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Associations between host migration and the prevalence of a protozoan parasite in natural populations of adult monarch butterflies

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Abstract. 1. Monarch butterflies *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae) are susceptible to infection by the obligate protozoan parasite *Ophryocystis elektroscirrha* (McLaughlin and Myers) (Apicomplexa: Neogregarinida). Because monarchs form resident and migratory populations in different parts of the world, this host–parasite system provides the opportunity to examine how variation in parasite prevalence relates to host movement patterns.

2. Parasite prevalence was evaluated using 14 790 adult monarchs captured between 1968 and 1997. Comparison of three populations in North America indicated that parasite prevalence is associated negatively with host dispersal distances. A continuously breeding, nonmigratory population in southern Florida showed high prevalence (over 70% heavily infected). The western population migrates moderate distances to overwintering sites on the Pacific Coast and has intermediate prevalence (30% heavily infected). The eastern migratory population, which travels the longest distance to Mexican overwintering sites, has exhibited less than 8% infection throughout the past 30 years.

3. Variation in parasite loads within North American migratory populations was investigated to determine whether the prevalence of heavy infection and average parasite loads declined during migration or overwintering. Average parasite loads of summer-breeding adults in western North America decreased with increasing distance from overwintering sites. This suggests that heavily infected monarchs are less likely to remigrate long distances in spring. No differences in the frequency of heavily infected adults were found among eastern or western North American monarchs throughout the overwintering period, however, suggesting that this parasite does not affect overwintering mortality.

4. Changes in the prevalence of monarchs with low parasite loads demonstrate that spore transfer occurs during migration and overwintering, possibly when adult butterflies contact each other as a result of their clustering behaviour.

5. This study of geographical and temporal variation in *O. elektroscirrha* among populations of *D. plexippus* demonstrates the potential role of seasonal migration in mediating interactions between hosts and parasites, and suggests several mechanisms through which migratory behaviour may influence parasite prevalence.

Key words. *Danaus plexippus*, disease transmission, migration, neogregarine, *Ophryocystis elektroscirrha.*

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Introduction

Theoretical and empirical studies have shown that the prevalence and spread of infectious diseases are influenced

by a variety of factors, including host density, parasite transmission mode, and the spatial structure of host populations (Getz & Pickering, 1983; Anderson & May, 1991; Antonovics & Thrall, 1995; Lockhart *et al*., 1996). In animal systems, patterns of host movement are also likely to affect pathogen prevalence. For example, seasonal migration has been implicated in the reduction of parasite prevalence in reindeer and baboons (Hausfater & Meade, 1982; Folstad *et al*., 1991; Shaw, 1994). The study reported here examines large-scale temporal and geographical variation in the prevalence of an obligate protozoan parasite, *Ophryocystis elektroscirrha*, in monarch butterflies *Danaus plexippus*. Monarchs form both migratory and nonmigratory populations, and within the migratory populations not all generations migrate similar distances. Thus, this system provides an opportunity to examine how both inter- and intra-population variation in parasite prevalence relate to host movement.

Within North America, three monarch populations show varying degrees of migratory behaviour. Eastern North American monarchs migrate up to 5200 km to coniferous forests in the trans-volcanic mountains of central Mexico (Urquhart & Urquhart, 1978; Brower & Malcolm, 1991; Calvert & Lawton, 1993; Fig. 1), arriving from late October to November, and overwinter in densely populated roosting sites that harbour tens of millions of individuals (Brower & Malcolm, 1991; Calvert & Lawton, 1993). In February and March, these same individuals break diapause and mate before flying north to recolonise their breeding range (e.g. Brower & Malcolm, 1991; Van Hook, 1993). Three or four summer reproductive generations breed each year between phases of migration and overwintering. Western North American monarchs migrate a shorter distance to the coast of California in September and October (Nagano *et al*., 1993; Brower, 1995; Fig. 1). Individuals in a nonmigratory population in southern Florida appear to move very little during the year, but recent evidence indicates that this population has a significant influx of autumn migrants from the larger eastern population (Knight, 1997). Monarchs also populate parts of Australia, Central and South America, and many Pacific and Caribbean Islands (Ackery & Vane-Wright, 1984; James, 1993), with varying degrees of seasonal movement.

The protozoan parasite *O. elektroscirrha* has been reported in both eastern and western populations of North American monarchs and in *Danaus gilippus*, the Florida queen butterfly (McLaughlin & Myers, 1970; Leong *et al*., 1992, 1997a), although recent work suggests that the same parasite strains do not infect both monarchs and queens (Leong *et al*., 1997a; S. M. Altizer, unpublished). The life history of *O. elektroscirrha* is correlated closely with host development. Asexual vegetative replication occurs within larvae and pupae, and sexually produced spores are found in the developing adult integument. *Ophryocystis elektroscirrha* spores must be ingested by larvae before they can break dormancy and cause new infections. Parasites are vertically transmitted (from infected females to their offspring) when spores are scattered on milkweed leaves and on the surface of eggs during oviposition (McLaughlin & Myers, 1970; Leong *et al*., 1997b). Spores lyse in the larval gut and migrate to the hypoderm, where they undergo vegetative replication. Before adults eclose, the parasite forms dormant spores around the developing integument of the adult butterfly, with the highest density of spores occurring on the distal third of the abdomen (Leong *et al*., 1992). Laboratory studies have demonstrated that heavy infection with *O. elektroscirrha* increases mortality of adult monarchs, particularly under dry conditions where rates of water loss are greater (McLaughlin & Myers, 1970; Leong *et al*., 1992; Altizer & Oberhauser, 1999). Heavily infected adults have difficulty expanding their wings and may die shortly after emergence, although adults with low parasite loads appear normal (McLaughlin & Myers, 1970; Leong *et al*., 1992, 1997b). In addition to maternal parasite transmission, spores can be transferred horizontally between adults during mating or other contact (Altizer, 1998).

The primary objective of the study reported here was to quantify variation in parasite prevalence among monarch butterfly populations with different migratory patterns. Samples from monarchs collected over the past three decades were examined to determine historical patterns of parasite prevalence. Short-term changes in parasite prevalence within migratory populations were examined to test the hypothesis that heavily infected hosts suffer higher mortality during migration or overwintering. Finally, changes in the proportion of monarchs with low parasite loads were examined to investigate whether horizontal spore transfer occurs among adults during migratory and overwintering periods, when monarchs cluster in dense aggregations.

Materials and methods

Comparison of assessment techniques

Parasite prevalence was assessed among adult *D. plexippus* captured from three populations in North America (Fig. 1) between September 1968 and September 1997, several locations in Australia in 1996, and in South America and Cuba in 1995 and 1996 (Appendix, Table A1). Adults were placed in individual glassine envelopes and either held in a freezer for long-term storage or released immediately following data collection. Butterflies sampled before 1994 were collected by Lincoln Brower and his associates for other purposes and stored until sampled; more recent collections were made explicitly for the purposes of parasite assessment. Parasite loads were evaluated for 12 000 adults from the eastern North American migratory population, 2141 adults from the western North American migratory population, 446 adults from southern Florida, 105 adults from Central and South America, and 108 adults from Australia.

Many samples listed in Table A1 represent collections from multiple dates or locations. For example, monarchs were collected from the eastern and western North American migratory populations at monthly or semi-monthly intervals during both reproductive and overwintering periods to assess short-term changes in parasite prevalence. In summer 1997, breeding adults were collected at 10 locations throughout western North America to determine whether parasite loads declined with increasing distance from overwintering sites.

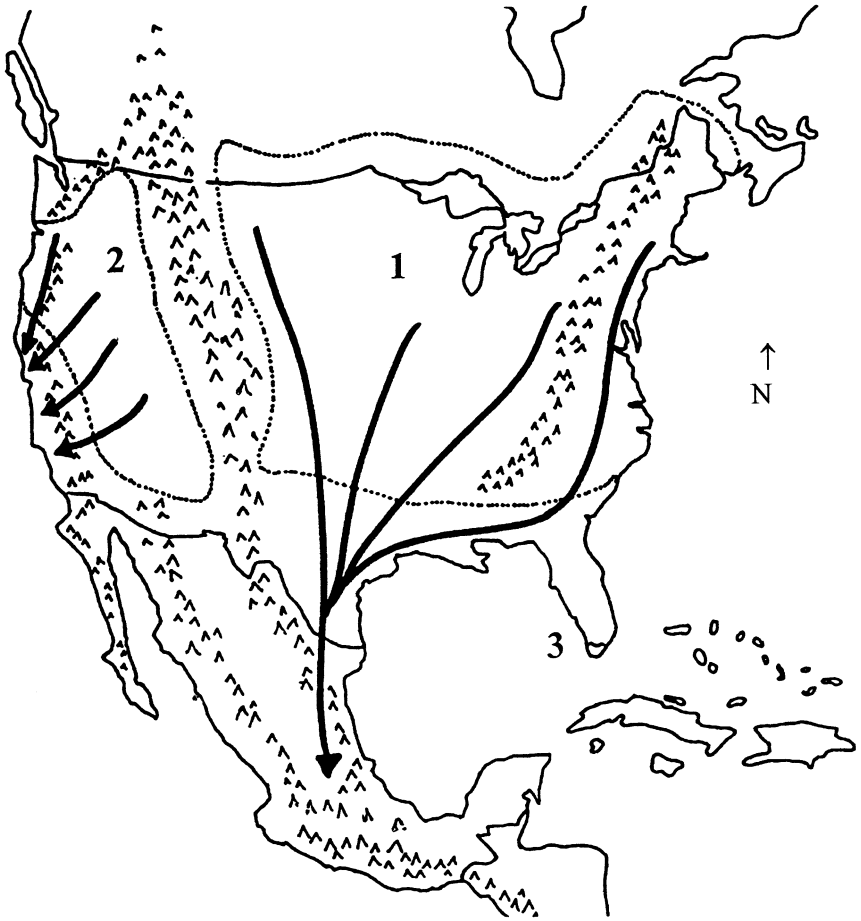

Fig. 1. Summer breeding ranges and major migratory routes for three North American monarch butterfly populations: 1, eastern migratory population; 2, western migratory population; and 3, southern Florida population (modified from Brower, 1995).

Dates and locations of samples used to evaluate fine-scale variation in prevalence are shown in the Appendix in Tables A2 and A3.

Sampling techniques

To examine butterflies for parasite loads, transparent Scotch^{TM} tape was cut into approximately 1 cm^2 units. This tape was held with fine forceps and pressed against the ventral abdomen to remove a sample of abdominal scales. Each tape sample was placed on a clear glass microscope slide or white index card, and viewed under a microscope at 400 ×. Spores appear as dark brown, oval-shaped bodies approximately 1/50th the size of a butterfly scale (Leong *et al*., 1992). All spores on the tape were counted, and butterflies were assigned to parasite load classes according to the following scale: 0, no spores; 1, one spore; 2, 2–20 spores; 3, 21–100 spores; 4, 101–1000 spores; and 5, > 1000 spores. To limit accidental spore transfer during handling of the butterflies, latex gloves were worn and all objects contacting the monarchs were rinsed periodically with a solution of 95% ethanol or 15% chlorine bleach.

Because two less extensive surveys of disease prevalence used a wash-and-count method for parasite assessment (Leong *et al*., 1992, 1997a), the correspondence between the Scotch^{TM} tape technique and the previously published method was examined. In July 1995, approximately 200 adults were reared in each of three calibrated inoculation treatments: 0, 10, 100, or 1000 spores administered per larva. Inoculum was prepared by vortexing the abdomens of infected adults in deionised water; a haemacytometer was then used to estimate the number of spores per volume of inoculum. Inoculum was passed through a dilution series to obtain the desired number of spores per 10 µl, and this volume of suspension was dropped onto a 1-cm^2 piece of milkweed leaf. Individual first-instar larvae were placed in sterile Petri dishes with a contaminated leaf, and after consuming the leaf tissue, they were transferred to plastic containers and reared to adulthood.

Emerging adults were placed into glassine envelopes and frozen for later disease assessment. After obtaining a tape sample from the ventral side of each individual, the abdomen was removed and placed in a glass vial containing 10 ml deionised water. Vials were vortexed for 1 min, allowed to stand for 5 min, and vortexed for an additional 1 min to

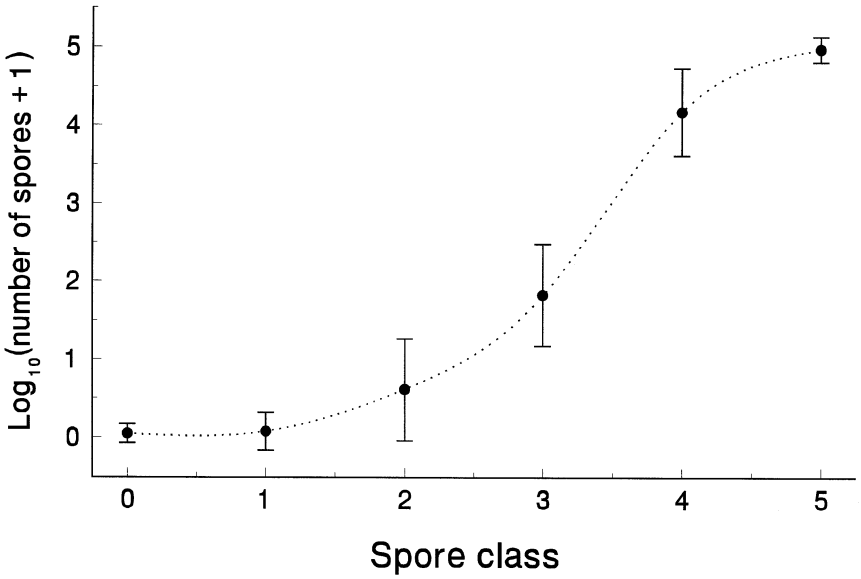

Fig. 2. Relationship between the $\log_{10}$ of haemacytometer spore counts and the measure of parasite load class using the tape method of disease assessment. This relationship was best described by a third-order polynomial regression model [$\log_{10}$ (spore count) $= \beta_0 + \beta_1 X + \beta_2 \chi^2 + \beta_3 X^3 + e$, where X = parasite load class and coefficients are as follows: $\beta_0 = 0.171$, $\beta_1 = -1.861$, $\beta_2 = 1.190$, and $\beta_3 = -0.124$]. Error bars represent 95% confidence intervals. Sample sizes are: class 0, 37; class 1, 11; class 2, 25; class 3, 34; class 4, 43; class 5, 76.

dislodge spores from the abdomen. The haemacytometer method outlined in Leong *et al*. (1992) was then used to estimate the total number of spores per vial.

Combining parasite load categories for statistical analysis

Logistic regression (GENMOD procedure, SAS/STAT Software, 1997) was used to determine whether the proportion of monarchs with different parasite loads varied with time, location, or other explanatory variables. For these analyses, infection status was treated as a binary response variable (where 1 = infected monarchs and 0 = all other categories). Two types of infected monarchs were recognised: those with high parasite loads (heavily infected, classes 4 and 5) and those with low parasite loads (classes 1, 2, and 3). These categories were selected because previous work indicated that high and low parasite loads result from qualitatively different transmission modes and have different consequences for host fitness. For example, laboratory experiments indicated that uninfected female monarchs acquire low numbers of spores by mating with heavily infected males (samples from females mated to infected males contained from 1 to 258 spores, mean = 85 spores, $n = 12$ matings; Altizer, 1998). Monarchs that consume spores as larvae almost always emerge heavily infected (Altizer & Oberhauser, 1999). Therefore, monarchs with low parasite loads are likely to have acquired spores as adults, whereas monarchs with high parasite loads are most likely to have become infected as larvae. Laboratory experiments have also demonstrated that heavily infected monarchs (classes 4 and 5) experience measurable fitness declines in comparison with uninfected monarchs or those with low parasite loads (Altizer & Oberhauser, 1999). For all logistic regression analyses, likelihood ratio tests were used to evaluate the significance of regression coefficients and test for model fit. Unless otherwise stated, likelihood ratios were constructed using the change in deviance between the null model (fitting the intercept only) and the more complex model (including explanatory variables), and were compared with the χ^2 distribution for hypothesis testing (Agresti, 1996; Hardy & Field, 1998).

Results

Comparison of assessment techniques

Parasite loads derived from the tape method of disease assessment were correlated highly with loads assessed using the haemacytometer technique (Fig. 2). Linear regression (STATISTIX Software, 1994) was used to quantify the relationship between these two methods. Prior to analysis, haemacytometer spore counts were log transformed, and 1 was added to each count to avoid taking the log of 0. Parasite load class was a significant predictor of the $\log_{10}$ of haemacytometer counts, and comparison of nested regression models indicated that this relationship was best described by a sigmoid (third-order polynomial) curve (Table 1). This nonlinear relationship suggests that parasite load categories based on the tape method recognise more variation in low parasite loads than the haemacytometer technique, but may underestimate the large difference in haemacytometer counts between parasite load classes 3 and 4. From a pragmatic perspective, major differences between the two techniques are the increased time

Table 1. Least-squares regression of $\log_{10}$ (haemacytometer spore counts) on parasite load class for laboratory-infected monarch butterflies. Parasite load classes derived from the tape method are described in the text. A third-order polynomial regression model provided the best fit to the data ($R^2 = 0.81$, MSE = 1.07, d.f. = 222). For comparison of nested models, the regression MS (d.f. = 1) was calculated as MS_{reg} = RSS(null model) – RSS(alternative model). F-values were calculated as MS_{reg}/MSE, where the MSE (and d.f. for the denominator) was based on the alternative model.

Model	d.f.	RSS	MSE	*F*	*P*
$Y = \beta_0 + \beta_1 X + e$	224	340.56	1.52	602.16	< 0.0001
$Y = \beta_0 + \beta_1 X + \beta_2 \chi^2 + e$	223	267.30	1.19	61.03	< 0.0001
$Y = \beta_0 + \beta_1 X + \beta_2 \chi^2 + \beta_3 X^3 + e$	222	238.456	1.07	28.84	< 0.001

and the destructive sampling required by the haemacytometer method. All data described below are based on parasite load categories using the tape method.

Recent and historical variation in parasite prevalence among North American monarchs

Monarch populations within North America show large differences in the proportion of adults with different parasite loads. In the eastern migratory population, very few adults were infected with any number of *O. elektroscirrha* spores (Fig. 3a), whereas most adults in the western migratory population showed intermediate or high parasite loads (Fig. 3b). All the monarchs captured in a continuously breeding population near Miami, Florida had *O. elektroscirrha* spores, and most of these adults were heavily infected (Fig. 3c).

Examination of butterflies in past collections suggests that population differences in the prevalence of heavily infected monarchs have persisted over time (Fig. 4), although changes in prevalence may have occurred during the large time intervals between samples. Logistic regression was used to determine whether the proportion of heavily infected adults (class 4 and 5) varied significantly across years for each of the three populations (model: infection status = year). To test the hypothesis that prevalence varied with year as an unordered factor, dummy variables were used to denote year, and likelihood ratio tests were based on the change in deviance between the full model (with a separate coefficient for each year) and the null model (intercept only). To determine whether variation among years followed any consistent pattern of increase or decrease over time, a simpler model was analysed treating year as a quantitative predictor. Likelihood ratio tests compared the fit of this quantitative model to the more complex model (with separate parameters for each year); here, a NS test statistic indicates that the quantitative model is adequate. Within the eastern migratory population, prevalence varied significantly over the 30-year sampling period (Δ deviance = 43.052, Δ d.f. = 17, $P < 0.001$; Fig. 4), however the simpler model treating year as a quantitative variable was rejected (Δ deviance = 39.62, Δ d.f. = 16, $P < 0.001$), indicating

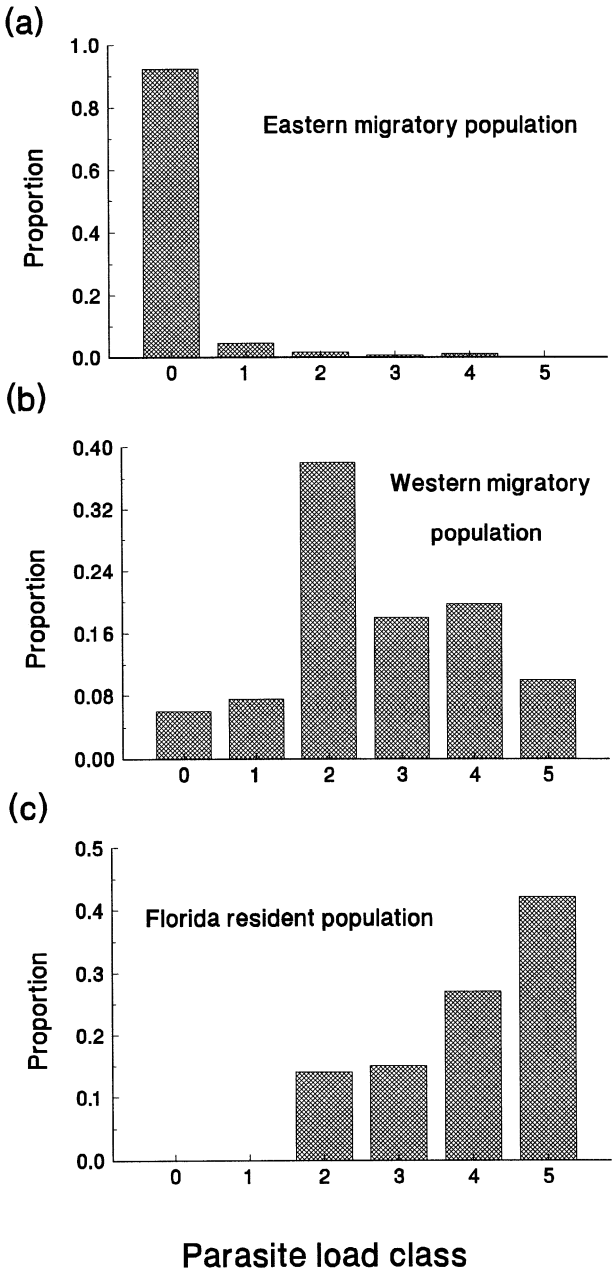

Fig. 3. Representative sample of the frequency distribution of parasite loads in three North American monarch populations: (a) eastern migratory population, Sierra Chincua, Mexico, March 1997, (b) western migratory population, Pismo Beach, California, February 1997, (c) southern Florida population, Miami area, July 1996. See text for description of parasite load classes. χ^2-analysis of the association between infection class (0–5) and population (a–c) shows that differences in the distribution of parasite loads are highly significant ($\chi^2 = 1087.5$, d.f. = 10, $P < 0.001$).

that prevalence did not change consistently over time. Across all years, the prevalence of heavily infected monarchs in the eastern migratory population was less than 8%, and this

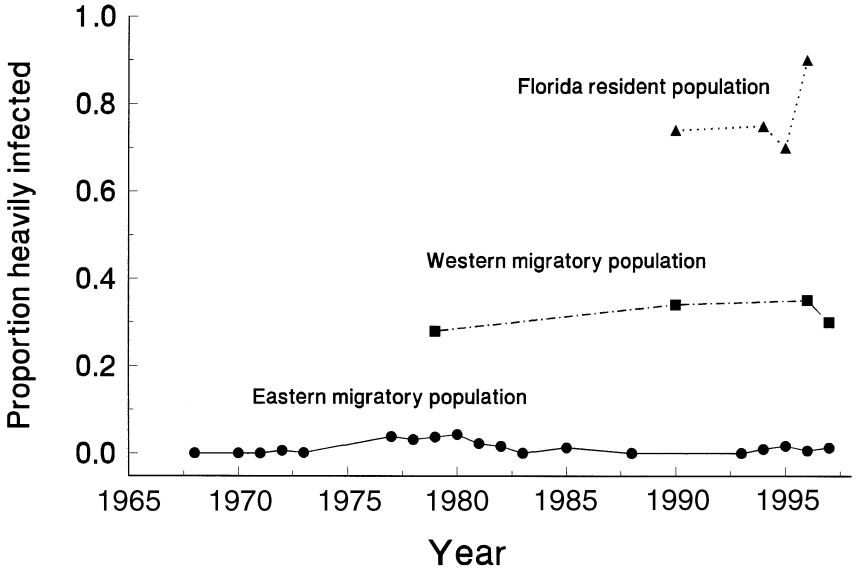

Fig. 4. Prevalence of heavily infected adults (parasite load classes 4 and 5) over time for each of three North American monarch populations. Sample sizes, locations, and dates are shown in the Appendix, Table A1.

prevalence was highest during the years 1977–1981. Year was not a significant predictor of prevalence in the western migratory population when treated as an unordered factor (Δ deviance = 3.3, Δ d.f. = 3, P = NS; Fig. 4), indicating that the prevalence of heavy infection remained relatively constant over time. It is important to note however that more than 10 years separate two of the western samples. In the southern Florida population, year was associated significantly with variation in parasite prevalence when treated as an unordered factor (Δ deviance = 21.59, Δ d.f. = 3, $P < 0.005$; Fig. 4), but this variation did not follow any consistent pattern of increase or decrease over time (Δ deviance = 19.42, Δ d.f. = 2, $P < 0.001$).

Short-term temporal and spatial variation in parasite prevalence in North American monarchs

Eastern migratory population. Logistic regression was used to examine whether the proportion of high and low parasite loads varied with seasonal host behaviour in eastern North America (model: infection status = behaviour). Samples collected from 1993 to 1997 were divided into the following three categories based on the location, date, and activity of adults at the time of capture: breeding, migrating south, and overwintering (Fig. 5; Appendix, Table A2). Although the prevalence of heavily infected adults varied among sampling dates, no association between behaviour category and the proportion of adults with high parasite loads was detected (Δ deviance = 2.41, Δ d.f. = 2, P = NS; behaviour treated as unordered factor), however behaviour category did have a significant effect on the proportion of monarchs with low parasite loads (Δ deviance = 58.88, Δ d.f. = 2, $P < 0.001$). The proportion of adults with low parasite loads was lowest among summer breeding monarchs and highest among those captured overwintering (Fig. 5). A simpler model treating behaviour as a quantitative variable (1, breeding; 2, migrating south; 3, overwintering) was adequate in comparison to the more complex model (Δ deviance = 0.365, Δ d.f. = 1, P = NS, $\beta = 0.62$, ASE = 0.096), indicating that the prevalence of low parasite loads increased throughout the monarchs' annual cycle.

During one overwintering season (1993–1994), adults were obtained from seven separate colonies in Central Mexico for each month (November to March). These samples were divided into three overwintering phases: early (November), middle (December, January), and late (February, March). The proportion of heavily infected adults was very small and remained nearly constant throughout the overwintering period, but the proportion of adults with low parasite loads increased between the early and middle overwintering phases (Table 2, Fig. 6a). For five colonies where collections spanned more than one overwintering phase (Table 2), the relationship between prevalence, overwintering site, and phase was investigated using logistic regression. Likelihood ratio tests were based on comparisons of deviance between pairs of nested models, beginning with the most complex model (parasite load = site + phase + site $\times$ phase; site and phase treated as unordered factors). Separate analyses were performed on adults with high and low parasite loads as described above. The prevalence of heavily infected monarchs did not change significantly with site, phase, or the two-way interaction (Table 3), however the prevalence of adults with low parasite loads was affected by site, phase, and their interaction (Table 3; Fig. 6a). Further simplification of the model (treating phase as a quantitative variable or eliminating the two-way interaction term) was not possible (Table 3), indicating that changes in prevalence varied among the different colonies.

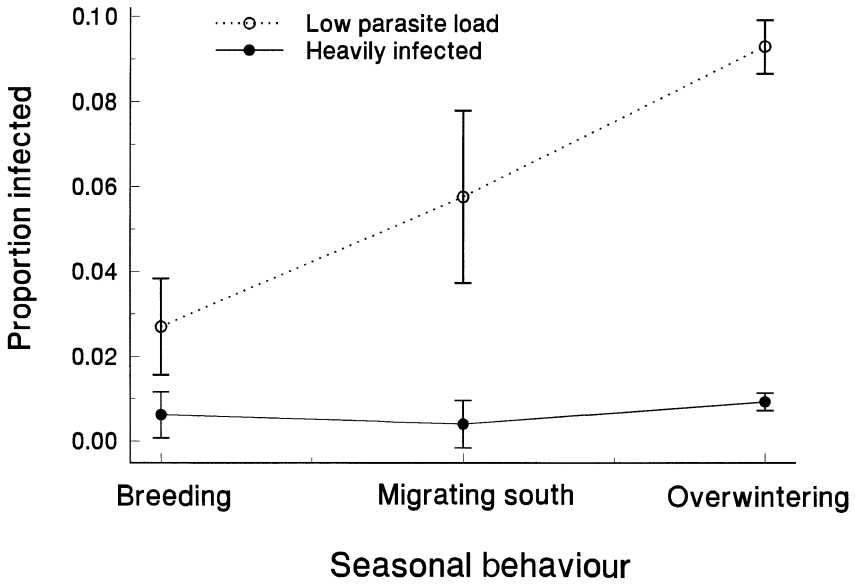

Fig. 5. Variation in parasite prevalence among adults in the eastern migratory population as a function of behaviour at time of capture (summer breeding, migrating south in the autumn, or overwintering in central Mexico). The solid line plots the prevalence of heavy infection (classes 4 and 5); the dotted line shows the proportion of adults with low parasite loads (classes 1, 2, and 3). Points represent weighted averages for samples collected from 1993 to 1997. Error bars represent 95% confidence intervals based on total number of butterflies combined across years. Sample sizes, dates, and locations are shown in the Appendix, Table A2.

Western migratory population. For one overwintering season, monarchs were collected at 10 separate colonies along the coast of California for 5 months (November 1989 to March 1990). The overwintering period was again divided into early, middle, and late phases, as above. The proportion of heavily infected adults declined slightly throughout the overwintering period, and the proportion of monarchs with low parasite loads increased from early to late overwintering phases (Fig. 6b). For the seven sites where collections spanned more than one phase (Table 4), the relationship between site, phase, and the proportion of infected adults was investigated (logistic regression model: parasite load = site + phase + site × phase). The prevalence of heavily infected adults was not affected by any of the explanatory variables (Table 3), however the prevalence of monarchs with low parasite loads varied with both site and phase (Table 3). In this case, model comparison suggested that the two-way interaction could not be eliminated, but that phase could be treated as a quantitative variable (with prevalence increasing consistently throughout the overwintering period, $\beta = 0.37$, ASE = 0.13; Table 3).

In summer 1997, breeding monarchs were sampled at 12 locations throughout western North America (Appendix, Table A3). Linear regression (STATISTIX Software, 1994) was used to quantify how average parasite loads (based on the 0–5 scale) were affected by the distance between breeding location and the closest overwintering site, measured to the nearest kilometre (Fig. 7). The response variable was the average parasite load of adults for each breeding location, and regression analysis was weighted by the number of observations within each location due to unequal sample sizes (Weisberg, 1985). Average parasite loads declined significantly with increasing distance from overwintering sites ($\beta = -0.002$, $T = -2.44$, $P < 0.05$; Fig. 7). A logistic regression analysis of the prevalence of heavy infection at each site (model: high parasite load = distance in kilometres) also indicated that the proportion of heavily infected adults was

Table 2. Temporal and spatial variation in the prevalence of monarchs with high and low parasite loads during the 1993–1994 overwintering season in Central Mexico. For locations, see Calvert and Brower (1986) and Alonso-Mejia (1996). Sample sizes range from 100 to 200 individuals per site per phase. Early, middle, and late phases are described in the text. Proportions represent the number of monarchs with either low (class 1, 2, and 3) or high (class 4 and 5) parasite loads over the total number of butterflies sampled. (The proportion of monarchs with no parasites is not shown.)

Colony	Parasite load	Early phase	Middle phase	Late phase
Sierra Chincua	High	0.02	0.01	0.00
	Low	0.10	0.11	0.13
Cerro Altamirano	High	0.02	0.02	0.04
	Low	0.10	0.28	0.32
Herrada	High	–	0.03	0.00
	Low	–	0.29	0.15
Cerro Pelon	High	0.01	0.01	0.01
	Low	0.07	0.26	0.08
Sierra El Campanario	High	0.00	0.00	0.02
	Low	0.02	0.06	0.12

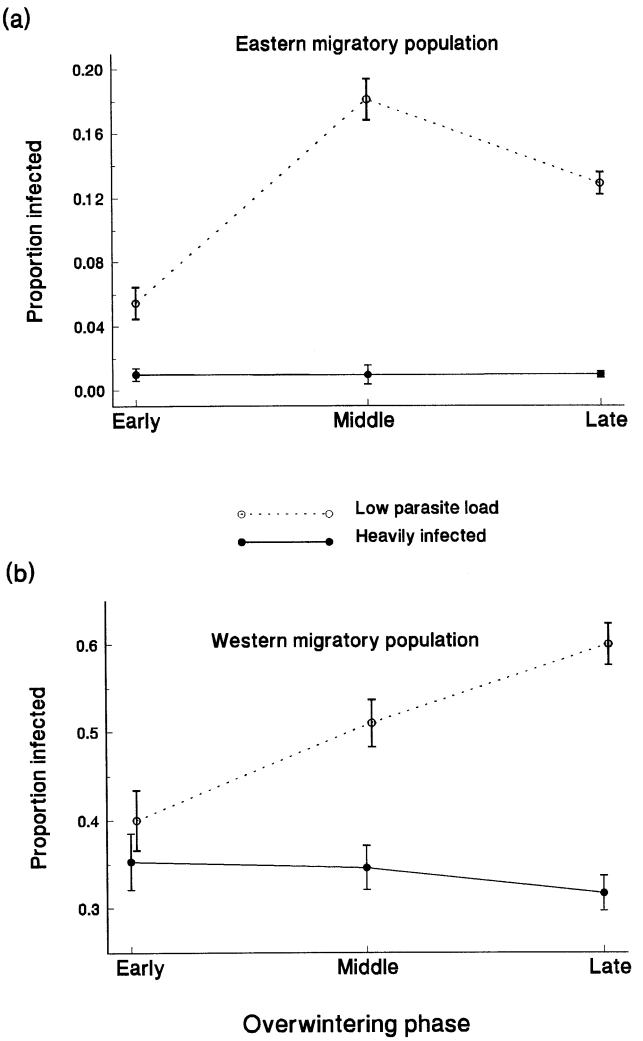

Fig. 6. Parasite prevalence in monarchs throughout early, middle, and late overwintering phases, shown as the proportion of heavily infected adults (solid line) and those with low parasite loads (dotted line). (a) Eastern migratory population, from five overwintering sites in central Mexico, November 1992 to March 1994. (b) Western migratory population, from seven overwintering sites along the California coast, November 1989 to March 1990. Error bars show 95% confidence intervals. See Tables 2 and 4 for a description of specific sampling locations.

lower at greater distances from overwintering sites (Δ deviance = 12.9, Δ d.f. = 1, $P < 0.001$; $\beta = -0.0013$, ASE = 0.0004).

Southern Florida population. Temporal variation in parasite prevalence in southern Florida was evaluated using monarchs collected at monthly intervals between December 1994 and November 1995 (Fig. 8). Logistic regression analysis demonstrated a significant association between month (treated as an unordered factor) and the prevalence of heavily infected adults (Δ deviance = 54.76, Δ d.f. = 9, $P < 0.001$). A simpler model treating month as a quantitative variable was not adequate (Δ deviance = 22.76, Δ d.f. = 8, $P < 0.01$), indicating that changes in prevalence did not follow a consistent temporal trend. The proportion of monarchs with high parasite loads was lower in October and November than at other times of the year (Fig. 8). This coincides with the time when the larger eastern population is migrating south. The proportion of heavily infected monarchs collected nearby in Cuba in November 1995 and 1996, and in southern Florida in November 1970, was statistically similar to the November 1995 Florida sample (χ^2 analysis: $\chi^2 = 4.32$, d.f. = 3, P = NS; Fig. 8).

Parasite prevalence in other populations

Samples of breeding monarchs collected in Australia and South America show that *O. elektroscirrha* has a wide geographical distribution, with infected monarchs present at all sampling locations (Fig. 9). Within Australia, monarchs from Sydney (SE Australia) showed the highest prevalence of heavy infection (0.39), while monarchs obtained farther north from Mt Crosby (near Brisbane, E Australia) had a lower prevalence of heavy infection (0.27). A sample of adults from Rockhampton (on the coast of Queensland, north of Brisbane) had the lowest prevalence of heavily infected adults (0.083). A χ^2 analysis of these three Australian populations showed that the proportions of heavily infected adults were significantly different ($\chi^2 = 6.87$, d.f. = 2, $P < 0.05$). Monarchs from northern South America (Colombia and Venezuela) had infection levels similar to those in Rockhampton, Australia (proportion heavily infected = 0.11 and 0.17, respectively). No monarchs collected in Trinidad in 1968 were infected with spores, although this sample was very small (11 adults).

Discussion

Ophryocystis elektroscirrha has a wide geographical distribution, and the prevalence of heavily infected adult *D. plexippus* is highly variable among populations (ranging from near zero to almost 100%). Within North America, parasites are most prevalent in southern Florida, where over 70% of the monarchs are heavily infected. Approximately 30% of the migratory population in western North America is heavily infected, whereas the eastern migratory population has < 10% heavily infected adults. Estimates of prevalence from older collections indicate that these differences may have persisted for up to 30 years. Although parasite prevalence varied among years within the eastern migratory population, there was no trend of progressive increases or decreases, as might be expected for a recently introduced pathogen or one that was unable to persist in a host population.

These data are consistent with previous measures of adult parasite loads in several monarch populations (Leong *et al.*, 1992, 1997a), and suggest an association between host migratory behaviour and parasite prevalence. The eastern migratory population in North America migrates the farthest distance each year and has the lowest prevalence of heavy infection. Monarchs west of the Rocky Mountains migrate

Table 3. Analysis of deviance likelihood ratio tests for effects of overwintering site and phase on the proportion of adults with high and low parasite loads. Predictors noted with {F} were treated as unordered factors using dummy variables to denote each level. The saturated model (1) was first checked against the null model (4) to evaluate the significance of model parameters. Given that predictors in the saturated model explained a significant amount of deviance, goodness-of-fit tests were used to determine whether a simpler model could be accepted as adequate (in this case, a NS test statistic indicated acceptance of the simpler model). Models that provided the best fit to the data are indicated by an asterisk.

Model	Deviance	d.f.	Models compared	Δ d.f.	Δ deviance	Probability
Eastern migratory population						
Response = high parasite load						
(1) Phase{F} + site{F} +phase{F} × site{F}	262.19	32	–	–	–	–
(4) Intercept only*	284.41	45	(4) – (1)	13	22.22	NS
Response = low parasite load						
(1) Phase{F} + site{F} + phase{F} × site{F}*	1800.86	32	–	–	–	–
(1) Phase{F} + site{F}	1837.43	39	(2) – (1)	7	36.57	< 0.001
(3) Phase + site{F} + phase × site{F}	1835.74	36	(3) – (1)	4	34.87	< 0.001
(1) Intercept only	1948.87	45	(4) – (1)	13	148.01	< 0.001
Western migratory population						
Response = high parasite load						
(1) Phase{F} + site{F} + phase{F} × site{F}	937.33	25	–	–	–	–
(4) Intercept only*	959.62	43	(4) – (1)	18	22.29	NS
Response = low parasite load						
(1) Phase{F} + site{F} + phase{F} × site{F}	1005.95	25	–	–	–	–
(1)Phase{F} + site{F}	1025.74	35	(2) – (1)	10	19.79	< 0.05
(3) Phase + site{F} + phase × site{F}*	1014.11	30	(3) – (1)	5	8.17	NS
(1) Intercept only	1042.43	43	(4) – (3)	13	28.32	< 0.01

considerably shorter distances (Fig. 1), and monarchs in southern Florida breed year-round and do not migrate (Knight, 1997). Another nonmigratory monarch population in Hawaii has been shown to bear extremely high parasite loads (up to 100% heavily infected; Brower *et al*., 1995; Leong *et al*., 1997a).

Many factors may be responsible for among-population differences in parasite prevalence, including genetic variation in host or parasite lineages (Read *et al*., 1995), environmental differences in temperature or humidity (Benz, 1987), and the different host migratory distances. Migration may affect parasite prevalence by influencing the mortality of infected hosts or by affecting disease transmission. For example, theoretical models of host–parasite interactions predict declines in parasite prevalence with increasing host mortality (Anderson & May, 1991). Therefore, if infected hosts suffer disproportionate mortality during migration, prevalence should decrease as migratory distances increase. Second, in the absence of host migration, parasites may accumulate in the hosts' environment over time (Shaw, 1994; Roberts *et al*., 1995), and hosts that undergo periodic migration may escape infection. Finally, migration and overwintering separate monarch reproductive intervals by up to 6 months, and could reduce the transmission of *O. elektroscirrha* by limiting the number of times that vertical transmission occurs each year.

If the mortality of infected hosts during migration is responsible for low prevalence in the longest-distance migrants, prevalence of heavy infection may increase in the summer generations due to biparental transmission (Altizer & Augustine, 1997), then decline during the autumn migration. In addition, if no parental transmission occurs at the overwintering sites (when hosts are not reproducing), the frequency of high parasite loads should decline during the overwintering

Table 4. Spatial and temporal variation in disease prevalence among monarchs in western North America during the 1989–1990 overwintering season (from L. P. Brower and W. H. Calvert, unpublished). Sample sizes range from 20 to 80 individuals per site per phase. Early, middle, and late phases are described in the text. Proportions represent the number of monarchs with either low (class 1, 2, and 3) or high (class 4 and 5) parasite loads over the total number sampled.

Colony	Parasite load	Early phase	Middle phase	Late phase
Cemetario	High	–	0.25	0.26
	Low	–	0.45	0.62
Ellwood	High	0.32	0.45	0.30
	Low	0.47	0.45	0.66
Moran Lake	High	0.55	0.53	0.38
	Low	0.45	0.47	0.55
Morro Bay	High	0.35	0.33	0.35
	Low	0.50	0.58	0.38
Pismo Beach	High	0.28	0.24	0.27
	Low	0.30	0.71	0.68
Refugio	High	–	0.38	0.38
	Low	–	0.54	0.57
Stinson Beach	High	0.29	0.38	0.25
	Low	0.47	0.44	0.65

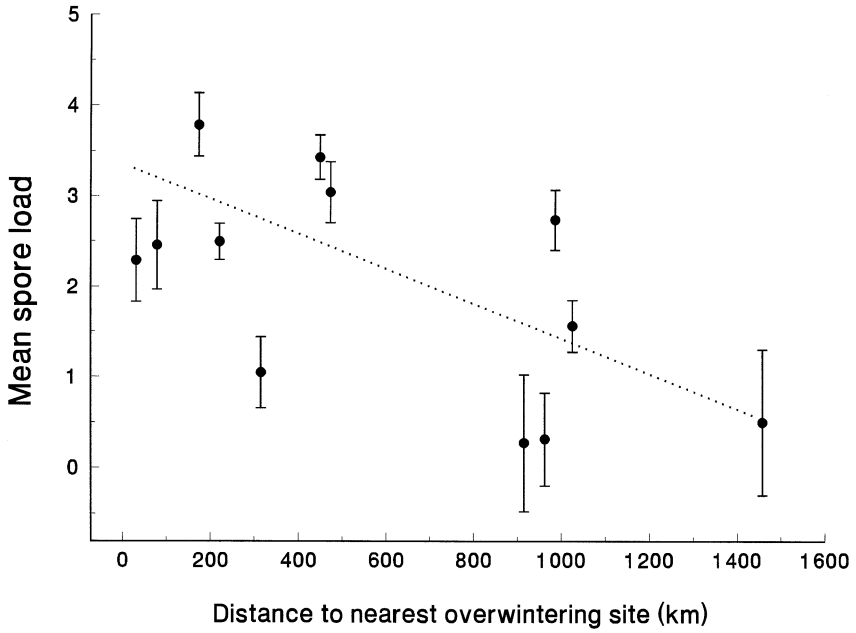

Fig. 7. Geographical variation in average parasite loads among summer breeding monarchs in western North America. Distance (km) was measured between the collection site and the nearest overwintering location. Error bars show 95% confidence intervals. Sample sizes and collection sites are presented in the Appendix, Table A3.

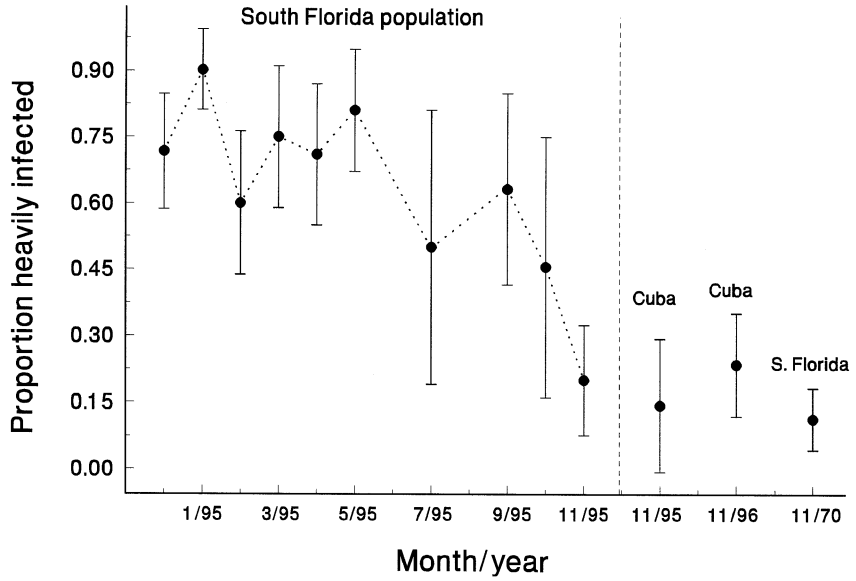

Fig. 8. Prevalence of heavily infected adults (classes 4 and 5) near Miami, Florida between December 1994 and November 1995. To the right of the vertical dashed line is shown the prevalence of heavily infected adults for three other samples collected in November in Cuba and southern Florida (showing disease prevalence similar to Miami for November 1995). Error bars represent 95% confidence intervals.

period due to the mortality of infected adults. No significant changes in the prevalence of heavily infected adults were detected among periods of breeding, migration, and overwintering in eastern North America (Fig. 5). Because prevalence in this population is so low and breeding monarchs were sampled near the northern limits of their summer range, these results must be regarded with caution, however observations from eastern and western migratory populations suggest that this disease does not cause increased mortality during the overwintering period alone, as no significant declines in prevalence were detected during overwintering (Fig. 6).

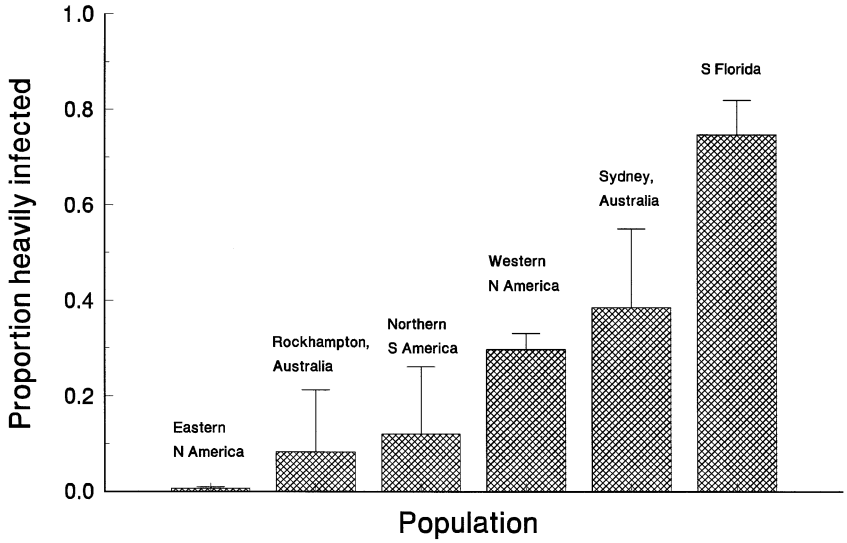

Fig. 9. Comparisons of parasite prevalence for North America, South America, and Australia. Bars show the frequency of heavily infected monarchs (classes 4 and 5) with 95% confidence intervals. Sample dates and sizes for Australian and South American data are shown in the Appendix, Table A1. Prevalence for the eastern migratory population in North America is for March 1996, Sierra Chincua, Mexico; prevalence for the western migratory population is combined across five overwintering colonies along the California coast in February 1997; prevalence for the southern Florida population is pooled for December 1994 to April 1995.

In contrast, parasite prevalence in summer breeding monarchs in western North America declined with increasing distance between breeding and overwintering sites. Monarchs collected from locations more distant from overwintering sites had lower average parasite loads and prevalence of heavy infection (Fig. 7). This pattern suggests that heavily infected monarchs may fail to reach breeding sites at the most distant extremes of their range. Deviations from this general pattern indicate that other factors influence the distribution of disease among breeding monarchs in western North America. One factor that may become increasingly important is the sale and transfer of live monarchs for special events by breeders in North America. Depending on the rearing practices involved, large numbers of healthy or infected butterflies may be released and artificially decrease or enhance parasite prevalence in local patches (Brower *et al*., 1995).

Laboratory studies of *O. elektroscirrha* transmission demonstrate that low parasite loads (classes 1–3) can result from mating or other contact between healthy and infected adults (Altizer, 1998). Temporal changes in the frequency of monarchs with low parasite loads indicate that spore transfer between adults also occurs during phases of migration and overwintering, when monarchs cluster in dense aggregations (Fig. 5). At overwintering colonies, the prevalence of adults with low parasite loads increased throughout the overwintering period (Fig. 6). This increase in the prevalence of monarchs with low parasite loads probably results from contact between healthy and heavily infected butterflies during intervals of high host density. Transfer via direct or indirect contact between adults may be important to the persistence of this parasite in the eastern migratory population, where heavily infected adults (and hence vertical transmission) are rare.

Temporal changes in the frequency of heavily infected adults in southern Florida show a decline in prevalence in October and November 1995, compared with high prevalence in other months of that year (Fig. 8). Recent work by Knight (1997) determined that this decline coincides with an influx of eastern autumn migrants into southern Florida. Moreover, most of the uninfected monarchs in the November sample were, as determined by thin layer chromatography, members of the eastern migratory population (A. J. Knight, pers. comm.). Two other samples of monarchs collected during November in Cuba also contained a mix of migrating and locally breeding butterflies (C. Dockx and L. P. Brower, unpublished) and showed prevalence similar to monarchs collected in November in southern Florida. This evidence suggests that uninfected migrating monarchs enter regions of high parasite prevalence in southern Florida and Cuba in the autumn, temporarily decreasing parasite prevalence.

Among Australian monarchs, prevalence of *O. elektroscirrha* followed a latitudinal gradient along the eastern coast. Monarchs sampled farther north in Rockhampton (Queensland) had lower parasite prevalence than those collected in New South Wales near Sydney (Fig. 9), and monarchs collected near Brisbane showed intermediate prevalence. The distribution of monarchs in Australia is confined largely to eastern regions of Queensland and New South Wales, and their winter range is restricted to the East coast (Zalucki, 1986). Although stable overwintering colonies have been observed in the Sydney Basin for many years (James, 1993), conditions in the Sydney area also support breeding monarch populations throughout the year (James, 1993). Conditions near Rockhampton (in Queensland, north of Brisbane), however, become too hot and dry in summer to maintain a continuous breeding population (M. P. Zalucki, pers. comm.). Thus, variation in

parasite prevalence observed in Australian monarchs may reflect host breeding ecology and migratory behaviour, with prevalence highest in areas where monarchs are present year-round and lower in regions where monarch populations are ephemeral.

To understand the role of parasites in regulating animal populations, or underlying factors that mediate parasite abundance, more investigations of natural populations are required (Dobson & Hudson, 1995; Gulland, 1995). Observations of population variation in prevalence show clearly that parasite prevalence and average parasite loads are lower in migratory than in nonmigratory monarch populations. Although fine-scale observations within populations do not indicate that differential mortality of infected hosts during overwintering generates this pattern, other processes related to host movement may still affect pathogen abundance. In particular, the observation that the distance from overwintering to breeding areas is correlated negatively with average parasite loads in western North America suggests that spring migration may be important in regulating disease prevalence. In addition, contact leading to spore transfer between adults at overwintering colonies is likely to sustain *O. elektroscirrha* in the eastern migratory population, where parental transmission is limited by the low frequency of infected adults.

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Appendix

Table A1. Location and date of monarch collections used to assess disease prevalence among populations. For samples marked with an asterisk, multiple dates and locations have been combined into a single entry. Note that Mexico is included in North American collections.

Population	Date	Location	Activity	*n*
Eastern North America	9/68	Massachusetts	Migrating	15
	9/70	Massachusetts	Migrating	36
	9/71	Massachusetts	Migrating	74
	9/72	Massachusetts*	Migrating	153
	9/73	Massachusetts*	Migrating	697
	11/77–3/78	Central Mexico*[1]	Overwintering	582
	9/79	Kansas	Migrating	54
	1/81	Central Mexico[1]	Overwintering	70
	11/81	Central Mexico[1]	Overwintering	90
	10/82	Texas*	Migrating	121
	12/83	Central Mexico[1]	Overwintering	84
	1/85	Central Mexico[1]	Overwintering	26
	10/85	N Florida	Migrating	50
	10/88	N Florida	Migrating	26
	4/93	N Florida	Migrating	42
	10/93	Texas	Migrating	134
	11/93–3/94	Central Mexico*[1]	Overwintering	3184
	4/94	N Florida	Migrating	55
	7/94	Minnesota, Wisconsin*	Breeding	183
	10/94	Texas, N Florida*	Migrating	320
	1/95–3/95	Central Mexico*	Overwintering	600
	7/95	Minnesota, Wisconsin*	Breeding	207
	10/95	Texas	Migrating	48
	11/95–3/96	Central Mexico*[1]	Overwintering	3393
	7/96	Minnesota, Wisconsin*	Breeding	56
	3/97	Central Mexico[1]	Overwintering	1309
	6/97–8/97	Minnesota, Wisconsin*	Breeding	370
Western North America	12/79	Santa Cruz, California	Overwintering	99
	11/89–3/90	California Coastline*[2]	Overwintering	946
	8/96	Davis, California	Breeding	40
	1/97–2/97	California Coastline*[2]	Overwintering	717
	7/97–8/97	California, Colorado, Nevada, Oregon, Utah, Washington*	Breeding	309
	9/97	Bolinas, California	Overwintering	30
South Florida	12/68	Miami, Florida	Breeding	7
	11/70	Flamingo, Florida	Migrating	80
	4/90	Miami, Florida	Breeding	46
	12/94–11/95	Miami, Florida	Breeding	292
	7/96	Miami, Florida	Breeding	21
Caribbean	11/95	Cuba		21
	11/96	Cuba		51
Northern South America	1/68	Trinidad	Breeding	11
	7/95	Colombia	Breeding	27
	11/95	Venezuela	Breeding	6
Australia	4/96	Sydney, New South Wales	Breeding	39
	7/96	Rockhampton, Queensland	Breeding	24
	7/96–10/96	Mt Crosby, Queensland	Breeding	45

[1]Represents from one to eight different overwintering areas in Central Mexico (including Sierra Chincua, Cerro Pelon, Palomas, Sierra El Campanario, Sierra Chivati-Huacal, Cerro Altamirano, and Herrada; for locations, see Calvert & Brower, 1986). [2]Represents from one to 10 different overwintering locations along the California coastline (including Bolinas, Stinson Beach, Moran Lake, Morro Bay, Pismo Beach, Ellwood, Gaviota, Leo Carillo, Cemetario, and Refugio; L. P. Brower and W. Calvert, unpublished).

Table A2. Samples of eastern North American migratory monarchs collected during breeding, migratory, and overwintering periods. Unless specified, overwintering sites in Central Mexico include Sierra Chincua, Cerro Pelon, Cerro Altamirano, Sierra El Campanario, Sierra Chivati-Huacal, Herrada, and Palomas. Breeding monarchs in Minnesota and Wisconsin were captured within a 250-km radius of St Paul, Minnesota. Migrating monarchs in Texas were captured in Central Texas.

Location	Date	Activity	*n*
Five sites in Central Texas	10/93	Migrating south	134
Five sites in Central Mexico	11/93	Overwintering	495
Seven sites in Central Mexico	12/93	Overwintering	648
Seven sites in Central Mexico	1/94	Overwintering	705
Seven sites in Central Mexico	2/94	Overwintering	698
Six sites in Central Mexico	3/94	Overwintering	594
Minnesota and Wisconsin	6/94–8/94	Breeding	183
Central Texas and N Florida	10/94	Migrating south	320
Four sites in Central Mexico	1/95	Overwintering	400
Sierra Chincua, Central Mexico	2/95	Overwintering	100
Sierra Chincua, Central Mexico	3/95	Overwintering	100
Minnesota and Wisconsin	6/95–8/95	Breeding	207
Central Texas	10/95	Migrating south	48
Sierra Chincua, Central Mexico	11/95	Overwintering	1000
Sierra Chincua, Central Mexico	1/96	Overwintering	1000
Sierra Chincua, Central Mexico	3/96	Overwintering	1393
Minnesota and Wisconsin	6/96–8/96	Breeding	56
Sierra Chincua, Central Mexico	3/97	Overwintering	1309
Minnesota and Wisconsin	6/97–8/97	Breeding	370

Table A3. Collection sites, dates, and sample sizes of breeding monarchs captured in western North America in July and August 1997.

Location	Date	*n*
Bay Area, California	7/97	11
San Luis Obispo, California	7/97	14
El Dorado Hills, California	7/97	63
Richvale, California	8/97	28
Gazelle, California	8/97	44
Minden and Reno, Nevada	7/97	21
Talent, Oregon	8/97	23
Salt Lake City, Utah	8/97	30
Grand Junction, Colorado	8/97	25
Denver, Colorado	8/97	4
Umatilla, Oregon	8/97	11
Outlook, Washington	8/97	32