PROPAGATION AND REPATRIATION OF THE REGAL FRITILLARY BUTTERFLY

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ABSTRACT

The regal fritillary (Speyeria idalia) is a species that has disappeared from its entire historic range east of Indiana, except for a single thriving population on the training areas of Fort Indiantown Gap Military Reservation (FIGMR). ZooAmerica has been involved as a partner with FIGMR biologists since 2011 in a joint project to rear regal fritillaries for reintroduction into new areas of Pennsylvania. The following paper discusses our progress thus far, and future goals for the project.

INTRODUCTION

Beginning in the summer of 2011, ZooAmerica North American Wildlife Park was invited to partner with Fort Indiantown Gap Miltary Reservation (FIGMR) in a project to rear regal fritillary butterflies for repatriation into suitable habitat within Pennsylvania. Regal fritillaries (*Speyeria idalia*) are a candidate species for listing under the Endangered Species Act. They were once fairly common throughout the eastern United States, where they thrived in meadows and cow pastures. Livestock grazing created disturbances and openings that allowed for the growth of violets, the larval host plants for regals. Buffalo wallows would have provided similar openings in the native tall grass prairies. A restored tall grass prairie, complete with bison and regal fritillaries from a successful reintroduction project, can be seen at the Neal Smith National Wildlife Refuge in central Iowa (Shepherd et al). In the eastern United States there is currently a single remnant population at FIGMR of an estimated 5000 regals. The nearest population of regal fritillaries to FIGMR is at Kankakee Sands, a Nature Conservancy property in western Indiana. Kankakee Sands is a restored tall grass prairie.

The firing ranges at FIGMR are a barren landscape. The ranges are subject to frequent burning and disturbance as a consequence of the training activities that take place. The soil is also thin and poor, as the training corridor is located in a fairly narrow valley between two mountains. These effects combine

to create a large area of suitable prairie habitat. Much habitat work has also been done there to provide more acreage for regals. Prescribed burns are scheduled to maintain optimal habitat, in areas that do not burn on their own from military activity. Altogether there are approximately 1000 acres of grassland habitat at FIGMR, of which 240 acres are managed specifically for regals. FIGMR employs butterfly specialists to study and monitor the population. The military biolgists also host regal tours during two weekends each July that draw hundreds of visitors to these normally off-limits areas. FIGMR biologists work jointly with ZooAmerica staff on the propagation and repatriation project.

In August of 2011 we collected three great spangled fritillaries (*Speyeria cybele*) at FIGMR, as a more common practice species to begin working with. Initial results with the great spangleds were very encouraging, in terms of the number of eggs being laid by the females. The biologists at FIGMR suggested that we collect two regal females that same year. Unlike great spangled fritillaries, regal fritilaries exhibit sexual dimorphism, which makes females easy to identify in the field. We used the husbandry manual for the related Oregon silverspot (*Speyeria zerene hippolyta*) as a starting point for developing our techniques (Anderson et al). The Oregon Silverspot Husbandry Manual is a joint work of the Oregon Zoo and Woodland Park Zoo. Mary Jo Anderson of the Oregon Zoo, and Erin Sullivan of the Woodland Park Zoo also provided much needed guidance via phone calls and emails. Fritillary husbandry is presented in detail in the silverspot manual, and will be covered in abbreviated form here.

PROPAGATION

Female regals are collected in mid-August. The timing is such that it is assumed they have been bred with, but have not yet started to lay eggs. They are housed in white paper bags, under artificial lighting that phases in and out in intensity. This is designed to mimic a natural day, with the maximum temperature of about 85 deg F achieved at mid-day. Inside the bag are roughly a dozen strips of brown paper towel, a cotton ball moistened with distilled water, and a violet leaf. The butterflies almost never lay eggs on the white bag itself, but rather on the paper strips and violet. Each female is fed daily, on a cotton ball saturated with a 10% sugar mix. A drop of egg white is added to provide protein to the mix. The bag contents are checked for eggs while each female is being fed, by gently inspecting them under a magnifying light. The cotton ball and violet leaf are changed daily.

Collected eggs are snipped from the paper strips, or the violet leaves, and placed on filter paper halves within petri dishes. Eggs laid on violet leaves are prone to mold issues, and are kept in separate dishes. Leaf sections with eggs attached are also dipped in a 5% bleach solution, and rinsed in distilled water, before placing them into petri dishes. Mold is the primary source of egg mortality. Vigilance is required to prevent mold issues from developing in the dishes. The egg dishes are hydrated daily, with 6-7 drops of distilled water spotted onto the filter paper between the snips of paper towel containing the eggs. We use a 1cc TB syringe to carefully place the drops and control the amount. Dishes containing eggs on violet are hydrated with only 3-4 drops of distilled water, spotted well away from the leaf snips to prevent mold. It is better to err on the side of dryness. Any eggs that begin to mold can often be saved by dabbing them with a 5% bleach solution using a fine tipped paintbrush. This does not seem to negatively affect their hatchability. Mold spots on the filter paper can also be treated in this way, to prevent them from spreading to the eggs.

Any eggs that do not darken from development within a week are considered infertile. These are removed whenever possible as potential mold vectors. Sometimes they are attached within clusters of fertile eggs, and must be left in the dishes. We have had eggs hatch between 16-48 days. Most eggs will hatch at 20 days. We move dishes containing eggs that are 18 days old to hatch bins. These are checked for hatchlings during their daily hydration.

Newly hatched regal fritillaries will consume their egg shell, take a drink of water, and then begin looking for a place to hibernate through the winter as first instar larva. We over winter, or diapause, freshly hatched larva in one of three ways. Initially all larva were diapaused in refrigerators in our butterfly lab. We still use this method for a minority of our larva, as a means of researching optimal survival conditions, and studying larval development in the lab. Hatchlings are encouraged to crawl under yurts, which are small squares of corrugated paperboard derived from insulated cup sleeves. They nestle into the grooves, where they hopefully remain for the winter. Hatchlings are seeking somewhere dark to hide. They can be led to settle in under the yurts by placing them into a petri dish on a white table, with overhead lighting. The dish is placed onto a small disk of black paper, so that the darkest place they can find is under the yurts, which are situated over the black paper disks. Roaming larva can be gently placed onto the yurts with a soft fine tipped paintbrush. This may need to be repeated several times until the worst offenders all settle in.

Once the larva in the dish are all settled into the yurts, the yurts are placed into Toledo tubes. The Toledo tube and jar concept was developed by the Toledo Zoo as a means of over wintering the second brood eggs of the endangered Karner blue butterfly (*Lycaeides melissa samuelis*) (Webb).
The tube itself is a section of acrylic tubing, with a shelf of silk near the top. The tube is placed within a canning jar that has gauze pads and distilled water in the bottom. The ring of the canning jar is used to secure a square of silk over the top of the jar, stretched over the tube top. Yurts with larva are arranged onto the silk shelf of the Toledo tube. They are thus contained within the jar in a situation that allows moist air to circulate. We place a dozed Toledo jars, with 20-30 larva per jar, into each bin in our refrigerators. The bins have about two inches of water surrounding the jars. Humidity in each bin is controlled by varying the amount of ventilation in the bin lid. In mid-winter we change the gauze and water in each jar to prevent mold growth. Bins are rotated weekly to balance out the inevitable temperature variations within each refrigerator. Over winter survival has been quite low under the best of conditions, generally less than 5%. The best survival seems to be in jars that are situated within the refrigerators such that they are as close to freezing as possible, without freezing solid. Rotating the bins weekly allows some jars to freeze into position, while others are allowed to thaw. The back wall of a refrigerator is the coldest region, while the front closest to the door will be the warmest area. Refrigerator thermostats are set so that ice forms on the water bath surrounding the jars in the back half of the bin.

ZooAmerica has an abundance of wood violet (*Viola sororia*) growing on our grounds, which is used for feeding the larva. In April, as soon as the wood violet begins to come up, we wake the larva in the lab to begin rearing. Yurts are sorted through to find survivors. Live larva are placed in 4 ounce glass jars, on a moistened square of paper towel with several tiny fresh violet leaves. Initially they are fed the smallest leaves we can pick. As they grow, we transfer larva to 12 ounce deli cups with ventilated lids. Filter papers can be obtained that are a perfect fit for the bottom of the deli cups. Fresh violets and bottom papers are provided as needed, which is usually on a daily basis. Cups and jars are checked daily, and each molt is recorded as the larva develop.

Regal fritillary larva pass through six instars before molting into the pupa stage. At room temperature, if fed on wood violet, the caterpillars may pupate within 35 days from the time they are removed from winter diapause. The number of days is sex-dependent, with females spending 3-4 days longer in the larval stage. They spend 17-22 days as pupa before eclosion into the adult stage. Females spend roughly one day longer as pupa than males. Once the pupa have hardened off, they are moved to our outdoor enclosures to complete their development.

In 2013 we decided to experiment with over wintering larva in outdoor violet beds. We were looking for an alternative to the Toledo jars, as diapuse mortality was very high in the laboratory refrigerators. Good success was reported with over wintering fourth instar Baltimore checkerspot larva (*Euphydras phaeton Drury*) in enclosed beds of their host plant, White Turtlehead (*Chelone glabra L.*). The larva would become active in spring, feed on the turtlehead, pupate and emerge as adults within the enclosure (Durkin et al). We thought that a similar tactic of placing first instar regal larva in a bed of their host plants should also be successful.

Beds containing roughly 500 wood violets, transplanted from concentrations occurring naturally on the zoo grounds, were enclosed within a two-foot high wall of plywood, buried several inches into the ground. This barrier was to discourage the tiny hatchlings from wandering out of the bed before finding a place to settle for the winter. Over the beds we installed a 28 foot long, 14 foot wide, eight-foot high greenhouse frame. A mosquito netting cover was custom made to stretch over the frame in the spring. The covering would both contain the adult butterflies when they emerged in early summer, and also exclude parasitic wasps from attacking the larva. The greenhouse frame was left uncovered over the fall and winter to prevent it from being damaged by storms and snow loads.

In the late spring of 2014 we were very encouraged to see violet leaves being eaten, and eventually many fat larva moving about and feeding. It was a great opportunity to observe caterpillar behavior in a natural setting. There was also a significant improvement in the percentage of larva surviving to adulthood. In 2014 we produced 64 adults in the lab from 4176 larva placed into Toledo jars, compared to 37 adults produced from 526 larva overwintered in the violet bed. This represented a 1.5% survival to adulthood indoors, compared to a 7% survival to adulthood for those larva placed in the outdoor violet bed.

In 2014 we added a second violet bed enclosure, and in 2015 we designed a graphic to educate our visitors about the project. The graphic panel contains information on the current status of the regal fritillary, our partnership with Fort Indiantown Gap, and the purpose of the project. A local nature photographer, Steve Rannels, designed and donated a life cycle collage for the graphic. The collage was a compilation of many photos which Steve shot in our lab, in the greenhouses, and in the field at Fort Indiantown Gap. The greenhouse enclosures and graphic can be viewed in the Big Sky Country section of ZooAmerica, which represents plants and animals found in western and mid-western North America.

REPATRIATION

Before adult butterflies are taken to release sites, measurements are taken of the body length, fore wing length, and head width. Identification markings are applied with a Sharpie marker to top and hind wing surfaces. Butterflies are transported to the release site in individual cups, and then placed directly onto nectar plants. The idea is to walk them calmly out of the cup onto a favorite nectar source, such as butterfly weed. This encourages them to settle in and feed, rather than immediately bolting from the release site.

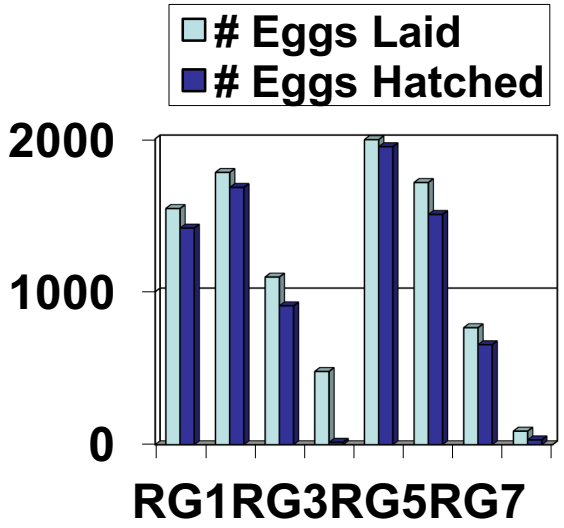
Release sites are chosen based on several criteria. Sites are surveyed for an ample supply of both nectar and host plants. Favored nectar plants include common milkweed (*Asclepias syriaca*), butterfly weed (*asclepias tuberosa*), monarda, and thistles. We also look for areas within the site with concentrations of violets. Two species of violets that we know to be used by regals are arrow-leaf violet (*Viola sagittata*), and common wood violet (*V. sororia*). Some of our sites also have good concentrations of primrose-leafed violet (*V. primulifolia*), which we suspect will also be fed upon. Sites with good nectar

plant density are more common than those with a good supply of violets. Violets can be difficult to find in summer, as they blend in well with the surrounding vegetation. Spring and fall are the best times to survey for violets. In spring their blooms make them conspicuous. In fall their leaves turn yellow for good visibility.

Two other release site criteria are a long term commitment to habitat maintenance, and security. In 2014 we partnered with the Pennsylvania Game Commission (PGC), which has created thousands of acres of habitat for grassland wildlife species. The PGC land managers in our area were enthusiastic about adding regal fritillaries into these ready-made prairies. The PGC grasslands that we currently use for release sites are either in remote locations, or in off-limits wildlife propagation areas. This addresses the security issue posed by the threat of irresponsible collecting. The PGC also has a long term commitment to maintaining these areas as grasslands. For each of our release sites, PGC land managers and FIGMR biologists have designed specific burn plans to meet the needs of both regals, and other wildlife using the habitat.

We have had good success at producing large numbers of caterpillars from relatively small numbers of collected females. One positive of working with regal fritillaries is that they are capable of laying very large quantities of eggs. Our record thus far was a female which laid 2969 eggs. However there is a great deal of variability between regal females in the number of eggs laid, and the percentage that will be fertile. For planning purposes, we project an average of 1000 eggs laid per female. We also plan on an 85% hatch rate, or 850 larva produced per female. In 2015 our eight females laid 9461 eggs, of which 8103, or 85.6% hatched. We would have had more eggs laid, but the females were released when we exceeded our goal of 9000 eggs for the year. Egg totals ranged from 89 to1994 laid per female. Hatch rates ranged between 3.3% and 97.7% for the various females.

Female ID	# Eggs Laid	# Eggs Hatched	% Fertile
RG-1	1544	1416	91.6
RG-2	1780	1682	94.5
RG-3	1096	908	82.8
RG-4	479	16	3.3
RG-5	1994	1948	97.7
RG-6	1714	1505	87.8
RG-7	765	654	85.5
RG-8	89	32	36



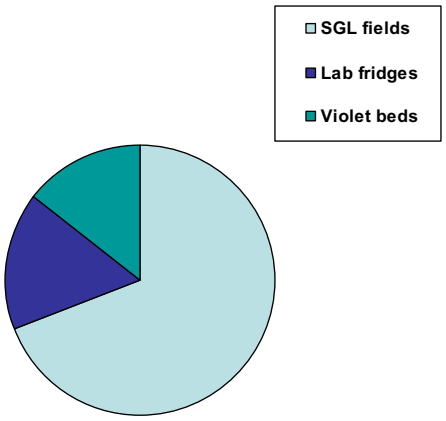
In 2014 we began to do direct field releases of newly hatched caterpillars. First instar larva were placed directly onto violets at our release sites using soft fine-tipped paint brushes. Each of our release sites are surveyed in spring, or the preceding fall, for violet concentrations. These locations are mapped for use in late summer when the caterpillars are hatching. Sometimes PGC land managers will mow the violet areas just previous to our releases. This makes the violets much easier to locate. Good regal

numbers were found at two of our four 2014 caterpillar release sites during the summer of 2015. In fall of 2015 we released more caterpillars at the two successful sites, and also at two new sites. Our plan is to continue to survey new promising locations to increase the number of release sites. We will continue releases each year at the successful locations to build in as much genetic diversity as possible. We plan to release larva at our successful sites for at least five years. Then we may halt releases and monitor each site to see if we have established a viable population. One of our sites is set aside for the release of any adult regals reared in the lab, or produced in the greenhouse enclosures at the zoo. All of our other sites will be larva releases only. This will allow for comparison between the effectiveness of adult versus larva releases for regal repatriation. Early indications are that there is better site fidelity with the larva releases. The adults seem more likely to stay in the area if they grew up there as caterpillars.

FUTURE GOALS

Going forward most of our effort will be devoted to caterpillar production for direct field releases. This seems to be the most effective way to reach our primary goal of establishing new regal fritillary populations. For research purposes, smaller numbers of larva will continue to be over wintered in Toledo jars, and in the violet beds at the zoo. In 2015 we placed 5593 larva, or 69% of our total larva production, directly onto violets at release sites. Into the lab refrigerators we placed 1334, or 16.5% of our caterpillars. And 1176 caterpillars, or 14.5%, were divided between the two outdoor violet beds.

Location	Total	Percent of Total
SGL fields	5593	69%
Lab Refrigerator	1334	16.5%
Violet beds at Zoo	1176	14.5%



Direct field releases also appear to provide the highest percentage of larva survival to adult stage. Of the 1663 larva over wintered and reared in the lab, only two, or 0.12% survived to adult stage in 2015. Of the 1998 larva placed in the two violet beds, 18, or 1.80% survived to adults. Compare this to the much higher survival rates at our two field release sites, based on mark and recapture data. At one site (SGL #1) an estimated 20.54 adults were produced from 604 larva released for a survival rate of 3.40%. At our second site (SGL #2) an estimated 27.52 adults were produced from 508 larva released for a survival rate of 5.42%.

Location	# Larva	# Adults	% Survival
Lab Fridges	1663	2	0.12%
Violet Beds	1998	18	1.80%
SGL#1	604	20.54	3.40%
SGL #2	508	27.52	5.42%

One of our research projects utilizing larva kept at the zoo is an iButton study in the Toledo jar bins. iButtons record temperature and relative humidity on an hourly basis. We hope the iButtons allow us to determine optimal temperature and relative humidity for larval diapause, based on which jars have the best over winter survival rates. We are also doing a comparative growth study using larva fed on three different violet species. This may be coupled with a nutritional analysis of the three violet species used in the study. Some of our release sites have multiple violet species present, while others may have only one type. The growth and nutritional study will allow us to fine tune our site selection and release locations. We also hope to determine if a fresh leaf is needed to stimulate egg laying. Does it need to be a violet leaf, or perhaps just the scent of a violet leaf? This study may shed light on the question of whether female regals actively seek out violets when depositing eggs. Or do they just scatter them randomly as some of the literature suggests? The answers to these questions will have implications regarding the minimum violet density required for a self-sustaining regal fritillary population. We are also preserving the spent females from the coming egg laying season for wolbachia testing. We would like to know if wolbachia is present in the source population at FIGMR. This may contribute to the radical variability in egg production and fertility that we find in the females we collect.

LITERATURE CITED

Anderson, Mary Jo, Mellissa Arnold, Elayne Barclay, Lorre Myers, David Shepherdson, and Erin Sullivan. 2010. "Oregon Silverspot (*Speyeria zerene hippolyta*) Husbandry Manual," First Edition.

Durkin, Pat, Jennifer Frye, Denise Gibbs, Rob Gibbs, and Matt Lustig. 2013. "Conservation and Management of the Baltimore Checkerspot (*Euphydryas phaeton* Drury) in Maryland: Strategies for Statewide Monitoring and for Wetland Restoration, Captive Breeding and Release in the Piedmont Region," Second Edition. pp 26-32.

Shepherd, Stephanie and Diane M. Debinski. 2005. "Reintroduction of Regal Fritillary (Speyeria idalia) to a Restored Prairie, Ecological Restoration," 23:4.

Webb, L. 2010. "Propagation Handbook for the Karner Blue Butterfly, Lycaeides melissa samuelis". First Edition. New Hampshire Fish and Game Department Nongame and Endangered Wildlife Program. Concord, NH. pp 30-31.