Migratory behaviour and host–parasite co-evolution in natural populations of monarch butterflies infected with a protozoan parasite

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ABSTRACT

The prevalence of the protozoan parasite, *Ophryocystis elektroscirrha*, varies dramatically among natural populations of monarch butterflies. One potential cause of this variation is that host resistance or parasite virulence differs among populations due to underlying variation in host migratory behaviour and parasite transmission. In this study, I examined the geographic variation in host and parasite characteristics using reciprocal cross-infection experiments, where monarchs from three North American populations were exposed to parasites from native and novel sources. I tested hosts and parasites from the following three populations: a continuously breeding population in southern Florida, a population in western North America that migrates relatively short distances and a population in eastern North America that migrates remarkably long distances. Cross-infection experiments using hosts and parasites from the eastern and western migratory populations demonstrated that western parasites caused higher mortality and parasite loads than eastern parasites, and eastern monarchs had higher survival and lower parasite loads than western monarchs. Eastern migratory and Florida resident monarchs performed similarly across all treatments, but parasites isolated from southern Florida caused higher parasite loads than those from the eastern population. Differences in parasite virulence among populations were also supported by sub-lethal effects of parasites on monarch wing-span, mass at emergence and rates of weight loss. Unlike other documented patterns of host–parasite specificity, *O. elektroscirrha* strains do not appear to be more infectious to their native hosts. Rather, geographic variation may be better explained by selection resulting from differences in host migratory behaviour and parasite transmission among populations. The results of this study are consistent with the hypothesis that seasonal, long-distance host migration is associated with higher host resistance and lower parasite virulence in North American monarch populations.

Keywords: co-evolution, *Danaus plexippus*, host resistance, neogregarine, *Ophryocystis elektroscirrha*, seasonal migration, virulence.

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INTRODUCTION

One theory of host–parasite co-evolution predicts that parasites are likely to become locally adapted, tending towards greater infectiousness and higher replication rates in sympatric than allopatric hosts. A possible explanation for local adaptation is that parasites have short generation times and can track common host genotypes (Lively, 1992; Lively and Apanius, 1995; Dybdahl and Lively, 1998). Examples include trematode and schistosome infections of snails (Lively, 1989; Lively and Jokela, 1996; Morand *et al.*, 1996), microsporidian parasites of *Daphnia* (Ebert, 1994) and trypanosome parasites of bumblebees (Imhoof and Schmid Hempel, 1998). However, time-lags in the co-evolutionary process and selection for host resistance alleles may lead to unpredictable compatibility for novel host–parasite combinations (e.g. Lively, 1999).

A second factor likely to influence the evolution of parasite virulence is the parasite's transmission mode (Ewald, 1983). For example, vertically transmitted parasites should evolve towards lower virulence because their rate of spread depends on the reproductive success of their hosts (Fine, 1975). Parasites with vertical transmission that cause substantial host damage may consequently decrease their transmission opportunities (e.g. Herre, 1993; Ebert, 1994). In contrast, parasites that are horizontally transmitted should evolve toward intermediate virulence depending on the relationship between within-host replication, host mortality and among-host transmission (Anderson and May, 1991; Lenski and May, 1994). If the relative importance of vertical and horizontal transmission varies among populations, then parasites might evolve different levels of virulence in different populations (Herre, 1993; Sorci *et al.*, 1997), with higher virulence coinciding with greater horizontal transmission opportunities.

Because of the rapid replication of most parasites, the host response to different parasite strains is often ignored. However, hosts should be under selection to minimize parasite infection and replication through a variety of mechanisms (Lively, 1987; Henter and Via, 1995; Ebert and Hamilton, 1996; Coltman *et al.*, 1999). As a result, parasite virulence may be a property of interactions with different host genotypes that show variable levels of susceptibility (Leonard and Czochor, 1980; Parker, 1992). In fact, many studies of plant pathogens in agricultural and natural settings have documented strain-specific host races in plant resistance to naturally occurring pathogens (e.g. Burdon and Jarosz, 1991; Alexander, 1992).

In this study, I examined the geographic variation in host resistance and parasite virulence in populations of monarch butterflies (*Danaus plexippus*) infected with the neo-gregarine protozoan parasite, *Ophryocystis elektroscirrha*. Monarchs and their protozoan parasites are distributed worldwide and parasite prevalence varies dramatically among populations (Ackery and Vane-Wright, 1984; Leong *et al.*, 1997a; Altizer *et al.*, 2000). One possible cause of this variation is that populations have co-evolved as a result of their different migratory behaviours. I therefore studied host survival and parasite replication in a reciprocal cross-infection experiment using hosts and parasites from three North American populations.

The *Ophryocystis elektroscirrha/Danaus plexippus* system

Monarch butterflies populate islands and continents worldwide, and migrate seasonally in parts of North America and Australia (e.g. Urquhart and Urquhart, 1978; James, 1993).

Three North American populations display varying amounts of migratory behaviour. Eastern North American monarchs migrate up to 5200 km to coniferous forests in the trans-volcanic mountains of central Mexico (Fig. 1). They arrive during late autumn and overwinter in densely populated sites that harbour tens of millions of butterflies (Urquhart and Urquhart, 1978; Calvert and Lawton, 1993). In February and March, these same individuals break diapause and mate before flying north to recolonize their breeding range (Brower and Malcolm, 1991; Van Hook, 1993). Western North American monarchs migrate a shorter distance to the coast of California (Nagano *et al.*, 1993; Brower, 1995; Fig. 1) and overwinter in less densely populated roosting areas. Individuals in a resident population in southern Florida breed continuously throughout the year, but recent evidence indicates that this population has an influx of autumn migrants from the larger eastern population (A.J. Knight and L.P. Brower, personal communication).
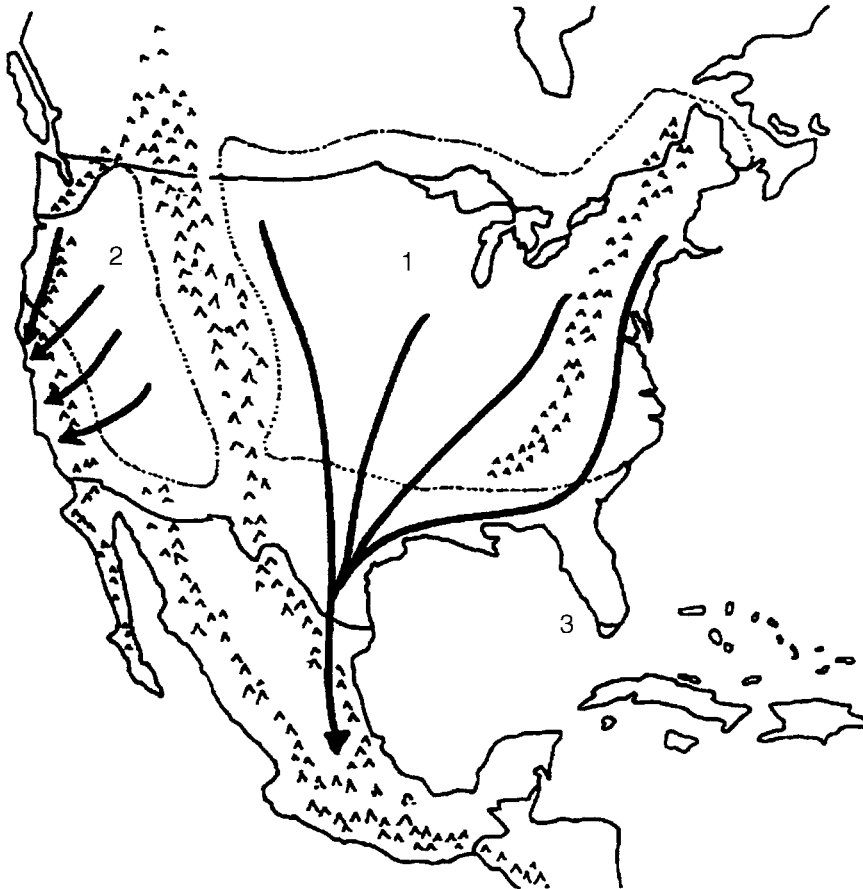

Fig. 1. Summer breeding ranges and major migratory routes for three North American monarch butterfly populations: (1) eastern migratory population, (2) western migratory population and (3) southern Florida population (modified from Brower, 1995).

The neogregarine protozoan parasite, *O. elektroscirrha*, is transmitted maternally when infected females scatter spores on eggs and milkweed leaves during oviposition (McLaughlin and Myers, 1970; Leong *et al.*, 1997b). Paternal and horizontal transmission also occur (Altizer *et al.*, 2000; S.M. Altizer, personal observation), although new infections require that larvae ingest spores by feeding on contaminated eggs or leaves (Leong *et al.*, 1997b). After ingestion, spores lyse in the larval gut and migrate to the hypoderm, where they undergo two cycles of vegetative replication. After host pupation, parasites undergo sexual reproduction and form dormant spores around the scales of the developing adult butterfly (McLaughlin and Myers, 1970). Most spores form on the abdomen, although spores also develop on the wings, head and thorax (Leong *et al.*, 1992). Negative effects of *O. elektroscirrha* on monarch fitness are dose-dependent and may result from initial gut wall damage and subsequent parasite replication. High parasite doses increase larval mortality, and heavily infected captive adults are smaller and shorter-lived than uninfected adults (Altizer and Oberhauser, 1999). Although heavily infected males have decreased mating success, no effects of *O. elektroscirrha* on female fecundity have been observed (Altizer and Oberhauser, 1999).

Ophryocystis elektroscirrha has a wide geographic range and parasite prevalence varies inversely with host migration distances (Leong *et al.*, 1997a; Altizer *et al.*, 2000). Monarchs in southern Florida and Hawaii that breed year-round bear the highest parasite loads (over 70% heavily infected). Approximately 30% of the migratory monarch population in Western North America is heavily infected. In contrast, less than 8% of the eastern migratory population, which migrates the farthest distance, is heavily infected. These differences in prevalence among populations have persisted for many years (Altizer *et al.*, 2000), and may result from host migration, environmental differences among locations or genetic differences in hosts or parasites.

Seasonal migration is likely to affect at least two variables that mediate host–parasite co-evolution: the relative fitness of infected hosts and parasite transmission. In migratory populations, monarchs breed for two to three generations each summer between intervals of migration and overwintering. Migration may be energetically costly and hosts in poor condition may have compromised migratory abilities. The negative consequences of susceptibility to infection may, therefore, be more severe in migratory populations, where monarchs with lower survival or migratory abilities are likely to die before reproducing the following spring. This high cost of susceptibility may select for higher host resistance in populations that migrate the farthest distance. Migration will also affect parasite transmission and could select for differences in parasite virulence. For example, parasite spores are likely to accumulate on plants in continuously breeding populations, leading to higher rates of horizontal transmission. Because greater horizontal transmission should select for higher parasite virulence (e.g. Ewald, 1983; Herre, 1995), virulence may be lowest in populations that migrate the farthest distances and highest in resident populations where both horizontal and vertical transmission occur throughout the year.

To examine the potential for geographic variation in host resistance and parasite virulence, I cross-infected monarchs and their parasites from three North American populations (Fig. 1). Because the virulence of many parasites results from their within-host replication (Anderson and May, 1991; Ebert, 1994), I measured the parasite loads of inoculated monarchs in addition to host survival and size at eclosion. Possible outcomes and their implications include: (1) higher virulence of parasites in their native hosts, indicating that parasites are locally adapted; (2) lower virulence of parasites in their native

hosts, indicating that hosts resist local parasites; (3) higher host resistance among the longest-distance migrants, resulting from an increased cost of infection; (4) higher parasite virulence in resident populations due to increased transmission opportunities; or (5) no effect of either host or parasite origin on host–parasite co-evolution.

MATERIALS AND METHODS

General methods

I performed cross-infection experiments between March and October 1996 to evaluate monarch susceptibility and parasite virulence in three different North American populations (Fig. 1). Migratory monarchs mix randomly during migration and overwintering and do not show site fidelity when recolonizing their breeding range (Eanes and Kohn, 1978; Wassenaar and Hobson, 1998). Moreover, because local sub-populations persist for only a few months each summer and do not show strong signs of inbreeding (Eanes and Kohn, 1978), I assumed an entire migratory population was one population. The timing of host collection and the availability of milkweed limited these studies to crosses between pairs of populations (rather than testing all three simultaneously). I performed two cross-infection experiments with hosts and parasites from eastern and western migratory populations; in the first I used a low parasite dose and in the second I used a 10-fold higher dose. A third experiment crossed hosts and parasites from the eastern migratory and Florida resident populations using a low parasite dose.

To obtain eggs, I placed 8–12 wild-captured, uninfected adult monarchs from the same population in 0.6 m^3 mosquito-net field cages and fed them *ad libitum* from sponges soaked in 20% honey-water. Immediately after mating, I transferred individual females to field cages with greenhouse-reared *Asclepias currasavica*. I removed or replaced the plants after 20–50 eggs had been deposited. Eggs for all treatments in each experiment were laid within a 5 day period, and larvae remained on natal host plants until they reached late second-instar.

I inoculated larvae with *O. elektroscirrha* as described by Altizer and Oberhauser (1999). Bulk inoculum was prepared by removing parasite spores from the abdomens of two to three wild-captured, heavily infected adults per population. Although more infected adults were not used because they are extremely rare in the eastern migratory population, the genetic diversity among parasites within a single host population is unknown. Control inoculum was prepared using two to three abdomens of wild-captured, uninfected adults. Larvae were inoculated individually in petri dishes by applying calibrated doses of parasite spores (in aqueous suspension) to 1 cm^2 pieces of sterilized milkweed leaves using a micro-pipettor. I removed larvae from these dishes after they consumed 80–100% of the leaf material, and transferred them to plastic containers ($11 \times 17 \times 30$ cm) with metal window-screen lids. Larvae were reared on fresh cuttings of *A. syriaca* or *A. curassavica* at densities of 8–12 larvae per container (as described in Altizer and Oberhauser, 1999). When all monarchs in a container had pupated, they were moved to another laboratory to avoid contaminating the larval rearing area. After adults emerged and their cuticles hardened, I placed them in individual glassine envelopes. To minimize accidental infection of larvae, I sterilized laboratory surfaces and tools using 20% chlorine bleach solution or 95% ethanol.

Parasite loads of all adults were evaluated within 48 h of emergence. I pressed transparent Scotch™ brand tape cut into 1 cm^2 units against the ventral side of the butterfly

abdomen. Based on the number of spores per cm^2, I scored butterflies for parasite loads according to the following scale: 0 = no spores, 1 = 1 spore, 2 = 2–20 spores, 3 = 21–100 spores, 4 = 101–1000 spores and 5 = more than 1000 spores. Parasite loads estimated in this manner were highly correlated with the log of hemacytometer counts estimating the density of spores on monarch abdomens (Altizer and Oberhauser, 1999).

Experiment 1: eastern *vs* western migratory populations (low dose)

Between March and April 1996, I cross-infected hosts and parasites from the eastern and western migratory populations. Adults were captured at overwintering sites in central Mexico (Sierra Chincua, Michoacan, Mexico) and coastal California (Pismo Beach State Park) in early March 1996. From each population, I obtained eggs from three uninfected females mated to separate uninfected males. Monarchs were inoculated with parasites from either the eastern or western population using a dose of 10 spores per larva, or were assigned to a control treatment as described above. Each female's offspring were divided equally among the three inoculation treatments. Because larvae hatched over a period of several days, approximately equal numbers of larvae from each treatment were inoculated 3 days apart and reared in two separate blocks. I inoculated 16–20 offspring per female per treatment per block, and reared larvae in two replicate containers per treatment within each block (for a total of 721 inoculated larvae). I chose to replicate offspring more intensely within each female rather than use fewer progeny from many females because of the need to replicate containers within treatments and to accurately measure within-female response variables.

   I recorded the number of offspring that successfully pupated and reached adulthood, and the parasite loads of emerging adults. For adults emerging in block 1, I also measured their mass 1 day after eclosion, and their subsequent mass each day for 3 successive days (to estimate their average daily rate of water loss per individual) using an analytical balance. During this time, adults were held in glassine envelopes in the laboratory at an average temperate of 21°C and were not handled other than for purposes of data collection.

Experiment 2: eastern *vs* western migratory populations (high dose)

In June and July 1996, I repeated the above experiment using a 10-fold higher parasite dose (100 spores per larva). To obtain parents for each host population, I selected uninfected males and females from the control treatment offspring of each female used in Experiment 1. Adults were paired in one of three cages with non-siblings of the opposite sex; females that mated were immediately placed in separate oviposition cages. I obtained eggs from four to five females mated to separate males from each host population. Offspring from each female were again divided equally among three inoculation treatments: eastern parasites, western parasites and control inoculum. Bulk inoculum was prepared from infected wild-caught monarchs as described above. I inoculated 16–36 offspring per female per treatment (for a total of 624 larvae) over 2 days. I reared larvae in two to three replicate containers per female (depending on the number of offspring inoculated). I measured the proportion of monarchs that survived to adulthood and the parasite loads of emerging adults. I also measured the mass of adults 1 day after eclosion using an analytical balance and their forewing length (from the point of wing attachment to the distal tip) using digital callipers.

Experiment 3: eastern migratory *vs* Florida resident populations (low dose)

In September and October 1996, I performed a third cross-inoculation experiment using hosts and parasites from the eastern migratory and southern Florida resident populations. Wild-caught adults from Minnesota and Wisconsin were used as parents for the eastern migratory population. To obtain uninfected parents from the Florida resident population, I collected monarch eggs from wild *A. curassavica* plants in Hialeah County, transferred them to sterilized milkweed and reared the monarchs in the laboratory. I divided the eggs from five mated females per source population evenly among three parasite treatments: eastern migratory parasites, southern Florida parasites and a control inoculum. Parasites from wild-captured adults were administered at a dose of 10 spores per larva. I inoculated 28–48 offspring per female per treatment over 2 days (for a total of 1163 larvae). Larvae were reared in two to four replicate containers per female per treatment; again I measured the survival and parasite loads of monarchs in each treatment, and the mass and forewing length of adults 1 day after eclosion.

Statistical analysis

I used analysis of variance (GLM Univariate procedure; SPSS, 1999) to test the effects of host origin, parasite origin, females nested within host origin and their interactions on monarch survival, parasite loads, wingspan and mass. Two proportional response variables were tested: the proportion of adults in each container that emerged heavily infected (levels 4 and 5) and the proportion of adults in each rearing container that survived to adulthood. Response variables measured as proportions were arcsine-square root transformed to normalize the error variance, and the analyses were weighted by the sample sizes to account for differences among containers. For tests of significance involving host origin, *F*-tests were constructed using the mean square for the female (host) or the parasite*female (host) as the error term, rather than the residual mean square (based on the nested experimental design). Because observations from the control treatments were included in the initial analyses as a separate level of parasite treatment, the significance of main effects and interactions involving parasite origin alone were later tested in separate analyses of variance that excluded control treatment observations (see Tables 1–6).

I also used analysis of variance to examine the effects of host and parasite origin on the parasite load, mass and wingspan of emerging adults. For tests of significance involving host origin, *F*-tests were constructed using the mean square for the female (host) or the parasite*female (host) as the error term. For all other tests of significance, I used the parasite*container*female (host) term as error to limit the contribution of larval rearing environment to the observed variation among individual monarchs. Separate analyses of variance (which excluded control treatment observations) were again used to explore the significance of main effects or interactions arising from parasite origin.

RESULTS

Experiment 1: eastern *vs* western migratory populations (low dose)

Monarchs from the western migratory population had higher pre-adult mortality across all inoculation treatments in Experiment 1 (Fig. 2a, Table 1a). However, parasite treatment
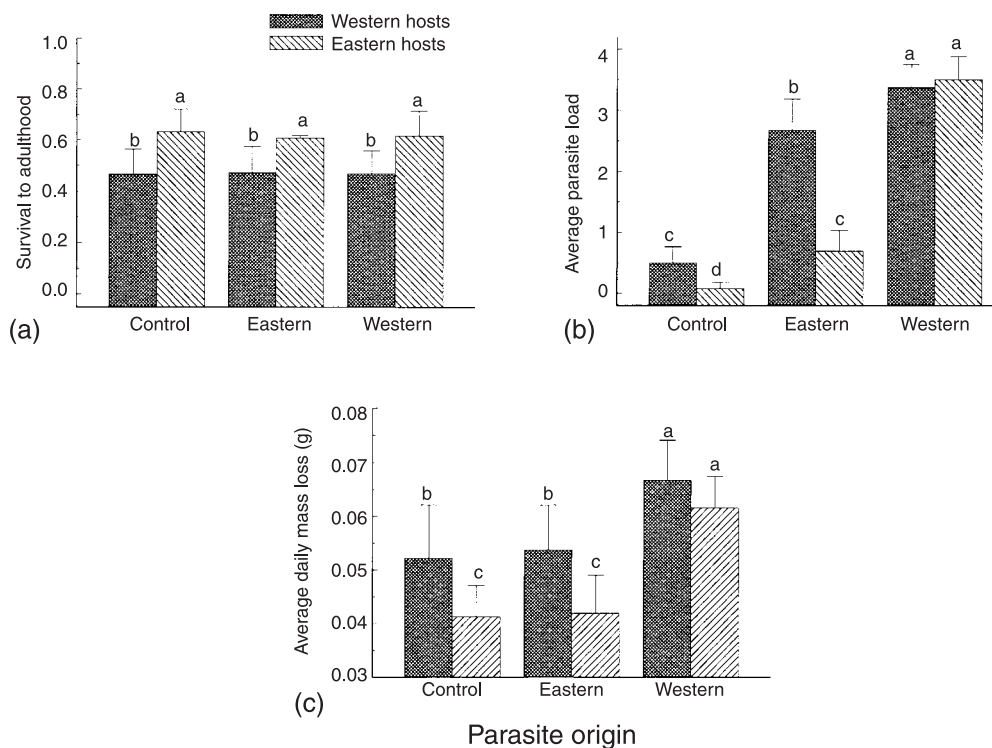
Fig. 2. Cross-infection results for eastern and western migratory populations (Experiment 1, low dose). (a) The proportion of monarchs that survived from inoculation to adulthood. Error bars represent 95% confidence intervals based on proportions and total sample sizes in each treatment. (b) Average parasite loads of emerging adults. Error bars represent 95% confidence intervals based on variation among tubs within treatments. (c) Average daily weight loss (over 3 successive days) of newly emerged adults. Error bars represent 95% confidence intervals based on variation among tubs within treatments. Letters represent means that are significantly different based on Tukey pairwise comparison tests ($a = 0.05$).

did not affect host survival (Fig. 2a), and no main effects or interactions involving parasite origin on host mortality were significant (Table 1a). Host survival was significantly higher in block 1 than in block 2, probably due to food limitations towards the end of larval development.

Eastern migratory monarchs inoculated with eastern parasites had lower parasite loads than any other host-by-parasite combination, and western parasites infected a higher proportion (approximately 70%) of both eastern and western hosts (Fig. 2b, Tables 1b and 2). The main effect of host origin on the proportion of heavily infected adults was not significant (Table 1b), but eastern monarchs had significantly lower parasite loads than western monarchs (Fig. 2b, Table 3a). Western parasites caused significantly higher average parasite loads and proportions of infected adults (Fig. 2b), and the host-by-parasite origin interaction was also significant (Tables 1b and 3a). Approximately 6% of western hosts in the control treatment emerged heavily infected (Table 2). This proportion was low relative to parasite-treated monarchs, probably resulting from undetected spores carried by

Table 1. Effects of host and parasite origin on the survival and proportion of heavily infected butterflies in Experiment 1 (eastern *vs* western migratory populations, low dose)

Source of variation	d.f.	MS	F	P	Error term used for *F*-test
(a) *Model*: survival = block + host + parasite + host*parasite + female (host) + parasite*female (host)					
Block	1	8.97	14.01	0.004	Residual MS
Host	1	5.13	9.83	0.035	Fem (host)
Parasite	2	0.31	0.49	0.616	Residual MS
Host*parasite	2	0.26	0.12	0.891	Parasite*fem (host)
Fem (host)	4	0.52	0.24	0.908	Residual MS
Parasite*fem (host)	8	2.18	3.41	0.003	Residual MS
(b) *Model*: proportion infected = block + host + parasite + host*parasite + female (host) + parasite*female (host)					
Block	1	0.042	0.10	0.765	Residual MS
Host	1	1.705	2.78	0.170	Fem (host)
Parasite	2	26.68	68.76	<0.001	Residual MS
Host*parasite	2	2.5	5.63	0.029	Parasite*fem (host)
Fem (host)	4	0.61	1.58	0.192	Residual MS
Parasite*fem (host)	8	0.44	1.14	0.350	Residual MS
Control treatments excluded					
Parasite	1	18.37	38.13	<0.001	Residual MS
Host*parasite	1	4.86	10.10	0.003	Parasite*fem (host)
Parasite*fem (host)	4	0.54	1.12	0.361	Residual MS

Note: Tests of significance used the effects in the far-right column as error terms. If differences among parasite treatments were significant, tests were recalculated excluding the control treatment. Proportions from each container were arcsine-square root transformed to normalize the error variance, and observations were weighted by the number of monarchs per container.

Table 2. Proportion of heavily infected adults (in parasite load classes 4 and 5) for each cross-infection treatment in Experiment 1

Parasite origin	Host origin	
	Eastern migratory	Western migratory
Control	0.01 (82)	0.06 (56)
Eastern migratory	0.11 (65)	0.48 (52)
Western migratory	0.72 (69)	0.69 (66)

Note: Sample sizes are shown in parentheses as the number of adults successfully emerging from all containers in each treatment.

wild-captured western females. Moreover, the average parasite loads of monarchs in the control group were extremely low (<1 spore per monarch) relative to monarchs inoculated with western parasites (>100 spores per adult; Fig. 2b).

I observed significant variation in the survival and average parasite loads of offspring from different females within each host population (Tables 1a,b, 3a). A wider range in average survival probabilities occurred among the offspring of different females from the

Table 3. Effects of host and parasite origin on the parasite load, eclosion mass and average daily weight loss of butterflies in Experiment 1 (eastern *vs* western migratory populations, low dose)

Source of variation	d.f.	MS	*F*	*P*	Error term used for *F*-test
(a) *Model*: parasite load = block + host + parasite + host*parasite + female (host) + parasite*female (host) + parasite*container*female (host)					
Block	1	6.45	4.55	0.047	Parasite*container*fem (host)
Host	1	59.59	7.77	0.049	Fem (host)
Parasite	2	281.20	198.93	<0.001	Parasite*container*fem (host)
Host*parasite	2	29.94	4.51	0.049	Parasite*fem (host)
Fem (host)	4	7.67	5.40	0.005	Parasite*container*fem (host)
Parasite*fem (host)	8	5.97	4.21	0.005	Parasite*container*fem (host)
Parasite*cont*fem(host)	18	1.41	0.75	0.764	Residual MS
Control treatments excluded					
Parasite	1	201.10	99.11	<0.001	Parasite*container*fem (host)
Host*parasite	1	49.81	7.40	0.052	Parasite*fem (host)
Parasite*cont*fem (host)	4	6.73	3.32	0.048	Residual MS
(b) *Model*: mass (g) = host + parasite + female (host) + host*parasite + parasite*female (host) + parasite*container*female (host)					
Host	1	0.0021	0.479	0.478	Fem (host)
Parasite	2	0.0003	0.218	0.859	Parasite*container*fem (host)
Host*parasite	2	0.0018	1.01	0.470	Parasite*fem (host)
Fem (host)	4	0.0045	0.3234	0.657	Parasite*container*fem (host)
Parasite*fem (host)	8	0.0173	1.257	0.311	Parasite*container*fem (host)
Parasite*cont*fem(host)	11	0.0138	0.993	0.465	Residual MS
(c) *Model*: Daily change in mass (g) = host + parasite + host*parasite + female (host) + parasite*female (host) + parasite*container*female (host)					
Host	1	$5.65E^{-04}$	6.81	0.048	Fem (host)
Parasite	2	$2.28E^{-03}$	17.80	0.005	Parasite*container*fem (host)
Host*parasite	2	$3.56E^{-05}$	0.477	0.657	Parasite*fem (host)
Fem (host)	4	$8.29E^{-05}$	0.646	0.611	Parasite*container*fem (host)
Parasite*fem (host)	8	$7.46E^{-05}$	0.581	0.649	Parasite*container*fem (host)
Parasite*cont*fem(host)	11	$1.28E^{-04}$	1.287	0.257	Residual MS
Control treatments excluded					
Parasite	1	$1.63E^{-03}$	9.50	0.014	Parasite*container*fem (host)
Host*parasite	2	$2.19E^{-04}$	1.87	0.228	Parasite*fem (host)
Parasite*fem(host)	5	$1.17E^{-04}$	0.68	0.522	Parasite*container*fem(host)
Parasite*cont*fem(host)	7	$1.72E^{-04}$	1.85	0.106	Residual MS

Note: Tests of significance were constructed using the effects in the far-right column as error terms. If differences among parasite treatments were significant, then test statistics were recalculated excluding the control treatment from analysis (to consider effects of parasite origin only).

eastern population (average survival = 53–75%) than among females from the western population (average survival = 41–56%). In addition, although average parasite loads of monarchs derived from different females varied for both eastern and western hosts, one western female's offspring had much lower parasite loads than the other two (1.75 *vs* 2.42, 2.76) averaged across all parasite treatments.

The mass of newly emerged adults (measured for block 1 only) was not affected by host origin or parasite inoculation treatment (Table 3b). However, the average daily weight loss (measured for 3 successive days after emergence) was significantly higher among western monarchs than those from the eastern population (Fig. 2c, Table 3c). In addition, monarchs from either population inoculated with western parasites lost weight faster than those from other inoculation treatments (Fig. 2c, Table 3c), indicating that higher parasite loads caused by western parasites are likely to increase desiccation among newly emerged adults.

Experiment 2: eastern *vs* western migratory populations (high dose)

The survival probability of monarchs in Experiment 2 (where hosts were inoculated with 100 spores per larva) was higher in the control treatments than for hosts inoculated with either parasite strain (Fig. 3a, Table 4a). Eastern monarchs had higher survival across all inoculation treatments and western parasites caused higher mortality than eastern parasites (Fig. 3a). In fact, 94% of the western hosts inoculated with western parasites died before reaching adulthood, and the interaction between host and parasite origin was also significant (after removing control treatments from the analysis; Table 4a). This suggests that western parasites were more lethal than eastern parasites (to western hosts) and that eastern hosts had similar survival when inoculated with either parasite strain. Inoculated butterflies emerged with dramatically higher parasite loads in Experiment 2 than Experiment 1 (Table 5; Fig. 3b). However, neither host nor parasite origin affected the average parasite loads or the proportion of heavily infected adults when control treatments were excluded (Tables 4b, 6a). In fact, 85–100% of hosts inoculated with parasites from either population emerged heavily infected (in parasite load classes 4 and 5; Table 5).

I again observed significant variation in the survival and parasite loads of offspring from different females in both host populations (Tables 4a,b, 6a). Average parasite loads were more variable among the offspring of different females from the eastern population than those from the western population. Interestingly, females whose offspring had higher average survival were associated with higher average parasite loads, and fewer monarchs in families with low average survival emerged heavily infected. One reason for this may be that heavily infected butterflies in families with low survival died before emergence, and the only hosts that survived in these treatments were the ones with lower parasite loads.

Eastern adults emerged with higher mass and larger wingspans than western monarchs (Fig. 3c,d). Western monarchs inoculated with western parasites had lower mass and smaller wingspans than adults in any other host-by-parasite origin combination (Fig. 3c,d). However, these trends were not significant at the 0.05 level (Table 4b,c), possibly due to the low survival (and subsequent sample sizes) of western adults inoculated with western parasites.

Experiment 3: eastern migratory *vs* Florida resident populations (low dose)

Monarchs from the Florida resident population had higher survival than eastern migratory hosts in both the control and eastern parasite treatments (Fig. 4a). Although the main
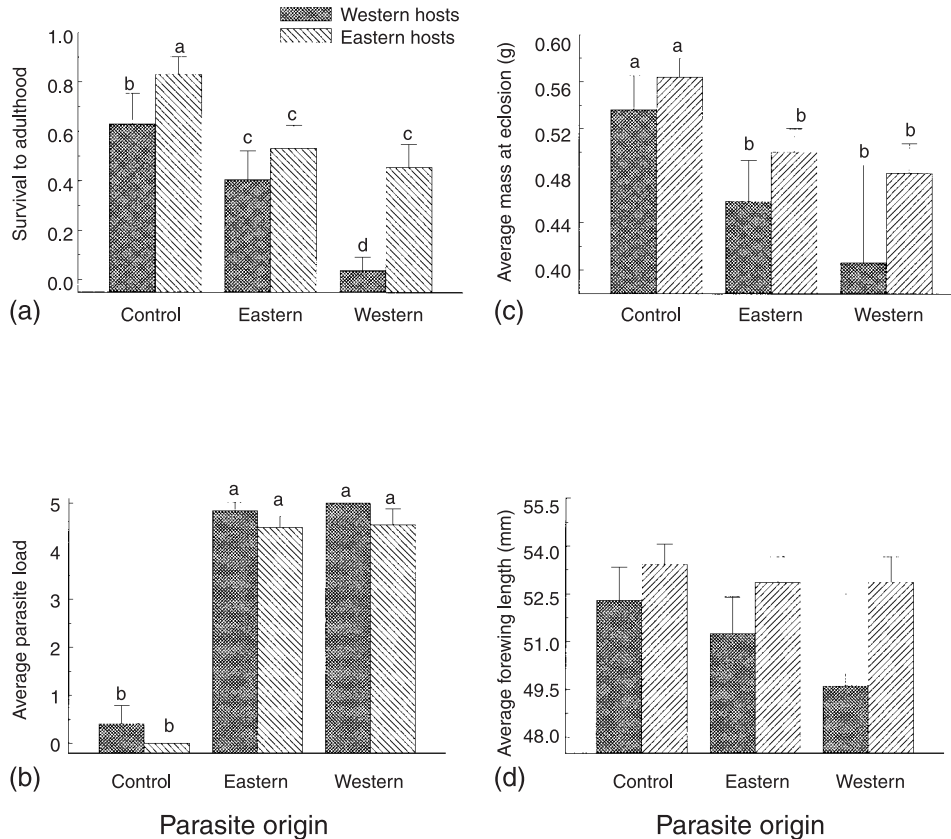
Fig. 3. Cross-infection results for eastern and western migratory populations (Experiment 2, high dose). (a) The proportion of monarchs that survived from inoculation to adulthood. Error bars represent 95% confidence intervals based on proportions and total sample sizes in each treatment. (b) Average parasite loads of emerging adults. Error bars represent 95% confidence intervals based on variation among tubs within treatments. (c) Mass of adults (g) 1 day after eclosion. Error bars represent 95% confidence intervals based on variation among tubs within treatments. (d) Wingspan (forewing length, mm) of newly emerged adults. Error bars represent 95% confidence intervals based on variation among tubs within treatments. Letters indicate means that are significantly different based on Tukey pairwise comparison tests ($a = 0.05$).

effect of host origin on survival was significant (Table 7a), no other main effects or inter-actions influenced host survival. Hosts from both populations performed very similarly across inoculation treatments with respect to average parasite loads and the frequency of heavy infection (Fig. 4b, Tables 7b, 8, 9a). However, parasites from the Florida resident population were associated with significantly higher parasite loads and probabilities of heavy infection than those from the eastern migratory population (Fig. 4b, Tables 7b, 8, 9a).

Average parasite loads varied among the offspring of individual females, an effect that was significant (Tables 7b, 9a). The average parasite loads and probability of heavy infection

Table 4. Effects of host and parasite origin on the survival and parasite load of monarchs in Experiment 2 (eastern *vs* western migratory populations, high dose)

Source of variation	d.f.	MS	*F*	*P*	Error term used for *F*-test
(a) *Model*: survival = host + parasite + female (host) + parasite*female (host)					
Host	1	12.09	8.53	0.022	Fem (host)
Parasite	2	20.22	50.81	<0.001	Residual MS
Host*parasite	2	1.60	0.92	0.423	Parasite*fem (host)
Fem (host)	7	1.42	3.56	0.008	Residual MS
Parasite*fem (host)	14	1.75	4.40	0.005	Residual MS
Control treatments excluded					
Parasite	1	10.22	22.03	<0.001	Residual MS
Host*parasite	1	2.75	5.61	0.050	Parasite*fem (host)
Parasite*fem (host)	7	0.49	1.05	0.431	Residual MS
(b) *Model*: proportion infected = host + parasite + female (host) + parasite*female (host)					
Host	1	0.29	2.05	0.196	Fem (host)
Parasite	2	33.93	89.24	0.001	Residual MS
Host*parasite	2	0.11	2.75	0.107	Parasite*fem (host)
Fem (host)	7	0.14	0.38	0.903	Residual MS
Parasite*fem (host)	11	0.14	0.38	0.950	Residual MS
Control treatments excluded					
Parasite	1	0.04	0.11	0.746	Residual MS
Host*parasite	1	0.33	2.35	0.200	Parasite*fem (host)
Parasite*fem (host)	4	0.14	0.35	0.841	Residual MS

Note: Tests of significance were constructed using the effects in the far-right column as error terms. If differences among parasite treatments were significant, then test statistics were recalculated excluding the control treatment. Proportions derived from each container were arcsine-square root transformed to normalize the error variance, and observations were weighted by the number of monarchs in each container.

Table 5. Proportion of heavily infected adults (in parasite load classes 4 and 5) for each cross-infection treatment in Experiment 2

Parasite origin	Host origin	
	Eastern migratory	Western migratory
Control	0.00 (108)	0.04 (54)
Eastern migratory	0.92 (68)	0.85 (34)
Western migratory	0.90 (60)	1.00 (3)

Note: Sample sizes are shown in parentheses as the number of adults successfully emerging from all containers in each treatment.

Table 6. Effects of host and parasite origin on the parasite load, mass at eclosion and forewing length of butterflies in Experiment 2 (eastern *vs* western migratory populations, high dose)

Source of variation	d.f.	MS	*F*	*P*	Error term used for *F*-test
(a) *Model*: parasite load = host + parasite + host*parasite + female (host) + parasite*female (host) + parasite*container*female (host)					
Host	1	0.030	1.18	0.314	Fem (host)
Parasite	2	0.110	18.38	<0.001	Parasite*container*fem(host)
Host*parasite	2	0.002	0.13	0.883	Parasite*fem (host)
Fem (host)	7	0.028	4.61	0.003	Parasite*container*fem(host)
Parasite*fem (host)	10	0.014	2.27	0.057	Parasite*container*fem(host)
Parasite*cont*fem(host)	20	0.006	1.18	0.269	Residual MS
Control treatments excluded					
Parasite	1	0.17	0.24	0.631	Parasite*container*fem(host)
Host*parasite	1	0.14	0.20	0.683	Parasite*fem (host)
Parasite*cont*fem (host)	3	0.70	1.00	0.425	Residual MS
(b) *Model*: mass (g) = host + parasite + female (host) + host*parasite + parasite*female (host) + parasite*container*female (host)					
Host	1	0.0421	1.696	0.118	Fem (host)
Parasite	2	0.1221	18.55	<0.001	Parasite*container*fem (host)
Host*parasite	2	0.0043	0.376	0.588	Parasite*fem (host)
Fem (host)	7	0.0248	3.77	0.014	Parasite*container*fem (host)
Parasite*fem (host)	11	0.0112	1.68	0.108	Parasite*container*fem (host)
Parasite*cont*fem(host)	20	0.0066	1.42	0.120	Residual MS
Control treatments excluded					
Parasite	1	0.0133	1.66	0.180	Parasite*container*fem (host)
Host*parasite	2	0.002	0.122	0.810	Parasite*fem (host)
Parasite*fem(host)	11	0.018	2.26	0.062	Parasite*container*fem(host)
Parasite*cont*fem(host)	14	0.008	1.546	0.108	Residual MS
(c) *Model*: wingspan (mm) = host + parasite + host*parasite + female (host) + parasite*female (host) + parasite*container*female (host)					
Host	1	67.02	3.64	0.062	Fem (host)
Parasite	2	19.65	2.45	0.092	Parasite*container*fem (host)
Host*parasite	2	4.18	0.529	0.556	Parasite*fem (host)
Fem (host)	7	18.41	2.28	0.081	Parasite*container*fem (host)
Parasite*fem (host)	11	7.89	0.98	0.435	Parasite*container*fem (host)
Parasite*cont*fem(host)	19	8.08	1.37	0.143	Residual MS

Note: Tests of significance were constructed using the effects in the far-right column as error terms. If differences among parasite treatments were significant, then test statistics were recalculated excluding the control treatment.

varied more among females from the eastern population than those from southern Florida (% heavily infected was 19–37% for southern Florida females and 19–58% among eastern migratory females). Although individual females did not significantly affect pre-adult
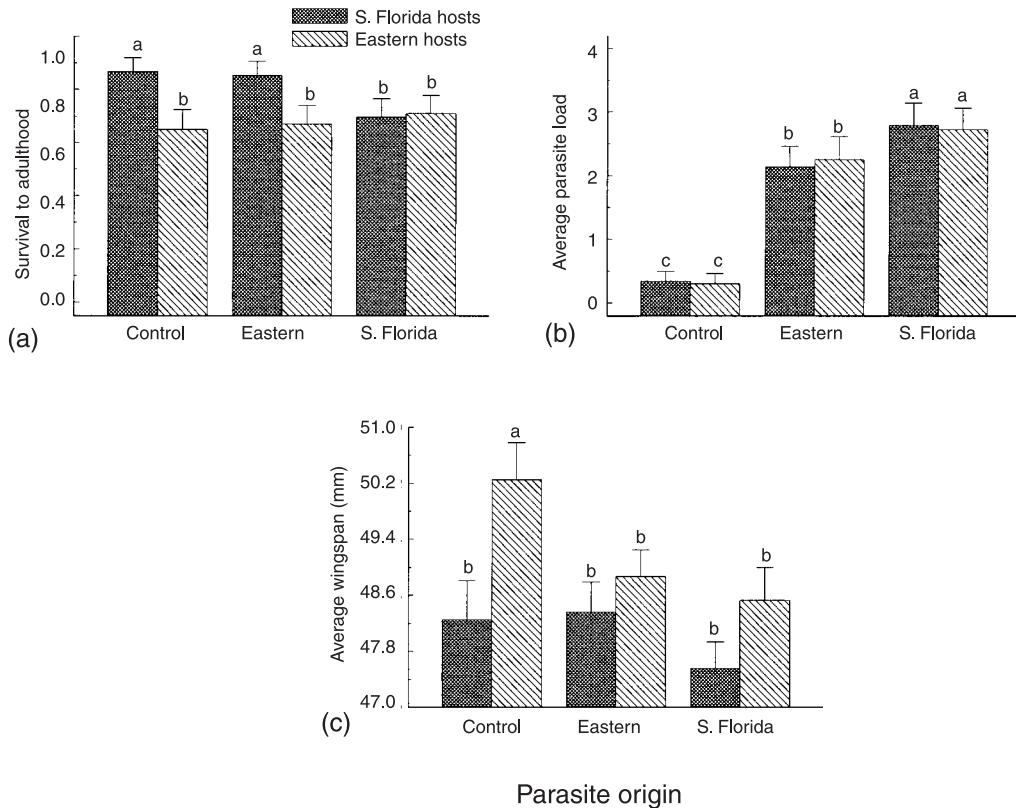
Fig. 4. Cross-infection results for hosts and parasites from the eastern migratory and Florida resident populations (Experiment 3, low dose). (a) The proportion of monarchs that survived from inoculation to adulthood. Error bars represent 95% confidence intervals based on proportions and total sample sizes in each treatment. (b) Average parasite loads of emerging adults. Error bars represent 95% confidence intervals based on variation among tubs within treatments. (c) Wingspan (forewing length, mm) of newly emerged adults. Error bars represent 95% confidence intervals based on variation among tubs within treatments. Letters indicate means that are significantly different based on Tukey pairwise comparison tests ($a = 0.05$).

survival, I observed slightly more variation in the survival probabilities of different females from the eastern population (range = 60–80%) than from the southern Florida population (range = 74–83%). In addition, the female lineages with the lowest average survival were associated with the highest parasite loads in both host populations.

The eclosion mass of adults in Experiment 3 was not significantly affected by host origin, parasite origin or their two-way interaction (Table 9b). Eastern monarchs emerged with larger wingspans than Florida resident butterflies (Fig. 4c) and the effect of host origin on adult wingspan was nearly significant at the 0.05 level (Table 9c). In addition, monarchs inoculated with Florida parasites had smaller wingspans than those inoculated with eastern parasites (Fig. 4c), a trend that was again nearly significant.

Table 7. Effects of host and parasite origin on the survival and infection of monarchs in Experiment 3 (eastern migratory *vs* Florida resident populations, low dose)

Source of variation	d.f.	MS	*F*	*P*	Error term used for *F*-test
(a) *Model*: survival = host + parasite + host*parasite + female (host) + parasite*female (host)					
Host	1	8.62	11.88	0.008	Fem (host)
Parasite	2	0.73	1.37	0.266	Residual MS
Host*parasite	2	1.78	2.22	0.141	Parasite*fem (host)
Fem (host)	8	0.73	1.37	0.241	Residual MS
Parasite*fem (host)	16	0.79	1.51	0.148	Residual MS
(b) *Model*: proportion infected = host + parasite + host*parasite + female (host) + parasite*female (host)					
Host	1	0.10	0.07	0.793	Fem (host)
Parasite	2	30.68	101.93	<0.001	Residual MS
Host*parasite	2	0.51	1.16	0.338	Parasite*fem (host)
Fem (host)	8	1.41	4.68	0.001	Residual MS
Parasite*fem (host)	16	0.44	1.47	0.165	Residual MS
Control treatments excluded					
Parasite	1	1.84	5.04	0.034	Residual MS
Host*parasite	1	0.28	0.94	0.361	Parasite*fem (host)
Parasite*fem (host)	8	0.30	0.82	0.595	Residual MS

Note: Tests of significance were constructed using the effects in the far-right column as error terms. If differences among parasite treatments were significant, then test statistics were recalculated excluding the control treatment. Proportions from each container were arcsine-square root transformed to normalize the error variance, and observations were weighted by the number of monarchs per container.

Table 8. Proportion of heavily infected adults (in parasite load classes 4 and 5) for each cross-infection treatment in Experiment 3

Parasite origin	Host origin	
	Eastern migratory	Florida resident
Control	0.02 (120)	0.04 (165)
Eastern migratory	0.41 (133)	0.39 (170)
Florida resident	0.50 (144)	0.51 (128)

Note: Sample sizes are shown in parentheses as the number of adults successfully emerging from all containers in each treatment.

DISCUSSION

Both parasite virulence and host resistance varied among three different North American monarch populations infected with *O. elektroscirrha*. Unlike other documented patterns of host–parasite co-evolution (e.g. Ebert, 1994; Morand *et al.*, 1996), *O. elektroscirrha* does

Table 9. Effects of host and parasite origin on the parasite load, mass at eclosion and forewing length of butterflies in Experiment 3 (eastern *vs* Florida resident populations, low dose)

Source of variation	d.f	MS	*F*	*P*	Error term used for *F*-test
(a) *Model*: parasite load = host + parasite + host*parasite + female (host) + parasite*female (host) + parasite*container*female (host)					
Host	1	0.03	0.00	0.972	Fem (host)
Parasite	2	401.90	91.88	<0.001	Parasite*container*fem(host)
Host*parasite	2	3.07	0.40	0.676	Parasite*fem (host)
Fem (host)	8	24.18	5.53	<0.001	Parasite*container*fem(host)
Parasite*fem (host)	16	7.67	1.75	0.079	Parasite*container*fem(host)
Parasite*cont*fem (host)	37	4.37	1.51	0.029	Residual MS
Control treatments excluded					
Parasite	1	40.19	6.49	0.017	Parasite*container*fem(host)
Host*parasite	1	4.41	1.06	0.333	Parasite*fem (host)
Parasite*cont*fem (host)	8	4.15	0.67	0.712	Residual MS
(b) *Model*: mass (g) = host + parasite + female (host) + host*parasite + parasite*female (host) + parasite*container*female (host)					
Host	1	0.0181	3.580	0.074	Fem (host)
Parasite	2	0.0042	1.990	0.210	Parasite*container*fem (host)
Host*parasite	2	0.0008	0.123	0.861	Parasite*fem (host)
Fem (host)	8	0.0054	2.847	0.015	Parasite*container*fem (host)
Parasite*fem (host)	13	0.0052	3.160	0.005	Parasite*container*fem (host)
Parasite*cont*fem(host)	25	0.0021	0.805	0.723	Residual MS
(c) *Model*: wingspan (mm) = host + parasite + host*parasite + female (host) + parasite*female (host) + parasite*container*female (host)					
Host	1	196.02	5.586	0.047	Fem (host)
Parasite	2	16.57	2.601	0.087	Parasite*container*fem (host)
Host*parasite	2	20.33	2.226	0.148	Parasite*fem (host)
Fem (host)	8	35.09	5.507	0.001	Parasite*container*fem (host)
Parasite*fem (host)	16	9.13	1.433	0.210	Parasite*container*fem (host)
Parasite*cont*fem(host)	37	6.37	1.852	0.052	Residual MS

Note: Tests of significance were constructed using the effects in the far-right column as error terms. If differences among parasite treatments were significant, tests were recalculated excluding the control treatment.

not appear to be more infectious to native host populations. Rather, variation in hosts and parasites may be better explained by selection resulting from differences in seasonal host migratory behaviour. In particular, monarchs and parasites from the longest-distance migratory population were on average more resistant and less virulent, respectively, than those from other North American populations.

Cross-infection experiments between eastern and western migratory populations demonstrated that western parasites were more virulent than eastern parasites, causing higher parasite loads at low doses and greater mortality at high doses (Figs 2, 3). Eastern

hosts were also more resistant to infection, emerging with lower parasite loads when exposed to low doses and having higher survival than western hosts when given a 10-fold higher parasite dose. The interaction between host and parasite origin also affected the outcomes of Experiments 1 and 2. For example, only when exposed to eastern parasites did eastern hosts appear significantly more resistant in Experiment 1 (Fig. 2b). In addition, the survival of eastern monarchs was similar for both parasite strains in Experiment 2, but a striking result was the extremely low survival of western hosts inoculated with western parasites (Fig. 3a). Together, these results suggest that, although eastern hosts are more resistant and western parasites are more virulent than their cross-population counterparts, the combination of host and parasite origins may interact to determine the precise extent of infection and mortality.

The effect of parasite dose on experimental outcomes is not surprising, as previous work has demonstrated that negative effects of *O. elektroscirrha* on monarch fitness are highly dose-dependent (Altizer and Oberhauser, 1999). Furthermore, transmission studies in captive monarchs indicate that high parasite loads and mortality occur among the offspring of heavily infected females, whereas low offspring parasite loads result from uninfected females that acquire spores by mating or other contact with infected adults (i.e. paternal or horizontal transmission; S.M. Altizer, unpublished). Therefore, it is likely that the low parasite dose chosen for this study is consistent with paternal or horizontal transmission, whereas the high dose captures infection via direct maternal transmission. Moreover, parasite loads observed in this study fall within the range of those reported in natural populations (Altizer *et al.*, 2000), suggesting that the experimental doses are representative of natural infections.

Sublethal effects of parasites on host body size and weight loss may translate into significant fitness costs in wild populations. In Experiment 1, western parasites caused higher rates of weight loss in newly emerged adults, probably due to host cuticle damage causing rapid desiccation. Although not significant at the 0.05 level, monarchs inoculated with western parasites in Experiment 2 emerged smaller and weighed less than those in other treatments (Fig. 3c,d). In particular, the few western monarchs that survived infection from western parasites were smaller and weighed less than monarchs in any other treatment, suggesting that they harboured low resistance or tolerance to western parasites.

In Experiment 3, eastern migratory and Florida resident monarchs performed similarly across all inoculation treatments and no differences in survival or parasite loads were due to host origin alone (Fig. 4a,b). As in Experiment 1, inoculation with a low dose of either parasite strain did not affect host survival (Fig. 4a). However, parasites isolated from southern Florida caused higher average parasite loads than those from the eastern migratory population (Fig. 4b). Although not significant, monarchs inoculated with Florida resident parasites also emerged with small wingspans and lower mass than those inoculated with eastern parasites, indicating that differences in parasite loads caused by different strains translated into sublethal effects on host fitness.

Host–parasite co-evolution in natural populations may be influenced by several processes, including the past history of disease in a population, local adaptation of parasites to hosts and resistance among hosts to native pathogen strains (e.g. Alexander, 1992; Ebert, 1994; Morand *et al.*, 1996; Lively, 1999). In addition, selection for host resistance and parasite virulence in monarch butterfly populations may be driven by host migratory behaviour. For example, monarch populations that migrate long distances may experience

stronger selection against susceptible genotypes if infected hosts have a drastically reduced probability of surviving the fall and spring migration. Thus, infected butterflies in the eastern migratory population that travel up to 3000 km each year may not survive to reproduce if parasitism significantly decreases their migratory ability and overwintering survival. In the western migratory population, monarchs migrate shorter distances and milkweed plants are abundant near overwintering sites (Nagano *et al.*, 1993, A.M. Wenner, personal communication). In southern Florida, milkweed plants are available year-round and monarchs can mate and lay eggs shortly after emergence. As a result of selection generated by long-distance migration, host resistance should be highest in the eastern population, which migrates the farthest distances.

Seasonal migration and host breeding ecology may also affect the evolution of parasite virulence. Previous studies have suggested that the evolution of virulence will be balanced by trade-offs between within-host replication (which should favour parasite transmission) and host mortality (which will decrease parasite spread by removing infected hosts from the population) (Bull *et al.*, 1991; Ebert, 1994; Lenski and May, 1994). Past theoretical and empirical work has indicated that parasites should be less virulent when transmission is limited (e.g. Ewald, 1983; Herre, 1995). Among monarchs, continuously breeding populations may offer parasites greater horizontal and vertical transmission opportunities because: (1) parasite spores that accumulate on milkweed in resident populations can generate high rates of horizontal transmission; (2) these populations have more breeding generations per year than populations that migrate long distances; and (3) females in resident populations may fly shorter distances between mating with infected males and ovipositing, thus increasing paternal transmission (S.M. Altizer, unpublished). In comparison, populations that migrate long distances should have more restricted transmission opportunities. Therefore, parasites in the eastern migratory population should exhibit the lowest virulence, because transmission in this population depends more strongly on the survival and reproduction of infected females. The results of this study are consistent with the above predictions, with parasite virulence being lowest in the eastern migratory population.

It is also possible that differences in parasite virulence among populations may reflect levels of inbreeding, or the overall genetic diversity of parasites within each host population. For example, parasites in the eastern migratory population, where most transmission is paternal or maternal, may be more highly inbred than those from the Florida resident population, where more horizontal transmission occurs (i.e. larvae may ingest parasite spores from multiple infected adults). However, dense aggregations of monarchs at overwintering sites and mass-mating activities before their spring migration could yield mixed genotypes of parasites on the abdomens of eastern migrating monarchs. At the present time, the population structure and genetic diversity of *O. elektroscirrha* within each host population remains unknown.

Better knowledge of the heritability of resistance in natural populations will improve our understanding of how hosts and parasites co-evolve. Previous studies of genetic mechanisms responsible for variation in host resistance have demonstrated that both major genes and quantitative variation underlie host resistance in natural populations (reviewed in Sorci *et al.*, 1997). The results of Experiments 1–3 suggest that variation in monarch susceptibility to different parasite strains may be heritable, because the offspring of different females within each population showed significant differences in parasite loads and host survival (Tables 1–9). In addition, the range of variation in host infection and survival was

greater among females from the eastern migratory population than among those from either the southern Florida or the western migratory population.

Not only does host resistance and parasite virulence vary among populations, but this study has suggested that other phenotypic traits differ as well. For example, the wingspan of eastern migratory monarchs in Experiments 2 and 3 was larger than that of adults from either the western migratory or Florida resident populations (Figs 3d, 4c), and may be associated with selection driven by migratory behaviour. However, the potential for gene flow or genetic divergence in any characters among North American monarchs is presently unknown. The Rocky Mountains may provide a natural barrier that limits gene exchange between the eastern and western migratory populations (Brower, 1995). In comparison, recent work has shown that a fraction of eastern migrants periodically enter Florida during autumn migration, and that some of these butterflies break diapause and join the resident breeding population (Knight, 1998). These migrants may result in gene flow that limits genetic divergence in host resistance between eastern migratory and Florida resident populations. However, because so few eastern migrants are actually infected (Altizer *et al.*, 2000), mixing between parasite populations may be less common.

Few, if any, previous studies have addressed host–parasite co-evolution in the context of host migratory behaviour. The results presented here show that monarchs in the eastern migratory population exhibited higher resistance to *O. elektroscirrha*, particularly to parasite isolates from their native population. In addition, parasites from western North America and southern Florida were more virulent, causing higher parasite loads, higher host mortality and more severe sub-ethal effects than parasites from the eastern migratory population. Together, these results are consistent with the hypothesis that seasonal, long-distance host migration affects the co-evolution of hosts and parasites, generating high host resistance and low parasite virulence in the population that migrates the farthest distance. These host–parasite differences probably influence the ecological dynamics and observed variation in parasite prevalence among populations.

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