Genetic factors on the second and third chromosomes responsible for the variation of amylase activity and inducibility in *Drosophila melanogaster*

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Summary

Using second- or third-chromosome substitution lines of *Drosophila melanogaster*, the genetic variation of inducibility and amylase specific activities in three media (starch, normal and glucose) were investigated. Genetic factors on both the second and third chromosomes were responsible for the variation in amylase specific activity and inducibility. In glucose medium, the genetic variance of amylase specific activity estimated for the second-chromosome substitution lines was larger than that for the third-chromosome substitution lines; however, for starch medium and inducibility, the variance was larger for the third-chromosome substitution lines. High correlations for the second-chromosome substitution lines and low correlations for the third-chromosome substitution lines were observed for amylase specific activities in different media. These results suggest that the genetic factor(s) responsible for inducibility or amylase activity variation in an induced medium such as starch should be on the third chromosome and those in the non-induced medium such as glucose should be on the second chromosome. The functional roles of the factors on the second and third chromosomes would be the repression and induction of amylase, respectively.

1. Introduction

α-Amylase (EC 3.2.1.1) hydrolyses the internal α-1,4 glycoside linkage of starch to produce maltose, glucose and α-dextrin. In *Drosophila*, expression of amylase is affected by environmental conditions; the amylase is induced when substrate starch is present (Abe, 1958; Yamazaki & Matsuo, 1983, 1984; Matsuo & Yamazaki, 1984; Inomata *et al.*, 1995) and repressed when end-product glucose is present (Hickey & Benkel, 1982; Benkel & Hickey, 1986, 1987; Inomata *et al.*, 1995). Amylase activity is also affected by the concentration of yeast (Hoorn & Scharloo, 1978). Therefore the level of amylase activity is regulated by the components and conditions of the medium food.

For many of the enzyme loci of *Drosophila melanogaster*, including G6PD, 6PGD, ADH and α-GPDH, activity variations have been studied for genetic factors that are either linked or not linked to the structural genes by using chromosome substitution lines (Laurie-Ahlberg *et al.*, 1980, 1982; Wilton *et al.*, 1982). However, the relative contributions of genetic

factors on the second and third chromosomes to *amylase activity* and *inducibility* are not known. The importance of genetic variation for regulatory factor(s) for amylase was demonstrated by analysing the relation with fitness or the selection in experimental cage populations (Yamazaki & Matsuo, 1984; Matsuo & Yamazaki, 1984).

Molecular analysis of the amylase genes in *D. melanogaster* and its related species has revealed the structure and nucleotide sequence of amylase genes and the mode of molecular evolution (Gemmill *et al.*, 1985, 1986; Levy *et al.*, 1985; Hickey & Benkel, 1990; Shibata & Yamazaki, 1995). The molecular mechanism of induction at the *Amy* locus has not been clarified, although a *trans*-acting genetic factor for amylase expression was shown (Matsuo & Yamazaki, 1986).

Therefore the amylase gene–enzyme system in *Drosophila* is a suitable system for studying the adaptation of organisms to environments at the molecular level (reviewed by Milanovic & Andjelkovic, 1993). Identification of the chromosomal location of the genetic factor(s) responsible for amylase activity and inducibility will provide useful information for the characterization of the genetic factor(s) related to

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adaptive evolution at the molecular level. In this report, the genetic factor(s) on the second and third chromosomes responsible for the variation in amylase activity and inducibility was investigated by using second- or third-chromosome substitution lines.

2. Materials and methods

(i) *Isogenic strains for the second or third chromosome*

Chromosome substitution lines of *Drosophila melanogaster* for the second or third chromosomes from natural populations (Lawrence, KS; Providence, RI; Cochrane, WI; and Raleigh, NC) on an isogenic background (Ho-R strain) were kindly supplied by Dr C. C. Laurie-Ahlberg at North Carolina State University. The second- or third-chromosome isogenic substitution lines, $(i_1/i_1; +_2/+_2; i_3/i_3)$ or $(i_1/i_1; i_2/i_2; +_3/+_3)$, were constructed with the balancer i_1/i_1; *Pm*, *CyO*; i_3/i_3 or i_1/i_1; i_2/i_2; *TM*6, *Ubx/Sb* for the second- and third-chromosome lines, respectively (Laurie-Ahlberg *et al.*, 1980). In this study, 45 second- and 32 third-chromosome substitution lines maintained on a cornmeal–molasses–sugar (normal) medium for approximately 7 years were used for analysis.

(ii) *Medium foods for* Drosophila melanogaster

Three medium foods – starch (S), normal (N) and glucose (G) – were used for the experiments. The starch medium contained 155 g of starch, 3 g of agar, 20 g of ebios and 4 ml of propionic acid per litre. The glucose medium contained 152 g of glucose, 6 g of agar, 20 g of ebios and 4 ml of propionic acid per litre. The normal medium contained 6 g of agar, 28 g of molasses, 14 g of sugar, 20 g of ebios, 110 g of cornmeal and 4 ml of propionic acid per litre.

(iii) *Activity assay for* Drosophila *amylase*

Enzyme activity for α-amylase was measured for each *D. melanogaster* strain with two replications, which were sampled from a different bottle. Therefore the error term is adequately estimated in the analysis of variance. Ten 4-day-old adult male flies were assayed for enzyme activity and protein content. The procedures for measuring amylase activity (Nelson, 1944) and protein content (Lowry *et al.*, 1951) were as described in Matsuo & Yamazaki (1984). The amount of reducing sugar groups released by amylase over 30 min was measured by the Nelson method (Nelson, 1944), and expressed as a glucose unit. Enzyme specific activity was defined as milligrams glucose per 10 min per milligram of protein. The activity assay was conducted under uniform conditions (i.e. on the same day) for all samples.

(iv) *Detection of the inducing factors for amylase* (*inducibility*)

Amylase specific activity in the induced medium such as starch was divided by that in the non-induced medium and this ratio was defined as inducibility, as indicated below (Yamazaki & Matsuo, 1983, 1984). Specific activity of the enzyme is expressed by the efficiency of the structure genes (ST) multiplied by the amount of enzyme produced (AM) as follows:

$$SP(i,k) = ST(k) \times AM(i),$$

where $SP(i,k)$ is the enzyme activity of allele k at the structure gene under environment i, $ST(k)$ indicates the efficiency of the product (enzyme) of the kth allele at the structure gene and $AM(i)$ indicates the amount of enzyme produced in environment i. Note that the efficiency of the enzyme $(ST(k))$ and the amount of enzyme produced $(AM(i))$ are determined by the amino acid (or coding) sequences and regulatory sequences or genes such as transcription factors, respectively. The effect of the structure gene can be eliminated by taking the ratio of specific activity of the same line in two different inducing environments $(SP(j,k)/SP(i,k))$ such as starch and glucose media:

$$SP(j,k)/SP(i,k) = (ST(k) \times AM(j))/(ST(k) \times AM(i))$$
$$= AM(j)/AM(i).$$

This value $SP(j)/SP(i)$ is called the 'inducibility' (Yamazaki & Matsuo, 1983). Inducibility measures the rate of increase (or decrease) of enzyme activity and does not contain any effect of the structural gene but only the effect of regulatory factors. Inducibility is obviously different from the interaction of genotype and environment. Since amylase activity is affected by the composition of media and the concentration of carbohydrates, inducibility is variable in the media used for the experiments. In this study, starch and normal foods were regarded as induced media and glucose food as a non-induced (repressed) medium because the average amylase specific activities in starch and normal media were higher than in glucose medium (Fig. 1). The amylase specific activity in normal medium food was also induced, probably because of the presence of starch in cornmeal. Even in ebios medium, which has been commonly used in many laboratories (50 g of ebios, 6 g of agar and 4 ml of propionic acid per litre), the induction of amylase activity was confirmed (Inomata *et al.*, 1995). The amylase activity in normal food may be affected by both induction and repression. However, the activities in starch and glucose foods may be affected mainly by induction and repression, respectively.

3. Results

(i) *Amylase specific activity and inducibility in the second- or third-chromosome substitution lines of* D. melanogaster

Amylase specific activity was measured for 45 second- and 32 third-chromosome substitution lines of *D. melanogaster* in three media: starch, normal and glucose. The means and standard errors of amylase specific activity in the three media are shown in Fig. 1. The mean amylase specific activities in the third-chromosome substitution lines were 0·53, 0·51 and 0·21 mg glucose/10 min per mg protein, and those in the second-chromosome substitution lines were 0·22, 0·21 and 0·15 mg glucose/10 min per mg protein in starch, normal and glucose media, respectively. For both second- and third-chromosome substitution lines, amylase specific activities in the starch medium were not significantly different from those in normal medium but were significantly higher than those in glucose medium ($P < 0.01$).

The inducibility of amylase was calculated for each measurement and averaged for the second- or third-chromosome substitution lines (Fig. 2). The average
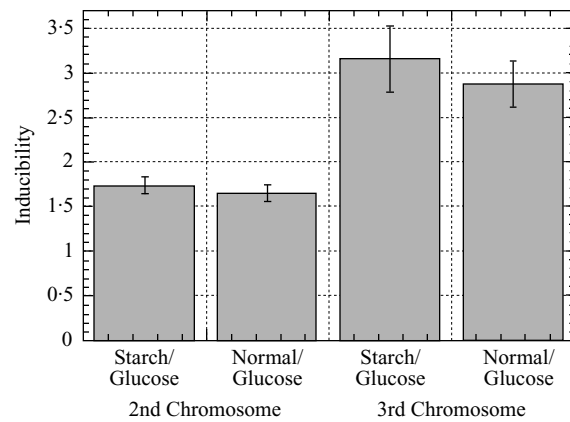
Fig. 2. Inducibilities for the second- or third-chromosome substitution lines. Average inducibilities for starch/glucose and normal/glucose are shown; bars indicate 1 standard error. Inducibility was calculated as the amylase activity in induced medium (i.e. starch or normal) divided by that in the non-induced medium (i.e. glucose).

inducibilities for starch/glucose and normal/glucose in the second-chromosome substitution lines were 1·73 and 1·64, respectively and those in the third-chromosome substitution lines were 3·15 and 2·87, respectively (Fig. 2). Inducibilities for starch/glucose and normal/glucose in the third-chromosome substitution lines were both 1·8 times higher than those in the second-chromosome substitution lines.

(ii) *Genetic variation of amylase specific activity and inducibility in the second- or third-chromosome substitution lines*

An analysis of variance (ANOVA) was conducted to investigate the genetic variation of amylase specific activity and inducibility for the second- and third-chromosome substitution lines (Table 1). All *F* values in Table 1 were statistically significant, indicating that both second and third chromosomes were responsible for the genetic variation of amylase specific activity and inducibility. Note here that the amylase structural genes are on the second chromosome (Kikkawa, 1964; Bahn, 1971), and therefore the genetic variation found in the third-chromosome substitution lines is due to the genetic factor(s) not being linked to the structural genes. The ratio of genetic variation (σ_{L}^{2}) to total variation ($\sigma_{L}^{2} + \sigma_{E}^{2}$) is also shown in Table 1. The percentage of genetic variation for amylase specific activity was 67–91 % in the second- and third-chromosome substitution lines except for the normal medium in the third-chromosome substitution lines, where the percentage was about 46 %. The percentage of genetic variation for inducibility in the third-chromosome substitution lines was 79·2 % and 79·3 % for starch/glucose and normal/glucose, respectively – higher than those in the second-chromosome substitution lines (41·5 % and 45·5 %, respectively).
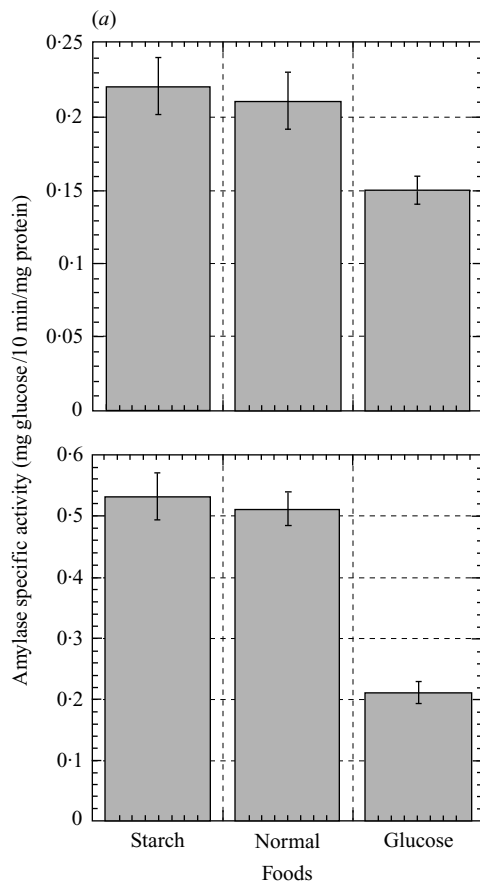

Fig. 1. Amylase specific activity for the second- or third-chromosome substitution lines in starch, normal and glucose foods. The bars indicate 1 standard error. (*a*) Average for the second-chromosome substitution lines. (*b*) Average for the third-chromosome substitution lines.

Table 1. *Analysis of variance for amylase specific activity and inducibility*

Medium food	Mean square of lines (d.f.)	Mean square of error (d.f.)	F value	Genetic variation (%)
Second chromosome				
Starch	0·0277 (42)	0·0045 (40)	6·15***	72·0
Normal	0·0292 (43)	0·0014 (44)	20·86***	90·8
Glucose	0·0302 (43)	0·0020 (44)	15·10***	87·6
Starch/glucose	0·8812 (42)	0·3643 (40)	2·42***	41·5
Normal/glucose	0·9040 (43)	0·3390 (44)	2·67***	45·5
Third chromosome				
Starch	0·0851 (30)	0·0104 (31)	8·18***	78·2
Normal	0·0529 (30)	0·0194 (31)	2·73***	46·4
Glucose	0·0169 (30)	0·0034 (27)	4·97***	66·7
Starch/glucose	7·7270 (30)	0·8953 (27)	8·63***	79·2
Normal/glucose	4·0302 (30)	0·4659 (27)	8·65***	79·3

Inducibility is expressed by the ratio of activities, starch/glucose or normal/glucose. The F value was calculated as the ratio of the mean square of lines divided by the mean square of error.
*** Significant at the 0·5% level.

Table 2. *Genetic variances and their standard errors for amylase specific activity and inducibility for second- or third-chromosome substitution lines*

Foods	Second chromosome	Third chromosome
Amylase specific activity		
Starch	0·0116 ± 0·0031	0·0374 ± 0·0111
Normal	0·0139 ± 0·0032	0·0168 ± 0·0073
Glucose	0·0302 ± 0·0033	0·0068 ± 0·0022
Inducibility		
Starch/glucose	0·2585 ± 0·1044	3·4159 ± 1·0050
Normal/difficult	0·2825 ± 0·1040	1·7822 ± 0·5241

Values are the mean ± standard error.

The relative contribution of the genetic factors on the second and third chromosomes was investigated by comparing the genetic variances. Table 2 and Figs. 3 and 4 show the estimated genetic variances and their standard errors for amylase specific activity and inducibility. The estimated genetic variance of amylase specific activity for glucose medium was significantly larger in the second-chromosome substitution lines than in the third-chromosome substitution lines (0·0302 ± 0·0033 $v.$ 0·0068 ± 0·0022, $P < 0·01$); however, the variance in starch medium was significantly larger for the third-chromosome substitution lines (0·0116 ± 0·0031 $v.$ 0·0374 ± 0·0111, $P < 0·05$). The differences in normal medium were not significant (0·0139 ± 0·0032 $v.$ 0·0168 ± 0·0073). The estimated genetic variances of inducibility for starch/glucose and normal/glucose in the third-chromosome substitution lines were 3·4159 ± 1·0050 and 1·7822 ± 0·5241, which are 13·2 and 6·3 times larger than those in the second-chromosome substitution lines (0·2585 ± 0·1044 and
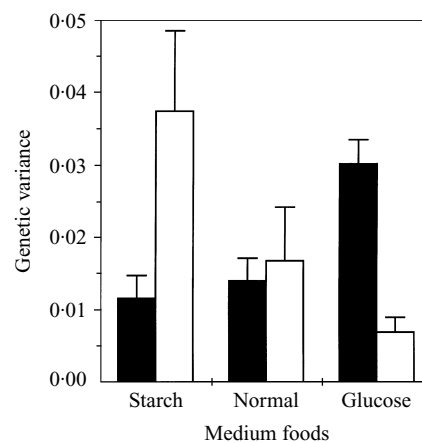

Fig. 3. Genetic variances estimated from analysis of variance for amylase specific activities in starch, normal and glucose media. The black columns show the estimated genetic variances for the second chromosome, and the open columns the estimated genetic variances for the third chromosome. The bars above the columns indicate 1 standard error.
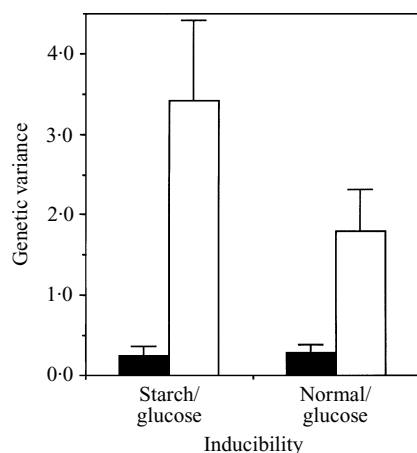
Fig. 4. Genetic variances estimated from analysis of variance for amylase inducibility in starch/glucose and normal/glucose. The black columns show the estimated genetic variances for the second chromosome, and the open columns the estimated genetic variances for the third chromosome. The bars on top of the columns indicate 1 standard error.

0.2825 ± 0.1040, respectively). The differences were both statistically significant at the 1% level.

These results suggest that genetic factors on the second chromosome affect amylase activity in a non-induced environment such as glucose, while those on the third chromosome will affect inducibility or amylase activity in an induced environment such as starch.

(iii) *Correlations among amylase specific activities in starch, normal and glucose media*

The relation between amylase specific activities in three medium foods was analysed by correlation (Fig. 5). Correlation coefficients among the amylase specific activities in three media for the second-chromosome substitution lines are shown in Fig. 5. All three correlation coefficients for the second-chromosome substitution lines were high (0.74–0.75) and statistically significant ($P < 0.005$), indicating that even in the different media the effects of genetic factor(s) on the second chromosome were similar. However, the correlation coefficients for the third-chromosome substitution lines were low (-0.06–0.46) and not statistically significant except for one case: between the specific activities in the induced media, starch and normal ($r = 0.46$, $P < 0.01$). This indicates that in different media as starch and glucose, the effects of genetic factor(s) on the third chromosome were independent.

Therefore we conclude that the factor(s) responsible for the genetic variation in the inducible environments for amylase loci must be on the third chromosome.

4. Discussion

Although the existence of genetic variation for amylase activity or inducibility in natural populations of

Drosophila melanogaster has been reported (Doane, 1969; Hoorn & Scharloo, 1980; Tejima & Ohba, 1981; Yamazaki & Matsuo, 1984; Matsuo & Yamazaki, 1984), the contributions of the second and third chromosomes to the amylase activity have not previously been analysed. Using chromosome substitution lines, the genetic factors responsible for the variation in amylase activity and inducibility could be analysed separately for second-chromosome factor(s) and third-chromosome factor(s).

The results of an analysis of variance showed that both second- and third-chromosome components are responsible for genetic variations in amylase activity and inducibility. The average activity of amylase for the second-chromosome substitution lines was lower than that for the third-chromosome substitution lines. The difference in average activity was caused by the effects of both substituted chromosomes and other genetic background. For analysing the relative contribution of chromosomes, the degree of genetic variation (not the average activity) is informative. By comparing the estimates of genetic variance and the correlation coefficients among amylase specific activities in different media, the characteristic properties of genetic factor(s) on the two chromosomes have been revealed. The genetic factor(s) on the second chromosome, which includes the amylase structural genes and their 5′ and 3′ flanking regions, have an effect on amylase specific activity in *glucose* medium and smaller effects in starch and normal media. The effects of genetic factors in different media are similar. The function of genetic factor(s) on the second chromosome is expected to be the repression of amylase activity in non-induced environments. In contrast, the genetic factor(s) on the third chromosome have a large effect on amylase specific activity in *starch* medium and on *inducibility* but a smaller effect in glucose medium. The effects of factors in starch and glucose media are independent. The function of the genetic factor(s) on the third chromosome is expected to be the induction of amylase activity in induced environments. Therefore the genetic factor(s) on the third chromosome will be important for environments where amylase should be induced, and the factor(s) on the second chromosome will be important for environments where amylase should be repressed. The experimental evidence for the former case has been presented in our previous results which indicated that the inducibility of amylase in *D. melanogaster* is an important factor for the fitness of individuals in starch medium (Yamazaki & Matsuo, 1983, 1984; Matsuo & Yamazaki, 1984). The latter case will, if possible, be examined experimentally. The properties of the genetic factors speculated above will be confirmed by molecular analysis of the factors on the second and third chromosomes.
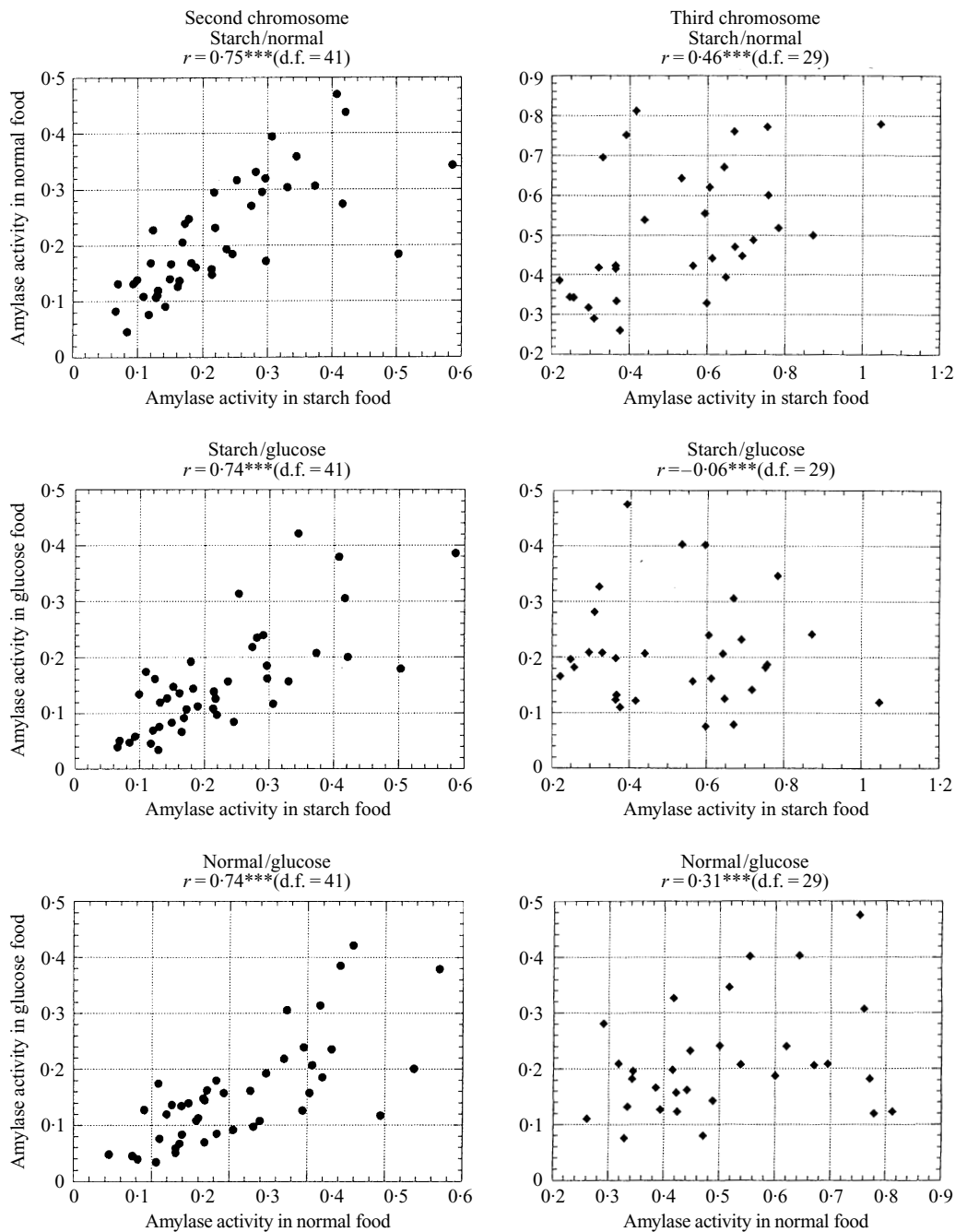
Fig. 5. Correlations among amylase specific activities in starch, normal and glucose medium foods. Correlation coefficients (*r*) with degrees of freedom (d.f.) are indicated above each figure. Correlations for the second-chromosome substitution lines are shown on the left and those for the third-chromosome substitution lines on the right. *Significant at the 1% level; ***significant at the 0.5% level.

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