ADDITIONAL NOTES ON REARING AND PRESERVING LARVAE OF MACROLEPIDOPTERA

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Since my earlier paper on this topic was published in this *Journal* (Vol. 18, no. 4: pp. 201–210), a number of miscellaneous details have accumulated and are brought together here under the appropriate headings. This paper is intended as a supplement to the earlier paper.

REARING

Feeding confined adult Lepidoptera while awaiting oviposition: Mr. H. Simmonds an entomologist in Suva, Viti Levu, Fiji, has developed a very useful, long-lasting medium (Honey-Agar) which is prepared as follows: (1) Dissolve 6–8 grams of agar-agar in 200 cc of water. (2) Add 250 to 320 cc of honey, and bring to a boil. (3) Pour out into a shallow plastic box or petri dish, and let cool. Store in refrigerator.

Use small pieces of honey-agar as needed for feeding confined Lepidoptera. A small amount of water may be placed on the piece to liquify its surface slightly, if this appears to be necessary. This substance may then be placed in the cage or jar with the confined insect, where it will serve as a food supply for many days. A small amount of water should be provided in another container nearby, but must be kept in such a way that the insect cannot fall into it or get its wings wet. Mr. B. O'Conner, Principal Entomologist at Koronivia Research Station, Nausori, Viti Levu, Fiji, told me that a slight modification of the above mixture, using 8 grams of agar-agar in 50 cc of water, with 300 cc of honey, seems to be more mould-resistent. In any event, the honey-agar method (or modifications of it) has obvious advantages over any method that requires the use of a wet or sticky solution needing to be frequently replaced. Difficulties with mould are also minimized.

Keeping newly hatched larvae alive until a suitable foodplant is located: Mr. N. B. Tindale of the South Australian Museum, Adelaide, informs me that the following technique will save many small larvae for an additional few days, which may make all the difference in getting them started, when it is not possible to locate the foodplant immediately. Place a piece of cut apple in a small box with the newly hatched larvae. Recut a thin slice off the cut surface each day, so that the surface continues to give off moisture. This slows down desiccation of the small larvae, and at the same time, they will often nibble at the cut surface of the apple, thus obtaining some nourishment.

Larvae requiring sunlight for stimulation of feeding should be provided

with it every day if possible; electric light is only a second choice, and will not produce such good results (in as short a time) as will a little daily sunlight. However, if electric light must be resorted to, be certain not to use the "cool white" (daylight) fluorescent tubes. "Warm white" fluorescent tubes are very satisfactory, however. Incandescent bulbs are also satisfactory, but one must beware of overheating the larvae if they are employed.

Pupae in diapause: Certain fall or winter-emerging moths need exposure to gradually LOWERING temperatures prior to emergence. This is just the reverse requirement of those emerging in spring or summer, but again, outdoor conditions are definitely preferable to any artificially produced conditions indoors, particularly about the time of emergence.

Most pupae in diapause come through to emergence time in excellent condition if kept in empty (no soil, sand, or moss), clean, glass jars of small size, with tight-fitting lids without holes. Cheesecloth strips may be provided for any unexpected emergences.

PRESERVING

Further details on the preserving technique: It is well to have a SEPARATE jar for the killing-jar (into which the larva is first dropped), as the solution in it will rapidly become too cloudy for use in preservation, yet it will serve indefinitely for killing. This procedure will save preserving fluid to an appreciable extent.

After the larva has been injected and replaced in the preservation-jar containing the solution, it should lie on its side until such time as it is to be removed to 95% ethyl alcohol for "clean-up" and permanent storage.

Exceptions to the general rule of injecting larvae should be observed for all lycaenid larvae, and also for most geometrid larvae of small to medium size. Such larvae are simply killed and left in the preserving solution without being injected, and then are removed to 95% ethyl alcohol after sufficient time in the preserving solution.

Larvae feeding on *Quercus* (any oaks) or certain leguminous plants sometimes need to be starved for a day to give best results in preservation. Otherwise, internal discoloration (darkening) may occur even after injection. This is not usually the case, but it happens often enough to warrant mention. These remarks also apply to many skipper larvae.

Preservation of eggs: As the inner tissues of eggs pull away from the outer covering, in most solutions, Peterson (1960: *Florida Entomologist*, 43 (1): 1–7), recommends the use of "a standard K.A.A. mixture diluted four or five times with ethyl or isopropyl alcohol. A standard K.A.A. mixture consists of 1 part commercial kerosene, 2 parts acetic acid, and 10 parts ethyl or isopropyl alcohol. For many eggs, isopropyl alcohol in the K.A.A. solution produces the most satisfactory results." But, as Peter-

son states, bright colors, waxy coatings, and other surface characteristics of some eggs are apt to change upon standing in liquid preservatives.

The eggs can be stored in vials of 95% ethyl alcohol, once they have gone through a preservation solution, but changes may occur in some eggs; others will remain in rather good condition, and still others may show external or internal features that are not so readily seen in the living eggs. If possible, it is worthwhile to either photograph, draw, or describe the eggs several days before the day they hatch. At the same time, note color changes as the eggs age.

Preserving solution: As dioxane is highly toxic, it is advisable to use a preserving solution which omits it, or which contains only a very small amount of it (such as K.A.A.D.I.). J. S. Buckett, of Davis, California recently produced a preserving solution that totally omits dioxane, but gives excellent results which are comparable to K.A.A.D.I., or perhaps even better. This solution ("K.A.S.A.") is as follows:

Kerosene (as obtained at service stations)—3 parts ±
95% Ethyl Alcohol—9 parts
sec—butyl Alcohol, CH_3 CH_2 CH(OH) CH_3—5 parts ± (enough to "clear" the solution and make the kerosene miscible)
Glacial Acetic Acid—2 parts

In K.A.S.A., and in the other solutions given in the previous paper, the sec-butyl alcohol, iso-butyl alcohol ($(CH_3)_2$ $CHCH_2$ OH), or dioxane (depending upon the solution) must be varied slightly in quantity, its function being to "clear" the solution so that it is water-clear and no kerosene is on top. For example, if exactly five parts of sec-butyl alcohol do not totally clear the solution of K.A.S.A., keep adding small additional amounts (and stirring) until it becomes clear. All of these solutions should be used only under conditions of adequate ventilation. Contact with the skin by solutions should be avoided as much as possible.

Briefly, the functions of the components of the various preserving solutions are as follows: the kerosene causes inflation of the specimens. The dioxane (or, the iso-butyl or sec-butyl alcohol) makes the kerosene miscible in the rest of the solution, and so must be increased or decreased along with variation in the amount of kerosene used. The acetic acid helps prevent internal darkening or discoloration of specimens. In standard K.A.A.D., the amount of alcohol used can vary between 7–10 parts, and the dioxane can be replaced with sec-butyl or iso-butyl alcohol, but more than one part is needed when using one of these substitutes. K.A.S.A. appears to be the best of the solutions I have used so far, and it is easily prepared; also, it has the additional good feature that it contains no dioxane.

All of these solutions seem to work better when slightly dilute with larval fluids (*i.e.*, when light yellow-green in color); the fresh solution

used to replace old solution in the preservation-jar is often improved by adding to it a small amount of the old solution. Fats and oils that float on top of used solution are easily blotted up with a rolled piece of Kleenex or paper towel, and this should be done periodically.

Injection of pupae is usually a simple procedure but the following points should be kept in mind: Pupae should never be injected and preserved until they are at least several days old, and are thoroughly hardened. Injection should be in the abdominal region, between the segments, and it is sometimes desirable to inject through several different places in one pupa; this is particularly necessary with nymphalid pupae, which will often turn pinkish or red if not sufficiently injected with preservative. In the case of very soft pupae, or those in which the wing cases tend to break away somewhat after injection (some lycaenids and small geometrids, etc.), one light injection in the abdominal region is ample, and they should not be left more than a few hours in the preservative before being removed to 95% ethyl alcohol; in some cases, a few minutes is sufficient time in the preservative. If the wing cases collapse in a soft pupa, the pupa should be injected again upon REMOVAL from the preservation-jar, this time using 95% ethyl alcohol; then place it in 95% ethyl alcohol for permanent storage (after a period of soaking in the "clean-up jar," as described in the earlier paper).

Stoppers and vials: The size-numbering system for neoprene stoppers is not the same as that used for standard cork stoppers. The following sizes of neoprene stoppers fit the sizes of vials given in parentheses: No. 00 stopper (1 and 2 dram homeopathic vials); No. 1 (4, 6, and 8 dram homeopathic vials); No. 3 (6 dram shell vial); No. 4 (8 dram shell vial). The stoppers should fit tightly, needing to be twisted in while letting out the trapped air with a bent insect pin. Neoprene stoppers come in various colors, including gray.

Both the "long form" and the "short form" of the eight dram homeopathic vial are useful; although the former is difficult to obtain, it is just right for storing many sphingid larvae or other long larvae that will not quite fit into the usual (short form) eight dram vial. For very large larvae, it is necessary to use olive bottles or similar containers. To save metal screw-caps, thin sheet polyethylene can be placed over the bottle top before screwing on the cap.

The various flexible plastic "push-in" or "snap-on" caps for vials are not as satisfactory as neoprene stoppers in the prevention of evaporation over long periods. Furthermore, neoprene stoppers will not harden in the way that some plastic caps will, and the neoprene stoppers can always be forced back in tightly after removal from vials. It is possible to write directly on neoprene stoppers, using permanent ink, which is a convenience.