Cabbage Aphid: Effect of Isolation on Form and on Endocrine Activity

Abstract. *Isolated wingless cabbage aphids,* Brevicoryne brassicae *(L.), produced only wingless young. Aphids isolated from birth all became wingless, whereas over 50 percent of grouped controls developed wings. Isolation caused hyperactivity of the corpus allatum; this hyperactivity may have caused the wingless form.*

Conditions occurring before or after birth may determine whether aphids will be winged or wingless. Embryos commence development as the more primitive winged form but may develop as the wingless form (*1*). Various environmental factors may induce the production of one form or the other, but the physiological mechanisms are unknown. Winged adults almost invariably produce wingless young, whereas wingless adults may produce either or both forms, depending on environmental conditions.

Crowding of parents or of young is an important factor in production of the winged form of the cabbage aphid *Brevicoryne brassicae* (L.) (*2*). In the aphids *Megoura viciae* (*3*) and *Aphis craccivora* (*4*), relatively brief contact between wingless adults causes them to produce winged offspring even after the parents have been reisolated, but my experiments indicate that the cabbage aphid does not respond to contact with other individuals in the same way that these aphids do.

Cabbage aphids, reared as described (*5*), were removed from the culture plants and placed on artificial diet (*6*) in perspex tubes of 1-cm internal diameter. The tubes were kept in an incubator at 20°C, 70 percent relative humidity, and 14-hour photoperiod. The food was renewed every 2nd day.

Newborn aphids whose parents (wingless) had been crowded on plants or on artificial diet were caged individually or in groups of ten per cage. In every case, aphids isolated from birth became wingless, whereas 50 to 70 percent of those reared in groups became winged. Aphids reared singly developed more slowly than those reared in groups (Fig. 1). When previously crowded aphids were isolated at the beginning of the second instar stage, both forms developed, but the duration of the second instar was greater in isolated aphids (mean 80 hours for grouped aphids, 126 hours for isolated aphids). Thus, isolation in the first instar suppressed development of wings, but ability to respond in this way had been lost by the beginning of the second instar.

When wingless adults were isolated on artificial diet, all their young were wingless. This was true both when the parents had been isolated from the third instar and when they had been taken directly from crowded conditions on plants or artificial diet. Young produced by parents caged in groups of five were usually more than 50 percent winged. If a long series was born to an isolated parent, a few of the later ones became winged, presumably because the mother had been stimulated by the presence of the first young. If young born to isolated parents were crowded soon after birth, a small number (15 to 20 percent) became winged; hence, determination of the wingless form is not always completed before birth when parent aphids are isolated.

A high concentration of juvenile hormone may cause the determination of the wingless form in aphids, and it is known that the corpus allatum is involved in phase polymorphism in locusts and probably caste polymorphism in social insects. Because isolation had such a marked influence on production of wingless aphids, tests were made to see whether this influence was correlated with activity of the corpus allatum.

Wingless adults which had been isolated or caged in groups of five for 5 days and young aphids which had been isolated or reared in groups from birth were fixed in alcoholic Bouin's fluid containing 0.5 percent trichloroacetic acid, sectioned frontally at 4 μ and stained with celestine blue and Mayer's hemalum. The diameters of nuclei in the corpus allatum were observed with an oil-immersion objective and measured with a screw-type micrometer built into the eyepiece of the microscope. The mean maximum diameters of nuclei were compared by *t*-tests, and the standard error of each mean was computed. The mean maximum diameters of nuclei give a more sensitive estimate of activity of the corpus allatum in the cabbage aphid than the volume of the corpus allatum calculated from the measured axes of the gland gives. Wingless adults that had been isolated had more active corpora allata than grouped wingless adults (mean maximum nuclear diameter ± S.E.: isolated 7.8 ± 0.25 μ, grouped 6.2 ± 0.20 μ; $P < .001$).

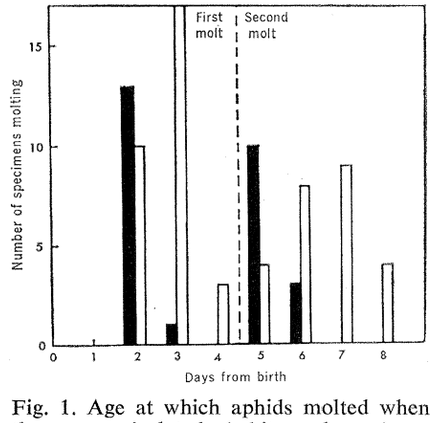

Fig. 1. Age at which aphids molted when they were isolated (white columns) or reared in groups (black columns) from birth.

Young aphids isolated from birth have greater activity of the corpus allatum by the middle of the first instar ($P < .05$) than crowded young, and the difference is greater by the end of the first instar ($P < .01$). During the second instar, no difference in corpus allatum activity was observed. In the third instar and until the end of nymphal life, nymphs with developing wing pads have much lower activity of the corpus allatum than nymphs without wing pads have. All these results were confirmed by a separate series of preparations in which the volume of the corpus allatum was calculated.

The evidence indicates that isolation (an unusual situation for the gregarious cabbage aphid) has immediate and far-reaching physiological effects. These effects are probably all ascribable to endocrine changes mediated by the central nervous system. Hyperactivity of the corpus allatum was observed in both adults and young nymphs when they were isolated. Isolated parents produced wingless young, and isolated young became wingless. This strongly suggests that the suppression of wing development before birth is associated with the increased activity of the corpus allatum in the mother. If the suppression is not completed before birth, it may be reinforced by rearing the young in isolation (or, to a smaller degree, in uncrowded conditions); in this case, it is the higher activity of the corpus allatum of the young aphid itself which brings about its determination as the wingless form. The activity of the corpus allatum was also estimated in the

offspring of winged parents. The wingless characteristic of these offspring was already determined before birth, but their corpora allata were less active at birth than those of the young of wingless parents were ($P < .001$). This may indicate that they are determined prenatally by a high maternal titer of juvenile hormone (*7*) rather than by maternal activation of the corpus allatum in the developing embryos (*8*).

There is apparently a major difference between the response to crowding and isolation of *B. brassicae* and the responses of *M. viciae* and *A. craccivora*. Adults of the last two species can be crowded briefly and then isolated, and as a result of the crowding they produce winged offspring over a considerable period. If the cabbage aphid is isolated, it immediately switches to the production of wingless young, even though it may have been crowded all its life. This response to isolation is so powerful that it overrides, to a great extent, the effects of other conditions which induce the development of the winged form in other species. In experiments on aphid form determination, it has seemed preferable to cage each treated adult separately and to record the effect of the treatment in terms of the number of parents which respond by producing one form of young or the other form (*3, 5*). Our work indicated that use of this method with the cabbage aphid may obscure the effects of other environmental factors; for example, in earlier experiments (*5*), no response to short-term crowding was detected because of subsequent isolation, and the effects of different temperatures were significant only at a low level.

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Amnesia: A Function of the Temporal Relation of Footshock to Electroconvulsive Shock

Abstract. *When rats received a brief footshock upon stepping off an elevated platform, and an electroconvulsive shock 30 seconds or 6 hours afterward, amnesia was not observed 24 hours later. If a second footshock (noncontingent) was delivered 0.5 second before the electroconvulsive shock, amnesia was observed. The amnesia was temporary if conditioning was strong and permanent if conditioning was weak.*

Compelling arguments have been raised for using a procedure of one-trial learning and a single electroconvulsive shock (ECS) to study the effects of ECS on memory. One-trial learning permits accurate control of the training-ECS interval (*1*), and the single ECS avoids the aversive (*2*) and physiological aftereffects (*3*) that accompany multi-ECS treatments. One of the most common single-trial, single-ECS procedures (*4*) uses as its base-level measure the rat's tendency to step down rapidly from an elevated platform. If the animal receives footshock (FS) immediately upon stepping down or an immediate FS followed 30 seconds later by ECS, it retains the avoidance response and remains on the platform during subsequent trials. However, if it receives an immediate FS followed 0.5 second later by ECS, amnesia is produced and the rat tends to step off the platform on subsequent trials.

The temporal relation between FS and ECS is clear: the shorter the interval between FS and ECS, the less retention observed. There are, however, at least two properties of FS that could interact with ECS: FS-produced learning and FS-produced arousal. Thus the question at issue is whether the differential amnesic effect of varying the interval between FS and ECS is a function of the relation between FS-produced learning and ECS or is due to the relation between FS-produced arousal and ECS. If arousal is critical, and if arousal can be manipulated independently of initial learning, it should be possible to deliver ECS 30 seconds after learning and still produce amnesia as long as FS-produced arousal precedes the ECS by 0.5 second.

To test this prediction, we employed an experimental procedure in which two brief FS's were delivered, one contingent upon the step-down response, the other (noncontingent) delivered 0.5 second before ECS. A 30-second period intervened between the offset of the contingent FS and the onset of ECS. If the critical relation is between FS-produced learning and ECS, the 30-second interval between the contingent FS and ECS should permit retention. If, on the other hand, the critical relation is between FS-produced arousal and ECS, the 0.5-second interval between the noncontingent FS and the ECS should produce amnesia.

Male albino rats (total $n = 220$ in the four studies to be described) of the Sprague-Dawley strain, weighing 225 to 275 grams, were trained in an apparatus patterned after one described by Chorover and Schiller (*4*). The apparatus consisted of a 50-cm square box with Masonite walls 37.5 cm high and a grid floor of 0.6-cm stainless steel rods spaced 1.25 cm apart. Located in the center of the box was a 12.5-cm square platform 5 cm high. The grid floor was connected to a Grason-Stadler shocker that was set to deliver a 0.8-ma FS for 2 seconds. The ECS (35 to 50 ma) was administered through ear clips for 0.3 second.

Each rat received one trial per day with the ear clips attached. On each trial the rats were placed on the platform and their step-off latencies were recorded. The first 3 days consisted of habituation trials in which the rats were permitted to step off and to explore the test chamber for 10 seconds. On day 4, 70 rats were divided into five groups. Three of the groups received two FS's (FS--FS conditions), one FS immediately upon stepping off and another FS approximately 27.5 seconds later: the FS--FS group received no ECS; the FS--FS,ECS group received ECS 0.5 second after the second FS; and the FS--FS--ECS group received ECS 30 seconds after the second FS. The rats remained on the grid floor during the 27.5-second FS-FS interval after which the second FS was delivered automatically. Two control groups were employed: the ECS-alone group received ECS 30 seconds after step-off (both FS's were omitted); the NT group received no treatment upon