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Strength in numbers: high parasite burdens increase transmission of a protozoan parasite of monarch butterflies (*Danaus plexippus*)

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Abstract Parasites often produce large numbers of offspring within their hosts. High parasite burdens are thought to be important for parasite transmission, but can also lower host fitness. We studied the protozoan *Ophryocystis elektroscirrha*, a common parasite of monarch butterflies (*Danaus plexippus*), to quantify the benefits of high parasite burdens for parasite transmission. This parasite is transmitted vertically when females scatter spores onto eggs and host plant leaves during oviposition; spores can also be transmitted between mating adults. Monarch larvae were experimentally infected and emerging adult females were mated and monitored in individual outdoor field cages. We provided females with fresh host plant material daily and quantified their lifespan and lifetime fecundity. Parasite transmission was measured by counting the numbers of parasite spores transferred to eggs and host plant leaves. We also quantified spores transferred from infected females to their mating partners. Infected monarchs had shorter lifespans and lower lifetime fecundity than uninfected monarchs. Among infected females, those with higher parasite loads transmitted more parasite spores to their eggs and to host plant leaves. There was also a trend for females with greater parasite loads to transmit more spores to their mating partners. These results demonstrate that high parasite loads on infected butterflies confer a strong fitness advantage to the parasite by increasing between-host transmission.

Keywords Pathogen · Virulence · Evolution · Trade-off · *Ophryocystis elektroscirrha*

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Introduction

Many parasites undergo massive replication within their hosts, with small numbers of infectious particles giving rise to millions or even billions of parasites per infected host (e.g. Anderson and May 1992; Diffley et al. 1987; Ebert et al. 2000; Mackinnon and Read 1999; Turner et al. 1995). By consuming host resources or damaging host tissues, such replication can reduce host survival or reproduction (Lipsitch and Moxon 1997). Although host death is necessary for the transmission of some parasites (Ebert 1999; Ewald 1983), in many cases this can be detrimental to the parasite by cutting short the infectious period over which transmission can occur (Anderson and May 1982; Frank 1996; Fraser et al. 2007; Levin and Pimentel 1981). Despite apparent costs to the parasite, the ubiquity of highly replicating parasites suggests a substantial fitness benefit of producing high numbers of offspring (Anderson and May 1982; Ebert 1999; Jensen et al. 2006; Mackinnon and Read 1999, 2004), yet surprisingly few studies have demonstrated this for naturally occurring host–parasite interactions.

To examine the fitness benefits of high parasite burdens, we studied the protozoan parasite *Ophryocystis elektroscirrha* in its monarch butterfly (*Danaus plexippus*) host. The prevalence of this parasite in monarch populations can be high (Altizer et al. 2000; Leong et al. 1997b;

McLaughlin and Myers 1970) and infections are known to cause substantial virulence (Altizer and Oberhauser 1999; De Roode et al. 2007, 2008a, b). Parasites are transmitted to monarch larvae when infected adults scatter spores onto eggs and host plant leaves during oviposition. Infection follows when spores are ingested by caterpillars; parasites then traverse the midgut wall, migrate to the host's hypoderm, and undergo several cycles of replication to produce new sexual spores in the tissues destined to become the scales of the adult butterfly. Upon eclosion, adult butterflies emerge from their pupal cases covered with dormant parasite spores on the outside of their bodies.

Like many other parasites, *O. elektroscirrha* reproduces at very high rates, with single-spore infections giving rise to adult butterflies with 10^5 or more parasite spores; at higher doses, parasite numbers can reach over 10^7 spores per butterfly (De Roode et al. 2007). Such replication is detrimental to the host (De Roode et al. 2007; De Roode et al. 2008a), with higher parasite loads resulting in shorter adult lifespan, lower adult body mass and reduced eclosion and mating success (Altizer and Oberhauser 1999; De Roode et al. 2007, 2008b). Parasite infection also reduces monarch flight ability (Bradley and Altizer 2005), an effect that is particularly important given the two-way migration these butterflies undertake annually in eastern North America (Brower 1995; Urquhart and Urquhart 1978). Monarchs that emerge late in the summer and fall must survive this two-way journey to reproduce the following spring. Because parasites are transmitted from adults to offspring, the lifetime fitness of parasites will depend strongly on the reproductive success of infected butterflies and their survival between breeding seasons.

Here we quantified the relationship between the spore load of infected butterflies and parasite transmission through multiple biologically important routes. Transmission of *O. elektroscirrha* occurs in three major ways. First, parasites can be transmitted maternally when spores are scattered onto eggs or host plant leaves during oviposition (McLaughlin and Myers 1970). Second, parasites can be scattered onto host plant leaves on which monarchs alight without laying eggs; and this can lead to horizontal transmission if spores are consumed by unrelated larvae. Finally, spores can be transferred between adult monarchs (Vickerman et al. 1999), especially during mating (Altizer et al. 2004), which can involve lengthy struggles and prolonged contact between monarch abdomens (Van Hook 1993). In the latter case, adult monarchs receiving parasite spores cannot become infected, but can transfer spores to eggs or host plant leaves where they can infect new larvae.

Because monarch larvae ingesting higher doses of spores have a greater probability of infection (De Roode et al. 2007; Leong et al. 1997a), parasite transmission was measured by counting the numbers of parasite spores that infected monarchs transferred to their eggs, to host plant leaves, and to their mating partners. We also examined the survival and reproduction of monarchs for the entire duration of their adult life. We compared the relationship between parasite infection load and each measure of transmission, and also examined whether measures based on different routes of spore transfer were positively correlated. Because parasite spores on adult butterflies are dormant and do not continue to replicate, it is likely that infected butterflies lose spores over time, causing parasite numbers to decline over the host's lifetime. We therefore determined whether parasite transmission also decreased over time.

Materials and methods

Experimental design

Monarchs used in the experiment were the lab-reared grand-progeny of wild-caught female monarchs caught in Athens, Georgia (USA) in May 2006. Six non-inbred full-sib monarch families were derived and assigned randomly to parasite infection treatments. Groups of 40 replicate larvae were infected with one, ten or 100 parasite spores of one of three parasite clones (denoted E1, E11 and E12; derived from the eastern North American migratory monarch population); another 160 larvae were left uninfected to serve as controls and as male mating partners for experimental female butterflies (total $n = 520$ larvae). We exposed monarchs to different doses not to study the effect of parasite dose per se (De Roode et al. 2007), but to maximize the range of parasite burdens for which transmission parameters could be measured. Thus, the range of parasite loads studied served as a proxy for genetic variation in parasite spore load production. A potential problem with this approach is that observed variation in response variables could be caused by dose effects and not by quantitative variation in spore load per se (as determined, for example, by genetic variation in parasites). To control for this effect, we included dose as a continuous factor in analyses described below. We also note that the range of parasite loads observed in this study [$\log_{10}$(spore load) 4.97–6.18] falls within the range observed for 18 different *O. elektroscirrha* clones based on a constant (ten spores) dose: 4.56 ± 0.52 (mean $\pm$ 1 SEM) to 6.24 ± 0.11 for the lowest- and highest-producing clones, respectively (J. C. De Roode and S. Altizer, unpublished results).

Infection and rearing methods followed those used in previous studies (De Roode et al. 2007, 2008a). Briefly, second instar larvae were inoculated individually by feeding them a 0.5-cm^2 piece of swamp milkweed (*Asclepias incarnata*) to which parasite spores had been manually transferred. Upon inoculation, larvae were reared singly in

0.94-l plastic containers with mesh screen lids and lined with moist paper towels. Containers were checked daily and larvae provided with fresh cuttings of *A. incarnata* (greenhouse-grown and free of parasites) as needed until pupation. Prior to adult emergence, pupae were scored for parasite infection using discoloration of the pupal case on a scale of 0–5, with 0 being uninfected (no dark patches under the pupal integument) and 5 heavily infected (dark patches forming under the majority of pupal integuments). Pupae were scored daily, beginning 3 days before the expected eclosion date, and the final (highest) score was used for analysis. Pupal scores are highly correlated with spore load on adult butterflies (results provided below). After eclosion, monarchs were transferred to glassine envelopes and moved to an incubator at 12°C.

Monitoring of individual monarchs

We randomly picked 62 female monarchs and transferred them to one of six mesh outdoor field cages (0.61 m^3) 1 day after eclosion. Uninfected and genetically unrelated males were added to these cages to serve as mating partners for the females. Cages were checked twice daily, and mating pairs were transferred to individual cages (0.61 m^3); after the pair dissociated, we pressed clear pieces of tape (approximately 1 cm^2) to male abdomens for future quantification of the number of spores transferred during mating (using a dissecting microscope at 60×) and then returned males to their original mating cages. Mated females were left in their new cage, supplied with an ad libitum 10% honey water solution, and used to quantify fecundity, longevity and parasite transmission. We provided each female with a single stalk of milkweed in a plastic bottle of water for oviposition. Every morning, milkweed stalks were returned to the laboratory and new stalks were added to cages. To prevent the possibility of spore numbers building up inside cages and thereby affecting transmission measures, we sterilized cages every 3–4 days by spraying them with a 10% bleach solution.

In the laboratory, we counted the number of eggs that monarchs had laid on each milkweed stalk. Each egg was examined using a dissecting microscope (60×) to count the number of parasite spores transferred to it. We also removed six leaves of each plant stalk (divided between leaves with eggs and those without) and pressed clear packing tape on them; the tape was transferred to index cards for future quantification of the number of parasite spores transferred to each leaf, again using a dissecting microscope (60×).

Virulence and transmission measures

To quantify virulence, we measured monarch adult lifespan (days between eclosion and death) and female lifetime fecundity (cumulative number of eggs laid prior to death). A subset of 57 (46 infected) out of the initial 62 female monarchs was used to measure adult lifespan (the remaining five were removed prior to mating and death because no further individual cages were available to monitor them). A total of 11 infected females died before mating, leaving 46 mated females (35 infected) for which we could estimate lifetime egg production; 33 infected females laid eggs and were used to calculate the proportion of eggs that carried parasite spores; one female laid a single egg without any parasites on it, leaving 32 females for which the average number of spores deposited per parasite-positive egg could be calculated.

For each infected female monarch, we quantified parasite transmission in four ways. First, we examined a total of 6,243 eggs across all females, and calculated the proportion of eggs per female that had parasites on them ($n = 33$ monarchs). Second, we calculated the average number of parasite spores on parasite-positive eggs ($n = 32$ monarchs and 3,984 eggs). This number was calculated by taking the average of all parasite-positive eggs laid by a female over her lifetime. Third, we counted numbers of parasites on a subset of leaves ($n = 518$) collected during the experiment, divided between leaves with ($n = 27$ monarchs) and without eggs ($n = 33$ monarchs); these numbers were calculated by taking the average of all leaves examined over the lifetime of a monarch. Fourth, we measured the total number of parasites that females transferred to their mating partners. Because several males mated with multiple females during the experiment, we only included data for spores transferred during the first mating event for each male ($n = 22$ monarchs).

Quantifying parasite spore load

A total of 133 adult monarchs were monitored at 12°C in the laboratory. Upon death, parasite spore loads were quantified by vortexing monarch bodies in 5 ml H_2O and estimating total spore loads using a haemocytometer slide. Regression analysis was used to quantify the relationship between the pupal discoloration scores and parasite spore loads of these monarchs [$\log_{10}(\text{spore load}) = 4.21 + 2.53 \times \log_{10}(\text{pupal infection score})$; $F_{1,131} = 173$, $P < 0.001$, $R^2 = 0.57$]. We then used this relationship to derive estimated spore loads for the female monarchs held in outdoor cages (and for which pupal infection scores, but not adult spore loads, had been recorded). This resulted in five levels of parasite spore load (9.3×10^4, 2.6×10^5, 5.5×10^5, 9.5×10^5, 1.5×10^6). Finally, after all monarchs monitored in field cages had died, we quantified their remaining parasite spore loads using the vortex and haemocytometer method to estimate the loss of parasites over the lifetime of a monarch.

Statistical analysis

The aim of the statistical analysis was threefold. First, we tested whether parasite infection reduced monarch adult lifespan and lifetime fecundity, and whether among infected animals, higher parasite loads resulted in shorter lifespan and reduced fecundity. As part of this analysis we also investigated relationships between lifespan and fecundity. Second, we examined the relationships between female parasite loads and all four measures of parasite transmission: the proportion of eggs with spores, the average number of spores transferred to parasite-positive eggs, numbers of spores transferred to host plant leaves, and numbers of spores transferred to mating partners. When analysing spore transfer to leaves, we distinguished between leaves on which eggs were laid and leaves on which no eggs were laid. We also investigated whether the different measures of parasite transmission covaried (e.g. whether spores transferred to host plant leaves and mating partners covaried with transfer to eggs). Third, we analysed whether parasite spore loads on adult butterflies decreased over the butterflies' lifetime, and whether this resulted in a reduction in spores transferred onto eggs. In the latter analysis we averaged the numbers of spores deposited on eggs laid by all females within an infection-level category (as based on the pupal infection scoring method) over a period of 5 days (intervals 1–5, 6–10, 11–15 and 16–20 days since mating).

As outlined above ("Experimental design" section), the aim of our experiment was to examine how parasite spore load affects transmission, and to obtain a wide and natural range of spore loads, we infected monarchs with different doses. We expected that higher doses would increase transmission by causing greater spore loads, and we also included dose as a continuous factor in analyses (restricted to the subset of infected females) to control for effects of dose on response variables above and beyond the spore load effect.

Analyses were carried out in R2.4.0. (R Development Core Team). Monarch lifespan, fecundity, parasite spore loads and numbers of spores transferred to eggs, leaves and mating partners were analysed using GLM with normal error distributions. The proportion of eggs with parasites was analysed using GLM with a quasibinomial error distribution; in analyses where this proportion was the explanatory variable, it was arcsine transformed, and the analyses were carried out using GLM with normal error distributions. Explanatory variables were either categorical (uninfected vs. infected; eggs present or absent) or continuous (parasite spore load, monarch longevity, parasites per egg/leaf/mating partner, proportion of eggs with parasites, dose), depending on the particular analysis.

Models were tested for normality of error and homogeneity of variance, and variables log transformed where necessary. Because the relationship between the proportion of spore-positive eggs and parasite spore load indicated non-linearity, we used non-linear least-squares regression to derive a Hill function for the shape of this regression line.

Results

As expected, monarchs infected with higher doses had higher spore loads ($F_{1,49} = 5.8$, $P = 0.02$), but spore loads did not differ between monarchs infected with different parasite clones ($F_{2,48} = 0.70$, $P = 0.50$). Therefore, parasite clone was not included in the following analyses. Dose was included in analyses of infected monarchs, and all *F*- and *P*-values for these analyses are based on models in which dose is controlled for.

Effects of infection on monarch fitness

Parasite infection significantly reduced adult monarch lifespan (Fig. 1a; $F_{1,55} = 9.36$, $P = 0.003$), with infected animals experiencing a 38% average reduction in lifespan compared to uninfected animals. Control monarchs lived between 12 and 35 days, which is slightly shorter than the observed longevity in comparable studies (e.g. Altizer and Oberhauser 1999; Oberhauser 1997; Zalucki 1981), probably because of warmer ambient temperatures experienced by monarchs during this experiment. As a result of reduced longevity, 24% (11/46) of infected females died before mating compared to 0% of uninfected females. Among infected animals, adult longevity decreased significantly with greater parasite loads (Fig. 1b; $F_{1,43} = 5.0$, $P = 0.03$) and higher doses ($F_{1,43} = 12.0$, $P = 0.001$). The fact that dose remained a significant factor in these analyses indicates that reductions in lifespan with higher doses are not merely a result of greater spore loads, but are also due to a dose effect per se (De Roode et al. 2007).

The number of eggs laid per day varied greatly among females and over time, and on average was lower than values observed in other studies (Oberhauser 1997; Zalucki 1981). Similar to these studies, the number of eggs laid per day peaked at an intermediate age. Variable fecundity across studies could be due to differences in temperature, solar radiation and other environmental factors (Oberhauser 1997; Zalucki 1981). Despite this variability, our data showed that infected monarchs that lived long enough to mate and lay eggs laid an average of 41% fewer eggs during their lifetime than did uninfected monarchs (Fig. 1c; $F_{1,44} = 4.34$, $P = 0.043$). Among infected animals there was no relationship between parasite spore load and lifetime fecundity (Fig. 1d; $F_{1,32} = 0.49$, $P = 0.49$) or between dose and lifetime fecundity ($F_{1,32} = 0.36$, $P = 0.55$). In addition,

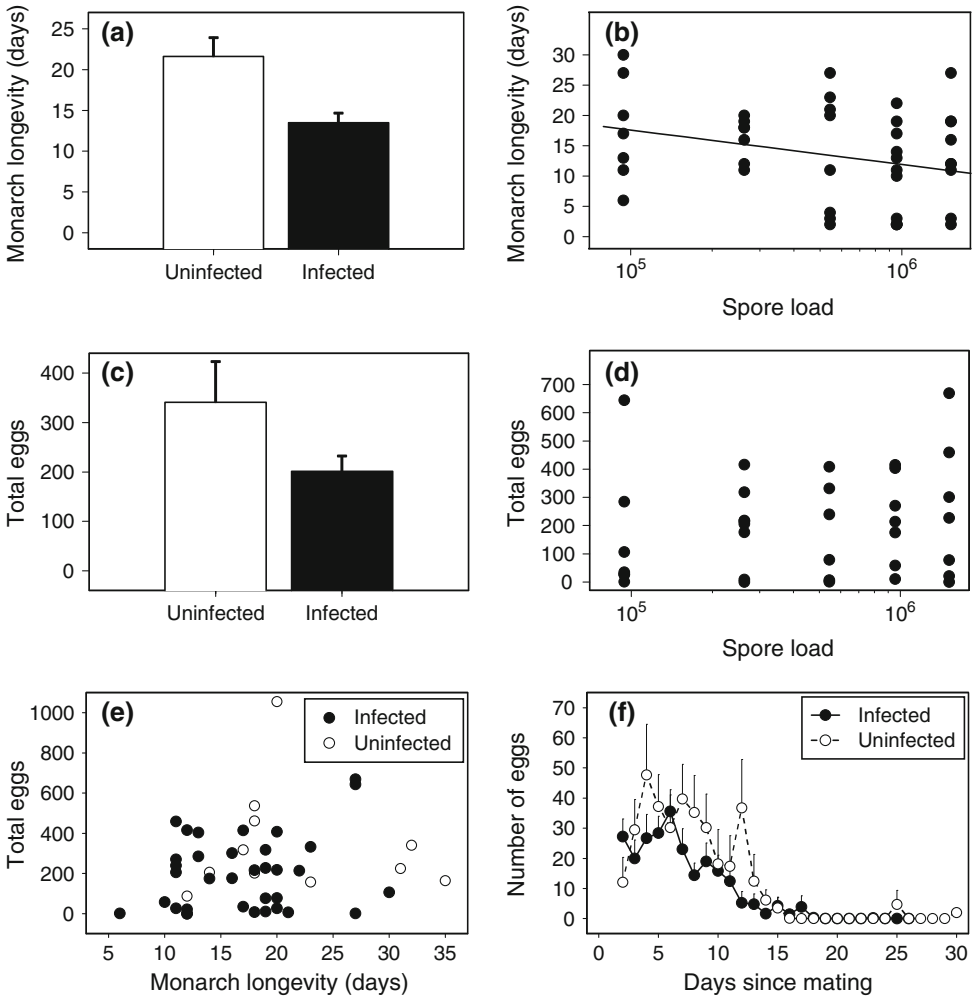

Fig. 1 Effects of parasite infection on monarch longevity and fecundity. Parasites reduced **a** monarch longevity, with **b** higher spore loads resulting in shorter adult lifespan. **c** Infection also reduced monarch lifetime fecundity, but **d** higher spore loads did not result in lower fecundity within the infected class. **e** There was also no relationship between monarch longevity and lifetime fecundity for monarchs that survived long enough to lay eggs, and **f** monarchs laid most of their eggs within the first 12 days post-mating. **a**, **c** and **f** *Error bars* are +1 SE; **b**, **d** and **e** *data points* are individual monarchs; **b** the *line* is the least-squares regression line. Note that monarchs that lived less than 5 days did not live long enough to mate and lay eggs; they are therefore not included in **d**, **e** and **f**

the lower lifetime fecundity of infected versus uninfected animals did not appear to result from their shorter lifespan (Fig. 1e; effect of longevity on lifetime egg production: $F_{1,43} = 2.09$, $P = 0.16$) because monarchs laid the majority of their eggs early on in life (Fig. 1f). Instead, the reduction in lifetime egg production resulted from fewer eggs laid per day (Fig. 1f).

Parasite transmission

High parasite spore loads resulted in greater parasite transmission to eggs and to host plant leaves. Females with low parasite loads transferred spores to only 20% of eggs on average, but females with high parasite loads transferred spores to almost all eggs laid (Fig. 2a; $F_{1,30} = 11.4$, $P = 0.002$). Females with higher parasite loads also transferred greater numbers of spores to parasite-positive eggs (Fig. 2b; $F_{1,29} = 45.7$, $P < 0.001$). We also found a strong positive relationship between parasite load and spores deposited onto host plant leaves (Fig. 2c; $F_{1,56} = 32.16$, $P < 0.001$). Leaves with eggs received much higher numbers of parasites than leaves without eggs (Fig. 2c; $F_{1,56} = 21.08$, $P < 0.001$). There was a trend for more heavily infected monarchs to transmit more spores to their mating partners, but this was not significant (Fig. 2d; $F_{1,19} = 3.71$, $P = 0.069$), perhaps due to the relatively small number of data points for this analysis. In all analyses of spore transmission, effects of dose were not significant ($P > 0.05$). Thus, higher doses resulted in greater spore loads—which resulted in greater transmission—but had no further effect on transmission when spore load was controlled for.

As expected, different measures of parasite transmission covaried strongly: there were significant relationships between the proportion of eggs with parasites and the number of parasites per parasite-positive egg ($F_{1,29} = 47.6$, $P < 0.001$), the proportion of eggs with parasites and the number of parasites on egg-positive leaves ($F_{1,24} = 22.71$, $P < 0.001$), the number of parasites on parasite-positive eggs and leaves (Fig. 2e; $F_{1,53} = 43.6$, $P < 0.001$), the number of parasites on egg-positive and egg-negative leaves ($F_{1,24} = 11.7$, $P = 0.002$), and the number of parasites on parasite-positive eggs and mating partners (Fig. 2f; $F_{1,18} = 5.46$, $P = 0.031$).

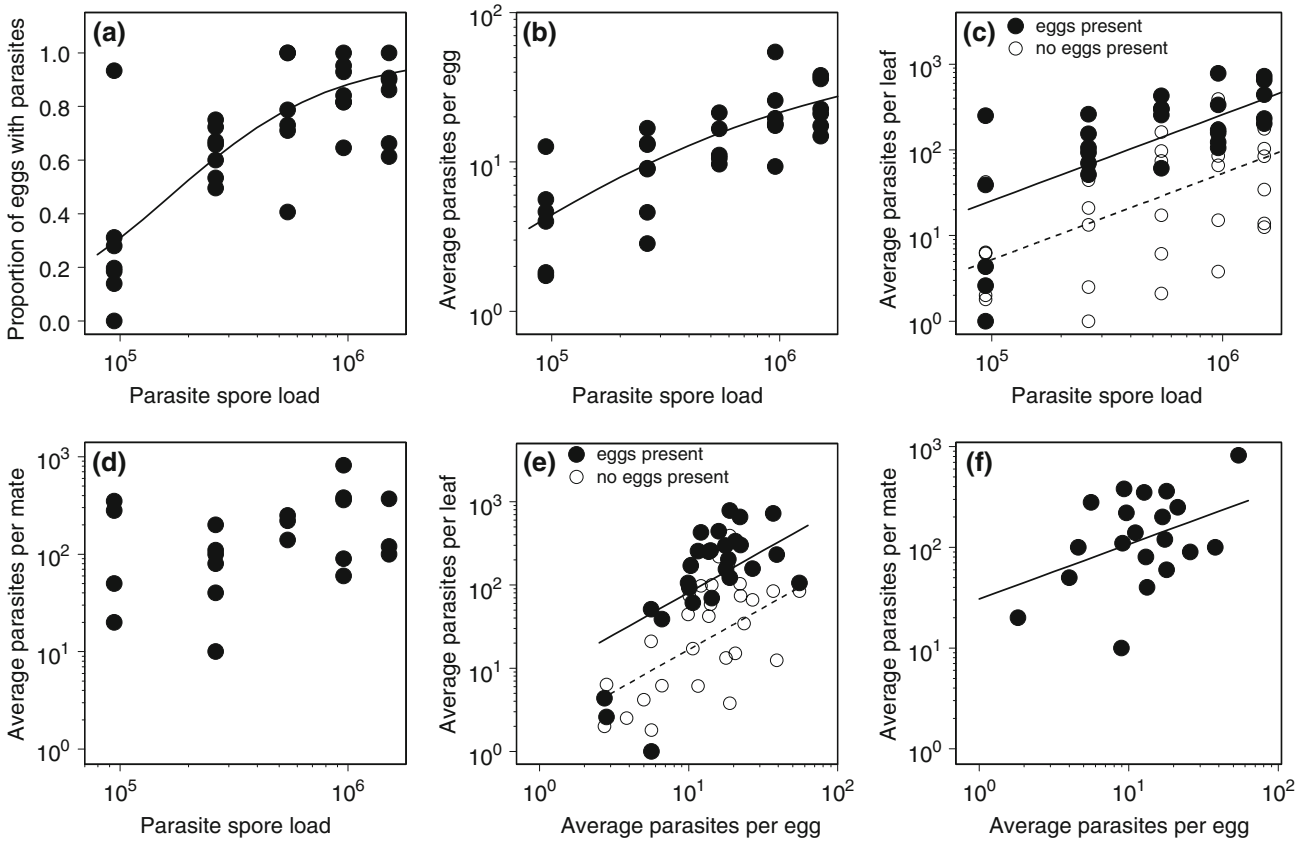

Fig. 2 Effects of parasite spore load on parasite transmission. Higher spore loads among infected females resulted in **a** a higher proportion of eggs with parasite spores, **b** higher numbers of spores transferred to parasite-positive eggs, **c** higher numbers transferred to leaves, and **d** tended to result in a higher number of parasite spores transferred to mating partners. Spore numbers transferred to parasite-positive eggs covaried positively with **e** numbers transferred to leaves and **f** mating partners. *Data points* are individual monarchs, and *lines* are least-squares regression lines. All relationships are statistically significant except for the relationship in **d** ($P = 0.069$)

Transmission over time

During their adult lifetime, monarchs lost on average over 90% of their parasite spores (Fig. 3a; $F_{1,67} = 55.1$, $P < 0.001$). However, it appeared that this had a limited effect on parasite transmission, because for most monarchs the average number of spores transmitted to eggs did not decrease over time (Fig. 3b). Only the most heavily infected monarchs showed a consistent trend of decreasing spore transmission over time (Fig. 3b). These results remained unchanged when we excluded spore count data based on ten or fewer eggs to reduce the bias introduced by means based on small numbers.

Discussion

This study confirms previous findings that infection with the parasite *Ophryocystis elektroscirrha* is detrimental to monarch butterfly hosts (Altizer and Oberhauser 1999; Bradley and Altizer 2005; De Roode et al. 2007, 2008a, b): infected monarchs had shorter adult lifespans and laid fewer eggs than did uninfected monarchs (Fig. 1). Moreover, within the infected class, adult lifespan decreased with increasing parasite loads and doses. Shortened lifespan and reduced fecundity clearly reduce monarch host fitness by reducing the number of mating opportunities and the number of offspring produced. Although the outdoor field cages used in this study were designed to expose monarchs to a range of environmental conditions experienced by wild populations, negative effects of parasites on monarch fitness will likely be amplified under natural scenarios where females must search actively for host plants and nectar resources, as compared with the ad libitum resources provided here. This is especially true for monarchs in the eastern migratory population in North America that emerge in late summer and fall; these adult butterflies must survive a long-distance two-way journey of up to 5,000 km to reproduce the following spring (Brower 1995; Urquhart and Urquhart 1978).

Parasite-mediated reductions of host fitness are not only harmful to monarchs, but also appear maladaptive to the

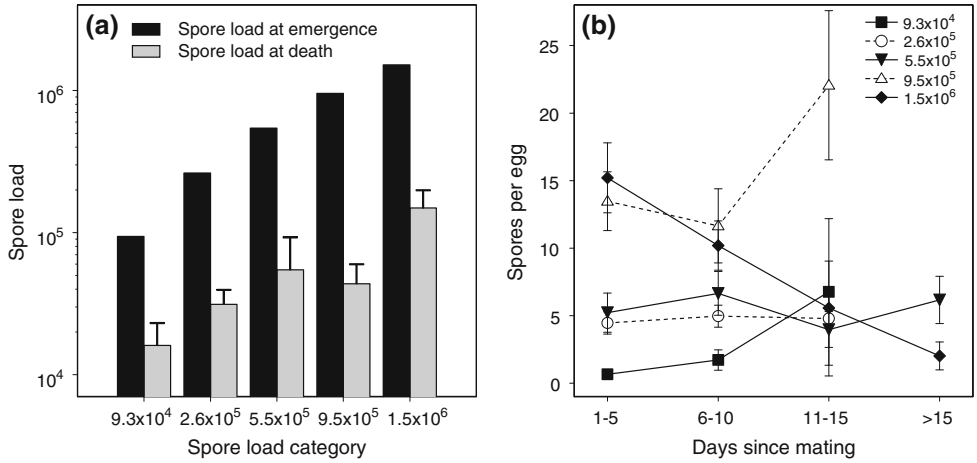

Fig. 3 Changes in parasite spore load of infected females from emergence to death and parasite transmission to eggs over time. **a** Monarchs lost over 90% of the parasites they carried during their adult lifespan, but **b** for most monarchs this did not result in a detectable reduction in the numbers of spores deposited onto eggs over time. *Data points* are means across all monarchs within a spore load category (with spore loads 9.3×10^4, 2.6×10^5, 5.5×10^5, 9.5×10^5, 1.5×10^6). **a**, **b** *Error bars* are +1 SE and ±1 SE, respectively. **a** *Black bars* do not have error bars because all monarchs within a spore load category were assigned exactly the same spore load as described in the text

parasite, because parasites require the host to survive long enough to mate and initiate oviposition, during which most parasite transmission occurs. Thus, hosts with high parasite loads that die quickly will contribute little or nothing to parasite fitness. However, our data show that there is also a clear benefit of high parasite numbers. First, high parasite loads are necessary to ensure transmission per se: our results suggest that parasite burdens $<10^5$ result in very few successful transmission events. This is true for both transfer of spores to eggs (based on both proportion of eggs with spores and number of spores per spore-positive egg) and transfer of spores to leaves. Second, beyond this minimum threshold, greater parasite burdens resulted in an increased transfer of spores to eggs and milkweed leaves. Thus, higher spore numbers are beneficial to the parasite, as they increase the chance of infection of the next generation of caterpillars ingesting them; doses of $\geq$10 spores are most likely to cause infections (De Roode et al. 2007).

In principle, it is possible that keeping monarchs in outdoor flight cages could artificially elevate the transfer of spores beyond that observed in nature. However, several lines of evidence suggest that this is not the case in the current experiment. First, we regularly sterilized cages and replaced milkweed stalks daily to limit the build-up of parasite spores. Second, the fact that plant leaves onto which eggs were laid had nearly an order of magnitude higher numbers of spores than those without eggs demonstrates that spore transfer is strongly associated with egg-laying behaviour, and is not a result of spores being scattered randomly. Finally, the mean number of spores transferred per spore-positive egg was similar for stalks with $\leq$4 eggs (mean ± SE = 8.24 ± 3.9) and stalks with >4 eggs (9.05 ± 0.36). This again suggests that eggs acquire the majority of spores during oviposition events and not from passive spore dispersal associated with female activity in the cages.

Our results have relevance for the evolution of parasite virulence, with the most popular theory predicting that virulence can evolve as a consequence of natural selection on parasite transmission (Anderson and May 1982; Antia et al. 1994; Bremermann and Pickering 1983; Frank 1996; Levin and Pimentel 1981; Van Baalen and Sabelis 1995). This theory assumes that greater within-host exploitation by parasites results in both a greater between-host transmission rate and a greater host mortality rate (virulence). Because parasite fitness depends on both the duration of infection (cut short by host mortality) and the transmission rate, mathematically it can be shown that parasites with intermediate levels of exploitation obtain greatest fitness. Unfortunately, the trade-off model in its strictest sense is of limited use to most host–parasite interactions, as it applies only to those with continuous transmission and infection duration cut short by parasite-induced host death. However, in a more general way the theory predicts that both virulence and transmission are positively related to parasite burdens (Fraser et al. 2007; Jensen et al. 2006; Mackinnon and Read 1999). By demonstrating that higher parasite burdens result in fewer transmission opportunities (due to the reduced probability of monarchs reaching reproductive age) and greater per event transmission probabilities (due to greater numbers of spores transferred per oviposition event), our results lend support to several predictions of this trade-off theory. To fully test the theory, we need to determine how parasite burdens affect other variables that shape lifetime parasite transmission, such as pre-adult host survival, mating ability, flight ability and the probability of host infection and survival following ingestion of transferred

spores; importantly, data on these variables from previous studies are also consistent with the trade-off theory (Altizer and Oberhauser 1999; Bradley and Altizer 2005; De Roode et al. 2007, 2008b). Thus, the current study must be seen as a necessary step towards a more comprehensive test of general trade-off theory.

Higher parasite loads resulted in greater spore transfer to eggs, host plant leaves and mates (Fig. 2). In nature, parasite transmission probably occurs as a mix of these three modes of transfer, with the relative importance of each mode differing between populations (Altizer et al. 2004). For example, in eastern North America, monarchs migrate annually to Mexico to escape freezing temperatures during the winter months (Brower 1995; Urquhart and Urquhart 1978). Because host plant availability in this population is seasonal and population densities in the breeding range are generally low (Altizer et al. 2004; Prysby and Oberhauser 2004), most transmission in this population probably occurs during oviposition. In contrast, in resident populations where monarchs breed year-round and do not migrate, such as in South Florida and Hawaii, breeding densities are generally higher. Because many monarchs may alight onto host plants in such populations and host plants do not die back seasonally, spores could accumulate on host plant leaves even in the absence of oviposition. Thus, a greater proportion of transmission may be tied to host reproduction in migratory than in resident populations. This could select for less virulent parasites in migratory populations (e.g. Bull et al. 1991; Day 2002; Stewart et al. 2005) because parasites in these populations require that their hosts live long enough to mate and lay eggs to achieve successful transmission.

In general, spore transfer during mating probably plays a small role in terms of the actual numbers of spores transmitted to monarch larvae. Although up to hundreds of spores can be transferred between mating adults, it is likely that only very few of these are transmitted on to eggs or milkweed leaves owing to a 10^3– to 10^4-fold reduction between spore numbers on the bodies of monarchs and the numbers transferred to eggs and leaves (Fig. 2b, c). On the other hand, Altizer et al. (2004) found that for females that mated with heavily parasitized males, up to 90% of the progeny were infected for eggs laid immediately (1 day) following mating. Although this percentage declined to zero by 5 days post-mating, mathematical models of host–parasite dynamics indicate that even low rates of paternal transmission can prevent a virulent parasite from going extinct when maternal transmission is high, and hence could be important for explaining parasite persistence in migratory populations, and the high prevalence observed in some resident populations (Altizer and Augustine 1997; Altizer et al. 2004).

One surprising finding of this study was that there was no consistent decline in the numbers of spores transferred to eggs over time, even though infected females had lost over 90% of the parasite spores from their bodies by the time they died (Fig. 3). Only the most heavily infected monarchs showed a trend of decreasing numbers of spores transferred over time. It is possible that a decrease in numbers was not detectable for less heavily infected monarchs because of the relatively large variability in the numbers of spores transferred to eggs, such that sample sizes were not sufficient to detect a significant trend (particularly for eggs laid later in life). In addition, females typically laid most of their eggs early in life, with very few eggs laid by females that were more than 10 days old. Because some females outlived their "egg-laying lifespan" by 10 days or more, during which time spores could have been continually lost from their bodies, declines in spore loads from emergence to death might not have correlated well with changes in spores deposited over time as expected.

Our results show that for monarchs that mated successfully, infected monarchs had lower average lifetime fecundity than uninfected monarchs. Surprisingly, this was not a result of their reduced lifespan, but appeared to be an effect of infection per se, possibly mediated through a reduced capacity to produce eggs or reduced flight activity necessary to alight on host plants. In nature, the cost of parasitism for lifetime fecundity could be much greater than the effect observed here because monarchs typically lay eggs singly and fly for variable distances between oviposition events (e.g. Oyeyele and Zalucki 1990; Prysby and Oberhauser 2004). When confined to a cage, monarchs also tend to lay a single egg at a time, but over the course of a day this can result in many eggs on single plants, sometimes exceeding 100 eggs/plant per day. Caged monarchs also spend less time searching for suitable plants for oviposition. Hence, in wild populations, parasite effects on female lifespan and flight ability could result in sharper reductions in female lifetime fecundity than observed here. It is important to test this hypothesis, not only to investigate the costs of the parasite to its host, but also to determine the costs to its own transmission.

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