GRASSHOPPER CULTURE IN THE LABORATORY

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INTRODUCTION

During the winter relatively few insects are available in the laboratory for experimental purposes. Grasshoppers are not generally thought of as suitable for such use since they naturally hibernate during the cold months, and but one generation a year can be procured in most species. There are some forms, however, whose life cycles can be greatly shortened by temperature control so as to yield several generations a year. Furthermore, the application of various temperatures to the pods permits retarding or accelerating their development at will, so that if pods of one laying are divided into groups, each of which is kept at a different temperature, the nymphs can be made to emerge at predetermined intervals over a period of many weeks instead of at one time. This treatment, when applied to several sets, each laid a month or two after the one preceding, ensures a governed supply of living insects in any desired stage of development throughout the year. Such control of the life history has obvious value for experimental purposes.

Carothers ('23)[1] gives a brief account of breeding methods, mentioning a number of species requiring diverse treatments. The purpose of the present paper, therefore, is to describe the breeding technique more fully, in sufficient detail that those unaccustomed to handling such insects may rear them with minimum waste effort. Attention is confined to several forms that require the same treatment, are viable under laboratory conditions, and thrive on lettuce. Their eggs do not require a period

[1] E. Eleanor Carothers: "Notes on the Taxonomy, Development and Life History of Certain Acrididae (Orthoptera)," Transactions of the American Entomological Society, Vol. XLIX, March, 1923.

of freezing, and the length of the life cycle may be modified by subjecting the eggs to various temperatures, a simple device for which is described. Appreciation is expressed to Dr. Carothers for the many suggestions she made personally concerning breeding methods.

Bodine ('25)[2] gives a detailed analysis of the effects of temperature upon the development of eggs of various species of grasshoppers. He calls attention to the consequent advantages of the material for general experimental purposes. He divides the eggs into three classes: first, eggs that require a period of freezing to undergo development; second, eggs that are frozen naturally but can hatch without freezing; third, eggs that are not frozen naturally. Species that are best adapted for general experimental purposes are obviously those belonging to the second and third classes where hatching is not dependent upon freezing. Bodine ('25, p. 95) lists such forms as follows: *Melanoplus differentialis, Melanoplus femur-rubrum, Chortophaga viridifasciata, Chortophaga australior, Dichromorpha viridis, Arphia xanthoptera, Encoptolophus sordidus,* and *Romalea microptera.* The species reported in the present study are: *Chortophaga viridifasciata, Encoptolophus sordidus* and *Romalea microptera.*

Chortophaga viridifasciata[3] was bred in large numbers. It hibernates as a third-instar nymph. This period can be eliminated, making possible about six generations per year (Carothers, '23, p. 13). The time of egg development can be extensively shortened or lengthened by the use of various temperatures. It is unusually viable in the laboratory.

Encoptolophus sordidus was also reared but only in a very limited way. Its hibernation period in the egg state can be suppressed (Carothers, '23, p. 13) increasing the number of yearly generations. The time of egg hatching can be modified in a man-

[2] Joseph Hall Bodine: "I. Effects of Temperature on Rate of Embryonic Development of Certain Orthoptera," Journal of Experimental Zoology, Vol. 42, May, 1925.

[3] Descriptions of grasshoppers are given by W. S. Blatchley, "Orthoptera of North-Eastern America." The Nature Publishing Company, Indianapolis, 1920.

ner similar to that possible with *Chortophaga,* but the details were not worked out.

Romalea microptera (= *Rhomaleum micropterum*) was bred in quantity. Its life history is much longer than that of smaller species, hence it is not as adaptable as they are for general experimental purposes. It was not possible to secure more than one generation a year, but the time of egg hatching is subject to wide modification by temperature control. *Romalea* has a high death rate, but it is as easily cared for as the other species and can be reared successfully in the laboratory. Its very large size recommends it for some types of work.

CARE OF NYMPHS AND ADULTS

An insectary is the ideal breeding place, but if one is unavailable a south window is selected for maximum sun. A window receiving over six hours' sun in December may get less than three hours in the late spring when the sun is higher in the sky. The cracks between the sashes and the frame should be carefully packed with cotton to prevent the entrance of cold air.

A simple type of cage is illustrated (Plate IV) together with a device for roughly controlling the temperature of eggs. If cages are kept on shelves, the latter should be wide enough to allow some working space in front. Shelves must be far enough apart to allow a six-inch space above the tops of the cages when set upon their trough, so that cages can be watered from above or the glass fronts can be elevated without moving them.

When cages are used for nymphs the front glass is cut to accommodate a thin layer of dry sand (Plate IV, cage 1, G–H). The air should be dry. The damp atmosphere of a greenhouse is dangerous, since mould is a serious enemy of many species of grasshoppers in captivity. Those here reported are not usually affected, a factor greatly simplifying their care. Ventilation is another primary need of many grasshoppers. In the first generation in captivity most species die if brought indoors but live a considerable time in cages out-of-doors. Those reported here, however, thrive in the first generation under indoor conditions.

Lettuce is a satisfactory food for *Chortophaga, Encoptolophus,* and *Romalea,* as shown by the fact that successive generations properly mature their germ cells, mate and lay viable eggs. This is another factor simplifying their care. Dr. Carothers ('23, p. 10) mentions lettuce, but she also describes the use of wheat and other grasses grown in small pots. The latter require a great deal of time and trouble, since successive plantings are necessary as each crop keeps fresh for only about five days and then turns yellow and becomes harmful. Such foods are necessary for some species, but where lettuce can be used much time is saved. In summer *Chortophaga* can be fed various common grasses and clover, while *Romalea* eats dandelion. It is to be noted that nymphs do not eat for several days after hatching.

Lettuce of the solidly headed type is placed in the cages in quarter or half heads which keep fresh for four or five days. Lettuce that is loosely headed or single leaves dry out in a short time and are troublesome when handling small nymphs. When changing food, especially for early instars, the fresh material is placed beside the old, and the latter is not removed till the next day, after the insects have transferred themselves. When the glass is raised to change food but few escape as they go toward the light which is opposite the raised glass. Any that may get out are returned by the corked hole in the top of the cage (Plate IV) so that the glass need not be lifted again. Adults are picked up by the wings. Small nymphs must be carefully handled, by catching them in a small net and then picking them out with delicate forceps by the foreleg. If a hind leg is caught it is apt to be kicked off, or one of its joints may be harmed and cause trouble in later moults.

If *Chortophaga* is collected as a third-instar nymph in the fall, it will undergo a period of arrested growth for at least a month even though brought indoors. It requires the same food and care as at other times but it is sluggish and the absence of moulted skins indicates cessation of growth. This period which would have lasted through the winter in nature is greatly shortened in the first generation of captivity. It will not appear at all in later generations if the nymphs are kept unchilled.

Records of over seven hundred *Chortophaga* hatched show a mortality rate of about twenty per cent. during the growth period due chiefly to difficulty in one of the moults. There is a somewhat higher death rate in the first generation in captivity because of parasitism in some cases and unknown conditions in others. Although they eat moulted skins and dead bodies it is thought that there is little if any cannibalism. About fifty adults or a larger number of nymphs are kept in one cage 10″ x 11″ x 7″ as this species thrives despite considerable crowding.

Chortophaga requires about seven weeks to become adult when kept in a south window receiving four to five hours' sun a day, in a room with a temperature of 22° ± C. during the day, and 18° ± C. during the night. The first instar requires 10 ± days; the second, 7 ±; the third, 8 ±; the fourth, 11 ±, and the fifth, 13 ±.

Records were not kept concerning mortality and lengths of instars of *Encoptolophus,* but as in the case of many small grasshoppers, it requires about seven weeks to become adult.

Romalea has a high death rate. Of over five hundred individuals hatched, sixty-eight percent died, and but thirty-two percent reached maturity. As in the case of *Chortophaga,* the *Romalea* nymphs eat moulted skins and dead bodies, but in addition, there is much cannibalism. There were numerous instances, where larger ones were seen attacking smaller individuals that appeared perfectly healthy. Two factors encourage cannibalism: first, the mixing of nymphs in various stages of development, when the smaller ones always disappear; and second, over-crowding. An attempt was made to lessen cannibalism by supplying animal food, such as boiled egg, cheese, etc., but without success. Cannibalism is slight in the first and second instars, greatest in the third and fourth, but it does not occur among adults. About ten adults or twenty nymphs were kept in a cage 10″ x 11″ x 7″.

In an experiment now in progress to study the inheritance of body color in the cross, *Romalea microptera* var. *microptera* (yellow) x *Romalea microptera* var. *macri* (black), it has been found that the yellow form is considerably more viable than the black one.

Romalea requires about fifteen weeks to become adult when kept in a south window receiving four to five hours sun a day, in a room with a temperature of 22° ± C. during the day, and 18° ± C. during the night. The first instar requires 13 ± days;

the second, 18 ±; the third, 20 ±; the fourth, 23 ±, and the fifth, 31 ±.

By elevating the room temperature to an average of 25° ± C. both day and night, the nymphal period of *Romalea* was reduced to eight weeks instead of the fifteen-weeks' period required at a temperature of 22° ± C. during the day and 18° ± C. during the night. The nymphal period of *Chortophaga* was lengthened to ten weeks by lowering the temperature to an average of 18° ± C. during the day and 14° ± C. during the night, instead of the seven-weeks' period required at a temperature of 22° ± C. during the day and 18° ± C. during the night. Such rough observations indicate that the growth period can be considerably shortened or lengthened by modification of the temperature, giving control of this part of the life cycle for experimental purposes. Control of temperature would also probably affect the period of several weeks between the last moult and the time of mating and egg laying. If cages are enclosed to elevate temperature the question of ventilation will probably require attention.

CARE OF EGGS

When grasshoppers are ready to lay their pods, they are placed in a cage with the front glass cut to accommodate deep sand (Plate IV, cage 2, G′–H′). Sand is better than soil as it does not pack, and can be washed and sieved to eliminate impurities and larger particles. Its depth must be somewhat more than the elongated abdomen, which is extended during oviposition, or egg laying will be hindered. Two inches is sufficient for *Chortophaga* and *Encoptolophus* and three and a half inches for *Romalea*.

After egg laying has begun it is best to remove pods at weekly intervals. This facilitates the different treatment required by eggs in contrast to that necessary for nymphs and adults. It also gives a relatively accurate record as to when a given group of pods were laid, as there is possible a maximum variation of but a week. Adults are temporarily removed by placing an empty cage face to face with the one from which pods are to be taken. After the glasses have been withdrawn the grasshoppers pass over, since their positive phototropism is used as an aid in their transfer. A quick way of obtaining pods consists of sliding the sand mass into a pail of tepid water since they float. The sand is gently stirred so that pods are not accidentally held at the bottom. Before placing them in a new cage its sand should be carefully sieved to eliminate stray single eggs that may have broken away from pods of former sets, as the hatching of such occasional eggs would interfere with the new hatching records. The pods should be placed in the sand in normal position at the depth in which they are naturally laid.

Sand containing pods must be kept moist (Bodine, '25, p. 92) or the pods dessicate and die. The sand is watered through the top netting with a rust-free container, care being taken not to dislodge the pods by the stream. When heat is applied to control the speed of egg development the sand must be watered frequently. Channels undetected by the eye may gradually be formed between surface depressions and the drainage holes so that the water passes off too quickly, wetting the surface to only a slight depth and leaving large regions of the deeper sand so dry that despite frequent surface wettings many of the pods dry out. If the top edge of the back glass (Plate IV, cage 2, E′–F′) as well as the top edge of the lower front glass (Plate IV, cage 2, G′–H′) are a little higher than the sand level, it can be well flooded, the water being held there between the glasses and slowly seeping down. If the sand is flush with the lower glasses, the water runs out through the back netting. When the sand is warmed the use of tepid water aids in maintaining a constant temperature. When nymphs appear, watering from above drenches them, hence remaining pods are watered by setting the cage for a short time in a vessel of water, the sand soaking it up through the drainage holes. Nymphs are not kept in a cage with unhatched pods since their droppings might affect the sand's acidity. Nymphs are also in less danger from mould if transferred to a dry cage.

The eggs of most grasshoppers, including those of *Chortophaga* and *Encoptolophus,* are pinkish, while those of *Romalea* are dark brown. The upper thicker cephalic end is marked by a small flat terminal region while the lower end is more slender and pointed. The top of the pod is marked by a mucus plug which fills up the hole after the abdomen has been withdrawn but this is easily broken off. When a pod is in proper moist condition the individual eggs are easily separated from each other. This becomes more marked as egg development proceeds so that just before hatching the pod may fall apart if disturbed. On the other hand, in pods which have been allowed to dry out slightly, the individual eggs adhere closely to each other and cannot be separated without tearing the outer coats. A single egg in good condition is solid and firm and if pricked during early development the yellow yolk material squirts out, while one that has dried is less firm, having a thick and gummy contents. The development of embryos can be examined by cutting the eggs down the dorsal convex surface and floating the contents out in normal salt solution.

Since *Chortophaga* hibernates as a nymph it naturally passes through its egg phase quickly. Although *Encoptolophus* normally hibernates in the egg state, if the pods are kept warm the hibernation period can be eliminated. Since *Romalea* has a southern range its pods are not naturally subjected to freezing.

By controlling the temperature of the sand containing pods, the rate of egg development can be accelerated or retarded, so that pods of one laying can be made to emerge over a considerable period. The temperature of the sand and its contained pods in a given cage is roughly controlled by the wattage of the electric bulb in the trough underneath. This is further modified by slightly elevating the cage to a greater or less degree with narrow strips, thus permitting the escape of various amounts of heated air (Plate IV). Such means of regulating temperature are modified by other factors such as: the temperature of the room, which is usually about 22° C. during the day, and drops at night to about 18° C.; the sun's shining on the cage, which temporarily raises the sand's temperature 5° to 8° C. in winter, and considerably more in spring and summer; the fact that the temperature of the sand is affected by whether it is quite wet or somewhat dried out; the temperature of the water with which the sand is moistened, etc. A thermometer is inserted in the sand through the corked hole in the top of the cage.

It is obvious that the methods of controlling temperature here mentioned are crude, as the purpose was not to study the effect of temperature upon development, but to control roughly the rate of development of various sets of pods in order to secure a continuous supply of living grasshoppers for experimental purposes.

Carothers notes ('23, p. 8) that under ordinary conditions sun is necessary for the emergence of nymphs. If the sand is heated, however, sun is unnecessary, and eggs develop normally, even though they have had no sun at all, and nymphs emerge without difficulty whether the sun is shining at that time or not.

If eggs are stored in unheated damp sand, in the shade, the evaporation keeps the temperature about 14° C., which permits only an exceedingly slow development of the embryos. If the cages are kept unheated but given normal daily sunshine, this intermittent heat accelerates development. Further speeding up is secured by the application of various amounts of continuous heat to the sand as described above. The pods may be laid in cold sand and stored in cold sand, to be placed later in the higher temperatures, a few at a time at various intervals; or they may be laid in warm sand and kept warm from the very beginning at higher temperatures. It is by different combinations of these methods that a given laying of pods can be made to emerge over a considerable period as desired. In applying such methods to grasshopper eggs generally, Bodine's ('25) observations must be kept in mind that certain species have definite rythms of susceptibility to low and high temperatures, and that there are wide differences in different species.

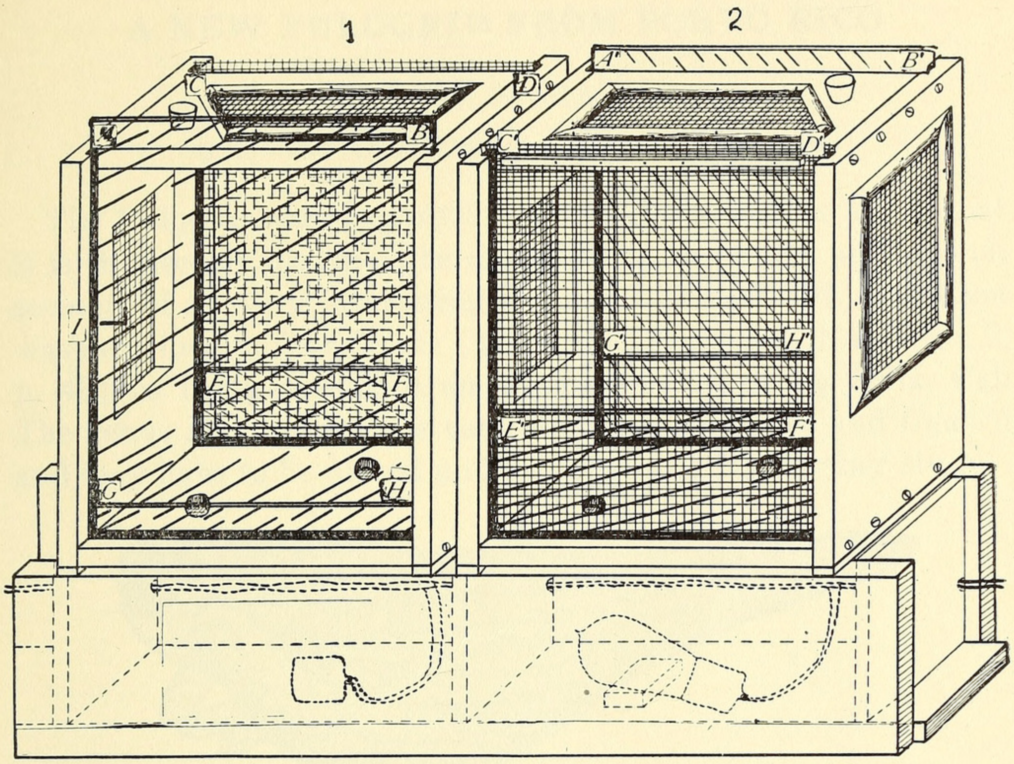
The shortest time for egg development of *Chortophaga* was four and one-half weeks at 34° ± C. Those which were laid in unheated sand, and within a week were transferred to sand kept at 25° C., hatched in about six weeks. This latter treatment was the one most commonly used. By keeping pods in cool sand for a shorter or longer period before applying heat, varying from 18° ± C. to 28° ± C., the period of development was lengthened to various points up to twelve weeks. It was not determined how long eggs can remain in cool sand and still remain alive, nor was refrigeration tried, but Carothers indicates ('23, p. 13) that at least this would be well over a year.

The effect of temperature upon the eggs of *Encoptolophus* was not studied in detail.

Romalea pods were laid during July and August in unheated sand and were allowed to remain there until mid October. They were then transferred, several at a time, at bi-weekly intervals, to sand heated at 25° ± C. Nymphs began emerging in late January and continued to hatch at intervals over a period of eighteen weeks. Whether or not an immediate application of heat would have produced an earlier emergence is not known.

PROPITIATION OF INSECTS

Frazer in "The Golden Bough" says that is the island of Oesel the Esthonian peasants "stand in great awe of the weevil, an insect which is exceedingly destructive to the grain. They give it a fine name, and if a child is about to kill a weevil they say, 'Don't do it; the more we hurt him, the more he hurts us.' If they find a weevil they bury it in the earth instead of killing it. Some even put the weevil under a stone in the field and offer corn to it. They think that thus it is appeased and does less harm." A German way of ridding a garden of caterpillars is for the mistress or another female member of the household to walk around the garden after sunset or at midnight, dragging after her a broom. "She may not look behind her, and must keep murmuring, 'Good evening, Mother Caterpillar, you shall come with your husband to church.' The garden gate is left open till the following morning."—Ed.



Cages and Device for Controlling Temperature of Eggs

The cages are shown in position on the trough for controlling the temperature of eggs. Cage 1 is seen in front view, with the front glass cut at G–H to accommodate a layer of shallow sand when used for rearing nymphs. Cage 2 is seen in back view, with the front glass cut at G′–H′ to accommodate deep sand when used for egg pods. When cages contain eggs, the sand is heated by electric lights placed in the compartments of the trough.

Cages for smaller species are 10″ wide x 11″ high x 7″ deep. They are made of cypress to prevent warping and are assembled with brass screws. The front (A–B and A′–B′) is of glass, sliding in a groove. The back (C–D and C′–D′) is of netting inserted in a similar groove, with an additional piece of glass, three inches high (E–F and E′–F′) across the lower portion, placed outside the netting. Sides and top have netted windows. All netting is of copper, 20 mesh to the inch, to prevent the escape of small nymphs. Drainage holes in the bottom have a piece of netting tacked over the outside and are filled with cotton to prevent the loss of sand. A half turn outward of the hook (I) holds the front glass up after it has been elevated.