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The alcohol dehydrogenase polymorphism in populations of *Drosophila melanogaster*

3. Differences in developmental times

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SUMMARY

From the F_2 ratios of crosses between *Drosophila melanogaster* strains homozygous for the $Adh^F(F)$ and the $Adh^S(S)$ allele it has been concluded that the developmental time of *FF* homozygotes is shorter than that of *SS* homozygotes. This difference is found to be reinforced by increasing levels of crowding. A further analysis of developmental times has been performed by the transfer of larvae to agar medium after they have stayed for periods of variable length on regular food. From the percentage of emerging adults it can be concluded that *FF* larvae and, to a lesser extent *FS* larvae, either reach their critical weights for pupation earlier than *SS* larvae or possess a lower critical weight. These differences in developmental time influence the course of allele frequencies. Between populations kept on a 2-week transfer schedule and on a 3-week schedule a divergence of allele frequencies is observed, in the former a decrease in *S* frequency occurs. The relevance of the observed differences in developmental time for the maintenance of the *Adh* polymorphism is discussed for laboratory populations kept on regular food and at varying densities.

1. INTRODUCTION

The alcohol dehydrogenase (*Adh*) locus in *Drosophila melanogaster* contains one of the few cases where the action of selection on an allozyme polymorphism has been unambiguously demonstrated. In the presence of substrates of the ADH enzyme (a variety of alcohols) reproducible changes in allele frequencies have been observed. Gibson (1970), Bijlsma-Meeles & Van Delden (1974), Van Delden, Kamping & Van Dijk (1975) and Van Delden, Boerema & Kamping (1978) have shown that in polymorphic populations kept on food supplemented with different alcohols a considerable rise in frequency of the *F* allele occurs. Fitness differences between the *Adh* genotypes on ethanol-supplemented food have been observed in different stages of the life-cycle (Morgan, 1975; Briscoe, Robertson & Malpica, 1975; Oakeshott, 1976; Van Delden *et al.* 1978). The enzymes of the *Adh* genotypes differ in a number of biochemical properties such as *in vitro* activity and temperature stability (Rasmuson, Nilson & Rasmuson, 1966; Gibson, 1970; Gibson &

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Miklovich, 1971; Vigue & Johnson, 1973; Day, Hillier & Clarke, 1974*a*, *b*; Van Delden *et al.* 1978). It is therefore plausible to relate these biochemical differences to the observed fitness differences under special conditions; for example, the higher mortality of *SS* homozygotes on ethanol-supplemented food compared to *FF* homozygotes is negatively correlated with the *in vitro* ADH activity in these genotypes. The relation between ADH activity and mortality is further confirmed by studies of Thompson & Kaiser (1977) and Kamping & Van Delden (1978). They found a negative correlation between ADH activity and mortality among strains of identical *Adh* genotype, which differed in ADH activity. The comparatively greater *in vitro* stability of ADH of the *SS* homozygotes at high temperatures has been described as the probable cause of a latitudinal cline in allele frequencies in the U.S.A., where a lower frequency of the *F* allele was found in hotter areas (Vigue & Johnson, 1973).

Bijlsma-Meeles & Van Delden (1974) and Van Delden *et al.* (1978) have reported that also on regular laboratory food consistent allele frequency changes occur in polymorphic populations, started from different initial frequencies. In these cases allele frequencies converge in the course of time to values equal to those in the base populations. This finding was interpreted as the result of some kind of balancing selection. Kojima & Tobari (1969) have reported the occurrence of frequency dependent selection at the *Adh* locus, leading to equilibrium allele frequencies. In the present paper the maintenance of the *Adh* polymorphism under regular laboratory conditions is the object of further study. Differences in developmental times between the *Adh* genotypes of the Groningen population are reported and further analysed. The role of these observed differences in the maintenance of the polymorphism in relation to factors such as generation time and population density are discussed.

2. MATERIALS AND METHODS

(*a*) *Strains.* Two *Drosophila melanogaster* strains, an Adh^F (*F*) and an Adh^S (*S*) strain, were used. Each strain was composed by intercrossing eight homozygous Groningen-B lines of the appropriate genotype (details on the Groningen-B lines in Van Delden *et al.* 1978).

(*b*) *Food and culture conditions.* The regular food consisted of a mixture of 1000 ml water, 19 g agar, 54 g sucrose, 32 g dried dead yeast and 13 ml nipagin solution (10 g nipagin dissolved in 100 ml ethanol 96%). The bottles used contained 30 ml food, the vials 9 ml food. Temperature was 25 °C, humidity ± 70% R.H.

(*c*) *Electrophoresis.* Polyacrylamide electrophoresis for *Adh* genotype identification was carried out as described by Van Delden *et al.* (1975).

(*d*) F_2*-crosses.* F_1 flies (25 pairs per bottle) from the crosses $SS \times FF$ and $FF \times SS$ were used as parents for an F_2 generation. Four different egg-laying periods were applied: 24, 64, 96 and 136 h, in order to obtain different levels of crowding. Each egg-laying period was represented by eight bottles (four with F_1

flies from the cross $SS \times FF$ and four with F_1 flies from the cross $FF \times SS$). The F_2 flies were collected 10, 11, 12 and 14 days after the cultures had been started, thus collection day 10 included the flies which emerged on days 9 and 10; collection day 11 the flies which emerged on day 11, etc. From the flies collected on a particular collection day a sample of 100 flies per cross (or less when the total number of flies present was insufficient) were electrophoresed for genotype identification. Particular samples are indicated by their egg-laying period, followed by the collection day, so 136–10 means a sample from an egg-laying period of 136 h, collected on day 10.

(*e*) *Allele frequency changes in bottle populations.* Eight bottle populations were started, each with 100 pairs of F_1 parents from the cross $FF \times SS$. The populations were continued for nine generations (the F_2 generation was denoted as generation 1). Each generation the parents were removed and discarded after 72 h. Four bottle populations were continued on a 14-day schedule: the offspring of the four bottles were collected and thoroughly mixed 14 days after the introduction of the parents, 400 of them were thereafter divided among four new bottles with fresh food (100 flies per bottle) to serve as parents for the next generation. The other four populations, kept on a 21-day schedule, were maintained in an identical way except that the transfer to new bottles occurred at 21-day intervals. Allele frequencies were determined in generation nine at the time of transfer to new bottles.

(*f*) *Larva-adult development.* Homozygous *SS* and *FF* larvae, derived from uncrowded cultures of the *S* and *F* strains and heterozygous larvae from the reciprocal crosses between these strains were picked up within 1 h after hatching from the egg and transferred to Petri dishes containing regular food. After a stay of variable length the larvae were again transferred, now to vials containing only agar (9 ml). The number of larvae per vial was 40. The number of vials per genotype per transfer time was generally 10, for the heterozygotes 5 for either reciprocal cross. In the first experiment the age of the larvae at the time of transfer to agar varied from 46 to 74 h, at 4 h intervals. In a second experiment, performed a week after the first, the ages were: 42, 44 and 46 h. The number of pupated larvae were recorded and the number of emerging flies were scored daily from the day the first flies emerged.

3. RESULTS

(*a*) *F_2-crosses.* The outcome of the F_2-crosses with varied egg-laying periods of the F_1 parents is given in Table 1, where the data for each of the four collection days are given separately. The F_2's of the reciprocal crosses were combined in the table because χ^2 tests did not reveal significant differences between them. The F_2 of sample 136–10 was only based on the $SS \times FF$ cross since the flies of the reciprocal cross were lost. Though males and females were scored separately, both sexes were combined as it was only in a single case (64–12) that a significant difference in the F_2 genotype ratio between males and females was found ($\chi_2 = 8{\cdot}89$, $P < 0{\cdot}025$). This difference was probably incidental since no indication

Table 1. *Outcome of the F_2's of crosses* FF × SS *and* SS × FF *(combined) at varying egg-laying periods and collection days*

Egg-laying period (h)	Collection day	n_1	n_2	Genotypes *SS*	*FS*	*FF*
24	10	370	200	35	106	59
	11	473	200	43	94	63
	12	490	200	47	103	50
	14	399	200	56	96	48
64	10	141	117	17	66	34
	11	284	187	31	97	59
	12	515	200	51	99	50
	14	1048	200	54	107	39
96	10	168	167	26	83	58
	11	312	200	37	103	60
	12	434	200	47	98	55
	14	953	200	54	95	51
136	10†	78	64	8	27	29
	11	319	200	30	97	73
	12	454	200	42	90	68
	14	785	200	39	104	57

n_1 is the total number of flies collected on a particular collection day; n_2 is the number of flies used for electrophoresis.

† Based on cross $SS \times FF$ only.

of a particular trend could be observed in the rest of the data. Inspection of Table 1 shows that considerable, systematic deviations from the expected 1:2:1 F_2 ratio were found. This is further elaborated in Table 2(A) where the χ^2 values for the deviation from the expected F_2 ratio are presented. Significant deviations were found, especially on collection days 10 and 11; furthermore, it appeared that the χ^2 values increased with the increasing length of the egg-laying period. The disturbed F_2 ratios were apparently caused by a relative excess of *FF* homozygotes, compared to *SS* homozygotes during the early collection days. This is shown in Table 2(C), where the observed *SS*/*FF* ratios are given (expected ratio 1:1). On the 2 last collection days, however, the numbers of both types of homozygotes became more alike and on day 14 even a small excess of *SS* homozygotes was observed, except for the longest egg-laying period. The difference in F_2 ratio between the early and late emerging flies was clearly demonstrated by calculation of the χ^2 values for a 2 × 3 contingency table of the numbers of the three genotypes and both extreme collection days 10 and 14. The χ^2's obtained for the egg-laying periods 24, 64, 96 and 136 h were respectively: 6·47 ($P < 0{\cdot}05$), 8·17 ($P < 0{\cdot}025$), 8·16 ($P < 0{\cdot}025$) and 6·48 ($P < 0{\cdot}05$). The data from Tables 1 and 2 suggest that the *FF* homozygotes had a shorter developmental time than the *SS* homozygotes and were therefore over-represented in the early collection days. The later emerging *SS* flies on the other hand provided an excess of this genotypic class in the last collection day. This picture is re-enforced by the effects of increased length

Table 2. *Analysis of data in Table 1*

Egg-laying period (h)	Collection day 10	11	12	14	T	T_c
	(A) χ^2 for deviations from 1:2:1					
24	6·48*	4·72	0·27	0·96	3·81	
64	6·86*	7·98*	0·02	3·23	4·03	
96	12·66**	5·47	0·72	0·59	9·49**	
136	15·34**	18·67**	8·76*	3·56	36·31**	
	(B) Frequency of S (p_S)					
24	0·445	0·450	0·493	0·520	0·476	0·477
64	0·427	0·425	0·503	0·538	0·479	0·505
96	0·404	0·443	0·480	0·508	0·461	0·481
136	0·336	0·393	0·393	0·435	0·419	0·427
	(C) Ratio SS/FF					
24	0·59	0·68	0·94	1·17	0·82	0·85
64	0·50	0·52	1·02	1·38	0·84	1·10
96	0·45	0·62	0·85	1·06	0·73	0·88
136	0·28	0·41	0·62	0·68	0·52	0·58
	(D) Ratio $SF/SS+FF$					
24	1·13	0·89	1·06	0·92	1·00	1·00
64	1·29	1·08	0·98	1·15	1·10	1·11
96	0·99	1·06	0·96	0·90	0·98	0·95
136	0·73	0·94	0·82	1·08	0·92	0·95

T gives totals over all collection days; T_c is the corrected T (details in text).
* $P < 0·05$; ** $P < 0·01$.

of egg-laying periods (which no doubt caused a higher level of crowding). Higher levels of crowding apparently exaggerated the effect of differential development times. The slowly developing *SS* homozygote was clearly at a disadvantage in this situation and in this case there was no recovery during the later collection days. The possibility is not excluded, however, that later collection days than those applied in this experiment would provide a further rise in the *SS*/*FF* ratio for these longer egg-laying periods too. The numbers of heterozygotes were generally not far from expected, though the ratio heterozygotes/homozygotes (Table 2D) tended to decline with increasing length of egg-laying periods.

The consequences of the observed differences in developmental time for the realized allele frequencies are clearly demonstrated in Table 2(B). At the early collection days much lower frequencies of the *S* allele were found than in the last collection days. Increasing levels of crowding also diminished the *S* frequency. In Table 2, column T, the allele frequencies (and other values too) are also presented for the total number of electophorized flies over all 4 collection days. These values, however, cannot be taken as the net result for all emergences over the whole period of 14 days because the total number of emerged flies per collection day and the pattern of emergence differed considerably between the different egg-laying periods (Table 1, column n_1). With the longer egg-laying periods

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relatively few flies emerged as early as collection day 10 and the majority of flies emerged on days 12 and 14, which did not hold for the 24 h egg-laying period. The allele frequencies, however, were based on a standard sample size of 200 flies (when available) for each collection day, irrespective of the total number of emerged flies present on that particular day. To obtain corrected overall mean allele frequencies, the frequencies have to be weighted for the number of emerged flies on the collection day involved. These corrected allele frequencies (and also the corrected ratios from Tables 2C and 2D) are also given in Table 2, under the heading T_c. It appears, from the overall allele frequencies for the whole period of 14 days, that crowding introduces differences in allele frequencies.

Table 3. *Numbers of each genotype and allele frequencies in samples of flies taken at generation 9 from populations kept under 2-week and 3-week schedules*

Schedule	Sample size	No. of flies			p_S
		SS	*FS*	*FF*	
2-week	132	12	70	50	0·356
3-week	150	38	85	27	0·537

Table 4. *Mean number of flies per bottle which emerged within 3 weeks* ($\bar{x}$) *and percentage of these flies which had emerged after 2 weeks*

Egg-laying period (h)	$\bar{x}$	Percentage emerged after 2 weeks
24	216·5	100
64	379·0	65·3
96	396·8	58·8
136	369·0	53·1

(*b*) *Allele frequency changes in populations with varying generation intervals.* The results from the previous section predict that variation in the generation time of laboratory populations (that means the time of transfer to new bottles with fresh food) would result in differences in allele frequencies in the course of time. Populations kept at a short generation interval (in our experiments 2 weeks) would undergo a decline in the frequency of the *S* allele, because the *SS* homozygotes emerge later than the *FF* homozygotes. A longer generation interval (in our case 3 weeks) would create a smaller decline in *S* frequency or no decline at all. The initial *S* frequency in our experiment was 0·50. The genotype and allele frequencies in generation 9 were determined at the time of transfer to new bottles. The results are given in Table 3. It appeared that on the 2-week schedule a drastic reduction in *S* frequency to $p_S = 0{\cdot}356$ had occurred, in contrast to $p_S = 0{\cdot}537$ on the 3-week schedule. Table 4 gives an impression of the number of flies which emerged after 2 weeks, compared to the number which emerged after 3 weeks. This table is, however, based on the data from Table 1 where the number of parents was 25 pairs, instead of 50 pairs in this population transfer experiment, while in the latter experiment the egg-laying period was 72 h.

(c) *Development from larva to adult.* The results from the previous sections lead to the hypothesis that the differences in time of emergence pattern in the three *Adh* genotypes could be ascribed to differences in time of development of the larvae. This was further investigated in a separate experiment where the larvae of the three genotypes were tested for the time needed to reach their critical weights. *Drosophila* larvae can only pupate after they have acquired a certain minimum weight (Bakker, 1961, 1969; Robertson, 1963). When food is withheld from larvae before this critical weight is reached they are able to survive for some time in the

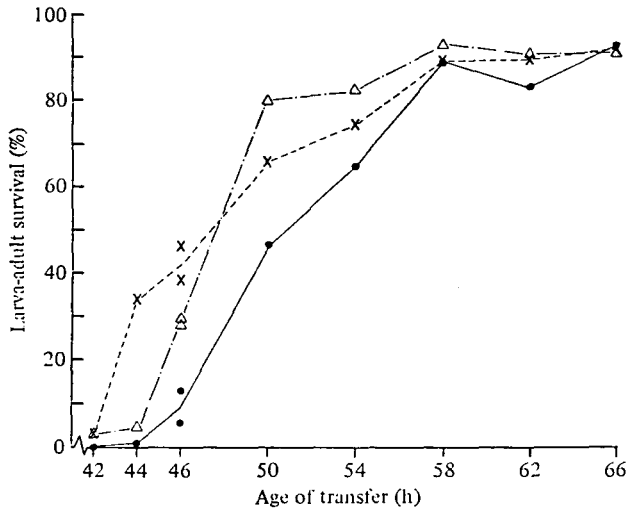

Fig. 1. The relation between larva-adult survival of the three *Adh* genotypes and the age of transfer from regular to agar medium. ●—●, *SS*; △–·–△, *FS*; ×·····×, *FF*.

larval stage, but will not pupate. In our experiment the larvae of the three *Adh* genotypes were at first kept on regular food under uncrowded conditions for periods of variable length, and thereafter transferred to pure agar medium. Larvae which had already reached their critical weight at the time of transfer will pupate and the majority become adults. In the first experiment the time of transfer to agar varied from 46 to 74 h with 4 h intervals. Since the earliest transfer time gave already rather high pupation percentages, at least for some genotypes, a second experiment was made, 1 week after the first, where transfer times of 42, 44 and 46 h were applied. The relation between the age at which the larvae were transferred from regular to agar medium and the percentage which emerged as adults is given in Fig. 1. The differences in survival between the genotypes are here clearly demonstrated. *SS* larvae have a lower survival than *FF* and *FS* larvae when the age of transfer is lower than 58 h. In Table 5, in addition to larva-adult survival, the percentage of larvae which pupate, the fly/pupa ratio and the sex ratio (♀♀/♂♂) of the adults are given. For each age of transfer a separate analysis of variance was performed both for pupation and for adult survival percentages after angular transformation of the survival percentages of the individual vials.

Table 5. *The effect of age at which larvae were transferred from regular to agar medium on survival*

(The percentages of larvae surviving to form pupae and flies are given separately, and also presented are the ratio of flies/pupae (E.R.) and the sex ratio (♀♀/♂♂); *n* is the number of replicate vials (each with 40 larvae). * Second experiment.)

Age (h)	*n*	Pupae	Flies	E.R.	Sex ratio
			SS		
42*	8	0	0	—	—
44*	8	1·6	0·9	0·60	—
46*	8	19·1	13·1	0·69	1·47
46	10	9·3	5·0	0·54	1·22
50	10	56·3	46·8	0·83	2·22
54	10	72·3	65·3	0·94	1·21
58	10	91·0	88·8	0·98	1·25
62	10	91·0	83·3	0·91	0·78
66	10	97·3	93·0	0·96	0·80
70	10	96·0	92·8	0·97	1·26
74	6	97·5	94·2	0·97	1·24
			FS		
42*	8	5·0	3·1	0·63	—
44*	8	5·3	4·4	0·82	1·33
46*	8	32·8	28·8	0·88	1·19
46	10	40·0	29·0	0·73	1·41
50	10	84·5	80·3	0·95	1·14
54	10	86·5	82·5	0·95	1·16
58	10	96·0	93·3	0·97	1·19
62	10	95·0	91·0	0·96	0·99
66	10	96·5	91·5	0·95	0·87
70	5	96·5	91·5	0·95	0·81
74	6	96·5	90·0	0·93	1·16
			FF		
42*	8	3·4	2·8	0·82	—
44*	8	37·8	33·8	0·89	1·25
46*	8	51·3	46·6	0·91	1·13
46	10	45·5	38·5	0·85	1·52
50	10	78·3	66·3	0·85	1·60
54	10	80·8	74·0	0·92	1·45
58	10	95·0	89·0	0·94	1·00
62	10	94·0	89·5	0·95	1·07
66	10	96·5	92·8	0·96	1·01
70	10	97·5	92·0	0·94	1·00
74	6	98·3	93·8	0·96	0·88

Tukey's multiple comparison test (see Li, 1964) was applied for a further analysis of the differences between the genotypes by using the error mean squares of these analyses of variance. The results are given in Table 6 where the F and P values of the analyses of variances are presented together with the genotypes ranked in decreasing order of survival percentage. Those genotypes are underlined which were found to be not significantly different at the 5% level. It appeared that, for

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Table 6. F *and* P *values from the analyses of variance of larva-pupa and larva-fly survival performed for each age of transfer separately after angular transformation of the survival percentages*

(The genotypes are arranged in decreasing order of survival. Genotypes not underlined by a common line are significantly different in survival at the 5% level (Tukey's multiple comparison test). * Second experiment.)

Age (h)	Pupae					Flies				
	F	*P*	Genotypes			*F*	*P*	Genotypes		
42*	16·56	< 0·005	*FS*	*FF*	*SS*	4·22	< 0·05	*FS*	*FF*	*SS*
44*	51·54	< 0·005	*FF*	*FS*	*SS*	58·18	< 0·005	*FF*	*FS*	*SS*
46*	19·28	< 0·005	*FF*	*FS*	*SS*	23·26	< 0·005	*FF*	*FS*	*SS*
46	11·48	< 0·005	*FF*	*FS*	*SS*	10·41	< 0·005	*FF*	*FS*	*SS*
50	33·43	< 0·005	*FS*	*FF*	*SS*	16·87	< 0·005	*FS*	*FF*	*SS*
54	5·51	< 0·01	*FS*	*FF*	*SS*	5·90	< 0·01	*FS*	*FF*	*SS*
58	4·72	< 0·025	*FS*	*FF*	*SS*	0·81	n.s.	*FS*	*FF*	*SS*
62	3·58	< 0·05	*FS*	*FF*	*SS*	4·39	< 0·025	*FS*	*FF*	*SS*
66	0·27	n.s.	*SS*	*FS*	*FF*	0·49	n.s.	*SS*	*FF*	*FS*
70	0·30	n.s.	*FF*	*SS*	*FS*	0·39	n.s.	*SS*	*FF*	*FS*
74	2·15	n.s.	*FF*	*SS*	*FS*	0·06	n.s.	*SS*	*FF*	*FS*

F = variance ratio; P = level of significance; n.s. = not significant.

survival till both the pupal and the adult stage, the lowest survival was found for the *SS* homozygotes up to a transfer period of 62 h. Thereafter no significant differences between the genotypes were found. Apparently the *SS* homozygotes needed more time to reach their critical weight than the other genotypes. The *FF* homozygotes had a lead at the earlier transfer times but were overtaken by the heterozygotes at intermediate transfer times. It further appeared (Table 5) that the *SS* homozygotes, in addition to lower larva-pupa ratios, also possessed lower fly-pupa ratios, therefore mortality in the pupal stage is also higher for this genotype at the lower transfer ages. The sex ratio decreased with increasing age of transfer. Dry weights of emerging adults were determined on samples of flies. Figure 2 shows the mean dry weights of female flies which emerged 9 days after the larvae had enclosed from the eggs, plotted against the age of transfer. Mean fly weight increased with age of transfer. The weights of *FF* flies were higher than of *SS* flies.

4. DISCUSSION

The outcome of the F_2 crosses (Tables 1 and 2) points to a more rapid development of *FF* homozygotes – and to a lesser extent of *FS* genotypes – compared to *SS* homozygotes. A further analysis of larval developmental time confirms this and shows (Table 5; Fig. 1) that the *FF* larvae reach their critical weight earlier than the *SS* larvae, while the *FS* and *FF* larvae are similar in this respect. This can be further elaborated by an analysis of the regression of the percentage of larvae emerging as adults (after probit transformation) on the age of transfer

from regular to agar medium (after logarithmic transformation), following the method of probit regression analysis devised by Finney (1947). Analogously to the median lethal dose (LD 50), a median age of transfer can be calculated, which is the age at which 50% of the larvae are able to emerge as adults. These values are given for each genotype in Table 7. There are more than 4 h difference in median transfer ages between *FF* and *SS*, while *FF* and *FS* differ by 1 h. It thus

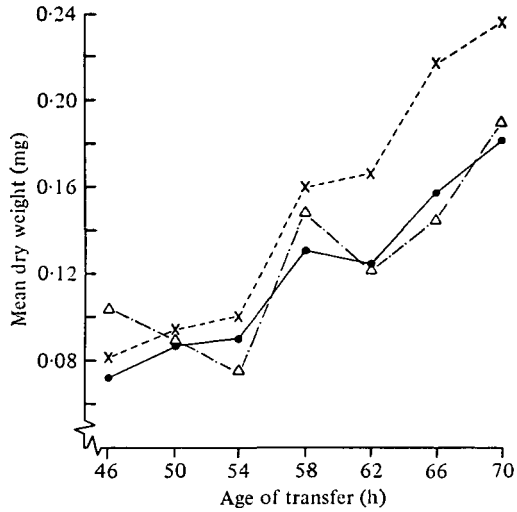

Fig. 2. The relation between mean dry weight of females (emerged 9 days after the corresponding larvae had eclosed from the eggs) and the age of transfer of the larvae from regular to agar medium. ●—●, *SS*; △–·–△, *FS*; ×----×, *FF*.

Table 7. *Median age of transfer: the age (in hours) at which 50% of the larvae are able to become adults without further food ingestion*

(The 95% confidence limits are given in parentheses.)

Genotype	Median age of transfer
SS	53·59 (53·24–53·95)
FS	50·44 (50·08–50·81)
FF	49·31 (48·91–49·71)

appears that the differences in emergence pattern observed in the F_2 crosses can be ascribed to differences between the genotypes in the time needed to reach their critical weights. Such differences could arise in two ways: either the critical weight for all genotypes is alike, while *SS* larvae need more time to reach it, e.g. because of slower feeding rates (Burnet, Sewell & Bos, 1977) or lower efficiency in the conversion of food; or *SS* has a higher critical weight. In our study no larval weight determinations at the time of transfer were performed so we cannot decide between these two possibilities. The higher weight of *FF* adults compared to *SS* is probably caused by the prolonged post-critical feeding period. This also causes the increase in adult weight when the age of transfer is prolonged (Fig. 2). The high sex ratios observed for the lower transfer ages are in agreement with the well

known fact that in *Drosophila melanogaster* the females emerge earlier than the males.

A critical question concerning the observed differences in the time of development between the genotypes is whether these differences can actually be attributed to the *Adh* locus itself or are connected with one or more closely linked loci. It is possible that in one of the original *F* lines used to establish the combined *F* strain the *F* allele was associated with an allele of another, linked, locus which is responsible for rapid development. Because of its apparent advantage in a laboratory situation where strains are transferred to new bottles every fortnight, such an association would be favoured by selection. Further research has to be expanded to more populations, from different geographic origin, to evaluate whether the more rapid development of *FF* and *FS* larvae is a general phenomenon and is therefore likely to be connected with the *Adh* locus itself. As long as the role of ADH in insect metabolism, except for the direct detoxication of alcohols, is still unknown, no direct functional relationship can be indicated for the observed differences in developmental time between the *Adh* variants.

Evidence for the generality of the faster development of *FF* homozygotes, however, comes from the experiments of Oakeshott (1976, 1977). He found that developmental time positively regressed on ethanol concentration and that the genotypes differed in the magnitude of the regression coefficients in the following order: *FF* < *FS* < *SS* < 0-mutant. No genotypic differences in developmental time were found on regular food, though it is possible that the method applied was not sensitive enough to detect small differences. The general increase of developmental time induced by ethanol addition to the food may have magnified the already existing small differences between the genotypes.

It can be asked in what manner the observed differences in developmental time are connected with the maintenance of the *Adh* polymorphism. Bijlsma-Meeles & Van Delden (1974) and Van Delden *et al.* (1978) have shown that experimental polymorphic Groningen populations kept on regular food and started with different initial frequencies ended all with the same allele frequency. This frequency corresponded to the frequency which was maintained in the Groningen base population for at least 3 years. This phenomenon was interpreted as being due to some kind of balancing selection and indications were found for heterozygote superiority in egg-to-adult survival. In the present set of experiments it is found (Fig. 1) that the graphs representing the survival of *FF* and *FS* genotypes cross each other: up to 48 h *FF* has a higher survival than *FS*, beyond that time *FS* exceeds *FF* in survival. This may be interpreted as follows: when only a very limited amount of food is available, as e.g. in high density situations, the *FF* homozygotes produce a higher percentage of adults; when, however, the amount of food is somewhat greater, but still limiting, the heterozygotes succeed in producing more adults. This can arise in a situation where the genotypes, though they need on the average about the same time to reach their critical weights, have different variances around that value (Bakker, 1961). A greater variance, as is here supposed for *FF* compared to *FS*, will then result in a greater survival

percentage when the food is extremely limited. We may then speculate that when the amount of food available becomes greater but is still insufficient for the development of all larvae, the less variable genotype, in our case *FS*, gets a relative advantage. This will create a situation of conditional overdominance dependent on the level of crowding. Though *FF* individuals will possess a relative advantage at high densities, the heterozygotes will have the highest survival at more moderate densities, as *SS* individuals possess an overall lower survival under crowded conditions. Such a system will result in density-dependent selection coefficients. Support for this hypothesis can be found in the comparison of the *FS* to *FF* ratio for the lower density situation (egg-laying periods 24 and 64 h combined) and for the high density situation (egg-laying periods 96 and 136 combined): these ratios are 2·10 and 1·66 respectively (expected ratio 2·00).

How does our view fit with that of Kojima & Tobari (1969) who claimed that egg-to-adult survival of *Adh* genotypes is frequency dependent? Their results, obtained by combining premated females of the three genotypes in three different proportions in order to get three different input ratios of eggs, resemble ours in that *FF* individuals at the two lowest *FF* frequencies (0·10 and 0·25) had a higher survival than expected. The latter frequency corresponds to our F_2 ratio. Only at their highest *FF* frequency (0·60) was a higher survival of *SS* observed. In this respect it has been shown by De Jong (1976) that viabilities become strongly frequency-dependent in a situation where the amount of food is restricted when the genotypes differ as to the time needed to gain their critical weights. De Jong has also given the conditions for equilibrium in such a situation. In our case, however, where the *SS* larvae lag behind considerably, these conditions are not fulfilled and no stable equilibrium due to frequency dependent selection can be attained. We therefore consider the heterozygote advantage in egg-to-adult survival at higher densities, shown in our present and earlier results, as a more plausible explanation for the maintenance of the *Adh* polymorphism. It should be stressed, however, that other fitness components, e.g. adult survival, mating success and fecundity, in which the genotypes may also differ, were not measured in our study.

A final conclusion is that also under regular laboratory conditions of food and temperature the *Adh* variants are not neutral. It is clear that the observed differences in developmental time have consequences for the course of allele frequencies in time, the effect being strongly influenced by the laboratory husbandry (Table 3). Unfamiliarity with these facts could lead to different conclusions about the selection mechanism dependent upon the transfer scheme of bottle populations or the replacement scheme of food cups in cages and upon the level of crowding. The limited knowledge about the population biology of *D. melanogaster* in nature, especially concerning generation time and level of crowding, makes it hard to decide what the significance of the described genotypic differences is for natural populations.

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