CAUSES OF DEATH ENCOUNTERED DURING REARING OF *DANAUS PLEXIPPUS* (DANAIDAE)

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In the process of rearing over 1600 *Danaus plexippus* (L.) during the summers of 1964 and 1965, we had an excellent opportunity to observe symptoms which repeatedly led to death during the course of metamorphosis of this insect. Rearing was done in Newton, Massachusetts by Mrs. Brewer, using the biology laboratory and research facilities of Newton Junior College. Diagnoses of abnormal conditions were made by Mr. Thomas at the University of California, Berkeley.

With the exception of a few eggs set aside for experimental purposes which have been previously described (Brewer, 1966), the insects were reared for purposes of tagging and release in migration studies, and every attempt was made to bring as many of them as possible to maturity. All insects reared in 1965 originated from field collected small larvae or from eggs deposited by newly collected adults, as we were unable to carry any individuals of the 1964 strain through the winter.

Only about a third of the original eggs survived to maturity in each year. The greatest loss, and the most difficult to relate to any specific cause, occurred up to and during the third instar. No cannibalism was observed at this stage, beyond damage wrought by newly hatched larvae which sometimes chewed into nearby eggs. This is understandable, since in nature usually only one egg is laid on a leaf by any one female, while in captivity there were up to fifteen on a leaf.

Some larvae were undoubtedly lost during the transfer to fresh food, some drowned, and some just simply disappeared. After the onset of the third instar, however, the symptoms accompanying death became relatively easy to recognize.

Parasitism was held to a minimum. Three or four larvae were parasitized by the tachinid fly *Lespesia archippivora* (Riley). In these cases the larvae suspended, but died prior to pupation. A white maggot issued from each larva, lowered itself to the floor of the rearing cage by a silken thread, and there formed a brown puparium. This was the only parasite we noticed.

Other tachinids and hymenoptera are known to feed upon *Danaus* (e.g., see Thompson, 1945 and Scudder, 1889).

A search of the literature shed light on only one of the conditions with which our animals were plagued—one which we labelled "black death." Symptoms of this condition became evident in these larvae just before

pupation. The larvae migrated to a horizontal surface in the usual manner. A few hours later they died clinging to the surface by one pair of prolegs while both head and abdomen hung limp on either side, and shortly thereafter turned black. Nine larvae in this condition were found in the field in 1963. Seven developed symptoms during rearing in 1964. The symptoms agree with a description by Brown (1927) of larvae of *D. plexippus* which he found had been infected with *Micrococcus flaccidifex danai* (Brown). In 1965 only one of our larvae exhibited these symptoms. Examination of it did not disclose the above microbe, nor did it disclose any polyhedral virus such as that defined by K. M. Smith (1950), which causes similar behavior and symptoms in many insects.

The following conditions were also present during both the first and second year (1964 and 1965).

Rusting.—This condition was so-called because after death the larvae became a rusty orange-brown color. Most were affected in the third instar. The larvae grew at a slow rate and behaved in a generally sluggish manner. Usually they had noticably deep yellow bands and often the thoracic filaments were either longer or shorter than usual. Some individuals showed another symptom which we called "black mask," because the head capsule, instead of being shed, remained glued to the face after moulting. The appearance of the animal in the initial stages of this condition was much the same as that of a normal larva about to moult. The whole area anterior to the thoracic filaments was swollen and misshapen, being at least three times its normal size. Some started to moult and some moulted completely, but in all cases the head capsule turned a glistening black and the larva was unable to cast it off. It soon became a tight button-like protuberance covering and constricting the eyes and mouthparts, while the posterior part of the head remained a bulge. These larvae became moribund, apparently could not eat, and soon died. Not all these larvae became rusty, and not all the rusty larvae bore "masks."

Blurred scaling.—Malformed adults with this condition could immediately be recognized by defective wing scaling. The black scaling of the veins was excessive and had an uneven, blurred, sometimes splotchy appearance. The veins themselves were often crooked. In most cases other abnormalities were also present including unpigmented antennae, malformed palpi, scarred abdomen and nonfunctional proboscis. In most individuals the apices of the forewings were either greatly shortened or greatly produced, and in some the wings were further deformed. The majority of these insects, but not all, emerged from irregular chrysalids which were roughened on the surface, uneven in shape, or whitish in

hue. Most of these adults expired within a few days, and the remainder appeared totally unequipped to survive under natural conditions. In several years of insect tagging we have never encountered any monarchs possessing these symptoms in the field.

Animals suffering from any of the four above described syndromes were found to be infected with *Pseudomonas aeroginosa* (Migula), a bacterium considered a potential insect pathogen in that it has no invasive powers of its own, but will enter the hemocoel and cause a fatal septicemia when an insect has been weakened by some other cause. It is a pathogen which commonly is responsible for secondary infections among insects reared in the laboratory, due to artificial physical conditions, wounding, or cannibalism which results from crowding.

A fifth condition, which was present in both 1964 and 1965, affected certain chrysalids in which the wing and abdominal sections were separated by deep clefts. In some of these it was possible to see the blood circulating beneath a thin membrane. Some of these pupae lived for as long as a week, but none reached the point of adult emergence. Dissection and examination by phase microscopy disclosed no apparent microbial etiology. There was no infection by nematodes, protozoa, fungi, bacteria or viruses. Cultures set in AC Medium[1] produced no microbial growth at all. It seems possible, therefore, that environmental factors may have been the cause of this condition. Malnutrition, insecticides, jolting of the cages at a crucial time, overcrowding, and lack of normal light, heat and humidity are all factors to be contended with in the rearing of insects, and none of these can be entirely eliminated in the cases under discussion except insecticides, against which the animals were zealously guarded.

Genetic factors may also have been involved. Most of the insects reared in 1964 were the second generation, and in 1965 the third generation, the original insects in both years having been found in the field as either eggs or very small larvae. The parent insects were probably, for each group, the offspring of a single female, so that in each case we had an inbred population which arose not by design, but owing to the scarcity of eggs. This may have had a debilitating effect, as indicated in Table I.

The animals collected in 1963 originated from breeding grounds in three states and were collected between July 19 and August 30 in all stages of development from egg to fifth instar. There were not more than 16 to a cage at one time, as opposed to 40 or more to a cage in the other two years.

[1] AC Medium: A Difco product, recommended for the cultivation of both aerobic and anaerobic microorganisms, and used for preliminary cultivation of microorganisms associated with insects.

TABLE I. RELATIVE REARING SUCCESS IN FIELD COLLECTED AND LABORATORY BRED GENERATIONS OF *Danaus plexippus*.

Date	Number	Emerged	Percent
1963 Collected in field	187 (eggs & larvae)	179	95.7
1964 (New stock) second generation inbred	567 (eggs laid by one female)	206	36.6
1965 (New stock) third generation inbred	599 (eggs laid by one female)	189	30.3

If environment and genetics were the primary causes of the high mortality and *Pseudomonas aeroginosa* a secondary cause, it would appear that we have not uncovered any disease of sufficient proportions to be responsible for the great fluctuations in numbers of the monarch from year to year. We hope that these symptoms and their causal agencies will prove useful to other investigators.

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