Monarchs Reproduced in Eastern Wyoming and Were Not Parasitized by *Ophryocystis elektroscirrha*

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Abstract - Monarch Butterflies are declining severely, partially due to the parasite *Ophryocystis elektroscirrha* (OE), which can decrease migration success. Little is known about the distribution, reproduction, or parasite loads of Monarchs at the junction of eastern and western populations. We investigated the presence of egg, larval, pupal, and adult Monarchs in east-central Wyoming. Additionally, we measured the parasite load of adult Monarchs. All 18 adult Monarchs lacked spores of the parasite OE. Monarchs have low abundances at the western edge of the eastern population, but they use Wyoming as breeding habitat. Our samples suggest very low rates of infection providing useful information for management decisions of this declining butterfly.

Introduction

Many butterfly species are declining, and little is known about the parasites that may affect them in the Rocky Mountain region. Butterflies play a role in pollination, are essential sources of prey, and have important cultural value (Hvenegaard 2016). Sharp declines in butterfly numbers are well-documented in Europe (van Swaay et al. 2006, Warren et al. 2021), and evidence for similar declines in North American is growing (Crossley et al. 2021). Several butterfly species are listed as threatened or endangered under the U.S. Endangered Species Act, including *Neonympha mitchellii mitchellii* French (Mitchell's Satyr Butterfly; USFWS 1998) and *Lycaeides melissa samuelis* Nabokob (Karner Blue Butterfly; USFWS 2003). Drivers for these declines include changes in temperature and precipitation (Forister et al. 2010), loss of breeding habitat, habitat degradation (Flockhart et al. 2014), and agricultural practices (Habel et al. 2019). Butterfly abundance declined 2.0% annually over a 21-year period in Ohio (Wepprich et al. 2019). Although some butterfly species are experiencing population increases (Crossley et al. 2021), over 3 times as many species are in decline (Wepprich et al. 2019). A better understanding of butterfly population trends is needed in many areas, including North America, to better identify management and conservation priorities.

Danaus plexippus Linnaeus (Monarch Butterfly) is one of the most well-known butterflies across North and Central America, and this species is in decline. Monarchs in North America are split into western and eastern flyways by the Rocky Mountains, and both populations have experienced severe declines in the past few decades (Brower et al. 2012, Schultz et al. 2017). Individuals from the eastern population overwinter in southern Mexico, and monitoring revealed an overall decline of ~85% since the mid-1990s (The Center for Biological Diversity 2022). Eastern Monarchs experienced a 22% decline between 2022 to 2023 at their overwintering sites (Xerces Society for Invertebrate Conservation 2023). Monarch Butterflies are a candidate species under the U.S. Endangered Species Act due to their decreasing numbers (USFWS 2020). In addition to the general factors causing butterfly declines, the loss of *Asclepias* spp. Linnaeus (Milkweed), loss

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of habitat (Flockhart et al. 2014), pesticides, climate change, logging of trees used for overwintering (Malcolm 2018), and the effects of the protozoan parasite *Ophryocystis elektroscirrha* McLaughlin and Myers (OE; Davis and de Roode 2018) have reduced the number of Monarchs.

The parasite OE affects Monarchs across their range and increases mortality in adult butterflies when parasite loads are heavy. Parasitized Monarchs can have deformed wings (Altizer and Oberhauser 1999), decreased mating success in male Monarchs (Babalola et al. 2022), and reduced migratory success (Bradley and Altizer 2005) due to decreasing adult wing mass and wing tear resistance (Davis and Roode 2018). Ultimately, OE decreases overwintering populations of Monarchs. Heavy parasite loads of OE can cause detrimental effects to Monarchs, while low and moderate parasite loads can decrease flight performance (Bradley and Altizer 2005), slow development, and shorten lifespans (Lindsey et al. 2009). Parasite spores of OE are transmitted vertically through the obligate host plant Milkweed, which Monarch larvae feed on. Spores of OE are deposited on Milkweed when female Monarchs lay their eggs, and caterpillars ingest the spores when they eat Milkweed (Alitzer et al. 2004, Leong et al. 1997). The parasite develops in caterpillars and completes its life cycle when the butterfly pupates (McLaughlin and Myers 1970). Spores are dormant when Monarchs emerge as adults, and the highest density of spores are found on the Monarch's abdomen (Leong et al. 1992). OE can also be transmitted horizontally by way of mating, and environmentally, when caterpillars consume spores left on milkweed by unrelated adults (Majewska et al. 2019).

Most Monarch Butterflies throughout North America are migratory. Migratory Monarchs have lower parasite loads than their non-migratory counterparts in states such as Florida, California, and Arizona (Altizer et al. 2000). Two hypotheses were proposed to explain this phenomenon. The Migratory Escape Hypothesis predicts that the act of migration allows individuals to escape areas with abundant parasites, thereby lowering parasite rates (Loehle 1995). The Migratory Culling Hypothesis predicts that the act of migration will cull infected individuals (Bradley and Altizer 2005). Currently, there is a gap in knowledge about parasite loads, distribution, and reproduction of Monarch Butterflies in the Intermountain West – including Montana, Idaho, Wyoming, Colorado, Utah, Arizona, and New Mexico. More information on OE loads in migratory populations is essential to advise management decisions.

The only peer-reviewed information on the distribution of Monarch Butterflies in Wyoming comes from a 2-year study in eastern Wyoming that found 31 Monarchs throughout the region in 2019 and 2021 (Crawford 2022). Only 1 data point exists for Monarch parasite loads in Wyoming (J. Berliner, Project Monarch Health, Athens, GA, 2023 pers. comm.); however, we have observed larval Monarchs in Wyoming, indicating they reproduce there (L.M. Tronstad, University of Wyoming, Laramie, WY, 2023 pers. obs.). Our study aimed to establish baseline information on the presence of each life stage and OE parasite loads of Monarch Butterflies in eastern Wyoming. To accomplish this, we surveyed for eggs, larvae, pupae, and adult Monarchs, and we tested adult Monarchs for the parasite OE. Our specific questions were: 1) Do Monarch Butterflies reproduce in east-central Wyoming, and can we find each life stage? and 2) What is the prevalence of OE in adult Monarchs in east-central Wyoming? We provide information about the distribution, reproduction, and parasite status of Monarchs which will inform management decisions.

Materials and Methods

Study sites

We surveyed for Monarchs near Douglas, Wyoming to investigate their reproduction and parasite loads. East-central Wyoming is largely composed of Sagebrush steppe and mixed grass prairie ecosystems covering rolling hills. The major land use in the area is livestock grazing (primarily cattle and sheep) and human densities are low (average 3.7 people/mi^2). We sampled 3 sites weekly from June 15, 2022 through August 16, 2022, for a total of 30 sampling events (Fig. 1). Sites were chosen for their large abundance of Milkweed plants. One site was ~12 km south of Douglas, Wyoming along the North Platte River. The North Platte site had a lush riparian area with Cottonwood trees and the dominant plants were *Asclepias speciosa* Torrey (Showy Milkweed), *Medicago sativa* Linnaeus (Alfalfa), *Sisymbrium altissimum* Linnaeus (Tumble Mustard), and *Centaurea* spp. Linnaeus (Knapweed). The other two sites were at Glendo State Park, which is a large recreational reservoir fed by the North Platte River, surrounded by riparian areas and foothills in a Sagebrush steppe ecosystem with conifers and Cottonwood trees. Glendo 1 was located at the southern tip of the reservoir and Glendo 2 was located on the northwestern side of the reservoir (Fig. 1). The most abundant flowering plants at the Glendo sites were *Grindelia squarrosa* Pursh (Gumweed), Knapweed, *Convolvulus arvensis* Linnaeus (Bindweed), Alfalfa, *Ratibida columnifera* Wooton and Standley (Prairie Coneflower), and Showy Milkweed. Temperatures in Douglas, Wyoming range from 10.7–28.5°C on

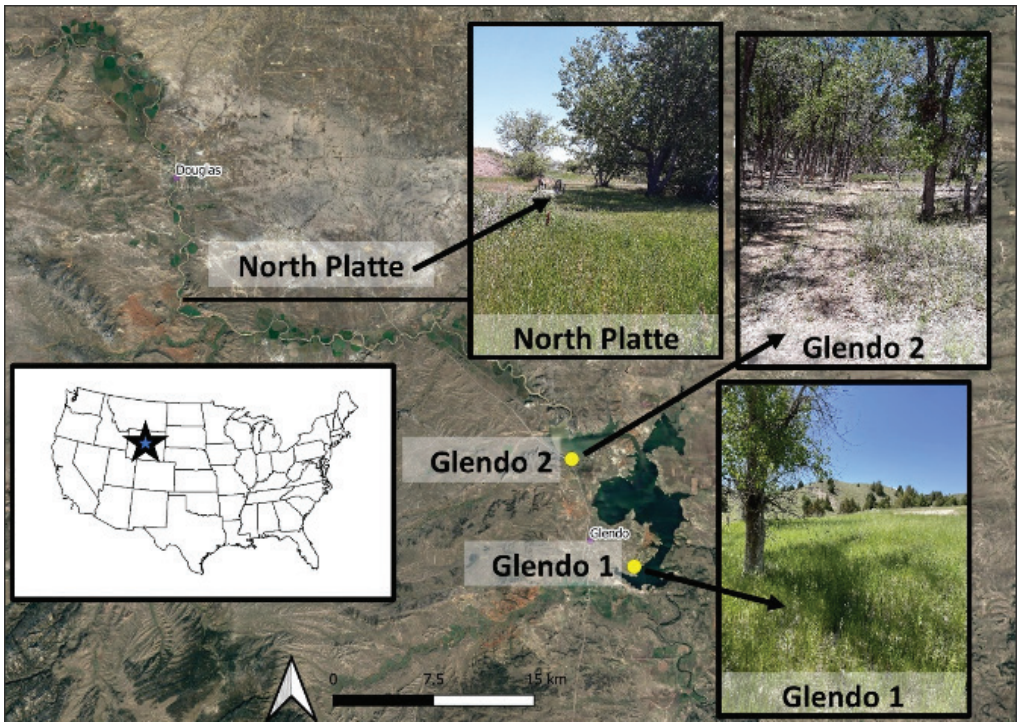

Figure 1. Map and photos of our study sites in east central Wyoming. The inset map shows the location of Wyoming in the USA. Glendo 1 and 2 were located in Glendo State Park, and North Platte was located along the North Platte River southeast of Douglas, Wyoming (location obscured to protect property owner).

average during the summer, with maximum summer temperatures at 30.7°C, and the area receives an average of 32.1 cm precipitation annually. Summer temperatures at Glendo State Park range from 10.9–29.4°C on average, with maximum summer temperatures at 31.2°C, and Glendo receives an average of 38.7 cm precipitation annually.

Adult butterfly surveys

One transect was surveyed at each site weekly to record the presence of adult Monarchs throughout the summer. Transects took the same route weekly, lasted 20–40 minutes (30 minutes on average), and covered 0.7–1.3 km (1 km on average). Average walking speed was 2 km/hr. Routes followed established dirt roads within sites. General site conditions were recorded, including air temperature, wind speed, and cloud cover.

Monarch egg, larvae, and pupae surveys

We surveyed for early life stages of Monarchs (Fig. 2)—egg, larvae, and pupae—to assess Monarch reproduction. Surveys were performed weekly from June 12, 2022 to August 16, 2022. Each site had 2–3 distinct patches of Showy Milkweed that we surveyed. Patches were between 20–750 m apart and ranged in area from 23–2,413 m^2. Every Milkweed plant in each patch was searched visually for Monarchs, including the undersides of leaves, where eggs are usually laid. We measured the length of larvae when we discovered them. At each visit, the number of blooming milkweed heads were counted.

Monarch Butterfly parasite testing

We collected samples from the abdomens of adults to investigate the prevalence of OE on Monarchs. Infection varies by life stage in Monarchs (Leong et al. 1997); however, spores are easily collected on the abdomens of adults during transmission. We collected parasites from adults caught in sweep nets during transect surveys. We also reared late instar larvae found on Milkweed plants at our sites to adults to test them for parasites. A clear sticker (25.4 mm diameter) was gently applied to the abdomen of the adult butterflies while they were firmly held at the base of their folded wings, following Project Monarch Health protocol (Project Monarch Health 2019). Parasite samples were collected from the sides and ventral abdomen, and the butterfly was released upon completion. Sticker samples were examined for spores under a dissecting microscope (magnification 94.5x). Monarch larvae were reared from August 16, 2022 to October 14, 2022 using the Project Monarch Health protocol. Larvae were reared in plastic containers with fresh Milkweed. Adult butterflies were tested for parasites within 24 hours after emergence.

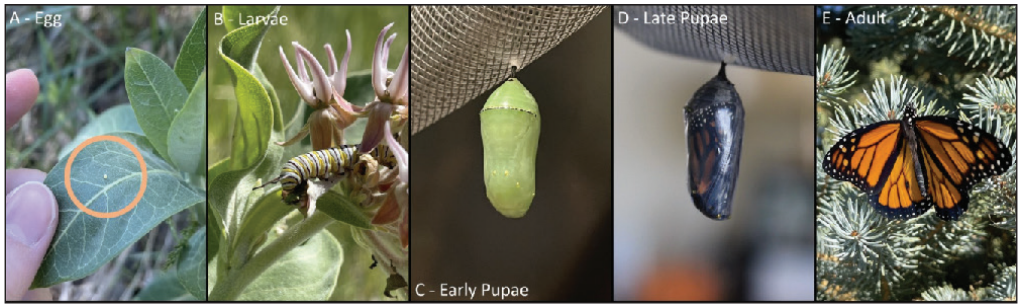

Figure 2: Monarch Butterfly life stages – (A) egg, (B) larvae, (C) early pupae, (D) late pupae, and (E) adult.

Results

During our surveys across 30 sampling events we identified Monarchs in many life stages, including 2 eggs, 29 larvae, 1 empty chrysalis, and 5 adults. We also identified 17 other butterfly species (Supplemental File 1, available online at https://eaglehill.us/prnaonline/suppl-files/prna-033-short-s1.pdf).We observed adult Monarchs at 2 of our sites, and larvae at all 3 sites, showing that Monarchs reproduce in eastern Wyoming. Showy Milkweed flowers ranged between 0.002–1.25 flower heads/m^2 in each patch. We identified 21 other plants at our sites whose flowers varied in density (1–1101 blooms/m^2; Supplemental File 2, available online at https://eaglehill.us/prnaonline/suppl-files/prna-033-short-s2.pdf). Of the 18 adults we tested for the parasite OE (12 males, 5 female), 3 were collected as adults in the field, and 15 were collected as larvae and reared to adults in the lab. Our samples did not contain any spores (0% infected; Fig. 3). Collected larvae were 12–50 mm in length at time of collection and spent an average of 11.8 days pupating.

Discussion

Increased parasitism by OE is a worrisome factor in the decline of Monarchs; however, we did not detect OE during our study in Wyoming. One other sample was collected near Lander, Wyoming; that sample also lacked spores (J. Berliner, Project Monarch Health, Athens, GA,

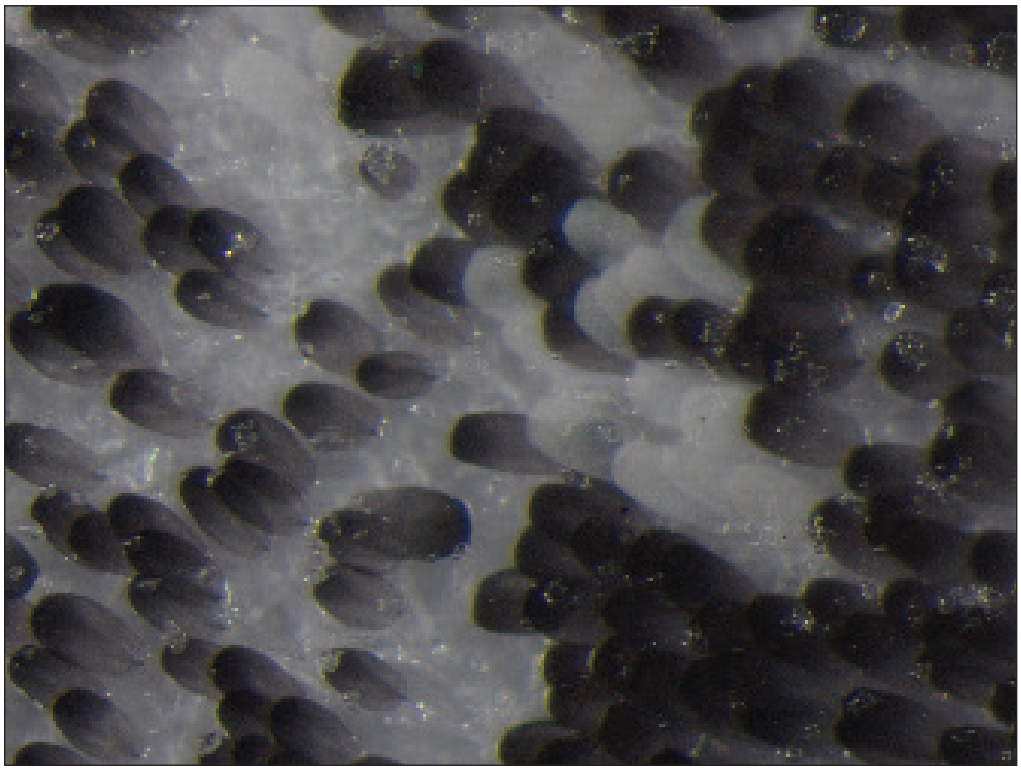

Figure 3. Sample from the abdomen of an adult Monarch to check for the parasite *Ophryocystis elektroscirrha*. The black structures are scales photographed under a dissecting microscope (magnification 94.5x). No spores were present in any of our samples.

2023 pers. comm.). The low abundance of Monarchs in Wyoming may lessen OE's detrimental effects (Bartel et al. 2011, Lindsey et al. 2009, Majewska et al. 2022). As predicted by the Migratory Escape Hypothesis, migratory Monarchs, such as those in Wyoming, have lower parasite loads than non-migratory Monarchs because migration allows Monarchs to escape parasites (Altizer et al. 2000). The eastern population of Monarchs are at the edge of their range in eastern Wyoming, potentially allowing them to reproduce in areas with lower Monarch abundance and lower parasite transmission rates. Further studies and a larger sample size are needed to estimate OE parasite loads in the Rocky Mountain region and measure infection at the junction of eastern and western populations.

Less than 10% of eastern Monarch Butterflies east of the Rocky Mountains are heavily infected with OE spores, while ~30% of western Monarchs are infected (Altizer et al. 2000). Our lack of OE detections aligns with another study at the edge of the Monarch's range in Ottawa, Canada that found extremely low parasite prevalence rates (Dargent et al. 2021). Our study supports the idea that butterflies at the edge of their range experience lower parasite loads than the middle of their range. Adult butterflies must have heavy parasite loads to successfully pass the parasite to their offspring (de Roode et al. 2009). The abundance of Monarch larvae was the best predictor of parasite prevalence in the Northeast and Midwest regions of the United States (Bartel et al. 2011). Low infection rates may be the product of low population abundances, as Monarchs experience higher infection rates when they are more abundant (Lindsey et al. 2009, Majewska et al. 2022). Therefore, Monarchs at the edge of their range may be less likely to transmit the parasite when infected. The main limitation of our study was the low sample size of Monarch Butterflies. While our sample size reflects the relatively low abundance of Monarchs in eastern Wyoming, we cannot definitively conclude that Monarchs in this region carry no parasite spores with such a small data set.

Air temperatures in eastern Wyoming probably do not reduce the viability of OE, but how the parasite responds to low humidity is largely unknown. Sánchez et al. (2021) found that sustained high temperatures (24-32 ºC over ≥35 weeks) reduced OE parasite persistence, viability, and transmission in Monarch Butterflies. These conditions do not typically occur in eastern Wyoming. Because Monarch Butterflies only live for several weeks during the breeding season, spores that persist for two weeks in extreme heat can likely still infect butterflies. Spores have thick walls (McLaughlin and Myers 1970) and can remain viable during short-term heatwaves. This thick wall may also prevent desiccation in semi-arid and arid environments, such as Wyoming; however, the ability of OE to remain viable in low moisture climates requires further investigation.

Milkweed contains a cardenolide, a toxic steroid produced by some plants, and may help explain the low parasite loads we observed. Female Monarch Butterflies tend to select certain Milkweed species when multiple species are present (Schultz et al. 2021) and infected females with OE tend to lay their eggs on Milkweed species containing high cardenolide levels as an anti-parasitic defense for their offspring (Lefèvre et al. 2010, Tao et al. 2016). The concentration of cardenolides varies with Milkweed species; *Asclepias speciosa* Torrey (Showy Milkweed), the species in our study, has an intermediate concentration (Decker and Hunter 2020). Intermediate cardenolide concentrations confer the highest fitness to infected Monarchs (Sternberg et al. 2012). The cardenolide concentration of Showy Milkweed may be at least partially responsible for the lack of parasite spores found in our Wyoming Monarchs. Further studies should investigate cardenolide concentrations of Showy Milkweed in Wyoming, to investigate the degree to which this species benefits breeding Monarchs and rearing programs.

The presence of eggs, larvae, and empty chrysalises indicate that Monarch Butterflies use habitats in eastern Wyoming to reproduce. Discovering new breeding habitat for Monarchs

is pertinent, given their declining numbers across their range. Habitat in Wyoming and other Rocky Mountain States may be critical for Monarchs because declining species tend to persist at the edge of their range (Channell and Lomolino 2000). New breeding habitat may provide refuge for these butterflies in the face of extirpation from their historically occupied areas and provide ideal regions to implement breeding, conservation, and reintroduction programs. Management strategies could aim to prevent habitat loss and provide Monarchs with more breeding opportunities at the edge of their range where parasitism is low, as observed in our study. Further studies are needed to estimate the abundance of Monarchs that use other areas at the junction of eastern and western populations as breeding habitat.

More data throughout the Intermountain West region is needed to quantify the prevalence of the parasite OE in Monarch Butterflies; however, these results are promising and provide baseline information on Monarchs in this region. Further studies should be done on the timing of Monarch migration, their abundance, parasite loads, and the potential crossover of eastern and western populations across the Rocky Mountains. Citizen science databases, such as iNaturalist, may be useful to investigate their abundance and distribution; however, a targeted study is needed to measure parasitism rates. Our results indicate that OE is largely absent in Monarchs from this region, which is encouraging, considering that rare species tend to persist at the edge of their range (Channell and Lomolino 2000). Our study provides a glimmer of hope for Monarchs, and we encourage others to investigate these butterflies at the junction of eastern and western populations to see if parasitism is generally low in those regions. If OE is seldom encountered, the Rocky Mountain region may be an area to focus conservation efforts. While the decrease of Monarchs is complex and multifaceted, the parasite OE contributes to their annual decline. Understanding the distribution and environmental requirements of this parasite is critical to best mitigate OE's effect on Monarch populations. Studying OE may further our understanding of parasites on other pollinators and help us implement management tactics to prevent future declines in insect populations.

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