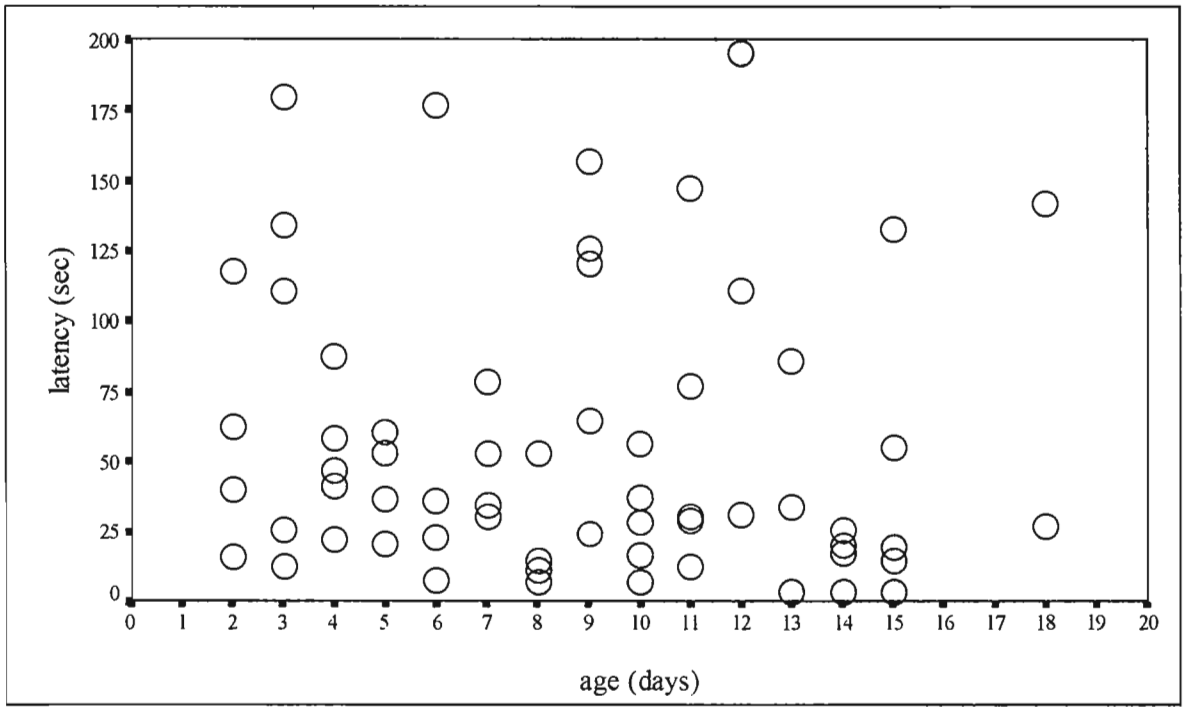


Figure 1. Age and latency to tarsal contact. Graph shows latency to tarsal contact in virgin males collected from a single population.

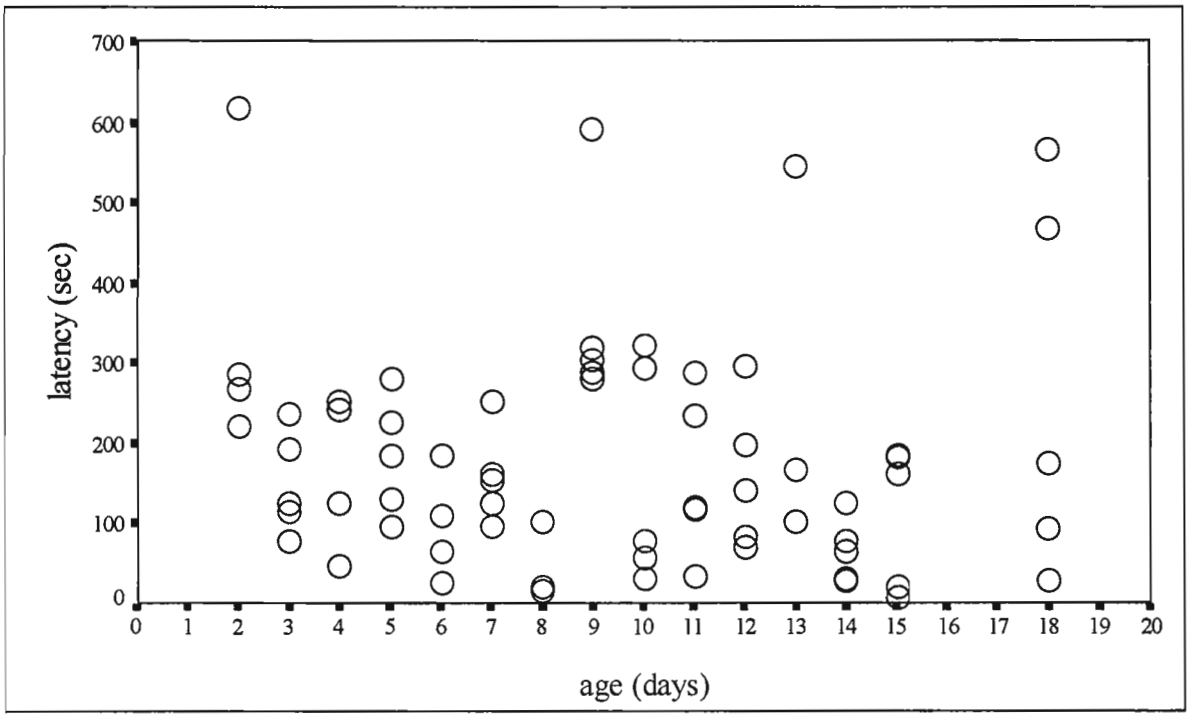


Figure 2. Age and latency to wing vibration. Graph shows latency to wing vibration in virgin males collected from a single population.

Table 1. Maturation and courtship behaviour. This table shows the results for a model I regression between age (2-15 days) and latency to each key behaviour.

| Behaviour | b | S.E. | t | p |
|---|---|---|---|---|
| tarsal contact | 0.078 | 0.168 | 0.465 | 0.645 |
| wing vibration | 0.085 | 0.164 | 0.520 | 0.605 |
| copulation | 0.190 | 0.194 | 0.979 | 0.332 |

Table 2. Maturation and courtship behaviour. This table shows the results for a least squares regression which was applied to a range of different ages between 12-17 days.

| Behaviour | b | S.E. | t | p |
|---|---|---|---|---|
| tarsal contact | - 1.202 | 0.780 | - 1.540 | 0.139 |
| wing vibration | - 1.733 | 1.059 | - 1.636 | 0.117 |
| copulation | - 1.090 | 1.222 | -0.892 | 0.382 |

were non-existent before 2 days. This avoided any bias that might result from the restricted sampling of the widely dispersed data. It was clear that sexual maturity developed within two days of emergence in both males and females and did not show any decline over the period that was examined. The results were generally consistent with other studies which have shown the rapid development of sexual maturity in this species over a range of conditions (Manning, 1959; Long *et al.*, 1980; Spieth, 1952, 1958). The period over which active mating occurred was, however, longer than the 12 days reported by Spiess (1970).

Analysis of the effect of age on the courtship behaviour of *D. virilis* showed that there was no significant relationship for any of the key behaviours that were measured between 12-17 days, as may be seen from Table 2 and Figures 4-6. There was, however, a long phase of juvenile development during which time no courtship or mating occurred, see Figures 5-6. There appeared to be a clear and parallel maturation period in both males and females up until nine days after emergence. This pattern was not apparent in the data for tarsal contact, although the variation between individual observations was higher during the initial period, see Figure 5. The single points at 900 seconds in Figures 5 and 6 represent five measurements in which the associated behaviour did not occur. They were scored at 900 as this was

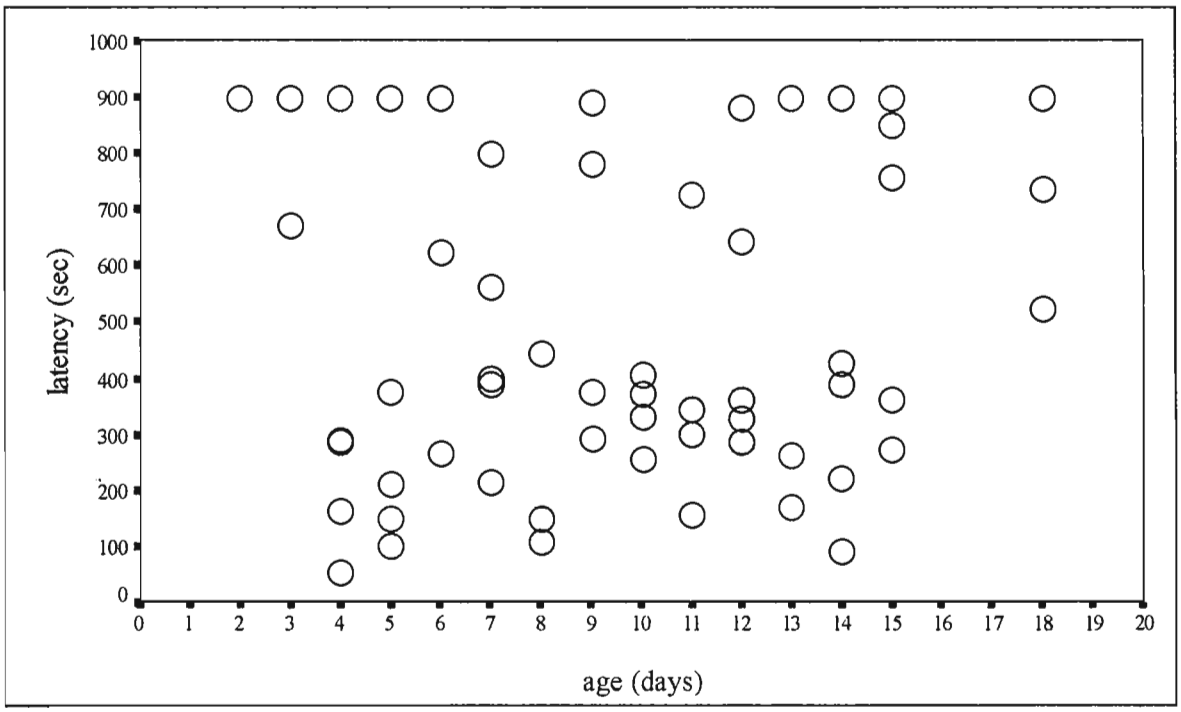


Figure 3. Age and latency to copulation. Graph shows latency to wing vibration in virgin males collected from a single population.

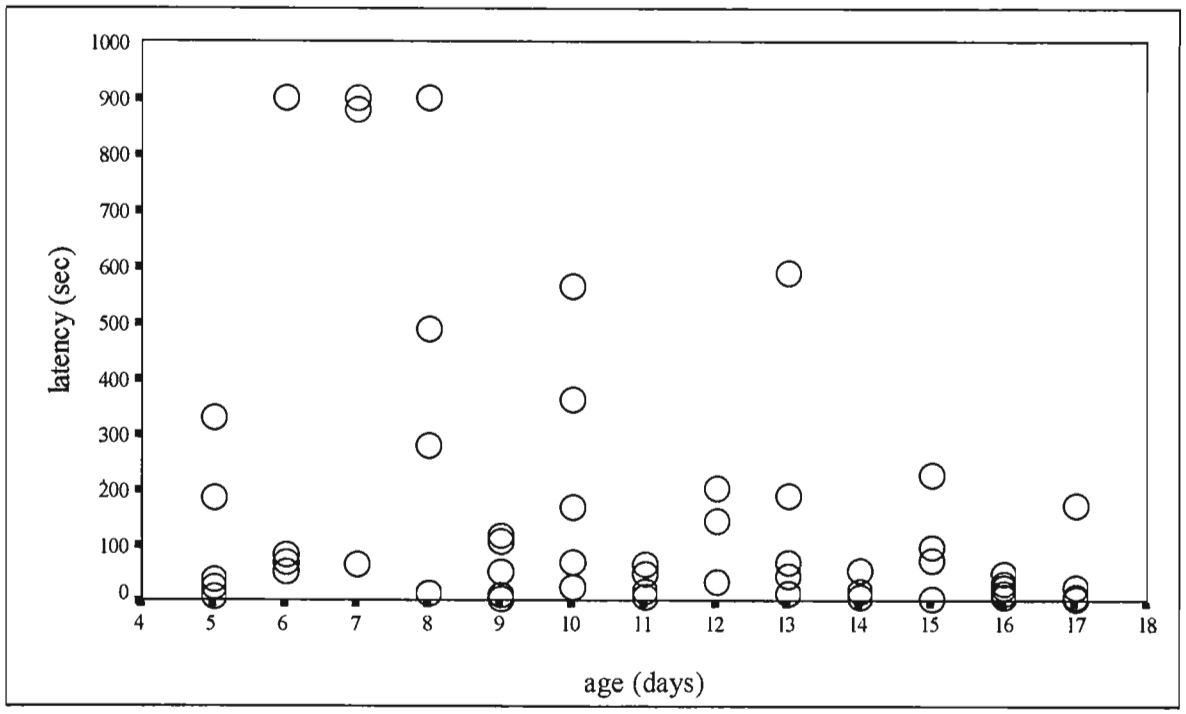


Figure 4. Age and latency to tarsal contact. Graph shows latency to tarsal contact in virgin males collected from a single population.

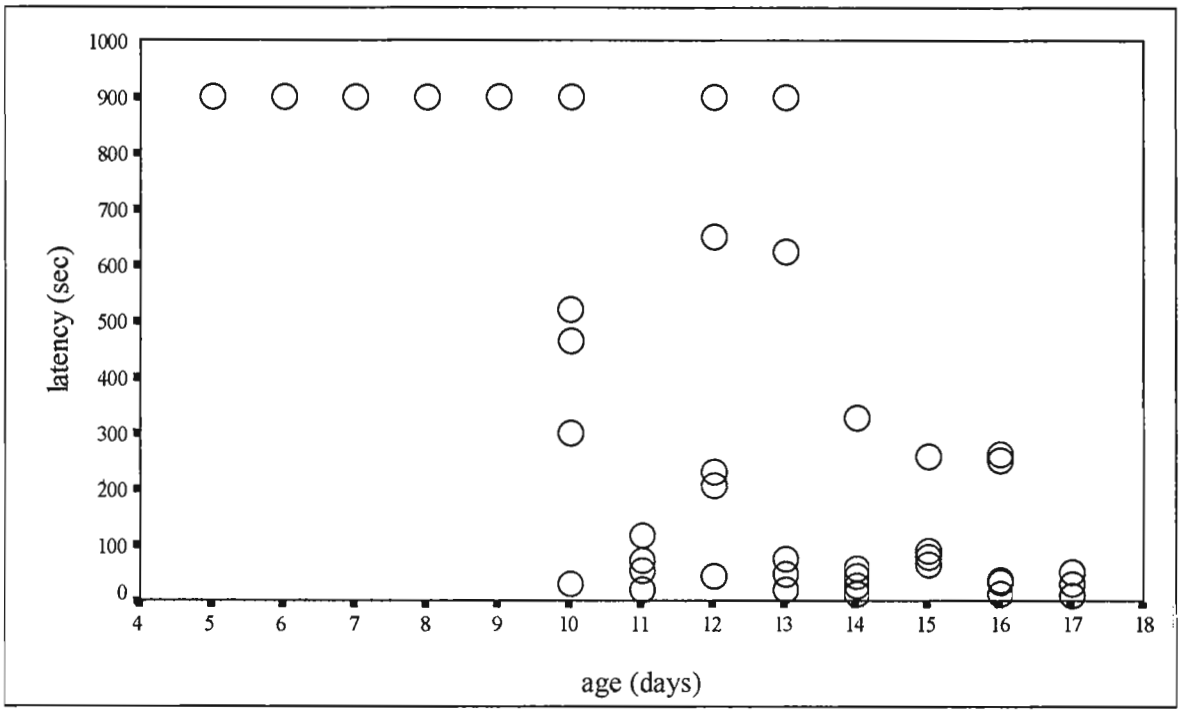


Figure 5. Age and latency to wing vibration. Graph shows latency to wing vibration in virgin males collected from a single population.

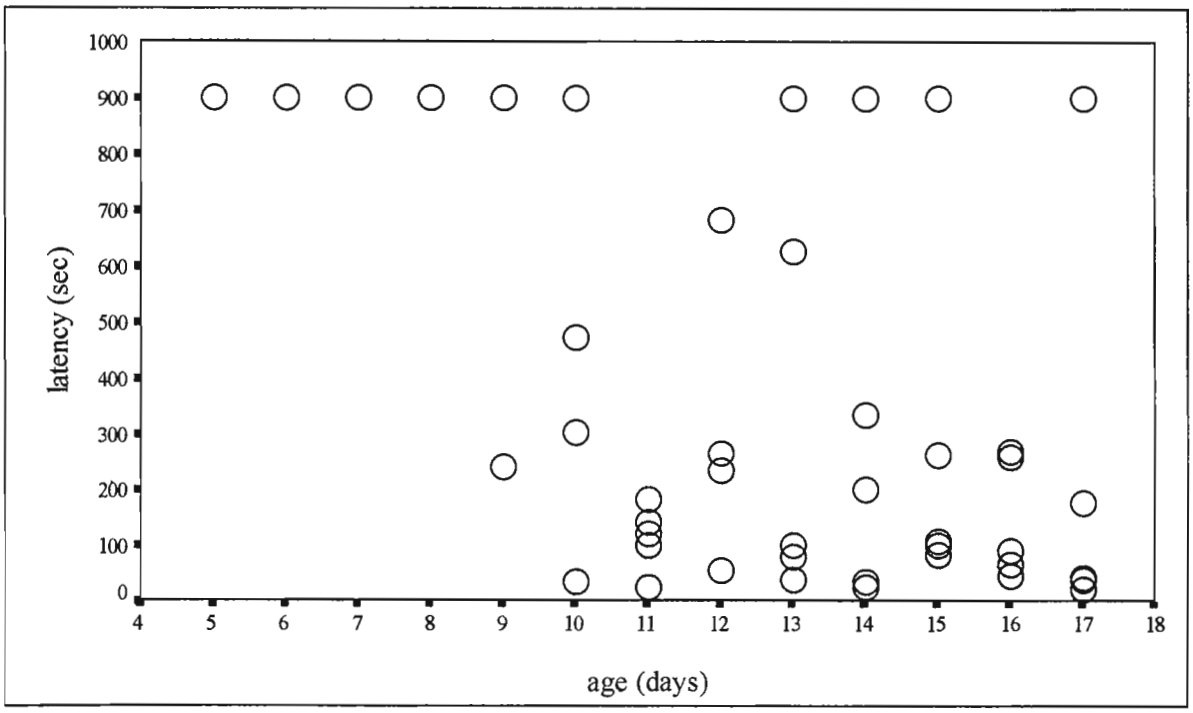


Figure 6. Age and latency to copulation. Graph shows latency to copulation in virgin females collected from a single population.

the end of the observation period; none of the behaviours of either species actually occurred at this time; therefore, all such values indicate a failure to observe any change. The results were not consistent with the work of Stalker (1942) who observed that substantial mating occurred for this species after only 4 days. Other workers have been more conservative and not used individuals younger than 10 days (Spieth, 1951) or even 14 days (Hoikkala and Lumme, 1984). Generally there appears to be a need to quantify the rate at which species mature under different conditions rather than assume that an arbitrary choice will be acceptable in courtship studies. Clearly the rates of development can vary greatly even within one species as this comparison has shown.

References: Bastock, M., 1956, Evolution 10: 412-429; Bastock, M. and A. Manning 1955, Behaviour 8: 86-111; Cobb, M., B. Burnet, and K. Connolly 1986, Behaviour 97: 182-212; Cowling, D.E. and B. Burnet 1981, Animal Behaviour 29: 924-935; Crossley, S.A., H.C. Bennet-Clark, and H.T. Evert 1995, Animal Behaviour 50: 827-839; Hoikkala, A. and J. Lumme 1984, Behavior Genetics 14: 257-268; Long, C.E., T.A. Markow, and P. Yaeger 1980, Behavior Genetics 10: 163-170; Manning, A., 1959, Behaviour 15: 123-145; Shorrocks, B., 1971, Dros. Inf. Serv. 46: 149; Spiess, E.B., 1970, Mating propensity and its genetic basis in *Drosophila*. In: *Essays in Evolution and Genetics in Honour of Theodosius Dobzhansky* (ed. M.K. Hecht and W.C. Steere), pp. 315-379. Wiley; Spieth, H.T., 1951, Behaviour 3: 105-145; Spieth, H.T., 1952, Bulletin of the American Museum of Natural History 99: 395-474; Spieth, H.T., 1958, Behaviour and isolating mechanisms. In: *Behavior and Evolution* (ed. A. Roe and G.G. Simpson), pp. 363-389. Yale University Press; Spieth, H.T., 1974, Annual Review of Entomology 19: 385-405; Stalker, H.D., 1942, Genetics 27: 238-257.

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Identification of genotype and its relationship with map gene pattern in a population of Korean *Drosophila melanogaster*.

In *Drosophila melanogaster*, the structural gene of alpha-amylase (E.C. 3.2.1.1; α-1,4-glucan glucanohydrolase) encoding a monomeric enzyme (54,500D) is controlled by allelic, codominant and duplicated genes located near site 78 of the second chromosome. In natural populations, eight variants of the amylase genotype have been reported (Lindsley and Zimm, 1992), and two regulatory factors in *Amy* gene expression have been well identified. Of them, *mapP*, a regulatory gene, affects the tissue- and age-specific expression of the *Amy* gene in the posterior region of the adult midgut (Klarenberg *et al.*, 1986). The other factor known as the dietary glucose repression depresses the level of *Amy* activity and its product in each developmental stage (Benkel and Hickey, 1987). In our study, the genotype and frequency of the *Amy* variants of *D. melanogaster* collected from a natural population were analyzed and the expression and genetic regulation of alpha-amylase were investigated at the tissue level.

**Materials and Methods**: The flies used in our study were collected at Cheon-An city near Seoul, Korea by sweeping net. To determine genotype and frequency of each variant, polyacrylamide (7.5%) gel electrophoresis was performed. After electrophoresis, activity staining of alpha-amylase needed incubation with separating gel in 2% starch and $I_2$-KI solution. The protein content was measured by the method of Bradford (Bollag and Edelstein, 1991). The specific activity of alpha-amylase of each *Amy* genotype was determined by the method of starch-iodine and DNSA (Doane, 1969). Pattern analysis of amylase activity in midgut (map) was carried out by the method of Abraham and Doane (1978) and each pattern was determined by the method of Doane (1980).

Table 1. Protein content and specific activity of amylase from each *Amy* genotypes in a natural population *of D. melanogaster.*

| Genotype | No. of Line | Frequency (%) | Protein content[1] (μg) | Specific activity (unit/min) |
|---|---|---|---|---|
| $Amy^{1}$ | 147 | 75.00 | 12.9453 | 2.0179 |
| $Amy^{1\cdot3}$ | 33 | 16.84 | 13.7170 | 1.8629 |
| $Amy^{1\cdot2}$ | 7 | 3.57 | 13.8156 | 1.8286 |
| $Amy^{1\cdot2\cdot3}$ | 4 | 2.04 | 13.1786 | 2.0662 |
| $Amy^{1\cdot3\cdot6}$ | 4 | 2.04 | 13.5442 | 1.3989 |
| $Amy^{1\cdot6}$ | 1 | 0.51 | 12.8061 | 1.2432 |
| Total | 196 | 100.00 | 13.1226[2] | 1.9695[3] |

[1]Protein content of crude extract; [2]Average of protein contents of six genotypes; [3]Average of specific activities of six genotypes

**Results and Discussion**: Frequency, protein content and specific activity of each *Amy* genotype are shown in Table 1. It was revealed that the population analyzed in this study consisted of six *Amy* genotypes designated $Amy^{1}$, $Amy^{1\cdot2}$, $Amy^{1\cdot3}$,