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Patterns of parasitism in monarch butterflies during the breeding season in eastern North America

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Abstract. 1. Migratory behaviour can result in reduced prevalence of pathogens in host populations. Two hypotheses have been proposed to explain this relationship: (i) 'migratory escape', where migrants benefit from escaping pathogen accumulation in contaminated environments; and (ii) 'migratory culling', where the selective removal of infected individuals occurs during migration.

2. In the host–parasite system between the monarch butterfly (*Danaus plexippus* Linn.) and its obligate protozoan parasite *Ophryocystis elektroscirrha* (OE), there is evidence to support both hypotheses, particularly during the monarchs' autumn migration. However, these processes can operate simultaneously and could vary throughout the monarchs' annual migratory cycle. Assessing the relative strength for each hypothesis has not previously been done.

3. To evaluate both hypotheses, parasite infection prevalence was examined in monarchs sampled in eastern North America during April–September, and stable isotopes (δ^2H, δ^{13}C) were used to estimate natal origin and infer migration distance. There was stronger support for the migratory escape hypothesis, wherein infection prevalence increased over the breeding season and was higher at southern latitudes, where the breeding season tends to be longer compared with northern latitudes. Little support was found for the migratory culling hypothesis, as infection prevalence was similar whether monarchs travelled shorter or longer distances.

4. These results suggest that migration allows individuals to escape parasites not only during the autumn, as shown in previous work, but during the monarchs' spring and summer movements when they recolonise the breeding range. These results imply a potential fitness advantage to monarchs that migrate further north to exploit parasite-free habitats.

Key words. *Danaus plexippus*, deuterium, infection prevalence, monarch butterfly, seasonal migration, stable isotopes.

Introduction

Elucidating the interactions between parasites and migratory animals could provide important insights into the ecological drivers of infectious disease dynamics as well as the evolutionary drivers of animal migration. In some cases, migratory animals may act as hosts or vectors that spread pathogens (Altizer *et al.*, 2011). For example, migratory birds are thought to be dispersal agents for avian influenza (Lycett *et al.*, 2016) and West Nile Virus (Rappole *et al.*, 2000; Owen *et al.*, 2006), and Ebola virus outbreaks in humans coincided with stopover of migratory fruit bats (Leroy *et al.*, 2009, but see Leendertz *et al.*, 2016). However, migratory animals may also experience lower infection risk due to migration if movements away from contaminated habitats decrease parasite transmission (Folstad *et al.*,

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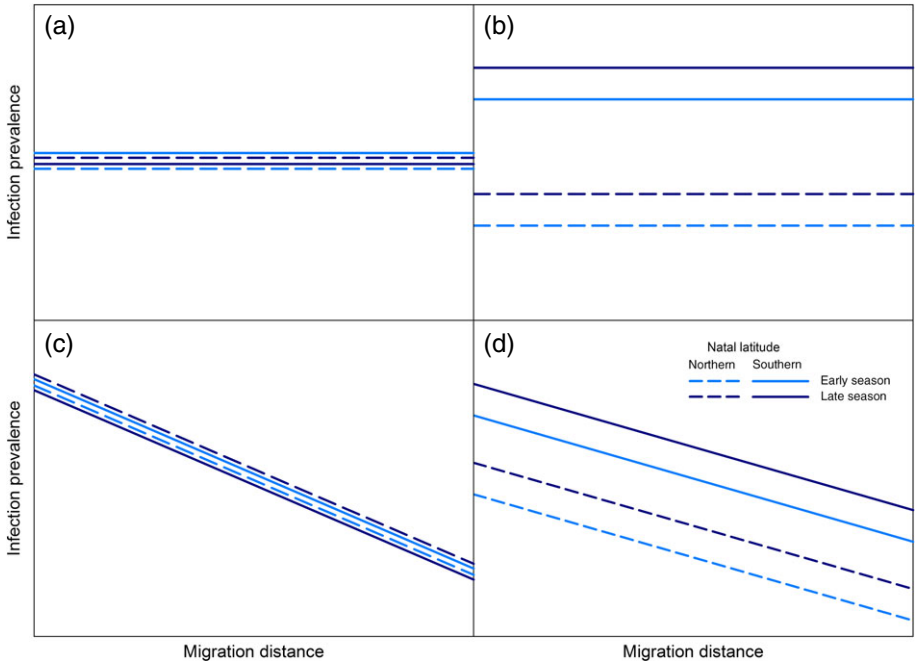

Fig. 1. Predictions of the four hypotheses to explain infection prevalence in monarch butterflies at southern (solid lines) and northern (dashed lines) portions of the breeding range, early (light blue) and late (dark blue) in the breeding season. (a) A null hypothesis predicts that infection prevalence is constant and does not vary over space and time. The order of the lines is not biologically relevant and the small separation between the lines is for aesthetic purposes only and reflects no significant differences between the factors. (b) The migratory escape hypothesis predicts a positive relationship between time of the breeding season and infection prevalence (shown here by different intercepts between coloured lines), and a negative relationship between breeding latitude and infection prevalence (shown here by different intercepts between dashed and solid lines). (c) The migratory culling hypothesis predicts a negative relationship between infection prevalence and migratory distance (shown here by negative slopes of all lines). The order of the lines is not biologically relevant and the small separation between the lines is for aesthetic purposes only and reflects no significant differences between the factors. (d) The joint migratory escape–culling hypothesis predicts a positive relationship between time of the breeding season and infection prevalence, a negative relationship between breeding latitude and infection prevalence, and a negative relationship between infection prevalence and migratory distance. [Colour figure can be viewed at wileyonlinelibrary.com].

1991). Thus, infectious disease processes may also feed back to shape migratory behaviour of animals.

Migratory behaviour can result in reduced infection risk in migratory host populations compared witih resident (non-migratory) populations (Altizer *et al.*, 2011). Two primary hypotheses have been proposed to explain this pattern. The 'migratory escape' hypothesis proposes that migratory populations have lower parasitism rates because migration can enable animals to leave contaminated habitats where parasites accumulate over time (Loehle, 1995; Bartel *et al.*, 2011). The predictions of the migratory escape hypothesis are that pathogens should accumulate in habitats over time and that hosts should move from contaminated habitats to less contaminated habitats (Fig. 1). For example, in reindeer (*Rangifer tarandus*), migratory distance from breeding grounds was negatively related to warble fly (*Hypoderma tarandi*) abundance during their post-calving migration, suggesting that individuals departing contaminated habitats experience reduced parasite transmission (Folstad *et al.*, 1991). The 'migratory culling' hypothesis proposes that migratory populations benefit from lower parasitism rates due to mortality of infected individuals during energetically costly migration (Bradley & Altizer, 2005; Bartel *et al.*, 2011; Altizer *et al.*, 2015). The predictions of the migratory culling hypothesis are that pathogen loads should be negatively correlated with migration distance (Fig. 1). For example, in Bewick's swans (*Cygnus columbianus bewickii*), initial migratory flight distance was shorter in individuals infected with low-pathogenic avian influenza (van Gils *et al.*, 2007). Both the migratory escape and migratory culling hypotheses are supported by independent studies in multiple host–parasite systems (reviewed in Altizer *et al.*, 2011). However, the influence of these two mechanisms can be difficult to discern from field datasets and these processes are not mutually exclusive. In order to gain a more complete understanding of the factors driving parasite–migration interactions, it is critical to test both hypotheses simultaneously. This knowledge could aid in making predictions about the degree to which animal migrations – or changes in such migrations – will affect host–parasite dynamics.

The host–parasite system for the monarch butterfly (*Danaus plexippus*) and its obligate protozoan parasite *Ophryocystis elektroscirrha* (OE) arises through successful infection transmission occurring during the monarch's complex multi-generational annual migratory cycle in eastern North America (McLaughlin & Myers, 1970). Infection occurs when monarch larvae ingest OE spores by consuming the egg capsule or host plant

tissue, which lyse and replicate in the hypoderm (Leong *et al.*, 1997), resulting in the production of dormant spores on the exterior of the adult butterfly (McLaughlin & Myers, 1970; Leong *et al.*, 1992). The infection of OE in monarch butterflies has two transmission modes: vertical and horizontal (Altizer *et al.*, 2000). Vertical transmission (parent to offspring) of OE occurs when spores scattered by female monarchs on eggs or the leaves are consumed by her larval offspring (McLaughlin & Myers, 1970; Leong *et al.*, 1997). Horizontal transmission occurs when spores from an infected adult are transferred to an unrelated larva (de Roode *et al.*, 2008); this happens if spores from an adult monarch, deposited during oviposition, are consumed by an unrelated larva, or if spores passively acquired by an adult monarch (from another unrelated monarch) are transferred to her offspring. Vertical transfer is probably the main route of transmission (Vickerman *et al.*, 2010). Monarchs infected with OE have reduced flight capabilities (Bradley & Altizer, 2005), reduced body size (Altizer & Oberhauser, 1999; de Roode *et al.*, 2007), lower mating success (Altizer & Oberhauser, 1999), and lower survival (Altizer & Oberhauser, 1999; Altizer *et al.*, 2015). Transmission of parasites occurs during the monarch breeding season, which starts as monarch return to the southern U.S.A. from Mexican overwintering sites during the spring. The monarchs' progeny and grand-progeny then migrate in a general northward direction to recolonise the breeding range in eastern North America over two to four generations (Cockrell *et al.*, 1993; Malcolm *et al.*, 1993; Miller *et al.*, 2011; Miller *et al.*, 2012; Flockhart *et al.*, 2013). Transmission of OE occurs throughout this recolonisation and breeding period, typically from April to August. Monarchs then migrate up to 4000 km in the autumn to central Mexico, where they spend several months as adults in a non-reproductive state. Thus, during the autumn migration and overwintering periods when breeding is not occurring, monarchs experience a pause in OE transmission (although passive transfer of spores between adults can occur), which is resumed the following spring.

Previous studies conducted on OE parasitism in monarchs in North America provide support for both the migratory culling and the migratory escape hypotheses. In support of the migratory culling hypothesis, Altizer *et al.* (2000) found lower infection prevalence among populations with longer migratory distances. Bartel *et al.* (2011) showed that infection prevalence tends to decline among eastern North American monarchs after autumn migration, suggesting that infected butterflies were removed from the population during strenuous journeys. Bartel *et al.* (2011) also provided evidence for the migratory escape hypothesis by showing a positive relationship between infection prevalence by site and the length of the breeding season at that location. Further, the loss of these processes can increase infection risk: in coastal areas of the southern and western U.S.A., monarchs that breed year-round (rather than migrate) in response to exotic milkweed plants experienced high OE prevalence, probably a result of the extended breeding season which allowed spores to accumulate in the environment (Satterfield *et al.*, 2015, 2016).

Understanding the relative influence of the hypotheses through which migration reduces infection risk in wild populations is fundamental. Previous studies focusing on monarchs and OE used different spatiotemporal datasets that precluded identifying which mechanism had a larger impact on monarch–OE dynamics. Here, we examine how OE parasitism varies by migration distance and natal origin of monarchs, as determined by carbon (δ^{13}C) and hydrogen (δ^{2}H) isotopes. We tested predictions from both the migratory culling and migratory escape hypotheses and, using Akaike information criterion (AIC) and model-selection procedures, examine the relative strength of evidence for each hypothesis. Additionally, by focusing on monarchs from the spring migration and the breeding season, our analysis augments previous work on infection dynamics during the autumn and summer. Investigations of host–parasite dynamics are rarely available across the full annual cycle for wild migratory populations, but such datasets enhance our understanding about how infection risk varies over time and space for highly mobile species.

Methods

Field sampling

The monarch specimens and stable isotope data used for this study are from Flockhart *et al.* (2013). Adult monarchs were sampled opportunistically between 13 April and 1 October 2011 across the breeding distribution in eastern North America along roadsides, milkweed patches, natural areas, fallow fields, and parks (Fig. 2a). For each monarch, we recorded the capture date, location and wing-wear score. Flockhart *et al.* (2013) reconstructed recolonisation patterns of monarchs over the entire breeding period. Here we analysed OE prevalence to evaluate the migratory escape and culling hypotheses, and updated our statistical procedure to assign individuals to their natal origin (see details later).

Our dataset represents monarchs over a large portion of their annual migratory cycle (spring through summer), and thus our specimens include: (i) spring migrants that overwintered in Mexico and began returning northward to the U.S.A. (which occurs as late as April); and (ii) their progeny represented in two to four subsequent generations that recolonise the breeding range (which occurs during approximately May–September). We assigned monarchs to one of these two groups of butterflies, for which we calculate migratory distance differently (described later). Monarchs collected early in the breeding season (April) at southern latitudes may be butterflies that overwintered in Mexico (Flockhart *et al.*, 2013). Overwintered butterflies were identified based on wing-wear condition and capture date (Flockhart *et al.*, 2013); these individuals were approximately 9 months old at the time of capture. The culling and escape hypotheses can be tested on overwintering monarchs if one can assign an approximate date that they eclosed the previous autumn. The literature suggests that the date of eclosion of the migratory generation varies based on several factors including day length, temperature, and food plant condition (Goehring & Oberhauser, 2002), which makes specifying time frames of eclosion challenging. Because date of eclosion was not known for overwintering monarchs, we assumed that the capture date of overwintered individual was equal to the latest capture date in our dataset of monarchs captured during the breeding

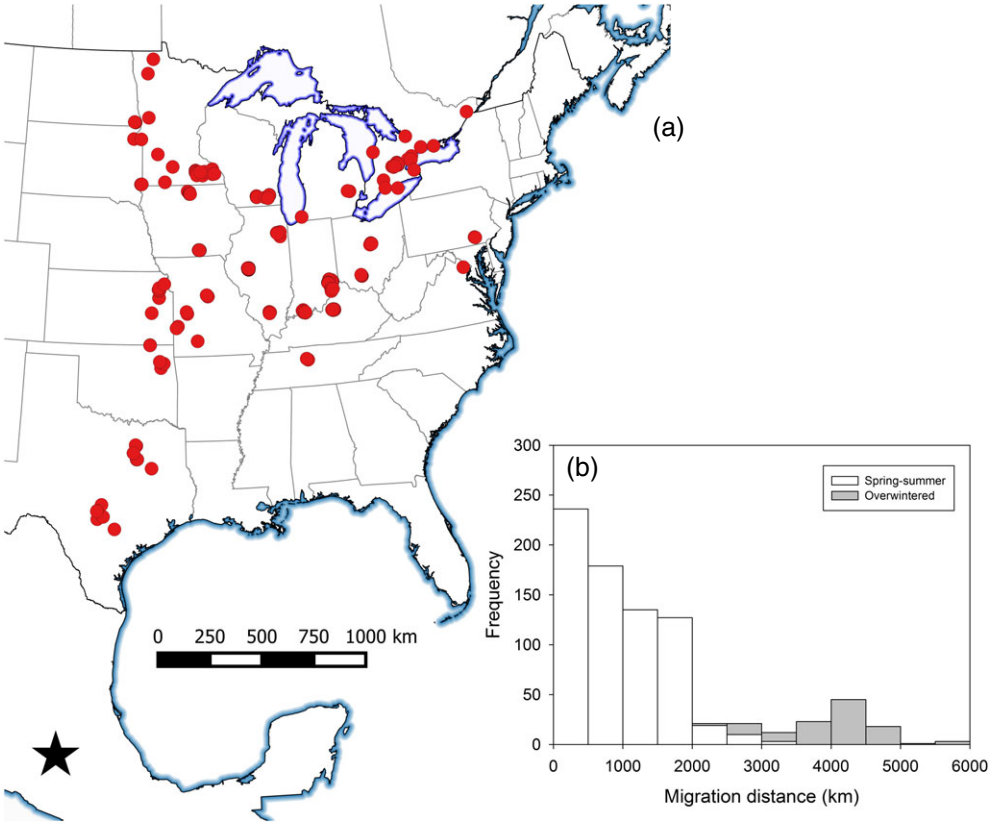

Fig. 2. (a) Map of locations where monarch butterflies were captured in 2011 (red dots) and the overwintering colonies (black star); (b) histogram of migratory distances based on Euclidean distances. Migration distance for spring–summer monarchs (white) was between capture location and natal origin. For monarchs that overwintered in Mexico (grey), migration distance was the sum of the distances between natal origins and overwintering areas in Mexico, and between overwintering areas and capture locations (see text). [Colour figure can be viewed at wileyonlinelibrary.com].

season (September 30),which should reflect infection prevalence at the natal latitude at the end of the breeding season (Bartel *et al.*, 2011).

Estimating migration distance from stable isotopes

Migratory distance was defined as the distance between the collection location and the natal origin of each monarch obtained by using wing carbon (δ^{13}C) and hydrogen (δ^{2}H) isotope measurements (Hobson *et al.*, 1999; Flockhart *et al.*, 2017a). We used multivariate normal distribution assignment models to calculate the probability of natal origin to each pixel in a continuous landscape that spanned the monarch breeding distribution (Royle & Rubenstein, 2004; Miller *et al.*, 2011). For each butterfly in Flockhart *et al.* (2013), the model calculated a probability of natal origin to each pixel (resolution: 0.1667°) in our study area based on the correspondence between δ^{2}H and δ^{13}C values in wing tissue to the isoscape-predicted values of monarch δ^{2}H and δ^{13}C wing tissue of each geographically indexed cell in the landscape from Flockhart *et al.* (2017b). Details of probabilistic assignment of natal origin follow those presented in Wunder (2010) and Flockhart *et al.* (2017b).

From this probabilistic surface, we calculated the latitude and longitude of natal origin in two ways. The high point method considered the latitude and longitude of the pixel with the highest likelihood of being the natal origin; we present the statistical results using the high point method in the main text of the manuscript. The centroid method calculated the mean latitude and mean longitude of the natal origin based on the odds that a given assigned origin was correct relative to the odds that it was incorrect as 2:1 and coded the upper 33% of the assignment surface probability of each butterfly as a binary surface following Hobson *et al.* (2009). The odds ratio approach is akin to selecting a type I error rate in a traditional statistical test and represents a compromise between having sufficient geographic structure in the assignments while correctly assigning the natal origin of an individual (Hobson *et al.*, 2012). In addition, this approach avoids problems in assignment noted by Wunder (2010) for cases with bimodal distributions of populations. The centroid method therefore accounts for uncertainty in the assignment of natal origin and reduces the potential effects of natal origins that are assigned near the edge of the breeding distribution based on a limited geographic extent of the isoscapes. To visualise the uncertainty in migration distance estimates, we present the minimum and maximum estimated migration distances of all pixels from the highest probability of origin (i.e the 2:1 odds ratio) for all monarchs used in the study (Appendix S2). The

statistical results using the centroid method are presented in the supplementary material. Migration distances were calculated as a straight-line distance from the latitude and longitude of the assigned natal origins to the capture location (rhumb line) using the *pointDistance* function in the raster package (Hijmans, 2015) of program R (R Core Team, 2014). For overwintered monarchs, we calculated migration distance as the sum of the distances between the natal location and the overwintering colonies (19°40′30″N, 110°18′15″W; Brower *et al.*, 2016) and between the overwintering colonies and the capture location.

Measuring infection prevalence

Following previous studies (Altizer *et al.*, 2000; Bradley & Altizer, 2005; Bartel *et al.*, 2011; Altizer *et al.*, 2015; Satterfield *et al.*, 2015), OE spores were collected by pressing transparent tape (mail sealing stickers) over the abdomen or thorax (if abdomen was absent) of the butterfly. The tape was then placed on an index card and viewed under a stereo microscope at 65× magnification. The spores were counted and assigned a parasite load using the following ordinal scale: 0, 0 spores; 1, one spore; 2, two to 20 spores; 3, 21–100 spores; 4, 101–1000 spores; and 5, >1000 spores (Altizer *et al.*, 2000). These six categories were reclassified on a binary scale: monarchs with an ordinal score of 0–3 were considered to be uninfected and those with an ordinal score of 4–5 were considered heavily infected (>100 spores; Bartel *et al.*, 2011; Satterfield *et al.*, 2015; Altizer *et al.*, 2015). These categories were based on the assumption that monarchs with >100 spores reflect a true infection (acquired at the larval stage), compared with lower spore numbers assumed to be a result of the passive transfer of spores between adults, which does not cause infection (Altizer *et al.*, 2004; de Roode *et al.*, 2007, 2009). To prevent cross-contamination, forceps used to handle specimens were rinsed in 15% bleach solution between each sample (Altizer *et al.*, 2000). A total of 18 individuals were excluded from the original sample of 839 individuals because the bodies were unavailable to be sampled for OE ($n = 821$).

Statistical analysis

We constructed models for each hypothesis and used information theoretic model selection procedures to determine which model best explained the data. For all models, we used generalised linear models with OE infection status (infected/uninfected) as a binary response variable. Because previous research has considered only OE infection status from samples take on the abdomen, we conducted each analysis with the full dataset and then again with only the subset of data taken from abdomen samples ($n = 574$). For analyses done on the full dataset, we included whether the sample was collected from the thorax or abdomen in all models to control for differences in the probability of infection dependent on where on the body the sample was collected. The null model was an intercept-only model that predicted the probability of OE infection to be constant (Fig. 1a). The model representing the migratory escape hypothesis had Julian date and natal latitude as independent variables, with the probability of OE infection predicted to increase with Julian date, as spores are thought to accumulate in the environment later in the breeding season (Bartel *et al.*, 2011), and to decrease with natal latitude, as breeding at higher latitudes begins later and ends sooner compared with lower latitudes (Fig. 1b). The model representing the migratory culling hypothesis included migration distance as the only independent variable, with the probability of OE infection predicted to decrease with migratory distance, as longer distances would be expected to increase culling of infected individuals (Fig. 1c). A joint migratory escape–culling model considered all three independent variables as additive effects with the same predictions as earlier (Fig. 1d). The four models represented the candidate model set.

For all models, we calculated the AIC corrected for small samples sizes (AICc), ranked them based on the difference between the AICc value for a given model and the model with the lowest AICc value (ΔAICc) and calculated the model weight (w_i), model likelihood (l_i) and model deviance. Given model selection uncertainty, we calculated parameter estimates for all variables with the *modavg* function in the *AICcmodavg* package (Mazerolle, 2014) which uses the model-specific AICc weights in the candidate model set to calculate the mean and 85% CI for each parameter (Arnold, 2010). As the migratory escape and migratory culling models were nested within the joint migratory escape–culling model, we omitted the joint migratory escape–culling model when calculating model averaging to avoid cannibalising model weight from simpler models (Arnold, 2010). All analyses were conducted in the program R version 3.1.0 (R Core Team, 2014).

Results

Migration distance ranged from 30 to 3236 km for monarchs classified as 'summer-breeding' (born within the year the samples were collected) and 2188–5875 km for monarchs classified as overwintered in Mexico prior to capture (born the previous year; Fig. 2b). Overall, monarchs in our collection had an OE infection rate of 14.3% ($n = 118/821$). The probability of infection was significantly different between samples collected from the abdomen (17.3%; $n = 97/547$) and those from the thorax (7.6%; $n = 21/274$), which supported the decision to control for this factor in statistical analyses.

The migratory escape hypothesis was the top supported model and was more than 30 times more likely than the null model (Table 1). The migratory culling hypothesis received no support (Table 1). The joint migratory escape–culling model was 2 AICc units from the top model so the addition of the extra explanatory variable (migration distance) did not improve the model fit. The model-averaged parameter estimates for both Julian date (positive) and natal latitude (negative) were in the predicted direction of the migratory escape hypothesis. However, the effect of migratory distance was positive and, therefore, in the opposite direction predicted by the migratory culling hypothesis (Table 2). Of the three independent variables we considered, only breeding latitude had coefficients that did not overlap with zero (Table 2), suggesting higher OE infection probabilities at southern latitudes compared with northern latitudes after controlling for Julian date and migration distance

Table 1. Models comparing the migratory culling, migratory escape, and joint migratory escape–culling models and a null model on the infection probability of monarch butterflies during the breeding season in eastern North America.

Model	AICc	ΔAICc	w_i	l_i	K	Deviance
Migratory escape	659.4	0.0	0.65	1.00	4	651.4
Migratory escape + migratory culling	661.4	2.0	0.24	0.37	5	651.4
Null	663.5	4.1	0.08	0.12	2	659.5
Migratory culling	665.4	6.0	0.03	0.05	3	659.4

AIC, Akaike information criterion; AICc, AIC corrected for small samples sizes.
The null model was an intercept-only model. The dependent variable in the migratory culling hypothesis was migration distance. The dependent variables in the migratory escape hypothesis included natal latitude and Julian date of capture. The dependent variables in the joint migratory escape–culling model included migration distance, natal latitude, and Julian date of capture. Note that all models included the location on the body where samples were collected to control for variation in the probability of infection between abdomen- or thorax-sampled individuals.

Table 2. Model-averaged parameter estimates, standard error and 85% CI of variables explaining disease probability of monarch butterflies during the breeding season in eastern North America.

Variable	Estimate	SE	85% CI
Julian date	0.0004	0.0026	−0.0033, 0.0042
Breeding latitude	−0.046	0.017	−0.071, −0.021
Migratory distance	0.00003	0.00008	−0.00009, 0.00014

(Fig. 3). There was strong agreement between estimates of natal latitude (Figure S1 in Appendix S1) and migratory distance (Figure S2 in Appendix S1) between the high point and centroid method. Model evaluation (Table S1 in Appendix S1) and model-averaged parameter estimates (Table S2 in Appendix S1) were consistent when using the centroid method compared with the high point method to estimate natal origin. Furthermore, analysing the data when only considering samples taken from the abdomen found equivalent results for model comparisons (Tables S3 and S5 in Appendix S1) and model-averaged parameter estimates (Tables S4 and S6 in Appendix S1).

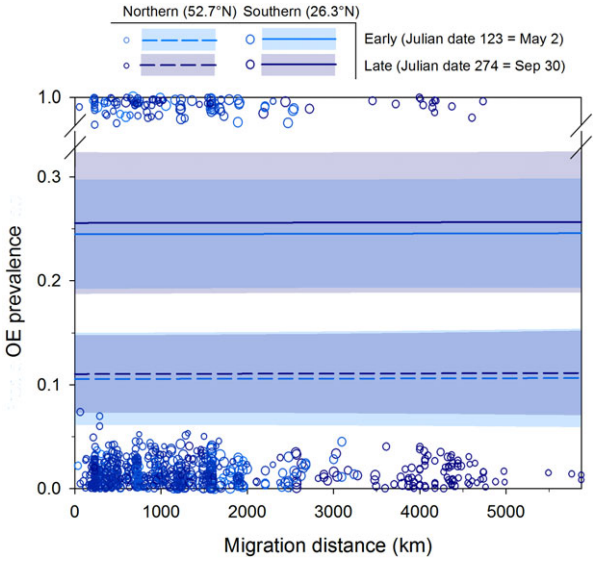

Fig. 3. Mean (lines) ± SE (shading) of model-averaged predicted *Ophryocystis elektroscirrha* (OE) prevalence and observed infection status (points) in monarchs in eastern North America. Samples of OE were taken from thorax or abdomen samples, but the predicted OE prevalence relationships reflect values sampled from the abdomen to allow comparisons with other studies that primarily use OE sampling derived from abdomens. Predictions are presented for breeding monarchs at the beginning (light blue; Julian date 123, May 2) and end (dark blue; Julian date 274, September 30) of the breeding season at southern (solid; 26.3°N) and northern (dashed line; 52.7°N) breeding locations. Raw data are represented as points that scale from northern (small points) to southern (large points) natal latitudes collected early (light blue) to late (dark blue) in the breeding season. Note that the data were binary (infected/not infected) and so the points are presented with jitter for display purposes. [Colour figure can be viewed at wileyonlinelibrary.com].

Discussion

Overall, the migratory escape hypothesis was the top model supported by our data, which suggests that, during the April–September breeding season, migrating monarch butterflies escape habitats with higher disease risk (Bartel *et al.*, 2011) and enter habitats with lower risk of disease to progeny. Consistent with the migratory escape hypothesis, monarch butterflies had higher disease prevalence in southern latitudes, where longer breeding seasons can allow spores to accumulate, than in northern latitudes. Further, infection prevalence increased over the breeding season, as found in previous studies (Bartel *et al.*, 2011). Migratory distance had no effect on infection prevalence, suggesting that monarch migration during the spring–summer breeding season is unlikely to cull diseased individuals from the eastern North American monarch population. These findings could have implications for how infectious disease risk for monarchs responds to longer migrations that may arise from expanding milkweed and monarch distributions (Lemoine, 2015) that are unlikely to remove infected individuals. Additionally, longer breeding seasons would probably enhance disease accumulation (Batalden *et al.*, 2007; Satterfield *et al.*, 2015) and could result in higher infection prevalence for monarchs in eastern North America.

Infection prevalence in monarch butterflies was highest in southern latitudes at the end of the breeding season, and lowest in northern latitudes at the beginning of the breeding season. Given these findings, monarchs that continue to migrate

north throughout the breeding season will encounter lower risk habitats. Spring migration in monarchs closely follows this pattern over the first generation (Cockrell *et al.*, 1993; Malcolm *et al.*, 1993; Miller *et al.*, 2012). Niche modelling suggests that, under predicted long-term climate warming, both monarchs and their milkweed host plants may continue to move northwards (Lemoine, 2015). If these scenarios play out, monarchs could colonise these new habitats. Movement during the middle portion of the breeding season is more stochastic (Flockhart *et al.*, 2013), suggesting that once monarchs colonise the northern portions of the breeding distribution, they may use local habitats that are less likely to harbour pathogens. However, disease risk is not the only factor that controls monarch migratory patterns during the spring and summer, because migration may allow monarchs to avoid density-dependence (Flockhart *et al.*, 2012) or to encounter suitable host plants (Baum & Sharber, 2012) which may increase individual fitness. For instance, breeding monarchs move south in the late summer (Flockhart *et al.*, 2013) to encounter re-emerging milkweed plants at the southern portions of the breeding range where climatic conditions promote development of the final generation of larvae (Calvert, 1999; Baum & Sharber, 2012). Thus, the fitness benefits of parasite avoidance at northern latitudes may be outweighed by recruitment benefits at more southern latitudes late in the breeding season.

As found in previous work, our data showed that infection risk accumulated over the breeding season – one premise of the migratory escape hypothesis (Altizer *et al.*, 2011; Bartel *et al.*, 2011) – although our parameter estimate of this effect did overlap with zero. In monarchs, infected adults deposit parasite spores as they interact with milkweed plants (McLaughlin & Myers, 1970; Loehle, 1995; Altizer *et al.*, 2004): females as they deposit eggs on milkweed plants, and males and females as they forage on nectar from milkweed flowers. Previous research suggested that longer breeding seasons lead to higher infection prevalence (Altizer *et al.*, 2000) and year-round breeding monarchs have higher disease rates compared with migratory monarchs (Altizer *et al.*, 2000; Satterfield *et al.*, 2015). Thus, an increase in residence time of monarchs along the Gulf Coast due to exotic milkweeds that grow year-round (Satterfield *et al.*, 2015), in association with an increase in OE at southern latitudes at the end of the breeding season, could constitute an ecological trap for migratory monarchs.

For monarchs in our dataset from spring and summer, we did not find evidence that heavily infected individuals were culled during their recolonising migration. Specifically, infection prevalence in monarchs did not decline with migratory distance, despite including overwintered monarchs that had travelled >3500 km. This finding contrasts with patterns in infection prevalence found during the autumn migration, when prevalence decreased as the migration progressed (Bartel *et al.*, 2011) and when overwintered monarchs from more northern locations were found to have lower infection prevalence (Altizer *et al.*, 2015). However, our data focused on a distinct phase during the monarchs' annual cycle, spring migration, when monarchs with the most severe infections may have already been culled during the autumn, such that the association between migration distance and infection prevalence might be less obvious. Further, our analysis allowed us to determine the natal origin of butterflies, enabling us to control for the distance that individual butterflies had migrated, to identify true regional differences in prevalence, and to avoid confounding associations among regional infection prevalence, regional productivity, and migratory timing. Migratory culling may not occur over the breeding season because the long distance and endurance required for autumn migration are not required in the summer, or there is a threshold distance after which monarchs have a higher probability of mortality (Bradley & Altizer, 2005). Consistent with this idea, a recent study showed that infected monarchs collected at overwintering sites in Mexico originated from more southern latitudes compared with healthy butterflies, suggesting that infected individuals survived the autumn migration when migration distance was relatively short (Altizer *et al.*, 2015). Collectively, these findings provide support that infected monarchs may have a maximum migration distance for endurance flying that, once surpassed, becomes fatal. Surpassing such a threshold may not often occur during the breeding season.

The finding that infection prevalence increases in monarch butterflies over the breeding season (in this study and in Bartel *et al.*, 2011) and is highest in year-round breeding monarchs (Altizer *et al.*, 2000; Satterfield *et al.*, 2016) suggests consequences for monarch host–parasite dynamics under global environmental change. Anthropogenic habitat changes due to the planting of non-native milkweed plants in Texas and along the Gulf Coast may result in a longer breeding season and high infection prevalence among monarchs at these sites (Satterfield *et al.*, 2015). As autumn migrants pass through these areas each spring and autumn, and breeding has been recorded on these milkweeds through the winter season, one concern is that breeding locations at southern latitudes will enhance exposure of migrants or their offspring to parasites. The consequence is that disease rates may be elevated in these modified habitats, particularly at lower latitudes. Furthermore, climate change predicts an expanded distribution of milkweed plants (Lemoine, 2015) and an increase in the number of breeding generations and the overall breeding season (Batalden *et al.*, 2007), which, given the findings here and previous findings, could lead to higher infection prevalence in monarchs. If monarchs are not culled during the breeding season or during longer migrations to overwintering colonies, disease levels may increase in this population. Given that monarchs face numerous varied threats to their viability (Flockhart *et al.*, 2015) and have been declining over the past two decades (Semmens *et al.*, 2016), an increase in infection prevalence may further challenge conservation of this species. By having a greater understanding of the relationship between migration and infectious pathogens across the full annual cycle for wild host populations, we can better predict the potential impacts of human activities and environmental changes on these systems.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12460

Appendix S1. Supplementary file of results.

Appendix S2. Data file to conduct analysis.

Appendix S3. R code to run statistical models.

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