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LETTER

Parasites hinder monarch butterfly flight: implications for disease spread in migratory hosts

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Abstract

Monarch butterflies (*Danaus plexippus*) are parasitized by the protozoan *Ophryocystis elektroscirrha* throughout their geographical range. Monarchs inhabiting seasonally fluctuating environments migrate annually, and parasite prevalence is lower among migratory relative to non-migratory populations. One explanation for this pattern is that long-distance migration weeds out infected animals, thus reducing parasite prevalence and transmission between generations. In this study we experimentally infected monarchs from a migratory population and recorded their long-distance flight performance using a tethered flight mill. Results showed that parasitized butterflies exhibited shorter flight distances, slower flight speeds, and lost proportionately more body mass per km flown. Differences between parasitized and unparasitized monarchs were generally not explained by individual variation in wing size, shape, or wing loading, suggesting that poorer flight performance among parasitized hosts was not directly caused by morphological constraints. Effects of parasite infection on powered flight support a role for long-distance migration in dramatically reducing parasite prevalence in this and other host–pathogen systems.

Keywords

Danaus plexippus, flight mill, host–parasite interaction, migratory culling, neogregarine protozoan, *Ophryocystis*, seasonal migration, vertical transmission.

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INTRODUCTION

Many animals migrate long distances to track seasonal changes in resources and habitats (Dingle 1996; Alerstam *et al.* 2003). Indeed, persistent, long-distance movements that require substantial energy allocation are pervasive among vertebrate animals and a variety of insects of several orders, including milkweed bugs, dragonflies, aphids, locusts and multiple lepidopteran species (Dingle 1996). Such periodic host movement could underlie variation in epidemiological patterns (Loehle 1995), although a little-studied consequence of animal migration is its effect on host–parasite interactions (Loehle 1995; Altizer *et al.* 2000). Several authors have provided indirect evidence that seasonal migration or periodic host dispersal might reduce the prevalence of parasites infecting mosquitoes, baboons, reindeer and fall armyworm moths (Schiefer *et al.* 1977; Hausfater & Meade 1982; Folstad *et al.* 1991; Simmons & Rogers 1991; Nilssen & Haugerud 1995). Two key mechanisms related to general principles of host–parasite ecology could cause this association. First, if parasites accumulate in the hosts' environment over time, migration might allow animals to leave behind contaminated habitats and hence will reduce parasite transmission (i.e. migratory escape; Loehle 1995). Second, if migration is energetically costly, infected animals might not migrate successfully and will thus be removed from the population (i.e. migratory culling).

Substantially lower migratory success among diseased hosts will reduce pathogen prevalence and rates of spread, and could lead to pathogen extinction. Indeed, migration in other host taxa is known to be stressful or energetically costly (Alerstam *et al.* 2003), and one recent study showed that migration resulted in flare ups of Lyme disease spirochaetes in migratory birds (Gylfe *et al.* 2000). However, despite the potential importance of this and other sublethal effects of parasites on their hosts, few researchers have directly examined the effect of parasite infection on the dispersal ability of insects or vertebrate animals. One notable exception is Akbulut & Linit's (1999) study on the effect of the pinewood nematode, *Bursaphelenchus xylophilus*, on the flight ability of its vector, the beetle *Monochamus*

carolinensis. They found that beetles carrying more than 10 000 juvenile nematodes had significantly shorter flights than those carrying less than 10 000, which could have a significant effect on the dispersal ability of this vector in areas sparsely populated by the host tree.

Native and introduced monarch butterflies populate islands and continents worldwide, and occupy a subset of the range of their larval host plants in the genus *Asclepias* (Ackery & Vane-Wright 1984). Like several other insect species that originated in the tropics but seasonally inhabit temperate environments, monarch butterflies cannot withstand prolonged freezing temperatures (Calvert *et al.* 1983), and have evolved a diapause generation to exploit fluctuating resources through a spectacular migratory behaviour in parts of North America and Australia (Urquhart & Urquhart 1978; James 1993; Brower 1995). The migratory patterns of North American monarchs are fairly well known (Brower 1995). Each autumn, eastern North American monarchs undergo the longest distance migration, traveling up to 5200 km from breeding to overwintering sites (Urquhart & Urquhart 1978; Brower & Malcolm 1991). In spring, the same butterflies that winter in Mexico mate and fly north to recolonize their breeding range (Van Hook 1993; Howard & Davis 2004). A second population in western North America migrates a shorter distance to overwinter along the coast of California (Nagano *et al.* 1993; Brower 1995). In tropical areas including southern Florida, Hawaii, Caribbean Islands and Central America, monarchs breed year-round and do not migrate.

The neogregarine protozoan parasite *Ophryocystis elektroscirrha* was first discovered in monarch and queen butterflies in Florida in 1968 (McLaughlin & Myers 1970). New infections occur when parasite spores are ingested by larvae that feed on contaminated eggs or milkweed (Leong *et al.* 1997a). Following ingestion, spores lyse and emerging sporozoites penetrate the intestinal wall, enter the hypoderm and undergo two phases of asexual replication. While monarchs are in their pupal stage, parasite spores form around the scales of the developing adult butterfly (McLaughlin & Myers 1970), and infected adults emerge covered with dormant spores on the outside of their bodies, particular towards the posterior end of their abdomens (Fig. 1; Leong *et al.* 1992). Vertical transmission occurs when females scatter spores on eggs or host plants during oviposition (McLaughlin & Myers 1970), and parasites can also be transmitted through paternal or horizontal routes (Vickerman *et al.* 1999; Altizer *et al.* 2004). Spores must be ingested by larvae to cause new infections, and active infections are not transmitted among adults. Parasites have been shown to have a variety of effects on host fitness, including reduced larval survival, smaller adult size, and shorter adult lifespans (Altizer & Oberhauser 1999; Altizer 2001). Most of these measurable fitness effects, however, are manifested when monarchs ingest the highest parasite doses (*c.* 1000 spores per larva), and monarchs inoculated with low parasite doses have similar fitness to unparasitized butterflies (Altizer & Oberhauser 1999).

All monarch populations examined to date are parasitized by *O. elektroscirrha*, and prevalence among regions varies inversely with host migratory tendency (Leong *et al.* 1997b; Altizer 2001). In eastern North America where monarchs undergo the longest annual migration, <8% of the

Figure 1 Spores of *Ophryocystis elektroscirrha* (smaller objects in photo to right) form around the developing abdominal scales of monarch butterflies (larger objects), and adult butterflies emerge covered with dormant parasites on the exterior of their bodies. [Photos by Karen Oberhauser and De Cansler].

butterflies are heavily infected (defined as adults with mean parasite loads ranging from 10^3 to 10^5 abdominal spores; Altizer *et al.* 2000). Approximately 30% of monarchs from a migratory population in western North America are heavily infected (Leong *et al.* 1992; Altizer *et al.* 2000). Monarchs in southern Florida and Hawaii that breed year-round and do not migrate bear the highest parasite loads (over 80% heavily infected; Leong *et al.* 1997b; Altizer *et al.* 2000). These differences in prevalence among populations with different migration strategies are further supported by additional samples of adult butterflies from Australia, South America and Caribbean Islands (Altizer *et al.* 2000).

The observation that striking variation in parasite occurrence has persisted for many years points to an association between host migration strategy, parasite transmission and prevalence. Moreover, within a migratory population, prevalence among breeding monarchs declined with increasing distances from overwintering sites (Altizer *et al.* 2000), suggesting that infected butterflies failed to reach the extreme limits of their breeding range. As parasite transmission in this system requires that larvae ingest spores scattered by adult monarchs, the loss of infected hosts between breeding seasons could substantially lower parasite prevalence in migratory populations. Differences in parasite transmission or host survival resulting from migratory behaviour could further select for differences in host resistance and parasite virulence among populations – an idea that has been supported by cross-infection studies showing that resistance to parasites is greatest and parasite virulence is lowest in the eastern North American population that migrates the farthest distances (Altizer 2001).

In this study, we tested effects of parasitism on the long-distance flight performance of monarch butterflies. As monarchs travelling between wintering and breeding habitats must fly for thousands of kilometres and are frequently exposed to unfavourable conditions, measures of long-distance flight endurance and sustained velocity should be crucial predictors for successful autumn and spring migration. We infected captive monarch butterflies derived from the eastern North America population with low and moderate numbers of *O. elekstroscirrha* spores and tested their powered flight using an automated flight mill apparatus. We measured a large number of flight parameters (including distance, time, speed and loss of body mass during flight) and further controlled for morphological variables (including wing size and shape and wing loading) that could represent physical and energetic constraints on flight ability (Dingle 1996; Kingsolver & Srygley 2000). We predicted that parasitized monarchs would fly shorter distances and at slower maximum and average velocity than uninfected butterflies, and that differences in flight performance should in part be explained by variation in butterfly size and wing loading.

MATERIALS AND METHODS

Butterfly and parasite sources

Monarchs used in this study were the progeny of wild adults captured between August and September 2003 from Atlanta (Georgia, USA) and Ithaca (New York, USA). These butterflies were from the migratory generation of the eastern North American population. Eggs were collected separately from each of 30 wild females mated to 10 different males, and remained on their natal plant until they reached second instar. Parasite inoculum was derived from a single heavily infected wild eastern monarch. Larvae were inoculated by feeding them low or moderate doses of 50 or 500 parasite spores per larva in individual Petri dishes at late second instar (Altizer & Oberhauser 1999). Parasite doses were chosen to capture the range of spore numbers deposited by infected females during oviposition events (S. Altizer, unpublished data), and are similar to 'low' and 'moderate' doses used in previous experimental studies of parasite effects on host fitness (Altizer & Oberhauser 1999; Altizer 2001). Larvae were reared to adulthood in plastic 4.7 L containers at densities of five larvae per container in our laboratory, and were exposed to natural light conditions from large windows facing south-east. Upon eclosion, live adults were examined for *O. elektroscirrha* (following protocols outlined in Altizer *et al.* 2000 and Davis *et al.* 2004) and their entire dorsal side (including wings) was scanned on a digital flatbed scanner. No physical signs of other parasites, including viral, bacterial or fungal infections were observed among larvae, pupae or adult monarchs in this study.

Of the adult monarchs that eclosed successfully, showed no wing deformities, and survived to 2 days post-eclosion, 50 parasitized and 50 unparasitized monarchs were selected for flight trials to reflect an even distribution among 29 different family groups. Infected monarchs were those found to be parasitized with 1000 or more *O. elektroscirrha* spores per cm^2 abdominal area, as these infection levels are similar to heavily infected monarchs captured in the wild (Altizer *et al.* 2000). We also obtained a continuous measure of infection status for each monarch using digital image analysis to estimate spore density on a segment of the abdomen as described in Davis *et al.* (2004). All monarchs were held in glassine envelopes at 12 °C and fed a solution of 20% honey water every 4 days for up to 20 days prior to preparation for flight trials.

Flight trials

Flight mills have been used in many studies to characterize insect flight ability or to examine the effects of sex, age or physical parameters on flight performance (Cooter & Armes 1993; Moriya 1995; Schumacher *et al.* 1997; Krell *et al.* 2003). We constructed an automated flight mill using a

lightweight carbon rod 120 cm in length and 3 mm in diameter threaded through a stainless steel pivot to provide a near-frictionless flight, and with movable counterbalances to account for variation in each monarch's weight. An infrared beam emitted by a photogate was interrupted by a flag attached to one end of the carbon rod. This photogate was connected to a PASCO PS-2000 datalogger (Pasco Scientific, Roseville, CA, USA) which recorded the time elapsed between each rotation (to measure instantaneous speed) and the cumulative flight time. A live monarch butterfly was attached to the rod and this design restricted the monarch's flight to continuous circles of 4.27 m circumference.

Flights trials were performed in a laboratory space maintained at 24 °C and controlled for light and air movement. Two days prior to flight trials, monarchs were removed in groups of eight and an ultra-light steel wire attachment (32 gauge, 9 cm long) was glued to the dorsal side of the thorax using rubber cement. The mass of each butterfly was recorded immediately before and after wire attachment, and the average wire mass was 0.245 g (range = 0.169–0.358 g). Following wire attachment, monarchs were held in a 0.6 m^3 flight cage to allow acclimation to the laboratory environment for 48 h. Infected and uninfected monarchs were placed in separate holding cages in the same room and were flown on alternating days to minimize parasite contamination in the laboratory.

Between 10 November and 5 December 2003, flight data were obtained from individual monarchs that were attached to the flight mill by securing the free end of the wire to the rotating arm. The mass of each individual was recorded using an electronic balance immediately before and after each flight. We counted the number of pauses in the butterfly's flapping during the first 10 rotations to compare the amount of active wing movement between individuals. Pauses were determined as an interruption in flapping and monarchs that paused more than 10 times during the first 10 rotations on two consecutive days were excluded from analyses. For each flight trial we recorded the total time, total distance and speed per rotation (Fig. 2). A flight was terminated when the monarch remained still (no wing movement and rotations completely halted) for more than 10 full seconds, and each individual butterfly was used only once in the data set.

It is important to note that our experiment was not designed to simulate natural conditions monarchs encounter during migration. As a case in point, during natural migration, butterflies must use alternating strategies to contend with cross-winds, head-winds or tail-winds (Schmidt-Koenig 1985; Davis & Garland 2002). Furthermore, during migratory flight, monarchs engage in a combination of flapping and gliding, can adjust flight altitude and vectors (Gibo & Pallett 1979; Gibo 1981; Schmidt-Koenig 1985), and butterflies might land on the ground when faced with adverse conditions (Davis & Garland 2004). However, there are times during which migrating butterflies are unable to land or are blown off-course and must continue flying (e.g. when crossing large water bodies, mountains or other unsuitable habitat; Brower 1995; Meitner *et al.* 2004). In these instances, monarchs that are unable to engage in sustained active flight will likely suffer reduced migratory success. Therefore, testing the velocity, flight distances and time to exhaustion for monarchs actively flying in the absence of wind should represent a component of flight performance that is important to long-distance migration.

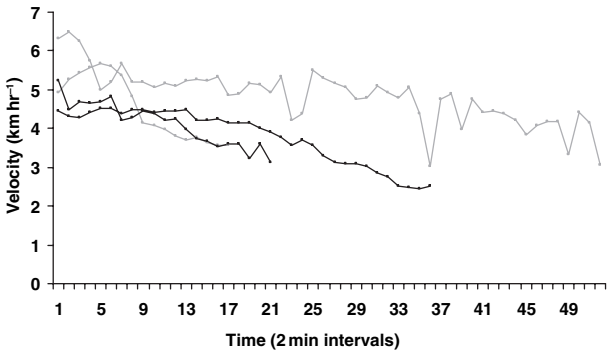

Figure 2 Flight trajectories for a representative sample of two parasitized (black) and two uninfected (grey) monarch butterflies used in this study. Velocity averaged across 2-min intervals is shown for each individual's flight trial. Flights were terminated when the rotating arm remained motionless for 10 s or longer (monarchs ceased flapping and glided to a halt).

Morphological constraints

We measured three morphological variables that have been shown to affect flight performance in butterflies and other insects (Dudley & Srygley 1994; Srygley & Kingsolver 2000; Berwaerts *et al.* 2002): wing area, wing loading and wing aspect ratio. Digital wing scans were made for each butterfly and we used Adobe Photoshop with the Image Processing Toolkit (Reindeer Graphics, Inc., Asheville, NC, USA) to measure forewing area (in mm^2), body area (in mm^2), and wing aspect ratio (length along long axis of forewing divided by width). Wing loading was estimated in two ways: wing $load_{area}$ = body area/wing area and wing $load_{mass}$ = body mass (in mg)/wing area. In the latter case, we used body mass immediately prior to flight trials excluding the mass of the wire.

Measuring flight parameters

We calculated four measures of flight performance: (i) endurance, (ii) speed, (iii) deceleration and (iv) loss of

body mass relative to total distance flown. Endurance was estimated by total distance flown in km (log-transformed), and this measure was highly correlated with total time in flight (in hours, also log-transformed; $r = 0.975$, $n = 73$; $P < 0.001$). Flight speeds (in km h^{-1}) were averaged across 2-min intervals for the duration of each flight (e.g. Fig. 2), and from these we computed three raw speed measures: (i) average speed across the entire flight, (ii) maximum speed as the greatest measure for any single 2-min interval, and (iii) initial speed averaged across the first 10 min of flight. These three measures were also significantly positively correlated (e.g. for average and maximum speed, $r = 0.621$, $n = 73$; $P < 0.001$). A composite measure of speed was obtained using a principal component (PC) analysis of initial, maximum and average speed. The first PC (hereafter called PC speed) explained 81.95% of the variance (contributions: average speed = 0.923, initial speed = 0.948, and maximum speed = 0.842).

Rate of deceleration for each individual was measured as the average change in speed between each 2-min interval throughout the duration of each flight. The absolute value of deceleration rate was log-transformed prior to analysis. Finally, we compared body mass before and after flight trials to determine whether monarchs lost more or less body mass than expected based on their total flight distance. Relative weight loss over the entire flight trial was computed as log-transformed total body mass change ($mass_{initial} - mass_{final}$). As described below, regression models focusing on loss of body mass included both initial mass and log-transformed total distance flown (in km) as additional covariates in statistical analyses, as these measures were significantly correlated with loss of body mass during flight (e.g. $r_{initial\text{-}mass} = 0.297$, $n = 71$; $P = 0.020$; $r_{log\text{-}distance} = 0.716$, $n = 71$; $P < 0.001$).

Data analysis

Analysis of variance (SPSS 2004) was used to examine the effects of infection status, sex and morphological covariates on each of the four measures of flight performance. We began each analysis with a full model that included main effects of each categorical variable, four continuous covariates, and two-way interactions between factors and continuous parameters (Model: flight performance = infection status + sex + infection status × sex + wing area + wing aspect ratio + wing $load_{area}$ + wing $load_{mass}$ + two-way interactions between infection status and morphological covariates + two-way interactions between sex and morphological covariates). We explored model fit and simplification (Crawley 2002) by first removing each non-significant ($P > 0.05$) interaction term and comparing Akaike information criteria corrected for small sample sizes (AICc) between the full model and the model with $k - 1$ terms. Interaction terms were excluded from a simplified model if (i) their removal resulted in a lower value of AICc, or (ii) their removal resulted in a small increase in AICc of <4.0, or (iii) if their associated P was greater than 0.2. Next, non-significant main effect terms were removed from the reduced model if they were not present in any significant interaction term and if their removal satisfied criteria (i) through (iii) above. We reported results from a final analysis that included main effects, covariates and interaction terms for those parameters that explained substantial variation in each response variable as determined by the model fitting exercise. We performed another set of ANOVAs to ask whether morphological constraints that might affect flight performance (wing area, body area, wing aspect ratio and wing loading) differed between parasitized and unparasitized monarchs.

RESULTS

General results

Of the 100 monarchs initially chosen for flight trials, data were obtained from 43 uninfected and 30 parasitized monarchs. Data were not available for eight parasitized butterflies that died prior to flight trials, and we excluded flight data from seven uninfected and 10 parasitized butterflies based on pause criteria described above. In general, monarchs flew long distances on the flight mill apparatus and averaged speeds of 4.18 km h^{-1} (maximum recorded speed was 8.40 km h^{-1}). Individual flight durations ranged from <10 min to 2.39 h (mean = 0.76 h) and total distances ranged from 0.12 to 13.37 km (mean = 3.17 km).

As parasite loads and flight performance of monarchs that had been inoculated with 50 and 500 spores were similar, we did not distinguish between these two treatments in the final data analysis. For example, of those infected monarchs for which flight data were obtained, mean quantitative measures of final parasite loads were similar for those inoculated with 50 (log-spore load = 3.15 ± 0.11 SE) and 500 (log-spore load = 3.44 ± 0.17 SE) spores, and this difference between mean values was not statistically significant ($t_{29} = -2.10$; $P = 0.06$). Similarity between dose treatments was probably related to the fact that two-third of monarchs used in flight trials had been inoculated with the lower dose, as many monarchs inoculated with the higher dose did not survive to 2 days post-eclosion, showed wing deformities, or were excluded from analysis because of pause criteria. Furthermore, using the subset of parasitized monarchs only, we found no significant relationship between quantitative parasite loads (log-spore load) and total flight distance in km ($t_{29} = -0.414$; $P = 0.682$) or flight speed (PC speed; $t_{29} = -1.136$; $P = 0.266$).

Infection status and flight performance

Parasitized butterflies had lower flight endurance than uninfected monarchs (Fig. 3a), flying for an average duration of 0.70 h ± 0.16 SE and mean distance of 2.79 km ± 0.47 SE per trial, relative to 0.81 h ± 0.11 SE and 3.44 km ± 0.47 SE for uninfected monarchs. This represents 14% shorter flight durations and 19% shorter flight distances among parasitized relative to unparasitized butterflies. For ANOVA focused on flight distance, comparison of AICc among models with different interaction terms and main effects supported the following simplified model: log(distance) = inf + sex + wing $load_{mass}$ + wing $load_{area}$ + inf × $wing_{load\ area}$ + sex × wing $load_{mass}$. Differences in flight distance relative to host infection status and sex were significant at the 0.05 level (Table 1), so that parasitized butterflies and males flew shorter distances than unparasitized butterflies and females. Flight distances also decreased significantly with wing loading as a continuous covariate, and interaction effects of sex × wing $load_{mass}$ and infection status × wing $load_{area}$ were significant and nearly significant respectively (Table 1). In this latter case, we observed a significant negative relationship between flight distance and wing loading for unparasitized monarchs but not for parasitized butterflies (Fig. 3a).

Table 1 Analysis of variance of log-transformed flight distance as a function of infection status, sex, wing loading (as a continuous covariate), and two-way interactions between factors and covariates. Comparison of model fit parameters (using minimum adequate model procedures based on AICc, and beginning with a full model with two factors, four continuous covariates and nine two-way interaction terms) supported the following final reduced model: log(distance) = inf + sex + wing $load_{mass}$ + wing $load_{area}$ + inf × $wing_{load\ area}$ + sex × wing $load_{mass}$

Effect	Mean square	d.f.	F	P-value
Infection status	0.67	1	4.32	0.042
Sex	0.77	1	4.95	0.030
Wing $load_{area}$	0.65	1	4.21	0.044
Wing $load_{mass}$	0.04	1	0.28	0.597
Inf × $load_{area}$	0.59	1	3.79	0.055
Sex × $load_{mass}$	0.95	1	6.12	0.016
Error	0.15	65		

Flight speeds were also lower among parasitized butterflies (Fig. 3b), averaging 3.88 km h^{-1} ± 0.19 SE over the course of each flight compared with 4.34 km h^{-1} ± 0.74 SE for uninfected butterflies. Percentage reductions

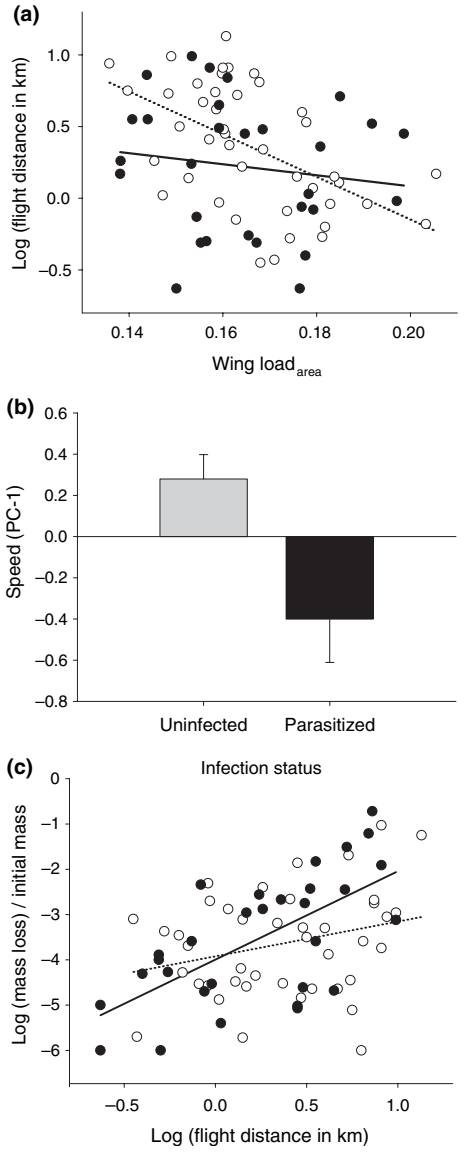

Figure 3 Differences in the flight performance of uninfected (open circles, grey bars) and infected (closed circles, black bars) monarch butterflies. (a) Flight endurance, measured as total distance flown in km (log-transformed) plotted against wing loading (wing area relative to body area). For unparasitized monarchs, the relationship between wing loading and flight distance was highly significant (e.g. linear regression slope = −14.95; $t_{42} = -4.0$; $P = 0.000$; $R^2 = 0.28$; dotted line), but for parasitized monarchs this relationship was non-significant (linear regression slope = −3.76; $t_{29} = -0.72$; $P = 0.48$; $R^2 = 0.02$; solid line); (b) PC speed was significantly greater among unparasitized relative to parasitized monarchs; (c) Mass loss during flight, measured as log(initial mass − final mass)/initial mass, plotted against total flight distance (in km, log-transformed). Parasitized monarchs lost proportionately more mass per km flown (e.g. linear regression slope = 0.92; $t_{29} = 6.4$; $P = 0.000$; $R^2 = 0.61$; solid line) relative to unparasitized monarchs (linear regression slope = 0.58; $t_{41} = 4.9$; $P = 0.000$; $R^2 = 0.38$; dotted line). Full statistical analyses for each flight parameter are described in the Results and in Tables 1 and 2.

Table 2 Analysis of variance of log-transformed total mass lost during flight as a function of infection status, sex, initial mass, log-transformed total distance flown, and the interaction between infection status and flight distance. Comparison of model fit parameters (using AICc, and beginning with a full model with two factors, six continuous covariates and 15 two-way interaction terms) supported the final reduced model: log-mass loss = inf + initial mass + log(distance) + inf × log(distance)

Effect	Mean square	d.f.	F	P-value
Infection status	0.06	1	0.75	0.390
Initial mass	2.32	1	27.42	0.000
Log-distance	7.44	1	87.81	0.000
Inf × log-distance	0.50	1	5.85	0.018
Error	0.09	66		

in initial, maximum and mean flight speeds of 16%, 14%, and 10%, respectively, were observed among parasitized relative to unparasitized butterflies. For ANOVA using PC speed, comparison of AICc among models with different interaction terms and main effects supported a reduced model with only a single main effect – infection status – and this factor was highly significant ($F_{1,70} = 9.57$; $P = 0.003$).

In the case of rates of deceleration, parasitized and unparasitized monarchs showed virtually the same mean values, and model simplification procedures supported a model with no main effects of factor, covariates or two way interactions (i.e. intercept only).

Finally, relative to their starting mass, parasitized monarchs lost proportionately more body mass per km flown (2.5%) relative to uninfected monarchs (1.6%). For ANOVA focused on loss of body mass, log(distance) and initial mass were included as covariates in the full model together with sex and infection status, morphological covariates, and two-way interactions between factors and continuous variables. Comparison of AICc supported the following simplified model: log(mass loss) = inf + initial mass + log(distance) + inf × log(distance). In this case, total weight loss was positively associated with initial body mass and increasing flight distances (Table 2; Fig. 3c). Although the main effect of host infection status was not significant in the final analysis, results showed a significant interaction between infection status and flight distance (Table 2), with parasitized butterflies losing relatively more body mass per km flown than unparasitized monarchs (Fig. 3c).

Parasite infection and morphological constraints

Analysis of variance showed that infection status was not significantly related to wing area ($F_{1,70} = 0.22$, $P = 0.645$), body area ($F_{1,70} = 0.05$, $P = 0.826$), wing aspect ratio ($F_{1,70} = 0.8$, $P = 0.775$), or initial mass ($F_{1,70} = 1.03$, $P = 0.313$). We also found no significant effect of infection status on wing loading when estimated as a function of body area ($F_{1,70} = 0.18$, $P = 0.674$) or body mass ($F_{1,70} = 0.77$, $P = 0.385$).

DISCUSSION

Seasonal, long-distance migration could influence multiple processes important to the dynamics of animal-parasite systems. Periodic movement has been suggested as a host strategy for reducing parasite occurrence, although the primary mechanism forwarded has been escape from parasites that accumulate in the hosts' environment (Loehle 1995). In support of this 'migratory escape' hypothesis, Folstad *et al.* (1991) proposed that the post-calving migration in reindeer allows them to escape warble fly infections by leaving behind areas contaminated with parasites, and yellow baboons have been shown to alternate their sleeping groves to avoid recently used sites, a behaviour that likely reduces their exposure to intestinal nematodes (Hausfater & Meade 1982). However, high prevalence among non-migratory populations could also result from the absence of 'migratory culling' of diseased animals between breeding generations, so that they continue transmitting infections without interruption. Empirical support for this second mechanism is limited, although patterns of infection by an ectoparasitic nematode in autumn armyworms (*Spodoptera frugiperda*) indicated that infected moths might have reduced migratory ability, in part because the first immigrant males to return north had few or no nematodes (Simmons & Rogers 1991) and also because adult parasite burdens increased along a gradient from temperate to tropical regions (Simmons *et al.* 1991).

As a result of their well known migration patterns, ease of manipulation in the laboratory and extreme variation in the prevalence of a commonly occurring parasite, monarch butterflies represent a model system for understanding the mechanisms linking long-distance migration to host–pathogen dynamics. Here we showed that monarch butterflies from a migratory population that were experimentally infected with the neogregarine parasite *O. elektroscirrha* had significantly poorer flight performances, including reduced flight speeds, lower flight endurance and greater proportional loss of body mass relative to their flight distances. Differences in the average performance of parasitized and unparasitized hosts on the order of 10–20% were significant in analyses that controlled for sex and morphological variables shown to be important for butterfly flight. Furthermore, analyses of total flight distance showed that whereas unparasitized monarchs demonstrated substantially greater flight endurance when wing loading was low, this relationship broke down among parasitized butterflies (Fig. 3a), and the interaction between infection status and

wing loading was marginally significant (Table 1). Finally, analyses showed that relative to their starting mass, parasitized monarchs lost weight at a faster rate per km flown than unparasitized butterflies (Fig. 3c). Our results represent the first experimental tests of parasite infection on the flight performance of a migratory insect host and are consistent with the hypothesis that long-distance flights could weed out infected animals and hence reduce parasite prevalence in the wild.

Comprehensive sampling of monarchs from multiple wild populations has previously shown that prevalence of *O. elektroscirrha* is dramatically lower in migratory relative to resident butterfly populations (Leong *et al.* 1997b; Altizer *et al.* 2000). Moreover, parasite prevalence among summer breeding monarchs declined with increasing distances between breeding and overwintering sites (Altizer *et al.* 2000), suggesting that heavily infected monarchs failed to reach breeding sites at the most distant extremes of their range. Despite this correlative evidence, field data supporting a mechanistic link between migration and parasite dynamics in monarchs have been elusive. For example, repeated sampling of eastern migratory monarchs during periods of breeding, migration and overwintering showed no systematic changes in prevalence between these three stages, as might be expected if parasitized monarchs are removed from the population during autumn migration (Altizer *et al.* 2000). As prevalence in this eastern migratory population is so low, however, field results must be interpreted with caution, because extremely large samples are required to detect relatively small changes in prevalence.

Past work demonstrated that experimentally infected monarchs reared in captivity experienced lower survival to adulthood, lower body mass upon eclosion, smaller forewings and had shorter adult lifespans than uninfected butterflies (Altizer & Oberhauser 1999; Altizer 2001) – but only for butterflies exposed to the highest parasite doses (1000 spores per larva). In fact, monarchs exposed to low or moderate spore numbers had similar body size and survival to uninfected adults. Our results here demonstrated the first negative effects of low and moderate parasite doses on monarch butterfly fitness (i.e. flight performance), and these sublethal effects should have major implications for divergence in prevalence in the wild. Thus, the most heavily parasitized adults should not survive long enough to reproduce or transmit the disease in any population, but monarchs infected with low or moderate doses (such as those used in this experiment) would probably have relatively high survival except under the demands of long-distance migration.

Past studies of other butterfly species have demonstrated that flight performance and mechanics are influenced by morphological variables (Dingle 1996; Srygley & Kingsolver 2000; Berwaerts *et al.* 2002). Surprisingly, we found no evidence that wing area, wing aspect ratio, initial mass or total wing loading differed between parasitized and unparasitized monarchs used in flight trials, indicating that differences in flight performance were not clearly attributed to parasite effects on these morphological constraints. A more likely explanation is that parasites influence the energetic resources available for powered flight or the total effort required to fly long distances. For example, Schiefer *et al.* (1977) found that *Anopheles stephensi* mosquitoes infected with *Plasmodium cynomolgi* showed poorer flight performances than uninfected mosquitoes and suggested that this might be the result of carbohydrate consumption by the developing parasites. Similarly, studies of dragonflies infected with gregarine gut parasites indicated that parasites affect the allocation of resources between muscle development and fat deposition, leading to poorer flight ability and lower territorial defence (Marden & Cobb 2004). In monarch butterflies, parasites could affect both stored energy (i.e. lipid accumulation) and host ability to absorb nutrients prior to flight. Furthermore, parasite-induced damage to host tissue (i.e. muscles, integuments or membranes) could demand greater effort from parasitized butterflies to accomplish the same powered flight. Finally, because parasite spores develop in clusters in the abdominal integument of adult butterflies (McLaughlin & Myers 1970), rates of water loss and dehydration could be higher among infected monarchs because of tissue damage, thus accounting for the greater proportional body mass loss per km seen among infected animals.

Although monarchs migrate through a combination of soaring and active flight (Gibo & Pallett 1979), measures of average speeds from our flight mill were similar to average daily velocities reported for migrating monarchs. If monarchs in eastern North America travel on average 2500 km from breeding to wintering sites in central Mexico (Urquhart 1987; Brower 1995), results from our study indicate that healthy monarchs actively flying 9 h per day would require *c.* 64 travel days to reach the overwintering sites, whereas monarchs infected with *O. elektroscirrha* would require 70 days. While this difference might seem small, infected butterflies would be exposed to fluctuating weather (including risk of frost and storms), predation and nutritional stress for a week longer than healthy butterflies. As our results showed that parasitized monarchs flew shorter distances per bout and lost proportionately more body mass per km flown, they might also stop more frequently and require more individual flights to reach the overwintering grounds, or might be less successful in crossing large expanses of unsuitable habitat (Schmidt-Koenig 1985). A related concern is that because monarchs are more likely to fly during favourable weather systems to minimize flying effort (Gibo & Pallett 1979; Davis & Garland 2002), monarchs grounded for any reason could miss favourable

flying days. Monarchs and other butterflies adjust their flight angles to correct for wind speed and direction (Schmidt-Koenig 1985; Srygley 2001), and butterflies that are too weak to fly against the prevailing winds are likely to be blown off course. For these reasons, even slight effects of parasitism on flight performance could translate to large differences in the probability of successful autumn and spring migration.

Finally, it is important to note that a number of parasitized butterflies demonstrated flight speeds, durations and distances that were well within the range of the uninfected class (e.g. Fig. 3a). As not all parasitized monarchs suffered substantial loss of flight performance, this could explain in part how *O. elektroscirrha* has persisted in the eastern migratory population, albeit at exceptionally low prevalence, for the past three decades (Altizer *et al.* 2000).

In summary, our research indicates that seasonal, long-distance migration should have important consequences for host–parasite interactions and could lead to wide divergence in prevalence across the range of environments inhabited by migratory animals. Understanding the mechanisms by which migratory behaviour affects epidemiological processes is also critical to understanding how hosts and their parasites have evolved in different parts of the world. Indeed, in monarch butterflies, mean levels of parasite virulence and host resistance have been shown to differ among the three North American populations, with host resistance highest and parasite virulence lowest in the eastern population that migrates the farthest distance (Altizer 2001). These patterns indicate that effects of migration on the survival of parasitized hosts and on parasite transmission could ultimately drive evolutionary divergence of hosts and parasites among populations.

Monarch butterflies in eastern North America undergo one of the longest annual migrations of any insect species, travelling distances of up to 5200 km from Canada to Central Mexico (Urquhart & Urquhart 1978; Brower & Malcolm 1991). This population is currently threatened by climate change (Oberhauser & Peterson 2003), severe weather patterns (Brower *et al.* 2004) and the destruction of the oyamel fir (*Abies religiosa*) forests that serve as wintering grounds for monarchs (Brower *et al.* 2002), to the point that monarch migration is considered to be a 'threatened phenomenon' (Brower & Malcolm 1991). Habitat degradation at overwintering sites and climate warming trends, combined with increased planting of tropical milkweed species in milder climates, could ultimately replace the large migratory populations with smaller remnant populations that breed year-round and do not migrate. Our results here suggest that these remnant populations are likely to become heavily parasitized, and an important question raised by this study is whether and how human activities will affect pathogen burdens in the wild.

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