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NOTE

Low prevalence of the parasite *Ophryocystis elektroscirrha* at the range edge of the eastern North American monarch (*Danaus plexippus*) butterfly population

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Abstract: Every year monarch butterflies (*Danaus plexippus* (Linnaeus, 1758)) from the eastern North American population migrate from Mexico to southern Canada in the spring. This northward migration has been shown to reduce monarch infection with the host-specific parasite *Ophryocystis elektroscirrha* McLaughlin and Myers, 1970 (OE); yet, the prevalence of OE at their range limits and the mechanism(s) responsible are unknown. We assessed OE infection levels of monarchs at the northern edge of the eastern population distribution around Ottawa, Ontario, Canada, and found extremely low levels of infection (~1% with upper confidence intervals close to 3%). Low OE infection levels are likely due to low densities of monarchs in this region and (or) migratory escape effects, where migrating individuals leave behind areas with high density of conspecifics and high potential for parasite accumulation and transmission. Future work should aim to disentangle the relative contribution of these two mechanisms for governing the decrease in parasitism at the range limits of migratory populations.

Key words: monarch butterflies, *Danaus plexippus*, OE, *Ophryocystis elektroscirrha*, range limit, host-specific parasite, prevalence, disease ecology, migration.

Résumé : Chaque année, des monarques (*Danaus plexippus* (Linnaeus, 1758)) de la population de l'est de l'Amérique du Nord migrent du Mexique au sud du Canada au printemps. S'il a été démontré que cette migration vers le Nord limite l'infection des monarques par le parasite spécifique *Ophryocystis elektroscirrha* McLaughlin et Myers, 1970 (OE), la prévalence d'OE aux limites de leur aire de répartition et le ou les mécanismes en cause demeurent inconnus. Nous avons évalué les taux d'infection à OE chez les monarques à la limite septentrionale de l'aire de répartition de la population de l'est autour d'Ottawa (Ontario, Canada) et relevé des taux d'infection extrêmement faibles (~1 % pour des intervalles de confiance supérieurs de près de 3 %). Les faibles taux d'infection à OE sont vraisemblablement dus aux faibles densités de monarques dans cette région et (ou) aux effets de la fuite migratoire, les individus qui migrent laissant derrière eux des zones de forte densité de conspécifiques et de potentiel élevé d'accumulation et de transmission de parasites. Des travaux futurs devraient s'affairer à départager les contributions relatives de ces deux mécanismes à la réduction du parasitisme aux limites des aires de répartition des populations migratrices. [Traduit par la Rédaction]

Mots-clés : monarques, *Danaus plexippus*, OE, *Ophryocystis elektroscirrha*, limite de l'aire de répartition, parasite spécifique, prévalence, écologie des maladies, migration.

Introduction

Every spring, monarch butterflies (*Danaus plexippus* (Linnaeus, 1758)) of the eastern North America population depart their wintering grounds in the highlands of central Mexico (Urquhart and Urquhart 1978) and engage in one of the most striking animal migrations in the world. Individuals born in the previous year leave Mexico in the spring and travel north towards their breeding grounds across the eastern US to southern Canada (including southern Quebec, Ontario, and Manitoba), an area covering over 4000 km (Urquhart and Urquhart 1978; Brower 1995; Flockhart et al. 2019*a*). This northward migration is completed through 3–4 generations. In contrast, the "return" fall migration southwards is generally completed in a single generation (Prysby and Oberhauser 2004; Solensky 2004).

Importantly, the migration process is influenced by interactions with the host-specific neogregarine parasite of monarchs, *Ophryocystis elektroscirrha* McLaughlin and Myers, 1970 (OE). Infection with OE can be detrimental to monarchs and may impact population density and persistence (Altizer et al. 2004). For example, infection has been shown to kill pupae, lead to unsuccessful eclosion, and shorter female life span (De Roode et al. 2008), as well as wing malformations and decreased flight ability (Bradley and Altizer 2005). It has also been shown to reduce body size and reproductive success (Altizer and Oberhauser 1999).

OE only infects its host when monarch larvae ingest dormant spores (i.e., gregarine oocyte stage) scattered on their egg, which larvae eat upon eclosion, or on milkweed (genus *Asclepias* L.), the host plants used by adult butterflies to oviposit (McLaughlin and Myers 1970; De Roode et al. 2009). After ingestion, OE sporozoites migrate to the hypoderm, reproduce, and spores are deposited in the butterfly cuticle, predominantly on the abdomen (McLaughlin and Myers 1970). Upon emergence, infected hosts may carry millions of oocytes (McLaughlin and Myers 1970; Leong et al. 1992). There are three hypothesized transmission pathways of OE. Maternal

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(vertical) transmission occurs during oviposition (McLaughlin and Myers 1970) and is thought to account for over 90% of OE transmission (Altizer et al. 2004). Female butterflies transmitting the spores could be infected themselves, and therefore may carry high loads of OE, or might be uninfected but still carry low spore loads following mating or contact with infected adults (i.e., adult transmission; Altizer et al. 2004). Alternatively, infected butterflies resting or nectaring on milkweed can release OE spores that accumulate and are later consumed by monarch larvae (i.e., environmental transmission; Altizer et al. 2004). Although OE is host-specific to monarchs, there is current debate on whether they also infect a species absent in our study area, Queen butterflies (*Danaus gilippus* (Cramer, 1775)) (Barriga et al. 2016).

While OE infection seems to occur in almost all monarch populations in the world (Altizer and de Roode 2015), the prevalence of the infection (i.e., the percentage of infected individuals) can vary considerably among populations anywhere from 5% up to 100% in some populations (Altizer et al. 2000). It has been noted previously that the eastern North America population has lower OE prevalence than the western North America population, which covers a shorter migratory range from Mexico along the coast of California (USA), and the non-migratory populations in the gulf of Mexico (Altizer et al. 2000).

There are two main theories thought to explain the variation in parasite prevalence within and among migrating monarch populations. The migratory escape hypothesis proposes that as individuals migrate, they leave behind areas where the density of conspecifics has been increasing and thus have higher potential for parasite accumulation and transmission (Folstad et al. 1991; Loehle 1995). Indeed, eastern monarchs travelling north during the breeding season are thought to benefit from migration-facilitated escape from contaminated habitats where OE accumulates over time due to increasing densities of hosts and parasite (Bartel et al. 2011; Flockhart et al. 2018). Alternatively, the migratory culling hypothesis contends that due to the high energetic demands of migration, infected individuals face higher mortality than uninfected individuals during migration and are therefore disproportionally removed from the population (Bartel et al. 2011). This process is thought to occur during the fall migration, when individuals travel longer distances and experience higher energetic demands, leading to lower prevalence in monarchs as they move southward (Bradley and Altizer 2005; Altizer et al. 2011, 2015; Bartel et al. 2011). Thus, northward and southward migrations are thought to support different parasite release processes.

Despite extensive study of the eastern North America monarch population (Altizer and de Roode 2015), the prevalence of OE at the northern range limit remains unclear. In particular, additional ecological mechanisms at range limits could influence OE prevalence in their monarch hosts. For example, the low density and decreased connectivity that is typical of subpopulations of organisms at their distribution edge (Sexton et al. 2009) has been shown to be insufficient to support stable parasite populations in other species (Gaston 2009) and can constrain re-colonization of parasites following local extinctions (e.g., Keeling et al. 2004; Kaunisto et al. 2015). Understanding infection patterns at the northern edge of the monarch distribution is important because these are sites predicted to have increased presence of monarchs and milkweed under some climate change scenarios (Lemoine 2015).

We surveyed monarch butterflies and their OE infection rates around Ottawa, Ontario, Canada, which is at the northern range limit of the eastern North American population. Migrating monarchs consistently reach this region every year, whereas the presence of monarchs in sites farther north is less reliable (Flockhart et al. 2019*b*). We predicted that monarchs in this region would have extremely low or no infection by OE based on previous support for the migratory escape hypothesis and the seasonality and length of growing season at this latitude. Monarch residency time is thought to be shorter in this region than farther south due to the distance from the overwintering grounds, the shorter growing season, and the earlier defoliation of milkweed in the fall (Bhowmik and Bandeen 1976). These factors reduce the accumulation time for parasites, restrict the host population density, and reduce the accumulation of OE spores through the season and eliminate them from 1 year to the next.

Materials and methods

Field collections

We collected eggs, larvae, and adults at 29 locations across the city of Ottawa and its surroundings (Fig. 1) between 18 June and 25 September 2019 (i.e., covering the monarch breeding season in our region). We collected these samples opportunistically as part of other projects looking into milkweed phenology and milkweed-specialist community diversity. All our collections were done at sites where common milkweed (*Asclepias syriaca* L.), the main host plant for monarch larvae in the region, was present. These sites covered diverse land-use types: urban, suburban, forested, and old fields. They spanned an east–west distance of approximately 70 km and a north–south distance of approximately 23 km (Fig. 1). At each site, we inspected at least 60 milkweed plants for eggs and larvae. We noted the instar at the time of collection and transported them to our laboratory at the University of Ottawa to be reared into adults (see below). We also attempted to capture adults that were within 10 m of us and we estimate that our capture success rate was about 30%. We trapped adults with butterfly nets, sampled OE in situ (see below), and then released individuals. In the rare instance when we sampled multiple adults at the same site and day, we kept track of each individual to avoid resampling.

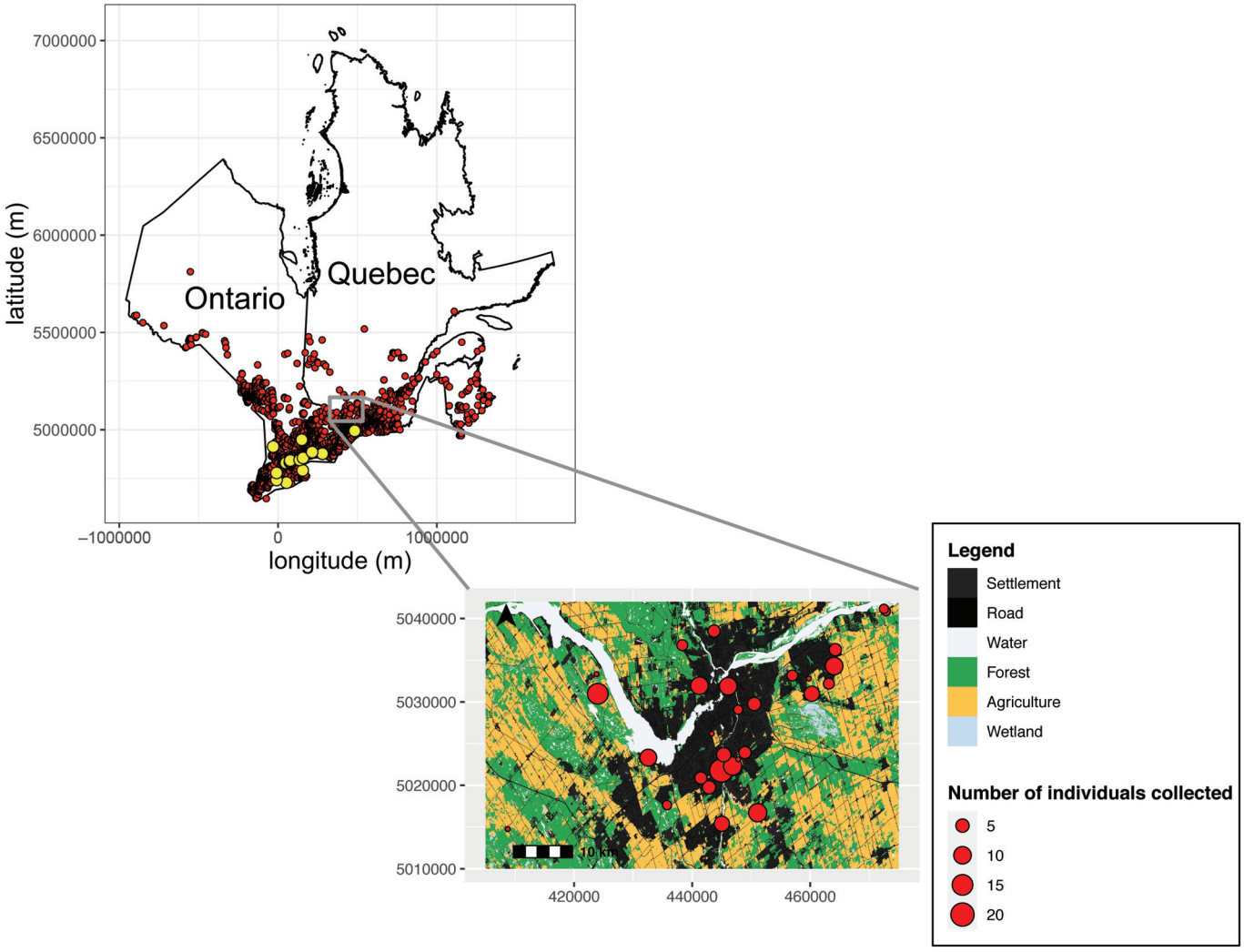
Larval rearing

Eggs and larvae were reared in a controlled environmental chamber (Biochambers Inc., model LTCB-19), with a 12 h light : 12 h dark photo cycle, constant humidity of 60%, and a temperature of 28 °C during the light cycle and 26 °C during the dark cycle, approximating the optimal temperature for larval development (i.e., 27 °C; Zalucki 1982; Nail et al. 2015) and OE reproduction (Lindsey 2008). That is, raising hosts and parasite at their optimal temperature increased our chances of detecting whether OE is present in our study area. Larvae were housed individually in 500 mL plastic or glass containers with a fine mesh lid and were fed ad libitum once a day with fresh common milkweed leaves collected from a single site in the field (site of collection varied daily). We checked that leaves were free of dirt but did not wash them with diluted bleach because we were more concerned by the potential negative effects of bleach on the larvae than possible cross infection from OE spores left by infected adults on our milkweed. We deemed the risk of OE accidental infection low because monarch densities in our study area are very low: based on 32 site visits (10 sites visited twice and 12 sites visited once) between 9 July and 17 September, we found a mean number of 0.016 monarch larvae per milkweed stem (no monarchs were found during 17 visits, 266 stems checked, on average, per site visit) (F. Dargent, S.M. Gilmour, E.A. Brown, R. Kassen, and H.M. Kharouba, unpublished data). We removed frass and cleaned cages every 2 days to maintain clean conditions. Upon formation of a chrysalis, we checked the cage daily until an adult emerged. Adults were then sampled for OE infection.

OE sampling

Following the methods of Altizer et al. (2000), we collected OE spores by placing a 1 inch (2.54 cm) clear envelope sticker (Pop Resin) on the abdomen of each adult and then placing it on a 3 inch × 5 inch index card. Cards were then inspected under a light optical microscope at 40× and the whole surface was

Fig. 1. Eastern Canada monarch (*Danaus plexippus*) sightings, location of collection sites, and sample size. Monarch sightings in Eastern Canada reported by citizen scientistis in eButterfly (Larrivee et al. 2018) and Mission Monarch (Larrivee et al. 2018) from 2016 to 2018 in red, and samples collected by Flockhart et al. (2018) in yellow. The inset shows our 2019 collection sites (red dots) in the Ottawa, Ontario, Canada, region relative to land-use types (Agriculture and Agri-Food Canada 2010). The size of the dots is scaled to the number of individuals that we collected at each site. This figure was created using package "raster" version 3.3-7 (Hijmans 2020) in R version 3.6.3 (R Core Team 2018) and assembled from the following data source (raster): Land Use 2010 (Agriculture and Agri-Food Canada 2010). Colour version online.



inspected for OE. When OE was detected, we estimated the number of OE spores following Altizer et al. (2000). All laboratory-reared adults were sexed and sampled for OE within a day of eclosion.

Analysis

We estimated 95% confidence intervals (95% CI) for prevalence using the adjusted bootstrap percentile (BCa) method with 1000 resamplings. We performed analyses using the "boot" package (Canty and Ripley 2019) in R (R Core Team 2018).

To provide a broader Canadian context to our results, we also calculated prevalence on a subset of the Flockhart et al. (2018) publicly available dataset collected in 2011 (Appendix S2 in Flockhart et al. 2018). Since we wanted to evaluate prevalence in the northeastern portion of the range (i.e., southeastern Canada), we used data north of 42.5777°N and east of 83.4511°W, which is roughly between the northeast point of Windsor, Ontario, and southwest of Ottawa (Fig. 1).

Results

We collected 140 pre-adult individuals that were reared in the laboratory (n = 65 males, 74 females, 1 unidentified) from 28 out of 29 locations surveyed (mean ± SE individuals per location: males = 2.5 ± 0.3; females = 2.3 ± 0.3; total = 4.8 ± 0.4) (Fig. 1, Supplementary Table S1[1]). We sampled 27 adult butterflies in the field (n = 12 males, 14 females, 1 unidentified) at 10 locations (mean ± SE individuals per location: males = 1.1 ± 0.3; females = 1.5 ± 0.3; total = 2.7 ± 0.4) (Fig. 1, Supplementary Table S2[1]).

Out of these 167 individuals, only one female, collected as an egg and reared in the laboratory, was infected with OE. It had a high infection (>1000 spores). Thus, if we consider our samples to be truly independent (i.e., each offspring laid by a different mother), OE infection prevalence in the city of Ottawa and its surroundings was 0.6% (95% CI: 0, 1.8). Alternatively, to account for potential pseudo-independence (each mother lays more than one egg per day and site), we also tested prevalence assuming that on a

[1]Supplementary tables are available with the article at https://doi.org/10.1139/cjz-2020-0175.

given sampling day, only samples from adults, and different pre-adult developmental stages from the same site, were independent (n = 90). For example, all first instar larvae collected on the same date and at the same location were treated as only one sample. With this conservative approach, we estimated prevalence to be 1.1% (95% CI: 0, 3.33) out of 90 samples.

In comparison, 59.1% of the butterflies (n = 98) from the Flockhart et al. (2018) dataset were infected with OE at a low level (i.e., <100 spores) and 14.3% at a high level (i.e., >100 spores). Spore loads of less than 100 may reflect the attachment of spores to an adult, which does not have any effect on the condition of that adult per se (i.e., not a "true" infection). A "true" infection follows spore consumption as a larvae (De Roode et al. 2007, 2009).

Discussion

We collected monarch butterflies from 29 locations at the northern edge of the distribution of the eastern North American population and found exceptionally low levels of infection (~1% with upper confidence intervals close to 3%) with the parasite *Ophryocystis elektroscirrha* (OE). Previous assessments of OE infection levels for the entire eastern North American population report the prevalence of infected individuals (i.e., carrying >100 spores) to be 7% (Altizer and de Roode 2015). However, infection levels range between 2.5% and 18% among years in the northeast part of the population (Bartel et al. 2011). Our OE estimates were also lower than the ones extracted from data collected south of our study area in southeastern Canada (Flockhart et al. 2018). Importantly, the northernmost collection site (45.10318°N, 75.22101°W) for the region sampled by Flockhart et al. (2018) is approximately 35 km south of our study area, and thus closer to the core of the monarch distribution than our samples. This could help explain why their estimate of prevalence was higher than ours (approximately 10% based on fig. 3 in Flockhart et al. 2018). Although variability in OE infection levels among years and locations can be high (e.g., Bartel et al. 2011), our comparatively low estimate of prevalence suggests that, at least within the year sampled, individuals at the northern edge of their distribution may experience lower infection levels than individuals closer to the core of their distribution.

There are two factors related to migration and edge effects that likely explain the low OE infection levels that we found at the northern range edge. First, previous studies have found infection patterns related to the northward migration of the eastern North America population during the spring–summer breeding period consistent with the migratory escape hypothesis. Specifically, that prevalence increases over the season in all areas (as monarch populations grow), southern areas have higher infection rates than northern ones (Bartel et al. 2011; Flockhart et al. 2018), and monarch larval density is positively correlated with OE prevalence (Bartel et al. 2011). Additionally, during the spring–summer migration, regional differences in prevalence are attributable to differences in local infection levels rather than due to differential migration distances between infected and non-infected individuals (i.e., migratory culling) (Flockhart et al. 2018). Our findings are consistent with these studies and suggest that the process of migratory escape leads to lower OE prevalence at the limits of the geographic distribution.

Second, the low density of monarchs at their range edge may be insufficient to maintain its host-specific parasite, as seen in other host species (e.g., holly leaf-miners attacked by parasitoids: Brewer and Gaston 2003; Asian house geckos infected by mites: Coates et al. 2017; bumblebees under nest parasitism by *Psythirus* spp. cuckoo bumblebees: Antonovics and Edwards 2011). Models by Flockhart et al. (2019*b*) using community science data show that monarch density is lower in this part of their northern range than in more southern areas. Indeed, we found lower density at our sites than Bartel et al. (2011) did in their northeastern region (>36°30′N). Additionally, since monarch adults cannot become infected (only carry spores if they become exposed) and infection can only occur through ingestion by larvae (McLaughlin and Myers 1970), transmission opportunities might be constrained even further than for other species living at their range edge. In combination, these two mechanisms could explain the exceptionally low OE prevalence at our sites. Future work should aim to disentangle the relative contribution of these two mechanisms for decreasing parasitism at the range limits of migratory populations.

Despite our estimate of prevalence being based on 167 individuals, we do not think our results reflect a limited ability to detect higher prevalence levels (i.e., low detection). Although we had a smaller sample than broader scale studies that rely on community science data (e.g., Bartel et al. 2011; Flockhart et al. 2018), we had a better sampling effort (e.g., number of collected individuals per km^2) than other studies done in nearby regions (e.g., Flockhart et al. 2018). This sampling effort, combined with our sampling during the breeding season in our region, increases our confidence that our prevalence estimate accurately reflects typical OE loads in our study area.

Our study suggests that the current prevalence of OE infection at the northern range edge of the eastern North American monarch population is very low. As our samples are from a single year in a relatively small area, longer term studies of OE infection across a larger geographical extent are needed to determine the temporal dynamics of OE infection in this region. Actions to protect monarch populations should integrate disease dynamics across the whole distribution range and evaluate the broader impacts of parasite evasion at the range limits.

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