REARING TECHNIQUES FOR SPECIES OF *SPEYERIA* (NYMPHALIDAE)

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Widespread interest in North American *Speyeria* has resulted in accumulation of considerable taxonomic and ecological information, yet problems in understanding species limits persist.

Rearing studies would be helpful in clarifying some of these problems, but until now no successful method of breaking larval diapause or of overwintering was known. This paper outlines rearing procedures developed by the authors during five years of combined research. We have used the method to rear more than 4,000 specimens, including all the Nearctic species. Although large scale rearing is described, its fundamentals can be reduced to the scale desired.

A search of the literature provides little information on rearing *Speyeria*. William Henry Edwards (1887) wrote of successfully overwintering larvae in an ice house. Grey, Moeck, and Evans (1963) reported a method of breaking diapause by periodic mechanical stimulation over an extended period. Little was learned concerning the origin of an idea which was developed into the overwintering procedure described in this paper as the "block method" of larval storage. The idea came to us through D. V. McCorkle of Monmouth, Oregon, who along with others explored it as a means of overwintering.

The Speyerian Life Cycle

A generalized life cycle for *Speyeria* may be useful in understanding the complexities of rearing. The one presented is based partly on our observations and partly on descriptions in literature.

Species of *Speyeria* are univoltine; adult emergence occurs between May and September, depending on the population. The males appear approximately a week before the females, which results from a disproportionate larval growth rate. Females often mate immediately, sometimes on or before their maiden flight. Egg laying begins within days or weeks depending on differing ovarian maturation rates between species. Food plants appear to be restricted to the genus *Viola* (Violaceae) with one doubtful exception offered by Durden (1965). Oviposition sometimes

occurs on the food plant, but commonly eggs are placed on other substrates near the host. More than 600 eggs may be deposited by a single female. The time from oviposition to eclosion under natural conditions ranges from 12 to 24 days depending on the species. We have noted a relatively uniform rate of development under laboratory conditions. Eggs held at temperatures between 24° to 32° C darken within 48 hours if viable, and hatch in approximately 9 days. After emergence, larvae immediately seek shelter in ground litter and hibernate. All *Speyeria* larvae overwinter in a state of diapause, remaining inactive for at least 8 months; mortality appears to be very high during this period.

Following appropriate stimuli in the spring, feeding starts, and the larvae grow to maturity in 6 to 10 weeks. The time from pupation to emergence varies from 7 to 22 days in the laboratory depending on the species and the temperature to which the pupae are exposed. The average length of the pupal period in nature is reported to be approximately 14 days, (Weed, 1927; Macy and Shepherd, 1941).

Field Storage and Handling of Live Females

Proper handling permits storage of live females for extended periods and successful shipping within North America in a condition to produce an ample supply of eggs. Field collected females should be placed with their wings closed in glassine envelopes without being stunned or anaesthetized. A portable ice chest should be taken where hot, arid conditions will be encountered, or on trips of more than one day. Enveloped females should be kept in tightly sealed glass jars stored in ice. Lengthy storage in ice will keep the insects immobile, and is not harmful as long as desiccation does not occur. The loss of body moisture is a great shipping and storage hazard and may be prevented by the addition of moistened paper towel or the equivalent to the jar. However, adding or accumulating too much moisture can drown the insects. Also, water can be accidentally drawn inside from the melting ice especially when a vacuum is created inside jars as they are cooled or by transporting them from a high to a lower altitude.

If storage will be longer than 3 days, females should be fed. One initial feeding will allow them to be carried for several weeks as long as jars are occasionally opened to replenish the oxygen supply and the cold is maintained. Similar success with storage has been obtained in the laboratory by holding the jars under refrigeration.

Shipment of Living Females

A double box method has proven to be safe and effective for shipping. The insects (in glassine envelopes) are packed in small loose groups in

dampened absorbent packing material in a durable, crushproof container. The container is sealed with waterproof packaging tape to help retain moisture, and is then surrounded with packing material in a slightly larger container. After packaging, shipments are held under refrigeration until air mailed. At destination, the females should be fed immediately or the package again refrigerated.

Adult Feeding Procedure

Females bagged for egg laying should be fed twice daily. The food solution and feeding procedure is similar to that given by McFarland (1964). The solution consists of 3 heaping teaspoons of granulated sugar to 8 ounces of distilled water.

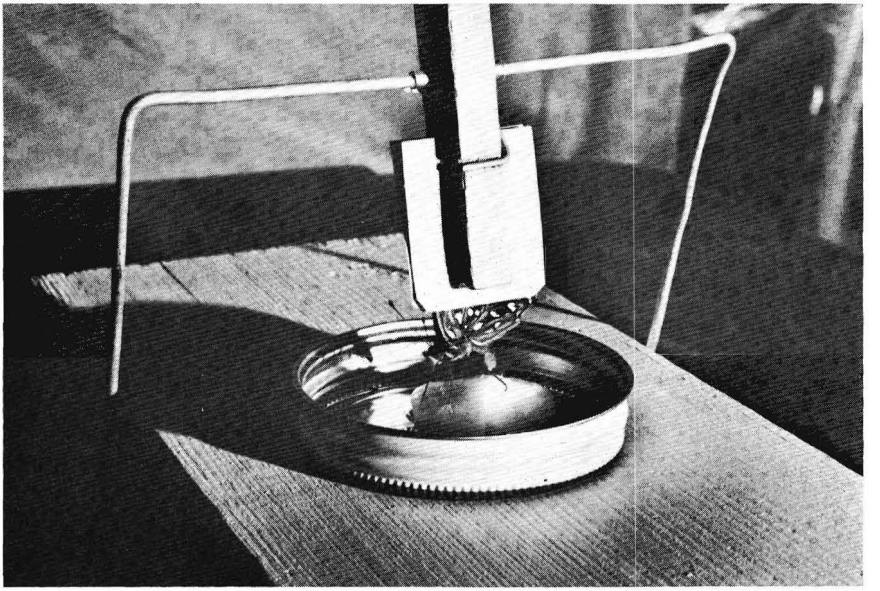
The butterfly, with its wings closed, is placed on absorbent cotton saturated with the solution. Organs of taste in the tarsi of the posterior pair of legs normally trigger a feeding response (Ford, 1945; Macy and Shepard, 1941; Oldroyd, 1959); however, if the response fails a pin can be inserted through the proboscis coil extending it into contact with the solution. Feeding lasts approximately 2 to 3 minutes with old, worn, or egg laying females. Unproductive individuals may not feed as long.

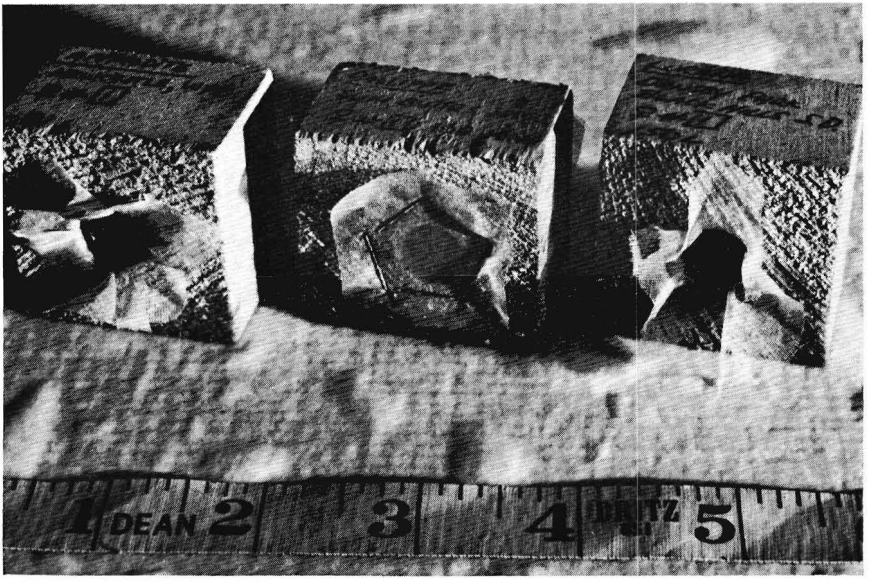
Adjustable holding devices like the one shown in Figure 1 are convenient when numerous females must be fed. In the one shown, a clothespin provided with cardboard grips can be adjusted to the height and angle necessary for holding any size specimen for feeding. Traces of food solution should be rinsed away from the insects tarsi and proboscis after each feeding, otherwise chrystallizing sugar may damage these organs.

Oviposition in Captivity

Following the method used by Grey, Moeck, and Evans (1963), egg laying can be promoted by enclosing the female in a brown paper bag exposed to sunlight. In the laboratory an incandescent light source is used. Size number 8 paper bags containing violet leaves are placed 12″ to 18″ from a 100 watt light. The best egg production is obtained when bags are exposed to a humid environment. Where dryness cannot be avoided, the paper bags can be enclosed in plastic bags and about 1 tablespoon of water added between them as needed to avoid desiccation of the females.

Generally most field collected females are gravid, but fresh specimens of some species must be bagged for many days before oviposition begins. Females are usually so worn by the end of the egg laying period that photographic records are necessary to compare progeny and parent.





Figs. 1, 2. 1, An adjustable feeding device for holding butterfly immobile during feeding; 2, dampened wooden blocks containing diapausing Speyerian larvae and showing two methods of attaching the nylon chiffon.

Method of Larval Storage

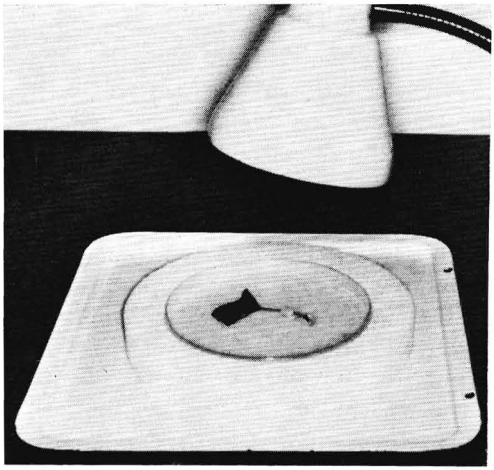
Newly hatched larvae are immediately removed from the bag and are housed in small wooden blocks. These blocks, which are approximately 1½″ cubes, are preferably made of basswood or other soft lumber. A hole ½″ diameter is drilled with the grain of the wood through the center of each block to form the storage chamber.

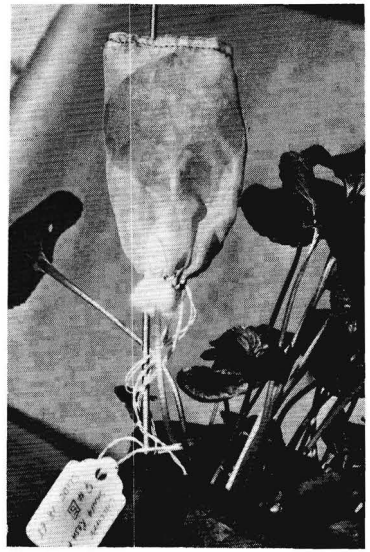
Up to 100 larvae may be housed in an individual storage block. The storage compartments are closed either by stapling nylon chiffon over the holes (chiffon is used because it allows ventilation while preventing larval escape) or by inserting it inside each end by use of plastic compression rings (Fig. 2). Parakeet banding rings adapt well for closing blocks because of their ability to expand and contract. Chiffon attachment by stapling when the blocks are dry will remain tight throughout periods of expansion and shrinkage resulting from periodic block soaking. Soakings are mandatory throughout larval storage, as desiccation is fatal to the larvae. The interval between soakings depends on dryness of storage conditions, but usually is not longer than a week. Blocks are soaked until partially wet by absorption in distilled water not deep enough to enter the storage chamber.

Blocks must be stored at all times under refrigeration at a temperature just above 0° C, as freezing of the blocks will cause high larval mortality. The blocks should be arranged in a tray or the refrigerator's vegetable pan so that adequate ventilation can pass through the storage chambers. Mold growth inside damp blocks is a serious problem as mold growing around larvae will usually cause death. Autoclaving of the blocks before use, and periodic inspections during storage is necessary. When mold is detected reblocking must be undertaken.

The overwintering larval condition referred to in this paper and in literature as "diapause," may ultimately prove to be quiescence. Throughout diapause, *Speyeria* larvae demonstrate an ability to repeatedly awaken from or return to dormancy in response to the application or removal of stimuli such as light, heat, and mechanical agitation. The ability to seek shelter from adverse environmental conditions may have an important influence in larval survival in nature.

Apparently, a correlation exists between larval metabolism and the agent controlling diapause. Apparently diapause cannot be permanently terminated until a given amount of stored energy has been expended. The rate of expenditure seems to regulate termination. Furthermore, the rate appears to fluctuate with temperature; thus, diapause ends more rapidly for larvae stored at room temperature (22°–26° C) than for those stored in the cold (1°–5° C). Carefully controlling the metabolic rate by refrigeration to approximate natural habitat temperatures is therefore necessary





Figs. 3, 4. 3, Humidity chamber apparatus for breaking larval diapause (feeding is promoted by the application of controlled heat, light, and humidity); 4, a bag type leaf sleeve of nylon chiffon is placed on violet leaf.

to extend diapause to its normal duration. Conversely, the rapid expenditure of energy through intense and continued artificial stimulation can rapidly terminate diapause in all *Speyerian* species.

Breaking Speyerian Diapause

Figure 3 shows an arrangement developed for breaking Speyerian diapause. This method employs light, heat, and humidity for stimulus and protection, and is designed for use at indoor temperatures (22°–26° C). The equipment consists of a white, enamel plated tray, or other reflective surface on which is placed a small, clear plastic box or glass petri dish. Paper towel or filter paper fitted in the bottom of the container is saturated but not flooded with distilled water. A young tender violet leaf with its stem held by foam rubber in a 8 × 25 mm water filled specimen vial is placed in the container. Using a camel hair brush, up to 30 larvae can be placed in the container. The larvae will partially awaken and contract into a C-shaped position in response to the handling. An adjustable gooseneck, hooded lamp using a 60 watt incandescent bulb is positioned approximately 8 inches from the container shining down on the larvae. After larvae uncoil and begin to crawl in response to the heat and light stimuli, they should be transferred to the leaf. A top is placed on the container to form a miniature escape proof humidity chamber in which the larvae

will not be harmed as long as the towel moisture is maintained. Reflective insulation material (such as expanded polystyrene) is cut to enclose the container exposing only the lid. This will substantially reduce condensation inside the chamber, in which wandering larvae can be trapped and drowned.

The time required for the first feeding response to occur is highly variable, and as previously stated, appears to depend on how much stored energy remains to be expended. During this interval the larvae may crawl extensively with intermittent periods of attempted sleep, often under the leaf or along the sides of the humidity chamber. The first indication of feeding is the appearance of small nicks along the margin of the leaf. Feeding occasionally occurs within 30 minutes, but normally takes from 1 to 3 days or longer. Feeding larvae usually display a gregarious tendency through the first 2 to 3 instars.

Host Plants

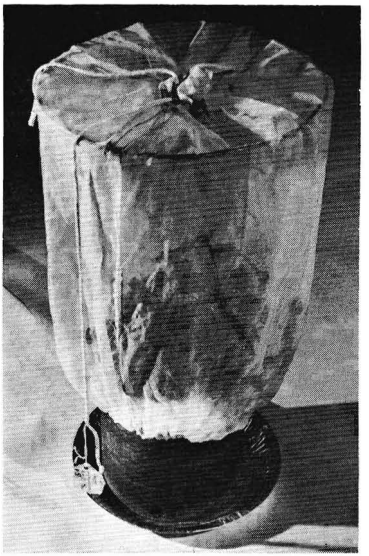
Many North American violets of the genus *Viola* serve as satisfactory hosts. Although there appear to be host preferences throughout the genus, indiscriminate feeding on any available *Viola* apparently occurs (Macy and Shepard, 1941, for *S. cybele*). The only unsuitable violets encountered are evergreen ornamentals of European origin. Although larvae of most species will accept an unsuitable host, symptoms possibly indicative of a nutritional deficiency or a toxic reaction, will develop in varying degrees depending on the species involved. Some species are quickly killed, while in others dwarfed adults have been obtained after an abnormally long development period.

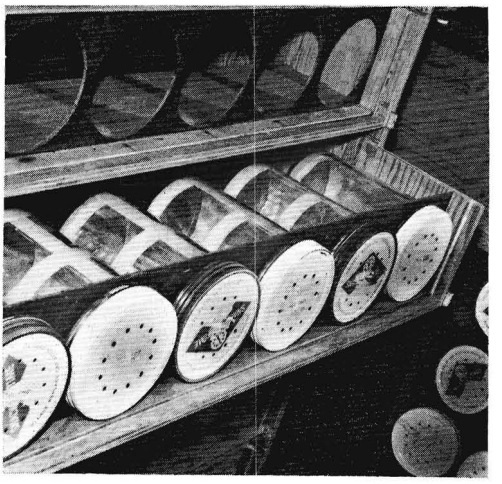
Viola papilionacea Pursh, and the partial albino form *V. priceana* Pollard have proven to be very successful hosts for all North American *Speyeria*. These large leafed deciduous plants are excellent seed and foliage producers which withstand repeated defoliation, and can be grown continuously in greenhouses with only a short winter dormancy.

Rearing Procedures

Larval losses are minimized by allowing completion of the approximate 2-day first instar period in the humidity chamber. They are then transferred to host plants where second and third instar development continues on young tender violet leaves in the confinement of a leaf sleeve. Figure 4 shows a nylon chiffon leaf sleeve securely closed by a drawstring which crimps cotton around the leaf petiole. Violets for sleeving are container grown for handling convenience. Leaf-sleeving becomes impractical by late third instar due to increased larval size and food consumption.

As shown in figure 5 the remaining larval development takes place in





Figs. 5, 6. 5, Nylon chiffon can-sleeve installed on container-grown *Viola;* 6, cabinet providing partial temperature and humidity control for storing pupation and emergence jars in dark.

a nylon chiffon can-sleeve. The wire legs of the sleeve support ring when pushed into the soil hold the sleeve in position. An elastic band in the bottom of the sleeve grips the can snugly while access is gained through the top by means of a draw string.

Violet plants can become infested with several common pest or disease organisms in addition to harboring hard to detect spiders which can kill early instar larvae. When pest control becomes necessary, washing the foliage or hand removal is recommended. *Speyeria* larvae exhibit a pronounced sensitivity to many commonly used home and garden pesticides, and any treatment should also include precautionary foliage feeding tests before plant reuse. Residual or systemic insecticides and those releasing fumes including household pest strips should be strickly avoided. Soil in the container may still be contaminated after the plant has proven safe. A layer of sawdust will prevent soil contact and will collect and help desiccate larval droppings for easy disposal.

The feasibility of conducting large scale rearing studies was greatly enhanced by learning that the normal more than 3 month larval-pupal development period could be significantly reduced. As with most insect development, heat is a major factor in shortening the maturation period. In nature, feeding is slowed or interrupted by the intensity of direct sunshine or nighttime cold. Where sleeved cans of larvae are placed under

continuous incandescent light at a temperature from 26° to 32° C with moderate humidity, the subdued light within the sleeve seems optimum for a rapid development of the normally nocturnal larvae.

An extreme example of accelerated development was achieved where normal males of *Speyeria callippe marcaria* were obtained in 20 days from first feeding to adult emergence, but the higher temperature levels needed are highly favorable for disease development.

Pupal Storage and Adult Emergence

Chrysalids should be stored in separate emergence jars during their development period. If full-grown larvae are transferred to jars for final development, the usual indications of oncoming pupation are discontinued feeding, rapid and continuous wandering, and finally a reddish discoloration to the larval droppings. If pupation occurs in the can-sleeve, masking tape can be used to easily detach and resuspend chrysalids in the jars without removing them from their silken attachment.

Newly emerged adults normally undergo nervous body movements which are apparently a functional part of the wing expansion process. Three-fourths of the inside circumference of the approximately 2 quart capacity wide mouth jars, are lined with moisture resistant Dacron curtain material held in place with "freezer type" masking tape. The lining provides an adequate foothold surface which reduces wing damage that results when adults accidently fall during emergence and cannot retain a hanging position. A gap is left in the lining large enough to lay a restaurant dispenser type paper napkin when the jar is on its side. The napkin, which is kept slightly damp to provide humidity for normal pupal development, also helps collect larval droppings before pupation and absorbs the reddish waste material released by the emerging adult.

Addition of water and finally the killing agent can be easily applied to the napkin with a plastic squeeze bottle through a hole drilled in the jar lid. Figure 6 shows a jar cabinet designed for large scale rearing. The storage drawers have removable fronts for easy access to the jars, while the cabinet provides temperature control and a darkened interior for emergence.

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