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Larval competition in *Drosophila melanogaster*: frequency-dependence of viability

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Summary. The application of the overfeeding technique (interruption of the competition during larval development) to the study of larval competition in two-strain cultures of *Drosophila melanogaster* demonstrates the following points: (1) viability is a function of competition time; (2) viability becomes more frequency-dependent as competition time increases; (3) the dynamics of the "inner" subpopulation (adults that have passed all their development in a crowded condition) and "outer" subpopulation (adults coming from larvae recovered by interruption of competition) vary with time as regards frequency-dependence; and (4) the wild type strain Oregon is the active agent in competition with the strain cardinal.

Key words: Larval competition – Larva-to-adult viability – Frequency-dependent selection – Overfeeding technique

Introduction

In *Drosophila melanogaster,* frequency-dependence of larva-to-adult viability among genotypes has been described in several ways (Bakker 1961; Snyder and Ayala 1979; Mather and Caligari 1981; Nunney 1983). Two types of explanation have been forwarded. Huang et al. (1971) investigated larval interference by waste products, using media conditioned by several genotypes. De Jong (1976), based upon Bakker's experiments (1961, 1969), concluded that frequency-dependent selection exists if a limited amount of food is able, whenever genotypes have different parameters of feeding behavior. This model, and the related model of Nunney (1983), assumes larval interference to be absent.

Literature about larval competition shows the importance of larval biotic residues for fitness (Weisbrot 1966; Dawood and Strickberger 1969; Huang et al. 1971), and of the intrinsic properties of genotypes (De Jong 1976; Nunney 1983). More information about the environmental and genetic causes determining the observed response in viability is needed. This kind of information can be obtained by means of the "overfeeding" technique (Moya and Ménsua 1983). The technique interrupts larval competition for food at different times in the development of larvae. It can give information about the mechanism of both density- and frequency-dependent selection. With this technique, density-dependent selection and frequency-dependent selection as functions of the deterioration of the environment and of the increase of competition with the larval development can be tracked.

The aim of this work is twofold: (1) to use the "overfeeding" technique in the study of viability in a mixed (diculture) population with larval competition; and (2) to explain different responses as a function of genetic composition and degree of environmental deterioration. For this purpose we use information available from studies on larval development in *Drosophila* (Robertson 1960a, b, 1961, 1963, 1964; Bakker 1961, 1969; Barker and Podger 1970; Ménsua and Moya 1983; Botella et al. 1985).

Materials and methods

Two strains of *D. melanogaster* are used: Oregon-R (Or-R), and the eye color mutant *cardinal* (*cd*, III-75.7), derived from a

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collection in a wine cellar near Requena (Valencia, Spain) in 1979. Both strains have been kept in the laboratory by a serial transfer at 25 ± 1 °C and 60 ± 5% relative humidity in 250 ml bottles supplied with 50 ml of Lewis' medium.

We use 40 × 8 mm vials with 0.50 ml of medium with 70 larvae; previous studies in our laboratory have established that this leads to strong competition without excessive mortality due to lack of moisture.

The large numbers of larvae needed were collected as follows: we exposed watch-glasses (with agar, water, acetic acid, ethyl alcohol, and seeded with a few grams of fresh yeast) for 12 h in the adult cultures. The food layers in the watch-glasses were cut into pieces with 150 to 200 eggs each, which were transferred to 250 ml bottles with 50 ml of fresh food. Five day old adults from these bottles were transferred to new bottles for 48 h and then exposed again to watch-glasses layers for 4 h. These food layers were kept for 18 h in Petri dishes; the larvae collected from them (which have parents of the same age and have started development under non-competitive conditions) were used for establishing the competition vials.

The cultures of each experimental block were studied simultaneously. Two genotypes (*cd* and Or-R) were combined in the following numbers: 0/70, 4/66, 10/60, 20/50, 35/35, 50/20, 60/10, 66/4 and 70/0 in each experimental block.

The overfeeding technique (Moya and Ménsua 1983) is as follows: seventy larvae (in one of the combinations listed) of 2 ± 2 h of age were placed in one of two kinds of vials: large (100 × 27 mm) containing 5.0 ml of Lewis' medium, and small (40 × 8 mm) with 0.50 ml of medium. One large and one small vial were used as controls for each genotypic combination. Eight small vials were used for "overfeeding" as follows: the first one was transferred to a large vial with medium slanted at an angle ("overfeeding" vial) on the 4th day of culture; the second one was transferred to another large vial two days later, and so on until the 18th day, when the eighth small vial was overfed. In each case, the small vial was removed from the large one after 24 h, by which time all larvae had migrated to the overfeeding vial. The larvae migrated spontaneously and rapidly. Only in the first two overfeedings (4th and 6th days of culture) did a few larvae remained in the small vials. Otherwise, all larvae migrated to the overfeeding vial in only one or two hours. The larvae from each small vial were classified into two groups: the "inner" subpopulation, composed of larvae near pupation, pupae, and adults emerged before the day when the vial was overfed; and the "outer" subpopulation, composed of larvae that migrate to the overfeeding vial. The emerging adults were collected and counted daily until the vials were exhausted.

An experimental block of 8 experimental vials and two controls for each of the seven mixed genotypes, and two single genotype combinations was replicated eight times, in intervals of about four days.

Table 1. Total numbers of emerging adults for both strains in inner and outer populations adding up the results of the 10 overfeeding days, the 9 genotypic compositions and the 8 replicates. The numbers between brackets show the same results but without taking both monocultures (genotypic compositions 0/70 and 70/0) into account

	Females	Males	Total (row)	
Cardinal	3,267 (2,175)	3,006 (2,085)	6,273 (4,260)	Inner
Oregon	2,825 (2,181)	3,452 (2,610)	6,277 (4,791)	
Cardinal	5,008 (4,961)	4,876 (4,825)	9,884 (9,786)	Outer
Oregon	4,504 (4,496)	4,323 (4,316)	8,827 (8,812)	
	Σ females	= 15,604 (13,813)	Σ males	= 15,657 (13,836)
	Σ inner	= 12,550 (9,051)	Σ outer	= 18,711 (18,598)
	Σ cardinal	= 16,157 (14,046)	Σ Oregon	= 15,104 (13,603)

Results

The overfeeding technique allows for observation of how far competition has progressed until a certain time. At any-one overfeeding time, fast developing larvae might have pupated or become adults in the inner vial. Larvae that are still in the third instar can migrate to

Table 2. Migration rates from the inner to the outer vial according to day of overfeeding and initial genotypic composition. The migration rates are added over both genotypes

Day of overfeeding	Migration rate							
	4	6	8	10	12	14	16	18
Genotypic composition Oregon/Cardinal								
70: 0	0.848	0.918	0.812	0.725	0.516	0.516	0.234	0.032
66: 4	0.820	0.877	0.820	0.728	0.561	0.443	0.212	0
60:10	0.707	0.848	0.768	0.666	0.527	0.447	0.205	0.018
50:20	0.821	0.850	0.802	0.684	0.559	0.438	0.157	0.009
35:35	0.827	0.900	0.811	0.668	0.495	0.361	0.155	0.011
20:50	0.759	0.848	0.777	0.698	0.536	0.420	0.205	0.002
10:60	0.770	0.825	0.777	0.718	0.486	0.371	0.152	0.016
4:66	0.812	0.846	0.902	0.704	0.512	0.341	0.100	0.018
0:70	0.762	0.821	0.773	0.616	0.536	0.400	0.086	0.023

Table 3. Mean and standard deviation of adult emergence in the inner population for the Oregon and cardinal strains

Day of overfeeding	4				6				8				10				12			
	Or		cd		Or		cd		Or		cd		Or		cd		Or		cd	
	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd
Genotypic composition Oregon/Cardinal																				
70: 0	5.10	4.24	–	–	0.70	1.13	–	–	3.60	1.98	–	–	9.40	3.68	–	–	12.90	3.68	–	–
66: 4	3.88	2.64	0.25	0.46	0.50	1.07	0.12	0.35	1.62	1.30	0.12	0.35	7.25	3.85	0.75	1.04	14.75	5.47	0.38	0.52
60:10	7.12	5.51	0.62	0.74	0.88	1.81	0.12	0.35	1.88	1.46	0.50	0.76	8.00	3.12	2.38	1.51	12.88	4.91	2.12	2.17
50:20	4.25	2.38	0.50	0.53	1.25	1.16	0.38	0.52	2.25	2.43	0.38	0.52	7.75	2.71	1.75	1.49	13.25	5.99	2.62	2.39
35:35	2.25	1.58	1.88	2.17	0.12	0.35	0.38	0.52	1.00	1.07	1.75	1.58	5.75	3.01	4.00	2.98	9.62	4.10	5.88	4.67
20:50	2.75	1.67	3.75	4.27	0.25	0.46	0.50	0.53	1.88	1.89	2.50	3.55	5.25	3.41	4.25	2.92	7.50	3.30	9.50	5.15
10:60	0.88	0.83	4.38	3.85	0.25	0.46	0.50	0.53	0.88	1.36	2.38	2.13	1.75	1.49	7.88	4.12	4.00	1.85	11.12	4.49
4:66	0.62	0.74	3.88	2.59	0.12	0.35	1.88	2.10	0.62	1.41	2.75	1.75	1.25	1.16	8.25	3.01	1.00	0.93	14.38	6.69
0:70	–	–	7.00	4.53	–	–	1.50	2.26	–	–	4.50	3.11	–	–	10.50	4.24	–	–	17.20	5.94

Day of overfeeding	14				16				18				0.5 ml control			
	Or		cd		Or		cd		Or		cd		Or		cd	
	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd
Genotypic composition Oregon/Cardinal																
70: 0	17.10	5.94	–	–	19.70	6.51	–	–	17.10	5.94	–	–	17.30	3.68	–	–
66: 4	16.88	6.17	0.88	0.99	18.25	6.07	0.50	0.76	16.88	6.58	1.25	1.58	20.38	5.53	0.62	0.92
60:10	18.12	3.74	2.25	2.55	16.00	5.24	2.12	1.89	16.12	4.29	1.88	1.96	16.88	5.11	2.00	1.77
50:20	13.25	4.71	3.12	2.30	14.62	3.20	3.63	3.50	13.88	6.60	3.75	1.67	15.48	5.73	3.75	1.83
35:35	10.38	4.60	9.12	4.94	12.62	5.22	9.62	5.71	10.75	6.04	6.75	4.46	12.00	5.50	9.00	6.50
20:50	7.00	4.00	11.12	6.62	8.25	2.96	12.50	9.62	8.62	3.29	14.38	8.02	7.62	3.96	14.62	7.03
10:60	3.88	2.03	15.12	7.57	4.00	2.00	19.88	9.57	4.00	3.25	20.12	6.62	3.88	1.55	16.38	7.87
4:66	1.62	1.60	16.75	9.42	2.00	0.93	25.88	8.56	1.75	1.16	26.88	6.24	1.50	1.19	24.75	9.84
0:70	–	–	19.50	9.05	–	–	24.00	7.92	–	–	29.10	6.22	–	–	27.40	9.90

Table 4. Mean and standard deviation of adult emergence in the outer population for the Oregon and cardinal strains

Day of overfeeding	5.0 ml control				4				6				8				10			
	Or		cd		Or		cd		Or		cd		Or		cd		Or		cd	
	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd
Genotypic composition Oregon/Cardinal																				
70: 0	55.20	3.96	–	–	54.40	5.37	–	–	57.10	3.39	–	–	49.60	5.94	–	–	43.70	5.66	–	–
66: 4	53.00	3.34	3.25	0.89	49.00	6.85	2.88	1.36	50.62	8.43	3.62	0.74	47.00	10.06	3.38	1.06	39.75	14.04	2.38	1.41
60:10	48.88	6.73	8.50	1.79	36.12	5.69	7.52	1.77	45.00	6.99	8.25	1.16	37.75	9.87	8.50	1.20	31.75	6.86	6.38	2.26
50:20	41.25	2.49	17.00	3.73	36.38	5.53	16.88	1.55	37.12	8.51	16.25	1.98	34.88	6.27	15.50	2.73	26.75	6.16	12.73	3.20
35:35	27.62	1.51	29.75	2.38	36.12	4.70	27.75	4.30	28.12	4.67	30.25	2.25	23.75	3.99	27.00	4.93	17.25	5.28	24.38	4.50
20:50	15.38	2.62	40.88	4.05	14.12	2.53	38.75	6.39	15.62	2.39	40.88	4.05	13.12	1.89	38.38	6.23	10.00	3.38	33.50	6.35
10:60	8.25	1.04	48.25	1.83	6.50	2.27	45.00	1.31	8.00	1.77	46.62	5.04	4.75	2.25	46.38	5.42	4.50	2.62	43.25	6.41
4:66	3.00	0.93	56.12	2.42	2.75	0.89	51.88	6.03	2.88	0.83	54.75	3.49	2.25	1.39	50.12	5.08	1.88	1.64	45.12	4.12
0:70	–	–	55.40	4.24	–	–	51.90	9.05	–	–	56.00	6.22	–	–	52.90	4.81	–	–	41.90	4.53

Day of overfeeding	12				14				16				18			
	Or		cd		Or		cd		Or		cd		Or		cd	
	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd
Genotypic composition Oregon/Cardinal																
70: 0	37.40	13.86	–	–	25.50	9.62	–	–	11.60	9.62	–	–	1.40	3.96	–	–
66: 4	29.75	6.02	2.25	1.67	17.50	5.07	1.38	0.92	8.88	7.12	0.62	0.74	0	0	0	0
60:10	22.12	5.49	5.00	2.62	11.38	5.15	2.50	2.27	4.75	4.77	0.88	1.64	0.25	0.71	0.12	0.35
50:20	19.25	5.90	11.88	3.52	13.75	4.98	9.12	4.09	3.38	3.46	3.25	4.40	0	0	0	0
35:35	12.88	5.89	15.12	8.90	6.75	3.99	8.88	9.72	2.38	2.62	3.00	3.85	0	0	0.12	0.35
20:50	6.00	2.98	24.75	10.05	3.38	3.34	18.75	7.40	2.12	2.47	6.88	5.19	0	0	0	0
10:60	3.12	1.81	26.62	6.09	2.12	1.89	15.50	11.38	1.50	1.69	6.38	5.18	0.25	0.71	0.25	0.71
4:66	1.25	0.89	32.25	6.96	0.50	0.53	18.75	8.33	0	0	4.88	4.91	0	0	1.00	2.07
0:70	–	–	36.10	6.79	–	–	26.10	9.33	–	–	5.00	4.24	–	–	0.90	1.41

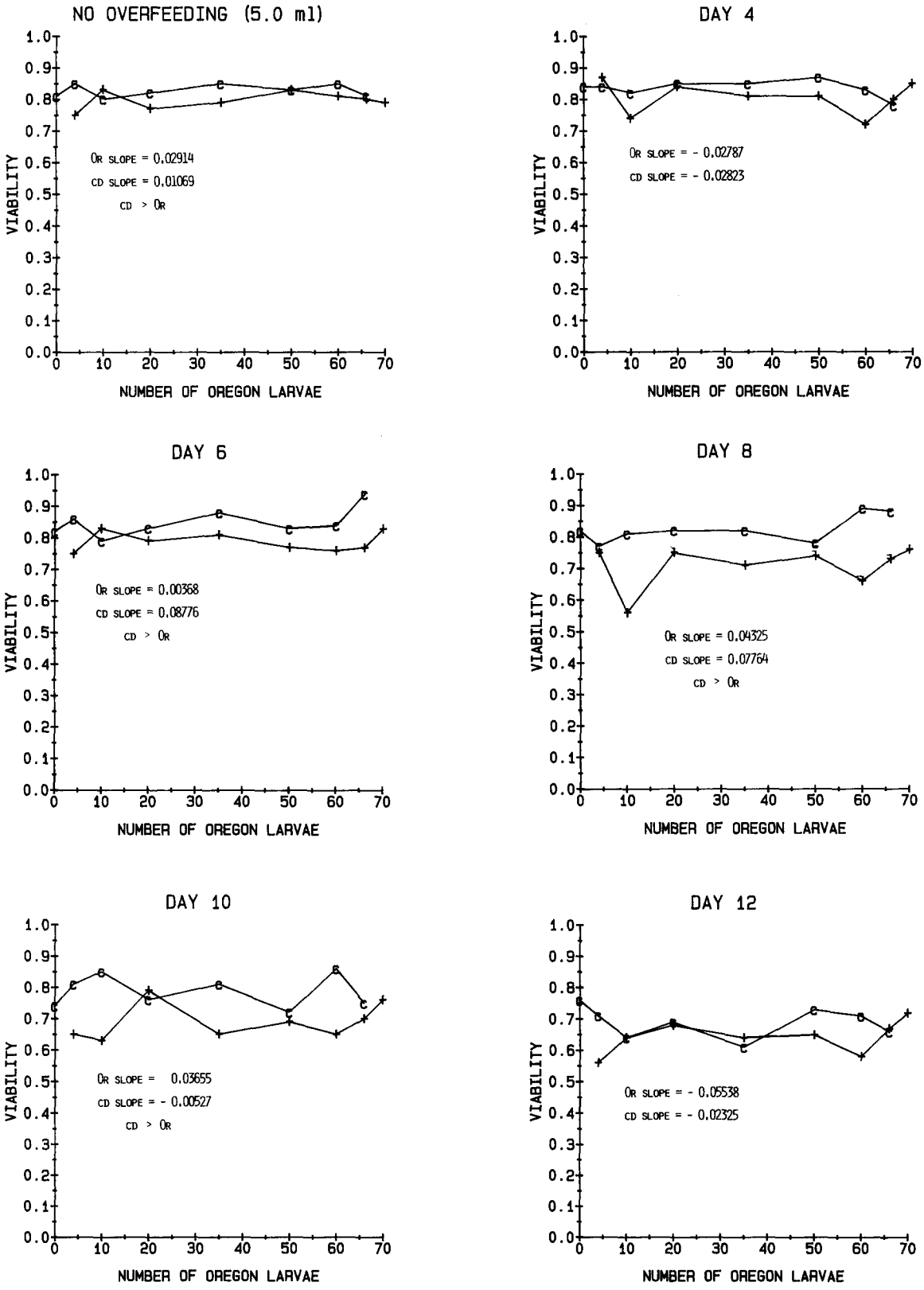

Fig. 1. Total viabilities (inner and outer) of both strains (cardinal: c; Oregon: +) for the different overfeeding days and both controls. The (*) after a slope indicates that it is significantly different from zero. When the mean viability of a strain is significantly higher than the other, it is indicated by a (>)

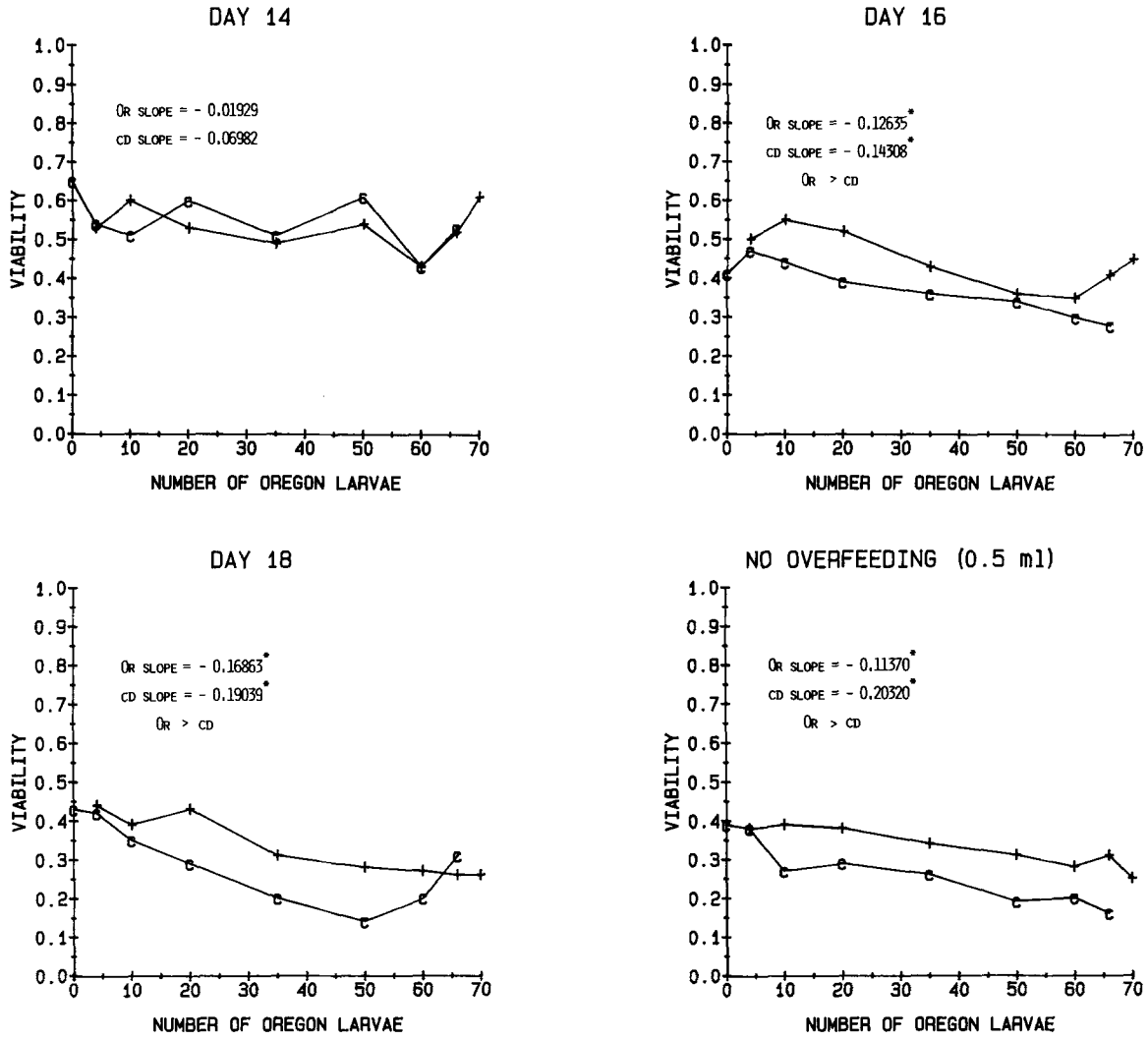

Fig. 1

the outer vial, where they either die as larvae (in a very small proportion) or pupate. Pupae in the outer vial might die or become adults. In Table 1 the total number of surviving adults of the total of 50,400 individuals initially seeded are shown. The very close 1:1 sex ratio obtained is remarkable.

As stated in the previous paragraph, an individual can either stay in the inner vial when overfed or migrate to the outer one. In Table 2, the migration rates for each overfeeding day and genotypic composition are shown. It is clear that more larvae migrate to the outer vial in the first days of overfeeding than in the later ones. For late overfeeding days, migration rates may depend on the culture composition.

The outcome of competition for an individual can be either its emergence as an adult or its death. Tables 3 and 4 show the numbers emerged of both strains in inner and outer subpopulations for each genotypic composition. Figure 1 shows the proportions of adults emerging for every genotypic combination of both strains, over both inner and outer populations, and for all the overfeeding days; 5.0 ml and 0.5 ml controls are also included. It is interesting to note that the emerging abilities of Or and *cd* are reversed between overfeeding days 12–14 as overfeeding is delayed. The number of dead larvae and pupae in both subpopulations can be computed from the results in Tables 2, 3 and 4.

The question arises as to whether the fate of an individual, regardless of its own genotype, is independent of genotypic composition of the culture and overfeeding day. For each combination of these factors, the number of individuals in the four classes (inner-deads, inner-survivors, outer-deads and outer-survivors)

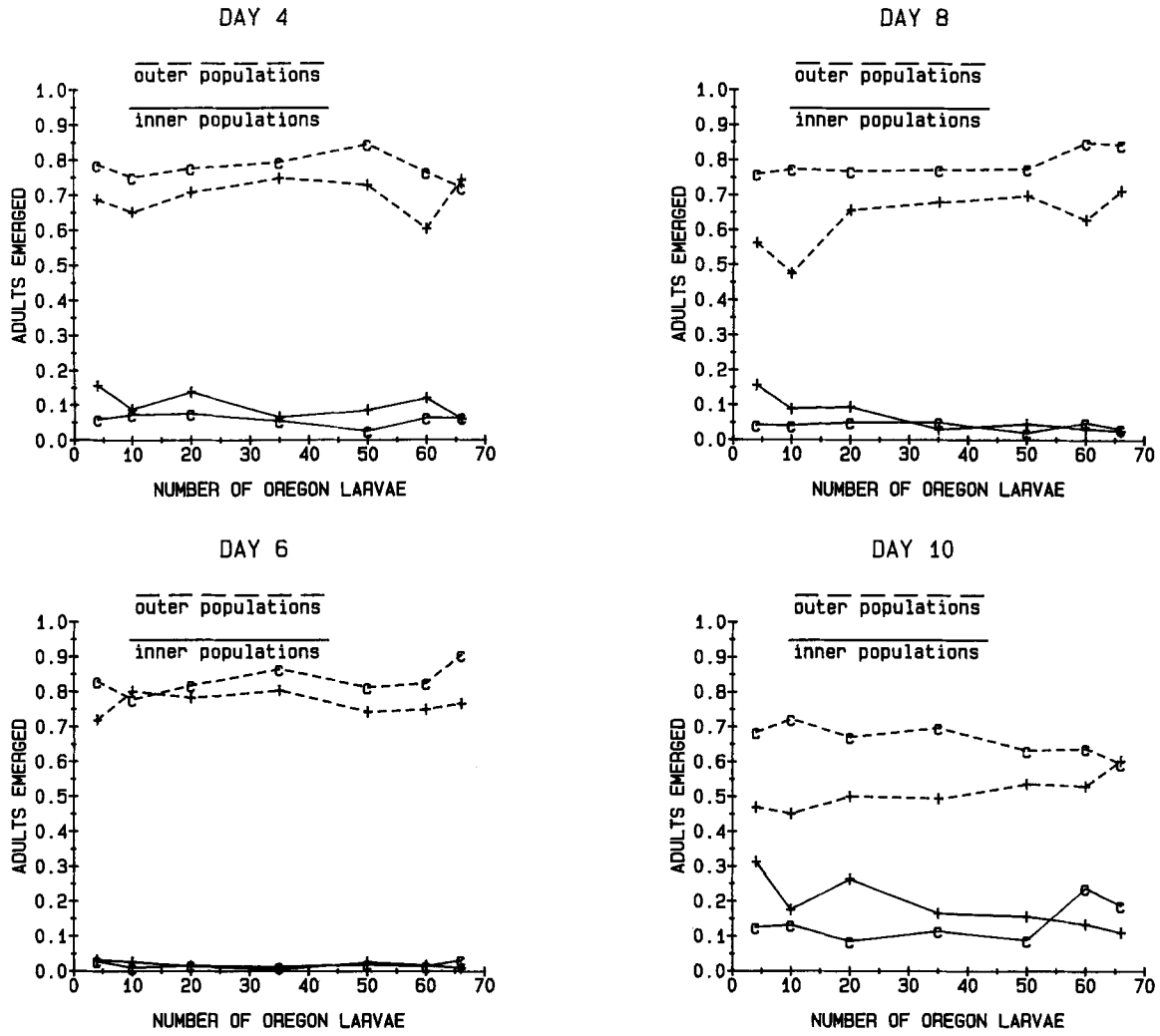

Fig. 2. Mean proportions of adults emerged in inner and outer populations for both strains (cardinal: c; Oregon: +). See Table 11 for values of slopes and their significances

is tested for independence in a three-dimensional contingency table (Sokal and Rohlf 1981). Table 5 shows this analysis of independence in the overall fate of the larvae. A noteworthy result is that there is no independence between genotype frequency and larval fate. If we particularize for the different overfeeding times, the same lack of independence between composition of the competition cultures and fate of the individuals is observed (Table 6). The lack of independence increases with the day of overfeeding, and a heterogeneity test shows those differences to be real. Table 5 also indicates a lack of independence between overfeeding time and larval fate, indicating a shift to more inner-dead individuals with later overfeedings.

The lack of independence among genotypic composition and larval fate presumably indicates the presence of frequency-dependent survival of the genotypes. To further analyze this, a three-way analysis of variance of percentage emergence was performed for the seven mixed genotypic compositions. Table 7 shows the results for both inner and outer populations together. Day of overfeeding (O) and genotypic composition (F) have a strong, significant influence on survival of larvae. Surprisingly, there is no significant difference for the main effect genotype (G); i.e., no difference in percentage survival between the strains can be detected. However, there is a strong genotype × genotypic composition interaction (G × F); the other interactions are not significant. This first order interaction indicates that the input composition influences the survival of both genotypes differently, thus indicating frequency-dependent selection. The strong G × F interaction prevents the detection of any differences in the overall survival between both strains.

The different behavior of Or and *cd* throughout the competition process is reflected in the two analyses of

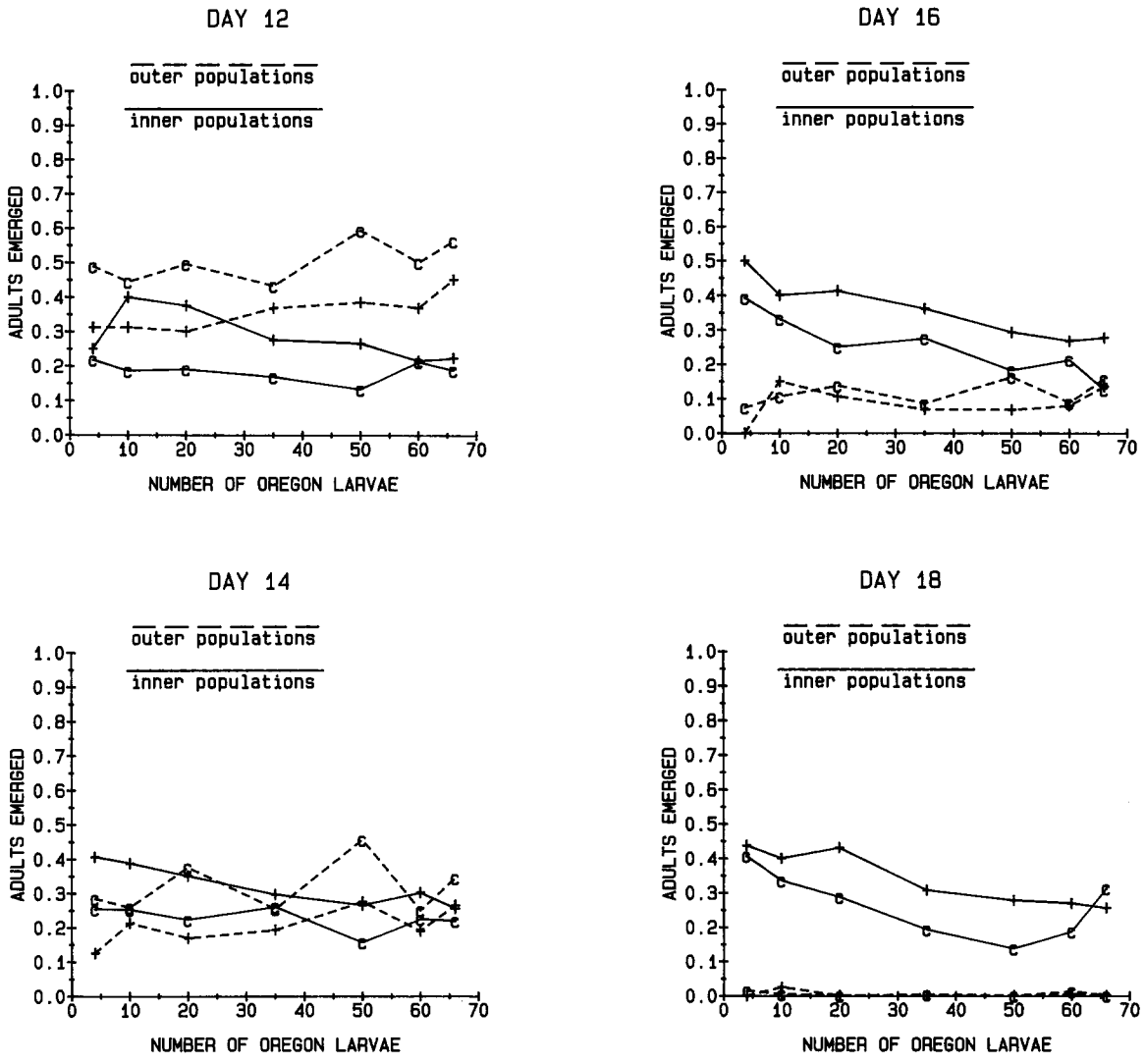

Fig. 2

Table 5. G-values for independence in three-dimensional tables with genotype frequency (7 classes), overfeeding day (8 classes) and larval fate (4 classes: inner-adults, inner-deads, outer-adults and outer-deads) as dimensions

Hypothesis	d.f.	G-value
Frequency × overfeeding	18	0.0[a]
Frequency × fate	42	365.6*
Overfeeding × fate	21	14,220.6*
Interaction	126	314.0*
Frequency × overfeeding × fate	207	14,900.2*

[a] For each genotypic frequency and overfeeding day the total number of individuals is always the same (70×8 replicates = 560). For that reason there is absolute independence between frequency and overfeeding

* $P < 0.005$

Table 6. G-values for two-dimensional contingency tables with genotype composition (7 classes) and larval fate (4 classes) as dimensions for each overfeeding day separately considered

Overfeeding day	d.f.	G-value
4	18	72.7*
6	18	79.1*
8	18	60.9*
10	18	94.6*
12	18	73.4*
14	18	90.7*
16	18	102.3*
18	18	106.0*
	144	679.7
Heterogeneity G	144–42 = 102	679.7–365.6 = 314.1*

* $P < 0.005$

variance that appear in Tables 8 and 9. Table 8 shows the same three-way analysis of variance as Table 7, but for the percentage of survivors in the inner subpopulations. Table 9 shows the same three-way analysis of variance as Tables 7 and 8, but for the percentage of survivors in the outer subpopulations. The effects of overfeeding day (O), genotype (G), and genotype frequency (F) are clear in both populations. The significant interaction that is found in Table 7 between genotype and genotypic composition disappears in both Tables 8 and 9.

The disappearance of the G×F interaction when the inner and outer populations are analyzed separately indicates that these populations are behaving differently with respect to both genotypes. In the inner population, the overall emergence of Oregon is higher than that of cardinal; in the outer population the difference is reversed (Table 1). Figure 2 shows that more cardinal than Oregon individuals emerge from the outer population, while the reverse is true for the inner population. Since in the early overfeeding days the total emergence corresponds to the emergence in the outer population, and in the later overfeeding days the total emergence corresponds to the inner populations (Fig. 2), and since the emergence on the later overfeeding days shows a slope with the input frequency of Oregon that is not present in the early days (Fig. 1), the G×F interaction for total survival must have been a consequence of the summation of survival over inner and outer populations, with their different composition of surviving adults. This differential distribution of Oregon and cardinal over inner and outer subpopulations is further analyzed in 2×2 contingency tables for each overfeeding and frequency combination (Table 10). This analysis shows a clear lack of independence at the later overfeeding days; for the more skewed genotype-frequency compositions, the power of the test is too low to show this lack of independence.

The time spent in competition conditions affects viability. The question is whether the degree of frequency-dependence of viability, as shown to exist in Tables 7, 8 and 9, depends on time spent in competition. In order to answer this question we analyzed the slopes of percentage of adults emerged per frequency composition for the inner and outer populations (Table 11). For the early days of overfeeding this slope was not significantly different from zero for both strains. From day 8, the slopes for the Oregon strain differed significantly from zero; this means that the number of Oregon adults emerging in the inner population decreased with the number of Oregon larvae seeded (negative slopes), whereas in the outer population this number increased significantly (positive slopes). The interpretation of this is that Oregon larvae start feeling the effect of competition between days 6

Table 7. Three-way analysis of variance, 10×2×7 with 8 replicates, arcsin transformed on percentage of adults emerged

Source of variation	S.S.	d.f.	M.S.	F
Overfeeding day (O)	68,785.5	9	7,462.8	17.35*
Genotype (G)	19.5	1	19.5	0.04 ns
Genotype composition (F)	15,463.5	6	2,577.3	5.85*
O×G	2,976.3	9	330.7	0.75 ns
O×F	6,087.3	54	112.7	0.26 ns
G×F	400,845.8	6	66,807.6	151.70*
O×G×F	2,974.0	54	55.1	0.13 ns
Error	431,580.3	980	440.4	
Total	928,732.2	1,119		

* $P<0.001$
ns: not significant

Table 8. Three-way analysis of variance, 9×2×7 (9=8 overfeeding days plus the 0.5 ml control; 2 genotypes and 7 genotypic compositions) with 8 replicates, on percentage of Or and *cd* adults emerged (arcsin transformed) in inner subpopulations

Source of variation	S.S.	d.f.	M.S.	F
Overfeeding day (O)	99,966.5	8	12,495.8	82.86*
Genotype (G)	8,118.2	1	8,118.2	53.44*
Genotypic composition (F)	8,391.2	6	1,398.5	9.21*
O×G	2,358.6	8	294.8	1.94 ns
O×F	4,905.3	48	102.2	0.67 ns
G×F	1,510.2	6	251.7	1.66 ns
O×G×F	3,695.4	48	77.0	0.51 ns
Error	133,976.3	882	151.9	
Total	262,921.7	1,007		

* $P<0.001$
ns: not significant

Table 9. Three-way analysis of variance 9×2×7 (9=8 overfeeding days plus the 5.0 ml control; 2 genotypes and 7 genotypic compositions) with 8 replicates on percentage of Or and *cd* adults emerged (arcsin transformed) in outer subpopulations

Source of variation	S.S.	d.f.	M.S.	F
Overfeeding day (O)	502,637.8	8	62,820.7	387.42***
Genotype (G)	10,243.3	1	10,243.3	63.16***
Genotypic composition (F)	3,134.0	6	522.3	3.22**
O×G	3,360.8	8	420.1	2.59*
O×F	5,939.3	48	123.7	0.76 ns
G×F	577.5	6	96.3	0.59 ns
O×G×F	5,617.0	48	117.0	0.72 ns
Error	143,036.5	882	162.2	
Total	674,546.2	1,007		

* $P<0.05$; ** $P<0.01$; *** $P<0.001$
ns: not significant

Table 10. G-test values from two-dimensional contingency tables with genotype (2 classes, Or and *cd*) and place of adult of emergence (2 classes, inner and outer) as dimensions for each overfeeding day and genotypic composition. The G-values are always for one degree of freedom

Overfeeding	Number of Oregon larvae						
	66	60	50	35	20	10	4
4	0.01	9.14***	0.34	0.24	12.84***	0.26	0.50
6	4.40*	0.43	2.23	0.18	0.00	0.00	0.32
8	1.28	0.01	0.02	0.54	7.95**	2.18	2.46
10	0.01	2.45	12.28***	7.16**	15.03***	0.01	0.83
12	0.35	3.19	9.51***	7.67**	32.38***	7.02**	2.99
14	0.13	6.59**	6.02*	2.39	30.33***	1.91	3.62
16	17.19***	0.04	9.68***	1.72	9.86***	0.01	7.91**
18	not sufficient data for analysis						

* $P<0.05$; ** $P<0.01$; *** $P<0.005$

Table 11. Slopes for mean percentage emergence of both strains vs. initial genotypic composition. Both types have been plotted with their own frequency as the X-axis. If slopes of cardinal vs. frequency of Oregon are wanted, change the signs on cardinal slopes. The (*) after a slope indicates that it is significantly different from zero. The first F-value column stands for the difference between Or and *cd* slopes plotted versus their own initial frequency. The second F-value column is for the same difference plotted versus the Oregon strain initial frequency

Day of overfeeding				
Inner populations	Cardinal	Oregon	F-value (own)	F-value (Or)
4	0.05490	−0.09080	6.671*	0.405
6	−0.00897	−0.04122	0.474	1.149
8	0.04018	−0.15633*	13.534**	4.728*
10	−0.04643	−0.14237*	2.187	8.470**
12	0.11018*	−0.11270*	13.715**	0.002
14	0.09122	−0.14950*	62.245***	3.649
16	0.21472*	−0.18463*	82.777***	0.470
18	0.18546*	−0.16898*	19.611***	0.042
0.5 ml control	0.20320*	−0.11370*	89.506***	7.139**
Significance of difference among 9 slopes	$P<0.001$	$0.10<P<0.25$		
Outer populations				
5.0 ml control	−0.01069	0.02914	1.195	0.256
4	−0.00655	0.04448	0.567	0.313
6	−0.07707	0.01701	3.117	1.270
8	−0.08895	0.12750*	14.730**	0.467
10	0.04500	0.14596*	2.850	10.197**
12	−0.06018	0.15609*	14.103**	2.774
14	−0.00460	0.14326*	2.392	2.104
16	−0.07335	0.14835*	3.930	0.450
Significance of difference among 8 slopes	ns	ns		

* $P<0.05$; ** $P<0.01$; *** $P<0.001$
ns: not significant

and 8. Oregon larvae that are still in their third instar on day 8 or later do not pupate, but migrate as soon as the overfeeding is made. Moreover, we can see that almost all the Oregon larvae that disappear from the inner subpopulations become adults in the outer subpopulations, as the difference between the absolute values of inner and outer slopes is almost zero.

Cardinal behaves differently from Oregon. The values of the slopes for cardinal in Table 11 are with the frequency of cardinal on the X-axis. Until day 12 the slopes are zero, but after day 12 they are positive in the inner subpopulation. This shows that cardinal larvae do better the more cardinal larvae there are, while the Oregon larvae do worse the more Oregon larvae there are. Therefore, competition is not simply a question of being inhibited by many larvae of your own type, but of being affected by strong competitors. The effect of increasing the frequency of Oregon is negative for both cardinal and Oregon.

The more Oregon larvae seeded, the fewer the number of cardinal adults which emerge in the inner population but, in contrast to the Oregon larvae, they are not recovered in the outer subpopulation. This is shown again by the difference among the absolute values of slopes in inner and outer populations for the cardinal strain. The initial frequency does not affect the recovery of cardinal adults in the outer populations, as the cardinal slopes are not significantly different from zero.

From Fig. 1 and Table 11 it becomes apparent that cardinal loses its initial advantage, in total survival, over Oregon by the same day (overfeeding at day 12) that the emergence of cardinal becomes frequency dependent in the inner population. Thus, the Oregon strain is the active agent in competition.

Discussion

The technique of overfeeding larvae from crowded competition cultures onto a surplus of food provides a tool for studying the process of larval competition in time. For a density of 70 larvae on 0.5 ml of food, no effects of competition can be discovered until overfeeding on day 8. The first indication of the adverse effects of competition is the decrease of the percentage of the Oregon larvae that emerge in the inner population, as a function of the input frequency of Oregon and cardinal larvae, and the increase of the percentage of the Oregon larvae that emerge in the outer population. The overall emergence of Oregon over both populations is only a little lower than on overfeeding days 4 and 6, and does not depend upon input frequency. From overfeeding day 10, the total number of individuals recovered as adults decreases with time spent in the crowded inner (0.5 ml) culture (Fig. 1). From overfeeding day 12, the number of individuals recovered as adults in the inner culture remains about the same, for both strains, while the number of individuals recovered as adults in the outer culture decreases progressively. The interpretation is that by day 12 all individuals that can succesfully pupate in the inner population have done so; the remainder have stopped growth (Ménsua and Moya 1983). The larvae that have stopped growth can survive, but as time goes on the probability of death increases, and by overfeeding day 18 few stopped larvae are in a condition to reach the adult stage, even if then presented with surplus food.

From the time that competition makes itself felt, the percentage of adult emergence in one of the subpopulations depends on the initial genotypic composition (Tables 7, 8 and 9). The strain Oregon is affected by competition by day 8; the strain cardinal by day 12. This frequency dependent survival will lead to frequency-dependent selection; only on day 10 is this frequency-dependent selection of the type that might lead to stability.

Since the input frequency of Oregon larvae affects Oregon and cardinal in the same way, Oregon and cardinal share the same niche. If Oregon and cardinal had separate niches, both would be adversely affected by increasing the frequency of their own type. Instead both are similarly affected, being depressed by high Oregon numbers. The strain cardinal has some advantage in viability over Oregon at short duration of competition (overfeeding days 4–8). This advantage under non-competitive conditions disappears under competition. At overfeeding day 12 and later overfeedings, an increase in input frequency of Oregon larvae depresses the emergence of the cardinal strain in the inner population. But no frequency effect can be found on the cardinal adults recovered from the outer population, and the conclusion seems to be that cardinal larvae are more likely to die under crowded conditions than Oregon larvae.

The time-series of duration of competition may very well mimic a density-series. In that case, frequency dependent effects on viability depend for their existence on higher densities.

We propose a hypothesis regarding the population dynamics in highly crowded conditions: with respect to the arrest of larval development, it is a common response in *Drosophila.* It occurs in all the strains and species examined in our laboratory (Botella and Ménsua 1986; Moya et al. 1986). It can be seen as an optimal response to competitive situations (Gill 1978) related to some selective strategy. This proposal is supported by the following: (1) there are larvae that develop fast and, hence, avoid the stop in development; (2) most other larvae enter into arrested development; and (3) the population whose development has been arrested pupates gradually. The developmental arrest may be followed by death during the larval stage, death during the pupal stage, or recovery and emergence of adults. Unfortunately little information is available about the population dynamics of arrested larvae. Environmental factors, partly determined by larvae (e.g. increasing concentration of biotic residues), as well as genetic factors (e.g. differences in "detoxication" ability) may determine the dynamics of the population (Botella et al. 1985).

We think that qualitative or quantitative differences in the biotic residues secreted by different genotypes may be a factor contributing to frequency-dependent viability, as argued by Huang et al. (1971) and Kojima and Huang (1972). However, this is not necessary. Frequency-dependent viability can be explained even if the differences between competing genotypes are only quantitative with respect to two processes: the rate at which the residues are secreted to the medium, and the rate at which detoxification is achieved. This explanation is similar to the one proposed by De Jong (1976), i.e., that the dynamics leading to frequency-dependent selection depend on the initial amount of food and on the genetic properties of the competitors (feeding rates and minimal requirements for pupation). The genotype influences the speed of exhaustion of the food and causes frequency-dependent viability. However, food is progressively depleted and degraded (Botella et al. 1985), and the response of the different genotypes is different as they ingest the available food.

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