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Rearing Pieris brassicae (L.) on a phospholipid and vitamin-supplemented semi-artificial diet

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The standard semi-artificial diet of a laboratory culture of *Pieris brassicae* was supplemented with phospholipid and vitamins B and E. Pupal yield, larval growth rate and pupal weight were followed over three generations and checked again in the 12th and 26th generations after continuous rearing on the supplemented diet. Larval growth rate became significantly faster, the average pupal weight decreased through several generations to a lower stable level, and mating activity showed an approximately fivefold increase. The youngest instars showed notable mortality, probably unconnected with diet.

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1. Introduction

The Large White, *Pieris brassicae* (L.), has been reared in our laboratory since 1960 (Junnikkala 1966). Originally the stock was maintained on cabbage, but since 1968 on the semi-defined diet of David & Gardiner (1966), using the type containing 1.4 % (w/w) of cabbage-leaf powder, 0.25 % (v/w) of linseed oil, and 0.21 % of veterinary grade aureomycin. In general, the semi-defined diet has proved a good substitute for cabbage. This has been checked with parallel rearings for several biochemical parameters of the haemolymph, e.g. proteins (van der Geest 1968), amino acids, and total and protein nitrogen (Junnikkala 1969). However, continuous rearing on the standard diet has possibly contributed to the low mating frequency observed in winter, as well as to slower larval development in comparison to cabbage-fed parallels, as has been noticed before (David & Gardiner 1966, van der Geest 1968, and Junnikkala 1969). Van der Geest & Sloog-Hoebe (1972) reported even slower development after continuous rearing of *Pieris brassicae* on the standard diet, and proposed that the vitamin content of the diet should be doubled. Turunen (1976) showed the beneficial effect of vitamin E in increasing linolenate accumulation in *Pieris* larvae. Further, Kastari & Turunen (1977) suggested that the present diet supplemented with seed oil has an unbalanced lipid composition for *Pieris* and suggested that the availability of essential seed oil fatty acids from the diet could be improved by addition of phospholipids.

The main purpose of changing the diet was to render the *Pieris* stock more homogeneous and to improve the readiness of the adults to mate.

2. Material and methods

The test animals were from our laboratory-reared stock of *Pieris brassicae* and were maintained at +23° ± 0.5 °C, 65 % ± 5 % R.H., and a daily 18L : 6D light regime (Junnikkala 1966, Turunen 1974). Effects of any thermal patchiness in the room were avoided by keeping the beakers for the tests and for their respective controls mixed on the same tray throughout the rearing period.

The linseed oil-supplemented standard diet of David & Gardiner (1966) was used to rear the controls and is here referred to as *diet S*. In addition the following modifications were prepared: *diet V* — as diet S, but with double the amount of B vitamins plus choline chloride, and with 0.03 % (v/w) of vitamin E (DL-α-tocopherol, Merck); *diet P* — as diet S,but with addition of 0.14 % (w/w) commercial grade lecithin (L-α-phosphatidyl choline from soy beans, Sigma No. P-5638); and *diet P-V* — as diet S, but including all the additions to diets V and P. All the diets for one generation were prepared at one time and stored in plastics bags at —18 °C until used within at most 3 weeks.

Scattered eggs and small clusters, which tend to be sterile, were discarded, and only relatively large, compact egg groups were chosen and photographed to facilitate accurate counting of the number of eggs per cluster. After hatching the groups of larvae were transferred to freshly thawed diets in 50-ml beakers. Larvae were transferred to fresh food in 250-ml beakers at the third instar and again at the beginning of the fifth instar, when the groups were divided to give a final density of 15 larvae or less per beaker. Pupation was observed twice a day and the pupae were weighed at the age of 4 days.

3. Results

In a preliminary test several batches of larvae were reared on the four test diets. The different batches seemed to show striking overall differences in ability to settle and grow on the diets. On all types of food, some batches manifested high mortality (especially among first instar larvae), postponed pupation, and a poor yield of pupae. This variability even on the same type of diet made comparisons difficult. However, even these preliminary rearings indicated certain tendencies. Thus, several batches of larvae grew faster on diets V and P than on the control diet S, and development was most rapid on diet P-V. On diet S the larvae proceeded to pupation in 17.5 days, and on diet P-V in 15.0 days on average. The same tendency was observed in pupal weights. Controls were generally lightest, and those reared on diet P-V were heaviest (334 mg and 358 mg, respectively).

Because these tests were devised for practical purposes only, the V and P diets were ignored in subsequent experiments, and further comparisons were made between cultures reared on the control diet S and on the most promising modification, diet P-V. The variability between individual batches made it necessary to devise a more rigorous comparison between the two diets. Therefore each large egg cluster from parents fed on diet P-V was divided by gently tearing the leaf in two to give approximately the same number of eggs on each half. These sub-batches were then reared on different diets (Table 1).

As a whole, the larvae from different batches reacted more uniformly than in the preliminary experiment. The results suggested no superiority of larvae fed on diet P-V; on the contrary, those of the same batch reacted in a remarkably uniform manner, irrespective of diet. The possibility of somewhat faster development and heavier pupae on diet P-V was tested in the following generation by separating the parent pupae according to the type of diet on which they were reared (Tables 2 and 3).

Table 1. Pupal yield, larval growth rate, and average pupal weights of *Pieris brassicae* reared on diet S or diet P-V. The batches of larvae compared were from divided egg groups. One parental generation had been reared on diet P-V. The number of eggs at the beginning of the experiment is given in parentheses.

Yield of pupae from 3rd instar larvae (%)		Time at 50 % pupation, days		Average pupal weight (mg) at age 4 days	
S	P-V	S	P-V	P-V	S
100 (22)	92 (32)	16	15	375	384
61 (31)	50 (38)	15.5	15.5	385	380
94 (39)	100 (39)	16	16	347	363
63 (52)	56 (51)	15.5	15.5	393	399
78 (31)	61 (28)	15.5	15.5	333	340
91 (42)	100 (41)	15	14.5	372	370
96 (38)	100 (21)	15.5	15.5	383	382
Mean					
83	80	15.5	15.4	370	374

Table 2. Pupal yield, larval growth rate, and average pupal weights of *Pieris brassicae* reared on diet S or diet P-V. The batches of larvae compared were from divided egg groups. Two parental generations had been reared on diet P-V. The number of eggs at the beginning of the experiment is given in parentheses.

Yield of pupae from 3rd instar larvae (%)		Time at 50 % pupation, days		Average pupal weight (mg) at age 4 days	
S	P-V	S	P-V	S	P-V
100 (30)	100 (32)	16	15	413	413
100 (39)	97 (45)	16.5	16.5	364	345
100 (36)	96 (28)	16.5	16.5	372	381
100 (57)	98 (68)	15	14	367	382
76 (40)	94 (34)	16	15.5	362	360
88 (27)	64 (30)	15.5	14.5	381	399
Mean					
94	92	15.9	15.3	377	380

Table 3. As Table 2, but the parental generation had been reared on diet S, the previous generation on diet P-V. The number of eggs at the beginning of the experiment is given in parentheses.

Yield of pupae from 3rd instar larvae (%)		Time at 50 % pupation, days		Average pupal weight (mg) at age 4 days	
S	P-V	S	P-V	S	P-V
88 (21)	84 (39)	17	16	370	402
96 (35)	92 (45)	16.5	16	361	347
46 (28)	57 (28)	18	15.5	303	389
100 (33)	100 (35)	17.5	17	365	330
90 (34)	96 (37)	16.5	15.5	352	373
100 (41)	93 (40)	16.5	17.5	353	335
Mean					
87	87	17.0	16.3	351	363

Tables 1 and 2 show the results for cultures differing only in the number of generations for which the parents had been reared on diet P-V. Parents reared on diet P-V produced offspring which reacted to the two diets in much the same way. However, if the same third generation is examined in Tables 2 and 3 the situation appears different.

Parents reared on diet S produced offspring that were clearly inferior when fed on diet S, as compared with the line reared continuously on diet P-V (Table 2). The difference is shown in larval growth rate, taking 15.3 ± 0.42 days on diet P-V, against 17.0 ± 0.25 days on diets S ($P < 0.01$). The same trend was seen in the fresh weights of 4-day-old pupae: 380 mg in animals reared on diet P-V against 351 mg in parallels reared on diet S ($P < 0.1$). The P-V diet also seemed to produce a slight improvement in the offspring of animals from the same parents reared on diet S (Table 3).

To find out whether the evidently beneficial effect would last for several generations, the same parameters were checked in the 12th generation after continuous rearing on diet P-V (Table 4).

If these results are compared with those for the corresponding parameters in Table 2, the yield of pupae can be seen to remain good. Larval growth rate is not significantly slower. Pupal mean weight, however, has undergone a statistically highly significant decrease from 380 mg in the 3rd to 324 mg in the 12th generation. In the 26th generation there were no further changes; larval development proceeded to pupation in 15.5 days, with a pupal mean weight of 322 mg.

In addition to these experiments, a preliminary test of the readiness to mate was made during the darkest season (December) when the stock reared on diet P-V was in its 9th generation. Daily matings were counted among some 40 potential pairs kept under identical conditions during a flight period of 2 weeks. Altogether 7 copulations were counted in the S group and 44 in the P-V group.

Table 4. Pupal yields, larval growth rates, and average pupal weights of *Pieris brassicae* in the 12th generation after continuous rearing on diet P-V. Number of 3rd instar larvae in parentheses.

Yield of pupae from 3rd instar larvae (%)	Time at 50 % pupation, days	Average pupal weight (mg) at age 4 days
95 (84)	15.5	345
94 (66)	15.5	346
97 (85)	16	295
98 (55)	16.5	313
100 (45)	16	320
Mean		
97	15.9	324

4. Discussion

The purpose of supplementing the standard diet was to achieve a higher degree of homogeneity in the growth of larvae of *Pieris brassicae* in our laboratory stock. The data presented clearly demonstrate the need for such homogeneity. Its lack is probably due in part to variation in larval settlement on and acceptance of the food. Well-synchronized hatching of the eggs is usually followed by successful and synchronous settlement on the food in dense aggregations. In *Pieris,* according to Long (1955) and Zaher & Long (1959), mass behaviour of this kind forms the basis for synchronous feeding, which in turn leads to faster development than in solitary larvae of the same species. Hence, the events in early larval life, including settlement on the food, are of crucial importance for later synchrony and homogeneity of the test material.

The larval growth rate appears to show a highly sensitive response to changes in the rearing method. Even on diet P-V the rate of larval development (15.3 days) is slower than on cabbage, but the difference is only about 1 day, and compared with the rate on diet S (17.0 days), the difference is highly significant. Measurements in the 12th and 26 th generations further showed that the larval growth rate had remained stable, and there was no gradual shift towards slover development (as found by van der Geest & Sloog-Hoebe, 1972, in larvae reared on the standard diet).

Pupal weight at a certain age is a poorer basis for evaluating development than the rate of larval growth, owing to the great variation among different batches. However, insects reared on diet P-V showed a definite trend towards lighter pupae. If comparisons are based on the mean weight of 380 mg in the 3rd generation (Table 2), a statistically highly significant reduction to 324 mg is seen in the 12th generation. The mean value of 322 mg in the 26th generation, however, indicates that pupal weight has already reached a stable level by the 12th generation, there being no further reduction. Such a trend may have been caused wholly by selection. According to Turunen (1976), Kastari & Turunen (1977), and Turunen (1978), added vitamin E and phospolipid both restrict accumulation of neutral lipids, so directing development towards lighter pupae. Whether a trend in this direction is considered good or not, is an open question, although it seems to be a step towards a more natural lipid balance.

The pupal yields in these experiments are low if estimates are based on numbers of eggs, but quite satisfactory if they are based on numbers of 3rd instar larvae. In both cases pupal yields are good enough to enable continuous maintenance of the stock. In the 26th generation mating activity is still as good as in the 9th generation. Hence breeding in captivity is successful at this most vulnerable stage even during the darkest season.

The present results suggest that the lecithin-vitamin supplemented diet accelerates larval growth, but after some generations produces lighter pupae. The benefit, or potential usefulness of these findings is difficult to evaluate. At least the effect on mating is favourable. Further efforts to improve the stock should probably concentrate on factors improving hatchability and early larval settlement, thus ensuring a firm basis for good synchrony in larval development.

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