Comparison of Nutrient Reservation in Apterous and Alate Pea Aphids, *Acyrtosiphon pisum* (HARRIS)¹

1. Developmental Time and Sugar Content

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Characteristics of crowded (mainly alate) and isolated (apterous) populations of the pea aphid *Acyrtosiphon pisum* were compared. The total developmental time in alatae was longer than that in apterae, the difference due to the extended fourth instar in the former. There was no significant difference in larval body weight between isolated and crowded insects in the first 3 instars, whereas the apterous fourth instar larvae and adults were heavier than the alate ones. Apterous parents produced a larger number of larvae than alate parents. No significant difference in total free sugar content was observed between isolated and crowded aphids. Crude glycogen content, however, decreased significantly near the time of adult emergence, especially in alatae. This may be due to glycogen utilization in wing development.

INTRODUCTION

Wing dimorphism is characteristic of aphids. Crowding of mothers and postnatal larvae invokes the appearance of alate aphids (SUTHERLAND and MITTLER, 1971; KAWADA, 1987). Not only tactile stimulation (LEES, 1967; KAWADA, 1987) but also the food quality is important in determining the wing forms in aphids (SUTHERLAND, 1969; FORREST, 1970; MITTLER and KLEIJAN, 1970; DIXON and GLEN, 1971). The relative proportion of apterae and alatae is influenced by the concentrations of sugar and amino acids in the diet which mothers and offspring receive (FORREST, 1970; RACCAH et al., 1972; PARRY, 1977; HARREWIJN, 1978). Phloem sap contains high concentrations of sucrose as a predominant carbohydrate (ZIMMERMANN and ZIEGLER, 1975; HAYASHI and CHINO, 1986), but most of it is excreted. Sucrose is also valuable as a phagostimulant; it augments food uptake at concentrations of 10–20% in synthetic diets (MITTLER, 1967). In *Myzus persicae* and *Brevicoryne brassicae* the high concentration of sucrose induces an increase of larviposition and also enhances wing production in the progeny (RACCAH et al., 1972; WHITE, 1972). Sugar is obviously important in the development, fertility and wing formation of aphids, and carbohydrate reservation in tissues may be influenced by the quantity of sugar in the diet,

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but the differences in the carbohydrate content of alate and apterous aphids are unclear.

To research the possible role of carbohydrates as a nutrient factor regulating wing form, carbohydrate content as well as the developmental time and fertility were compared between crowded (mainly winged) and isolated (wingless) populations of the pea aphid. Furthermore, carbohydrate content was compared between the apterous aphids produced by apterous and alate parents.

MATERIALS AND METHODS

Aphid rearing. An apterous adult was collected from a leaf of *Vicia sativa* L. on our institute ground in May, 1975 and cultured parthenogenetically on seedlings of the tick bean, *Vicia faba* L., at 12.5°C, 16L-8D photoregime as stock cultures. Bean seeds were soaked in tap water until germination, and then planted in sand. Five cm seedlings were used. They were pruned at the lowest modified stipple to prevent growth and transplanted into plastic pots (7.5 dia. × 8 cm ht.) filled with sand. Apterous parents taken singly or in groups of 20 from the stock cultures were placed on individual seedlings, which were then covered with glass tubes (2.4 dia. × 10 cm ht.) with nylon meshes on the tops to prevent contamination and kept at 12.5°C under 16L-8D. The newborn larvae produced by the apterous parents reared in crowded and isolated conditions were collected separately every 12 hr. The larvae taken in groups of 20 from the crowded batches and singly from the isolated batches were transferred to new lots of pruned bean seedlings. During maintenance, alate adults obtained from crowded rearing and dead insects were discarded and the same number of apterous aphids of the same age were introduced from spare crowded batches. Among the successive batches the newborn larvae collected 3 to 14 days after deposition of the first larvae were used for stock maintenance. Lineages thus propagated through apterous parents were maintained in either crowded or isolated conditions for more than 3 generations. Thereafter, new larvae produced by crowded and isolated apterous parents were reared one of the 2 condition types and used for experiments. The alatifform development rate in the crowded larvae ranged from 70% to 80%. Isolated and crowded day 0 larvae were offspring born within 15 and 18 hr, respectively, after individual parents had been placed on new plants. Since wing buds of alatifform larvae were clearly visible at the third instar, the third and fourth instar crowded-rearing larvae were divided according to the 2 wing forms and used individually. Apterous offspring produced by alate parents occurring in crowded conditions the fourth or fifth generation were reared singly on the pruned bean seedlings. Development was indicated by duration of each larval instar and body weight changes. Larval moults were checked by observing exuvium at 8 hr intervals. Isolated and crowded larvae at 2 days after each moult were used. Adults were used 2, 4, 8 and 21 days after emergence. Fertility was measured by the number of newborn larvae produced by an alate or apterous parent.

Carbohydrate contents. After weighing, 50 to 120 larvae or 25 to 55 adults were homogenized in 5 ml of 80% ethanol and heated at 80°C for 5 min. The mixture was then refrigerated overnight and centrifuged at 3,000 rpm for 10 min. The supernatant was withdrawn and washed with 5 ml of 80% ethanol. The precipitate was suspended in 5 or 10 ml of 5% trichloroacetic acid (TCA) and boiled in water for 10 min. The suspension was centrifuged as above and the pellet was discarded. The combined

ethanol supernatant and the TCA fraction were measured for sugar content by the anthrone-sulfate method (Tsumuki and Kanehisa, 1978). Results were expressed as the total free sugar and crude glycogen contents, respectively.

RESULTS

Developmental time and fertility

The larval developmental time during the first 3 instars was similar between the isolated and crowded conditions which induced predominantly apterae and alatae in the adult stage, respectively (Figs. 1 and 2). However, the duration of the fourth instar was longer in the alatiiform larvae, as was the alate pre-larviposition period after emer-

Fig. 1. Developmental time and fertility of crowded aphids.

Fig. 2. Developmental time and fertility of isolated aphids.

gence. In addition, the apterous offspring produced by alate parents reared in crowded conditions had similar developmental rates to offspring of the apterous adults reared in isolation. Apterous parents produced larger numbers of offspring than alatae (Figs. 1 and 2).

As shown in Fig. 3, there was no significant difference in larval body weight for the first 3 instars between the isolated and crowded aphids. Furthermore, the weights of crowded apteriform third and fourth instar larvae were similar to those of alatiform larvae at the same ages. However, significant differences in body weight were observed at the adult stage; the weight of apterous aphids was markedly elevated by the time of adult emergence, whereas the weight of alate aphids remained approximately constant. The body weights of offspring produced by alate parents reared in crowded conditions were almost at levels comparable to those from apterous parents reared in isolation, but the adults emerging from the latter group were slightly heavier (Fig. 3).

Carbohydrate contents

Figures 4 and 5 give the mean values of the contents of total sugar and crude glycogen, respectively, of isolated and crowded aphids obtained from 4 to 8 separate experiments. The total free sugar in larvae and adults was approximately constant in both conditions. Crude glycogen content fluctuated remarkably during larval and adult development. Glycogen content in both crowded and isolated larvae increased abruptly after the initiation of feeding. A drastic gap was observed between larvae

Fig. 3. Changing patterns in aphid body weight. Δ : alate aphids (crowded rearing, contained 20–30% apteriform larvae in the first and second instars) produced by apterous parents from crowded rearing (see text), $\circ$: apterous aphids (isolated rearing, 100% apteriform) produced by apterous parents in isolation, $\cdot$: apterous aphids (isolated rearing) produced by alate parents from crowded rearing, $\blacktriangle$: apterous aphids obtained in crowded rearing. Bars represent S.D. of mean.

Fig. 4. Changing patterns in total free sugar content of aphids. See Fig. 3 for symbols.

Fig. 5. Changing patterns in crude glycogen content of aphids. See Fig. 3 for symbols.

and winged adults; the content became as low as one-third after emergence in the latter. Glycogen content slowly decreased in apterous adults, occasionally fluctuated, finally reaching the same level as that in alatae on day 20 after emergence. No significant difference in carbohydrate content was observed between the apterous offspring produced by apterous and alate parents.

DISCUSSION

A. pisum has 4 larval instars, and the developmental time until emergence is longer in alatae than in apterae. The difference is due to the extended fourth instar. Furthermore, the pre-larviposition period is also relatively longer in alate adults, which may adapt for dispersal before larviposition (FRAZER, 1972; TSUJI and KAWADA, 1987). The cumulative number of newborn larvae produced until 35 days after hatch by apterous parents (heavy body weight) is larger than that of alate parents (light body weight). Crowding and diet composition have been defined as possible factors affecting the wing dimorphism of aphids (SUTHERLAND, 1969; MITTLER and KLEINJAN, 1970; SCHAEFERS and JUDGE, 1971; SUTHERLAND and MITTLER, 1971; HARREWIJN, 1978). Sugar and carbohydrates, however, have been given only scant attention. KUNKEL and HERTEL (1975) have reported that *M. persicae* alatae utilize much glucose to build up energy reserves. In our study, total free sugar content was approximately constant during the larval and adult stages, whereas crude glycogen content greatly changed. Although glycogen increased with larval growth, no significant difference was observed between isolated and crowded larvae at early ages including day 0. This result implies that development of wing buds may not burden energetic alate larvae. Consequently, the significance of carbohydrate reservation to the onset of wing formation cannot be discerned on the basis of our results. Glycogen content decreased around the period of larval-adult transformation. The possibility of glycogen being the first fuel utilized by the muscle in flight does not offer a likely explanation for its decrease after the emergence of alate adults, since flight begins 2 or 3 days after emergence (TSUJI and KAWADA, 1987). Therefore, the drastic decrease in glycogen which probably occurs prior to emergence may be caused by utilization of carbohydrates for wing development. This is compatible with the fact that a lower rate in decrease of glycogen was observed in the apterous adults reared in isolation. Approximate amounts of carbohydrate utilization for the wing production may be calculated from the differences in content of sugar and glycogen between new alate and apterous adults. The apparent initial slowing of weight increase of alate adults possibly reflects a higher metabolic rate or consumption of energy required for wing development.

Somewhat similar changes of the developmental time and carbohydrate content were observed between apterous offspring produced by apterous parents in isolation and offspring (fated to be apterae) produced by the alate parents reared in crowded conditions, showing that alate parents may produce offspring at the expense of fertility.

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