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Effects of the Protozoan Parasite *Ophryocystis elektroscirrha* on the Fitness of Monarch Butterflies (*Danaus plexippus*)

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We evaluated the effects of the protozoan parasite *Ophryocystis elektroscirrha* on the survival and reproduction of monarch butterflies. Because larvae in natural populations are likely to experience a wide range of natural parasite population densities, we examined the effects of increasing spore density (0, 10, 100, or 1000 spores per larva) on host fitness. Parasites had little effect on monarch survival or reproduction, except at the highest dose. Monarchs inoculated with 1000 spores per larva had decreased survival to eclosion, and this effect was more severe when larvae were inoculated at an earlier stage (first versus third instar). Monarchs inoculated with higher spore densities also emerged with smaller wingspans and lower body mass than noninoculated adults. Infection with the highest dose of *O. elektroscirrha* led to decreased male lifespan and reproductive success, but females infected with *O. elektroscirrha* did not experience a significant decline in lifetime fecundity. However, heavily infected females in outdoor enclosures were less active than uninfected females and gained weight during their adult lifespan. Among samples of adult monarchs captured in natural populations, parasite loads were associated with butterfly condition and activity. Heavily infected adults captured breeding in western North America and southern Florida were smaller than uninfected monarchs. Among overwintering adults in Mexico and California, mating activity was positively associated with higher parasite loads. In addition, the proportion of adults with low and intermediate spore loads (as opposed to no spores) was higher among adults with greater wing tatter and scale loss. Our findings of minor effects of *O. elektroscirrha* on the survival and reproduction of monarch butterflies are consistent with the expectation that maternally transmitted parasites should have little or no effect on host fitness compared with horizontally transmitted parasites. However, because our laboratory studies demonstrated that monarchs exposed to the highest parasite density experienced decreased larval survival, smaller adult size, and shorter adult lifespans, additional transmission routes are likely to be important for parasite maintenance in natural populations. © 1999 Academic Press

Key Words: protozoan parasite; virulence; insect disease; neogregarine; vertical transmission.

INTRODUCTION

The damage inflicted by parasites on their hosts is often a direct consequence of parasite replication within hosts and the production of transmission stages (Anderson and May, 1991). Although severe effects on host fitness have traditionally been viewed as maladaptive, recent theoretical and empirical work suggests that parasites may not evolve to become increasingly benign to their hosts (Herre, 1993; Lenski and May, 1995). The degree of virulence that maximizes parasite fitness should be influenced by several factors, including the mechanism by which new hosts acquire the disease (Levin, 1996). For example, diseases that depend strictly on vertical (parent: offspring) transmission are predicted to go extinct in the presence of any negative effects on host fitness, whereas pathogens that infect hosts via vectors or direct horizontal transmission may persist despite severe effects on host survival or reproduction (Fine, 1975; Ewald, 1983; Lipsitch *et al.*, 1995).

Negative effects of parasites on host fitness will influence the degree to which parasites depress host population size (Anderson and May, 1991). Increasing theoretical and empirical attention has focused on such population-level impacts of parasites (Fenner and Myers, 1978; Anderson, 1979; Onstad and Maddox, 1989; Myers, 1993; Dwyer, 1994; Dobson and Hudson, 1995; Gulland, 1995; Jaenike, 1998). However, estimating the effect of parasites on host population density poses many challenges due to fluctuations in parasite abundance and external factors affecting host fitness (Dobson and McCallum, 1995; Dobson and Hudson, 1995). One approach, therefore, is to estimate the relative survival and fecundity of infected hosts and use these values in simple models of host–parasite interaction to

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establish the potential for parasite regulation of host population size (McCallum, 1994).

We quantified fitness effects of the neogregarine protozoan parasite *Ophryocystis elektroscirrha* on monarch butterflies, *Danaus plexippus*. Neogregarines are members of the class Gregarinia (phylum Apicomplexa) which encompasses over 1450 species of entomopathogenic protozoa (Tanada and Kaya, 1993). Unlike other orders of gregarines, neogregarines can multiply within their hosts via vegetative merogony, and infections are often tissue specific. Thus, neogregarine infections are in general more virulent than those produced by other gregarines and have been shown to affect host development, survival, and reproduction (e.g., Weiser, 1963; Jouvenaz and Anthony, 1979; Cowley, 1989; Munster, 1991).

The neogregarine *O. elektroscirrha* was first recovered from monarch and queen butterflies in Florida in 1966 (McLaughlin and Myers, 1970) and has been reported in monarch populations in North America, Hawaii, New Zealand, and Australia (Leong *et al.*, 1997a; Altizer *et al.*, in press). Parasite life history is closely correlated with host development. Vegetative reproduction occurs in larvae and pupae, and spores are found on the exterior of adult butterflies. Parasite spores are transmitted maternally when infected females scatter spores on the egg chorion and host plant surface during oviposition (McLaughlin and Myers, 1970). Spores may also be transferred between adults during mating or other contact (resulting in paternal or horizontal transmission), although ingestion of spores by larvae is required to initiate new infections. Spores lyse within the larval gut, and emerging sporozoites penetrate the intestinal wall and enter the hypoderm. As monarch larvae pass through five developmental instars, parasites undergo two phases of vegetative, asexual replication. Following host pupation, the parasite completes a sexual phase and forms dormant spores around the scales of the developing adult butterfly. The highest spore density is found on the abdomen, although spores also develop on the wings, head, and thorax (Leong *et al.*, 1992; S. M. Altizer, pers. observ.).

Among monarch populations in North America, the prevalence of *O. elektroscirrha* is inversely related to host migratory distance (Altizer *et al.*, in press). A continuously breeding population in southern Florida shows nearly 100% prevalence, and approximately 60% of the individuals in a migratory population in western North America are infected with this disease. Less than 10% of the eastern migratory population, which travels the farthest distance to overwintering sites in central Mexico, is infected. One explanation for this pattern is that if parasites depress host fitness and infected hosts are less able to migrate long distances, then prevalence may decline as migratory distances increase. In support of this idea, the prevalence of *O. elektroscirrha* in wild-captured monarchs (within a migratory population) was higher among monarchs breeding close to overwintering sites than those breeding farther away (Altizer *et al.*, in press). In captive monarchs, heavily infected adults have difficulty expanding their wings and experience higher mortality than uninfected adults when kept in arid, warm conditions (McLaughlin and Myers, 1970; Leong *et al.*, 1992, 1997a,b; S. M. Altizer, pers. observ.).

The objectives of the present study were to determine (a) how increasing doses of parasite spores influence the parasite loads of emerging adults and (b) the consequences of this disease for host fitness. Because the transmission of *O. elektroscirrha* is primarily vertical, its persistence and spread should depend on host survival and reproduction, and virulence is therefore expected to be low. However, spore transmission between adults through mating or other contact or the density-dependent accumulation of spores in the hosts' environment over time will increase parasite transmission opportunities (S. M. Altizer, in preparation). Thus, measurable virulence may be maintained in natural populations, depending on host density and breeding ecology (Lipsitch *et al.*, 1995; Altizer and Augustine, 1997). We examined the combined effects of parasite dose and larval instar at the time of inoculation on the spore loads and survival of emerging monarchs. Because sporozoites penetrate the gut wall, high parasite doses fed to early-instar larvae may cause more severe gut wall damage and mortality than those ingested by late-instar larvae. Because parasite effects on larval development, adult survival, and adult fecundity can all influence population-level interactions, we measured several larval and adult traits likely to correlate with host fitness.

METHODS AND MATERIALS

Inoculation and Rearing of Captive Monarchs

During the summers of 1995 and 1996, we conducted several experiments to assess the effects of increasing doses of *O. elektroscirrha* on monarch fitness. Unless otherwise indicated, experimental hosts were first or second generation offspring from wild adult *D. plexippus* captured in east-central Minnesota and west-central Wisconsin. Eggs were obtained by enclosing 4–5 mated females per experiment in a 0.6-m^3 mosquito-net cage with potted *Asclepias curassavica* plants. All females from which eggs were obtained were uninfected with *O. elektroscirrha* (see below).

Inoculation techniques. All spore inoculum was derived from the abdomens of infected wild-captured monarchs that had been stored in glassine envelopes for less than 2 months. Abdomens from two infected adults were placed in glass vials containing 10 ml of

deionized water and vortexed for 2 min to dislodge the spores. We estimated spore concentration by counting the number of spores in eight 1×10^{-4}-ml grid-cells on four separate hemacytometer slides. Inoculum was then passed through a dilution series to obtain concentrations of 10, 100, or 1000 spores/10 µl. Control inoculum (0 spores/10 µl) was prepared by vortexing the abdomen of an uninfected wild-captured adult monarch in 10 ml of deonized water.

To inoculate larvae, we soaked *A. syriaca* leaves in a 10% bleach solution for 20 min, rinsed the leaves with deionized water, and cut them into 1-cm^2 pieces. Milkweed squares were placed onto dampened filter paper inside sterile 8.5-cm-diameter plastic petri dishes. When larvae reached the appropriate instar (see below), we placed them on the milkweed squares and added a 10-µl drop of the designated inoculum using a micropipette. Each larva fed on the inoculated leaf material until it had consumed at least 80% of the square. Note that the dose of spores is the average number of spores administered to larvae, and larvae that failed to consume the inoculum within 48 h were discarded.

Host rearing. Following inoculation, larvae were transferred to plastic $11 \times 17 \times 30$ cm containers with metal window-screen lids at densities of 10–17 larvae per container. Spores of *O. elektroscirrha* are not produced during larval phases, and horizontal transmission between larvae as a result of fecal contamination does not occur (McLaughlin and Myers, 1970; Leong *et al.*, 1997b). We fed larvae fresh *A. syriaca* leaves and removed frass from the containers daily. Containers were kept on a windowsill in a laboratory in which no infected adults were permitted. When all monarchs in a container had pupated, they were moved to another laboratory to prevent contamination of the larval rearing area. After adults emerged and their wings hardened (roughly 6 h postemergence), they were placed in individual glassine envelopes. The time from egg to eclosion in the laboratory was approximately 30 days.

To minimize accidental infection of larvae, all laboratory surfaces and tools that contacted larvae or adults were sterilized with 20% chlorine bleach solution or rinsed with 95% ethanol. Latex or polyvinyl-chloride gloves were worn when handling monarchs of any life stage in the laboratory, and gloves were discarded between handling larvae or adults from different treatments. We never handled larvae or milkweed after working with potentially infected adults and sterilized all oviposition cages and containers between experiments in a solution of 10–20% chlorine bleach for at least 2 h.

Disease assessment. We evaluated spore loads of all adults within 48 h posteclosion, using transparent Scotch brand tape cut into 1-cm^2 units. This tape was held with fine forceps and pressed against the ventral side of the abdomen to remove a sample of abdominal scales. Each tape sample was placed on a microscope slide and viewed at 400×. Spores appear as dark brown, oval-shaped bodies approximately 1/50 the size of a butterfly scale (Leong *et al.*, 1992). All spores on the tape were counted, and butterflies were scored for parasite loads according to the following scale: 0, no spores; 1, 1 spore; 2, 2 to 20 spores; 3, 21 to 100 spores; 4, 101–1000 spores; and 5, >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 *et al.*, in press).

Experiment 1a: Effects of Parasite Dose on Larval Development and Survival

In 1995, we examined the effects of increasing parasite dose on parasite loads and survival of monarchs using four inoculum concentrations (0, 10, 100, and 1000 spores per larva). We inoculated 45–52 third-instar larvae per dose in each of three blocks (separated by 1 day between inoculations), for a total of 150 larvae per treatment. Larvae were reared in three replicate containers per treatment per block, for a total of 36 containers. We recorded the number of larvae that successfully pupated in each container and the emergence date for each adult. Newly emerged adults were placed in glassine envelopes, individually numbered, weighed on an analytical balance 1 day posteclosion, and examined for the presence of *O. elektroscirrha* spores. Using calipers, we measured wing length as the distance between the point of thoracic attachment and the distal tip of the forewing.

Experiment 1b: Effects of Parasite Dose on Adult Fitness

Of the monarchs from the 1995 inoculation experiment that survived to 2 days posteclosion, we assigned a random subset of 50 adults per treatment to each of four 1.8-m^3 outdoor enclosures. Each screen enclosure had equal numbers of adults from each treatment and a 1:1 sex ratio. Cages were assembled on a grassy, partly shaded lawn and separated by a minimum distance of 5 m. Monarchs were fed from sponges soaked with a solution of 20% honey-water placed onto 0.75-m-high platforms inside each cage. We also added four flowering plants (asters, cosmos, and zinnias) to each cage and three to four stalks of common milkweed (*A. syriaca*) in glass bottles. New milkweed stalks and fresh honey-water were added to the cages daily, and sponges were cleaned with 10% chlorine bleach every 2nd day to limit fermentation.

We recorded the mass of each monarch every 3rd day as well as the identity of all mating individuals and

dates of all deaths. In a few cases, butterflies were missing on weighing days; for these we estimated the date of death as 1 day after the previous weighing. (This occurred when ants discovered dead monarchs before we did and carried them out of the cages.)

To measure female fecundity, we removed the first 12 females to mate from each inoculation treatment and placed them in 0.6-m^3 oviposition cages kept outdoors. Four mated females per treatment were placed in each of 3 oviposition cages, for a total of 12 oviposition cages. Every day we fed these females, provided a fresh stalk of *A. syriaca* for oviposition, and counted the number of eggs laid in each cage. To maintain even sex ratios in the large enclosures, we replaced all females removed from the larger enclosures with laboratory females from the same treatments.

Experiment 2: Effects of Instar at Time of Inoculation on Larval Development and Survival

In 1996, a 4 × 2 factorial design was used to evaluate the effects of parasite dose and larval instar at the time of inoculation on survivorship and adult parasite loads. We used the same four inoculation treatments as above and inoculated larvae at either first or third instar. All larvae were removed from plants 1 day posthatching. Those to be inoculated as first instars were transferred to petri dishes as outlined above. The remainder were reared in plastic containers until they reached the third instar, at which time they were inoculated and transferred back to the same containers. We inoculated 30 larvae for each stage-by-dose combination. Larvae were reared in 2 replicate containers per treatment (for a total of 16 containers and 240 larvae across all treatments). We measured the number of larvae alive in each container every 2nd day after inoculation and recorded pupation and eclosion dates. One day after eclosion, adults were weighed and spore loads assessed as described above.

Associations Between Spore Loads and Adult Condition in Natural Populations

The size, condition, and parasite load of wild-caught North American monarchs were assessed for five collection dates and locations (Table 1). We measured parasite loads and forewing length as described above. Wing scale loss was assigned using a 1–5 scale, with 1 assigned to newly emerged monarchs and 5 assigned to monarchs with the highest amount of scale loss (nearly transparent wings). Wing tatter was assessed as the number of wings with pieces missing (on a 0–4 scale). We also recorded the activity of many overwintering adults at the time of capture (mating vs roosting) and palpated the abdomens of females to determine the presence and size of spermatophores (an indicator of previous matings; Van Hook, in press).

TABLE 1

Dates and Locations of Monarchs Sampled in North America to Examine Association between Parasite Loads and Adult Condition

Population	Activity	Location	Date	*N*
Eastern migratory	Wintering	Sierra Chincua, Central Mexico	3/97	1309
	Breeding	Minnesota and Wisconsin	6/97–8/97	370
Western migratory	Wintering	Central California Coastline[a]	2/97	717
	Breeding	California, Nevada, Utah, Oregon, Washington, Colorado	7/97–8/97	309
Southern Florida	Breeding	Miami, Florida	7/96	21

[a] Represents six different overwintering locations along the California coastline, including San Leandro Golf Course (near San Francisco Bay), Moran Lake, Morro Bay, Pismo Beach, Ellwood, and Gaviota.

Statistical Analysis

Analysis of variance (GLM procedure; SAS, 1985) was used to test the effects of parasite dose, block, sex, and the dose × block and dose × sex interactions on the parasite load, mass, wingspan, and development time of monarchs in experiment 1. We used two-way ANOVA to examine the effects of dose, instar, and the dose × instar interaction on the parasite load, mass, and development time of monarchs in experiment 2. F tests were constructed using the mean square for the container (dose × block) in experiment 1 or the container (dose × instar) in experiment 2 as the denominator, rather than the mean square error. This limited the contribution of the larval rearing environment to observed variation between treatments. Two-way ANOVA was also used to examine the effects of dose and block (experiment 1) or dose and instar (experiment 2) on the proportion of monarchs that survived to eclosion. In this case, proportions surviving in each container were arcsin-transformed and observations were weighted by the inverse of the variance estimate ($p \cdot (1 - p)/n + 1/2n$, where n is the initial number of larvae in each container).

Analysis of variance was used to evaluate the main effects of dose treatment and cage replicate on the lifespan, mating success, and weight change of adults kept in outdoor enclosures in experiment 1. We used one-way ANOVA to examine the effect of dose treatment on female lifetime fecundity, using the total number of eggs laid per oviposition cage as our unit of measurement. Type III sums of squares were used in constructing all ANOVA tests of significance. Wherever data are presented for multiple comparisons among means, we used Tukey's test (significance level = 0.05). For data collected as counts (i.e., number of days, number of mating events), the square-root transforma-

tions of the response variable were used to normalize the error variance.

We used χ^2 analysis to investigate the association between parasite load and the following variables among wild-caught monarchs: wing wear, wing damage, sex, the presence and size of spermatophores carried by females, and activity class. ANOVA was used to evaluate the relationship between parasite load and wingspan of wild-captured adults.

RESULTS

Effects of Dose on Larval Survival and Adult Parasite Loads

The proportion of monarchs that survived from inoculation to eclosion was affected by the dose treatment and the larval instar at the time of inoculation (Fig. 1). For experiment 1 (in which all larvae were inoculated as third instars), survival declined for increasing dose treatments ($F_{3, 24} = 13.45$, $P < 0.001$). Survivorship exceeded 80% for the control (noninoculated) and 10-spores-per-larva treatments, but was significantly lower in the 100- and 1000-spore treatments (Fig. 1a). Although we observed no significant main effect of block on survival to eclosion ($F_{2, 24} = 1.37$, $P = 0.273$), our analysis detected a significant dose × block interaction ($F_{6, 24} = 6.74$, $P < 0.001$). Dose was more strongly associated with survival in blocks 1 and 2, but showed no clear relationship with survival in block 3.

In experiment 2, both dose and instar at the time of inoculation were significantly associated with survival ($F_{3, 8} = 11.95$, $P = 0.008$ and $F_{1, 8} = 12.84$, $P = 0.002$, respectively). Survival was higher among larvae inoculated as third instars versus first instars, and survival of larvae inoculated with the highest dose was significantly lower than survival in the other treatments (Fig. 1b). In addition, the dose × instar interaction was marginally significant ($F_{3, 8} = 3.66$, $P = 0.063$). No first-instar larva inoculated with the highest dose survived to eclosion, whereas 50% of the third-instar larvae inoculated with the highest dose survived (Fig. 1b).

Parasite loads of emerging adults increased with dose (Fig. 2), and almost 100% of the adults in the 100- and 1000-spore treatments were heavily infected (classes 4 and 5). In experiment 1, both dose and block significantly affected adult spore loads ($F_{3, 23} = 57.9$, $P < 0.001$ and $F_{2, 23} = 21.1$, $P = 0.014$, respectively). Comparison of means shows that spore loads increased significantly for each successive dose (Fig. 2a), and spore loads for adults inoculated in block 3 were lower than for adults inoculated in blocks 1 and 2. In addition, we observed no significant effects of sex ($F_{1, 23} = 0.00$, $P = 0.98$), dose × sex ($F_{3, 23} = 0.62$, $P = 0.61$), or dose × block ($F_{6, 23} = 1.20$, $P = 0.34$) on adult spore loads.

Because none of the first-instar larvae inoculated with the highest spore dose in experiment 2 survived to eclosion, we omitted the highest dose category from our remaining analyses. Instar at time of inoculation did not affect the parasite loads of surviving adults ($F_{1, 6} = 0.24$, $P = 0.64$), but we again observed a significant main effect of dose ($F_{2, 6} = 283.62$, $P < 0.001$). Comparison of means showed that adult spore loads in the control treatment were lower than those in the remaining dose treatments, but there were no significant differences between adult spore loads in the 10- and 100-spore treatments (Fig. 2b). We observed no

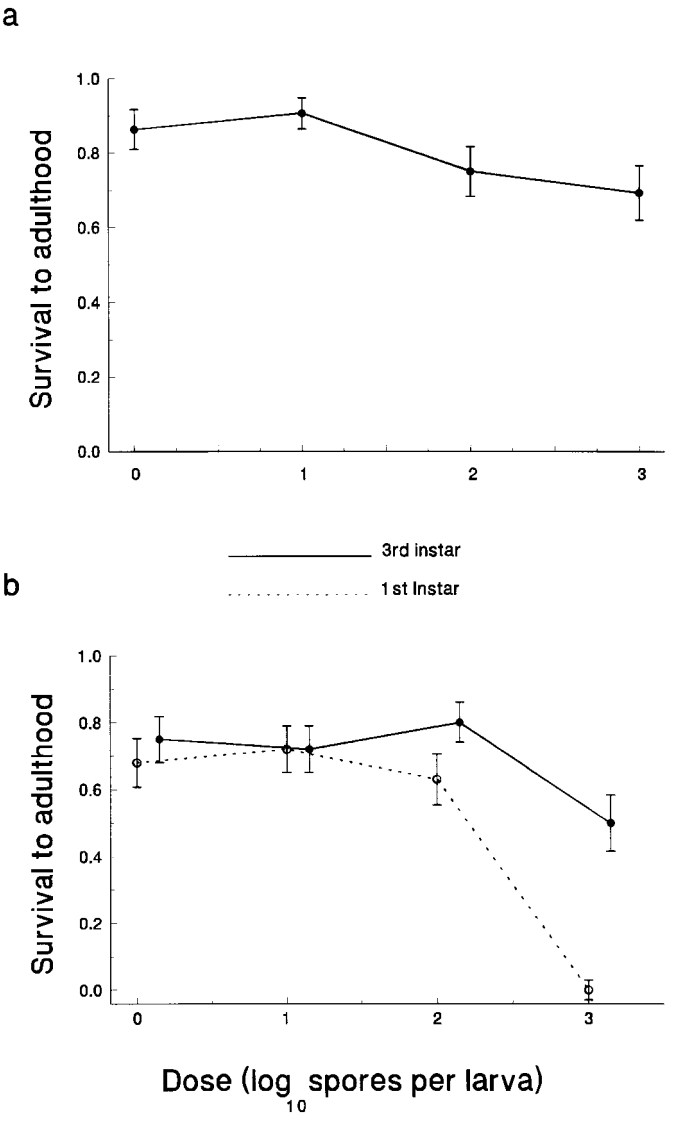

FIG. 1. The proportion of monarchs that survived from inoculation to adulthood for increasing doses of *O. elektroscirrha* spores. (a) Survival for experiment 1, in which larvae were inoculated as third instars only. (b) Experiment 2, in which larvae were inoculated as either first or third instars. Error bars represent 95% confidence intervals; data were combined across rearing containers for each treatment. Results for larvae inoculated as third instars are shifted to the right to prevent overlap of error bars.

significant interaction between dose and instar on adult spore loads in experiment 2 ($F_{2, 6} = 0.53$, $P = 0.61$).

Effects of Dose on Size and Development Time

The mass of emerging adults in experiments 1 and 2 declined with increasing dose treatments (Fig. 3). In experiment 1, inoculation with the highest dose (1000 spores) resulted in significantly lower mass at emergence compared with the other three treatments ($F_{3, 23} = 4.19$, $P = 0.017$). Males were significantly heavier than females in experiment 1 ($F_{1, 23} = 40.21$, $P < 0.001$), although we observed no significant dose × sex interaction ($F_{3, 23} = 0.07$, $P = 0.98$). Emergence mass was not affected by block ($F_{2, 23} = 2.83$, $P = 0.079$) or the dose × block interaction ($F_{6, 23} = 1.67$, $P = 0.17$). Although the highest dose was excluded from our analysis of experiment 2, larvae inoculated with 100 spores weighed significantly less upon emergence than those inoculated with 0 or 10 spores (Fig. 3b; $F_{2, 6} = 9.97$, $P = 0.012$). Surprisingly, emergence mass was significantly higher for larvae inoculated as first instars versus third instars (Fig. 3b; $F_{1, 6} = 17$, $P = 0.006$), but we observed no significant interaction effect of dose × instar on emergence mass ($F_{2, 6} = 1.31$, $P = 0.34$).

The wingspan of emerging adults in experiment 1 varied significantly with parasite dose (Table 2a), and

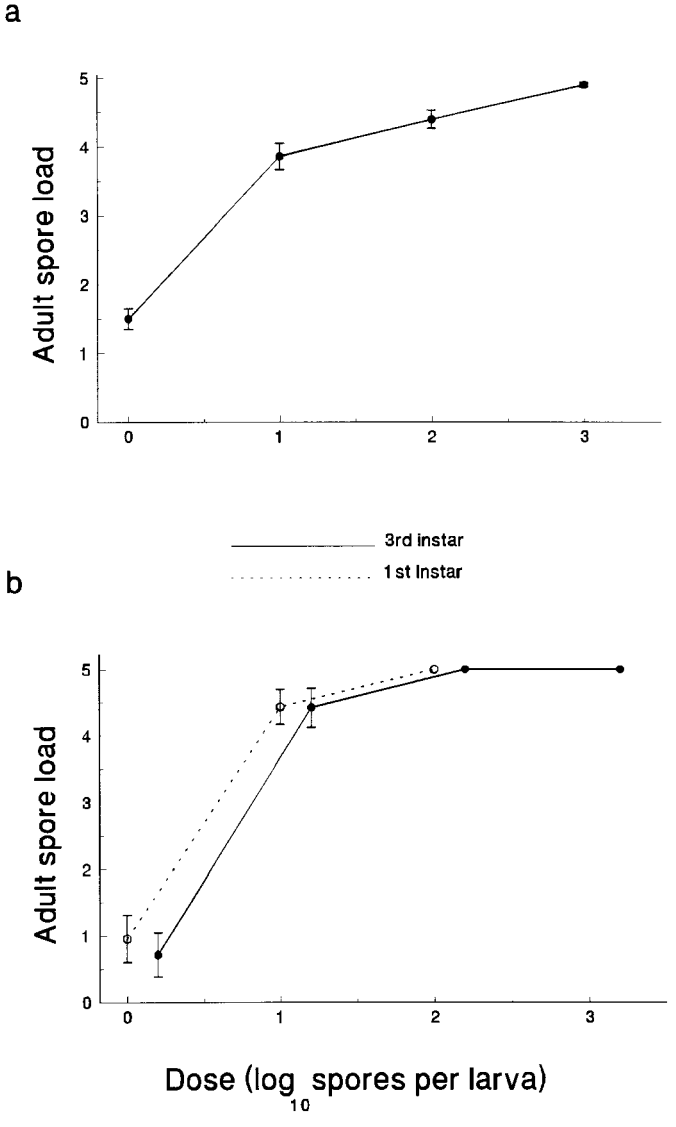

FIG. 2. Effects of parasite dose on the spore loads of emerging adults for (a) experiment 1, in which larvae were inoculated at third instar only and (b) experiment 2, in which larvae were inoculated at either first or third instar. Error bars represent standard errors for each treatment. Results for larvae inoculated as third instars are shifted to the right to prevent overlap of error bars

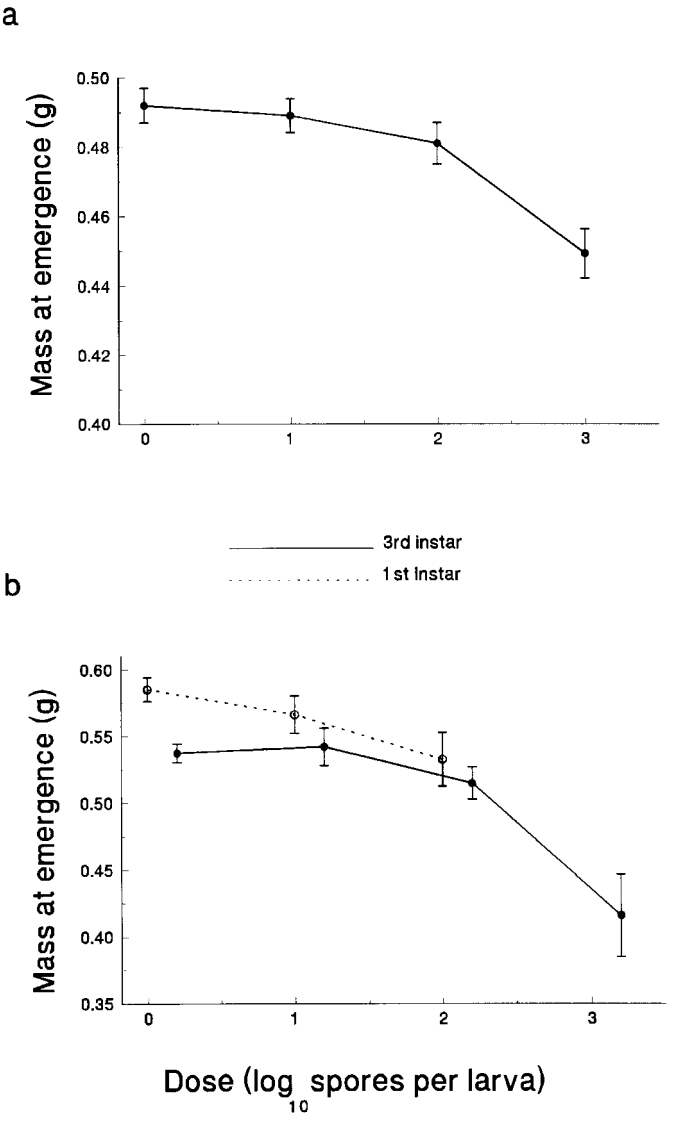

FIG. 3. Effects of parasite dose on the mass of adults 1 day postemergence for (a) experiment 1 and (b) experiment 2. Error bars represent standard errors for each treatment. Results for larvae inoculated as third instars are shifted to the right to prevent overlap of error bars.

wingspans of adults in the control treatment were significantly larger than those inoculated with 1000 spores. Males were significantly larger than females, although we observed no effect of the dose × sex interaction (Table 2a). In addition, adults in block 3 had significantly larger wingspans than those in blocks 1 and 2. There were no significant effects of any treatment variable on forewing asymmetry in experiment 1 (based on the absolute difference between left and right forewing lengths; Table 2b). Inoculation with *O. elektroscirrha* did not affect the development time from hatching to adult emergence in experiment 1 (Table 3a). In experiment 2, monarchs in the control treatment emerged an average of 0.5 days sooner than those in the 10- and 100-dose treatments, but this effect was not significant (Table 3b).

Effects of Dose on Adult Fitness

The lifespan, mating success, and weight loss of adults in outdoor enclosures were affected by infection with *O. elektroscirrha* (Fig. 4). Because these effects were different for males and females, each sex was separately analyzed. Male lifespan was shortest in the highest dose treatment ($F_{3, 98} = 7.15$, $P < 0.001$; Fig. 4a), although female lifespan was not significantly affected by dose ($F_{3, 100} = 1.44$, $P = 0.23$; Fig. 4a). Different spore doses also influenced the number of times males mated ($F_{3, 98} = 2.96$, $P = 0.036$; Fig. 4b), but not females ($F_{3, 100} = 2.19$, $P = 0.093$). Among males, those in the control treatment had significantly more successful matings than those inoculated with the highest dose. However, we found no effects of dose on the rate of mating (i.e., the average number of matings per butterfly per day) for either males or females ($F_{3, 98} = 1.64$, $P = 0.183$ and $F_{3, 100} = 1.95$, $P = 0.121$, respectively). In addition, the cage to which a butterfly was assigned did not influence the lifespan or mating success of males or females in any of our analyses.

Infection with *O. elektroscirrha* influenced the lifetime change in mass for females, but not males (Fig. 4c). Infected females actually gained weight during the course of the experiment ($F_{3, 100} = 3.08$, $P = 0.031$), and comparison of means shows that the change in mass for the control group was significantly less than that for the highest dose treatment. Males lost weight during the course of our experiment, but average weight loss did not vary among dose treatments ($F_{3, 98} = 0.21$, $P = 0.89$). For females used to examine the effects of dose on lifetime fecundity, we found no effects of increasing spore doses on the total number of eggs laid in each cage over a 25-day period (Fig. 5; $F_{3, 8} = 0.52$, $P = 0.67$).

Parasite Loads and Adult Condition in Natural Populations

Monarchs with high parasite loads had smaller wingspans in two of the five samples of wild-caught adults. Among breeding monarchs collected throughout western North America, higher spore loads were significantly associated with smaller wingspans ($F_{5, 329} = 2.96$,

TABLE 2

Effects of Dose and Sex on the Wingspan and Forewing Asymmetry of Emerging Adults in Experiment 1

Source of variation	*df*	MS	*F*	*P*
		(a) Response variable is left forewing length		
Sex	1	44.0	26.31	<0.001
Block	2	18.90	11.29	<0.001
Dose	3	12.10	7.19	0.001
Block × dose	6	12.39	7.41	<0.001
Sex × dose	3	1.32	0.78	0.516
Container (block × dose)	24	1.67		
Source of variation	*df*	MS	*F*	*P*
		(b) Response variable is absolute forewing asymmetry		
Sex	1	0.469	2.30	0.142
Block	2	0.044	0.22	0.805
Dose	3	0.067	0.33	0.803
Block × dose	6	0.302	1.48	0.227
Sex × dose	3	0.055	0.25	0.804
Container (block × dose)	24	0.204		

Note. Only the subset of adults used in the outdoor enclosure study were measured and included in the analysis. ANOVA tables were constructed using the container (block × dose) term as the error for tests of significance.

TABLE 3

Effects of Dose and Instar at Time of Inoculation on Development Time (Measured as Days from Hatching to Adult Emergence)

Source of variation	*df*	MS	*F*	*P*
		(a) Experiment 1		
Sex	1	0.213	1.82	0.190
Block	2	0.682	5.86	0.009
Dose	3	0.163	1.40	0.268
Block × dose	6	0.091	0.78	0.592
Sex × dose	3	0.001	0.01	0.999
Container (block × dose)	23	0.117		
Source of variation	*df*	MS	*F*	*P*
		(b) Experiment 2		
Instar	1	0.024	0.83	0.397
Dose	2	0.022	0.73	0.519
Instar × dose	2	0.003	0.002	0.945
Container (instar × dose)	6	0.029		

Note. The response variable was square-root transformed before analysis. ANOVA tables were constructed using the tub (instar × dose) or tub (block × dose) term as the error for tests of significance.

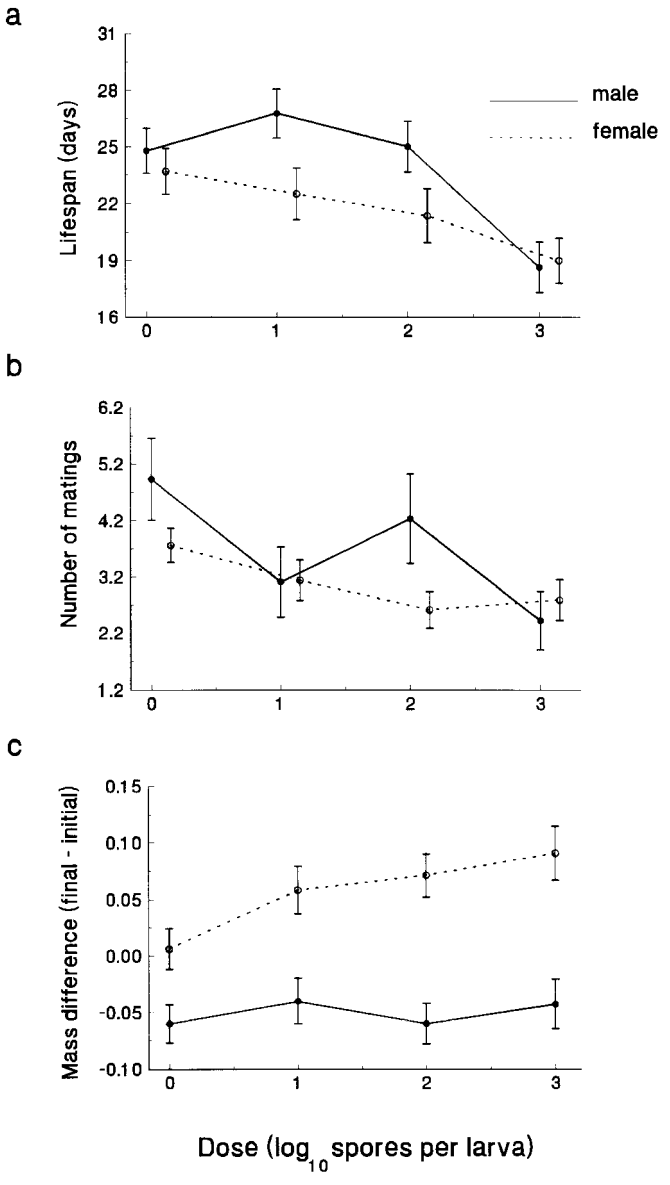

FIG. 4. Effects of parasite dose on the fitness of adults in outdoor enclosures. Data are shown separately for males and females. (a) Adult lifespan measured in days postemergence, (b) number of successful matings throughout lifetime, and (c) total weight change, measured as the difference in grams between the final mass (the last mass recorded before death) and the initial mass (at emergence). Error bars represent standard errors for each dose treatment. For graphs (a) and (b), lines for females were shifted to the right to prevent overlap of data points.

$P = 0.013$), and a comparison of means showed that wingspans of spore classes 0 and 5 were significantly different (Fig. 6). Monarchs in southern Florida with high parasite loads also had smaller wingspans (Fig. 6); this effect was significant despite a small sample size and low numbers of uninfected adults ($F_{2, 20} = 4.98$, $P = 0.038$).

Among monarchs overwintering in Mexico, adults

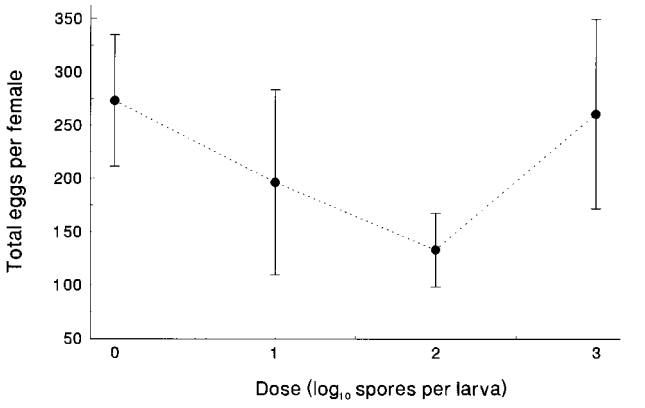

FIG. 5. Effects of *O. elektroscirrha* on female fecundity, measured as the total number of eggs laid per female in each of three replicate cages per dose treatment. Error bars represent standard errors based on estimates of among-cage variance.

with greater degrees of wing scale loss showed higher frequencies of spore loads 2–4 ($\chi^2 = 49.92$, $df = 20$, $P < 0.001$; Fig. 7a). Adults from this sample that showed the greatest degree of wing tatter were also more likely to be infected with any number of spores ($\chi^2 = 32.71$, $df = 20$, $P = 0.036$; Fig. 8a). For monarchs captured breeding in western North America, adults with greater degrees of scale loss were more likely to carry spores ($\chi^2 = 34.88$, $df = 20$, $P = 0.02$; Fig. 7b). A similar association was observed between spore loads and wing tatter ($\chi^2 = 61.2$, $df = 20$, $P < 0.001$; Fig. 8b), such that uninfected adults had less wing wear and damage.

Associations between sex and spore loads were detected in only one sample: breeding season males in western North America had higher than expected frequencies in spore classes 2–5, whereas observations of uninfected females were higher than expected

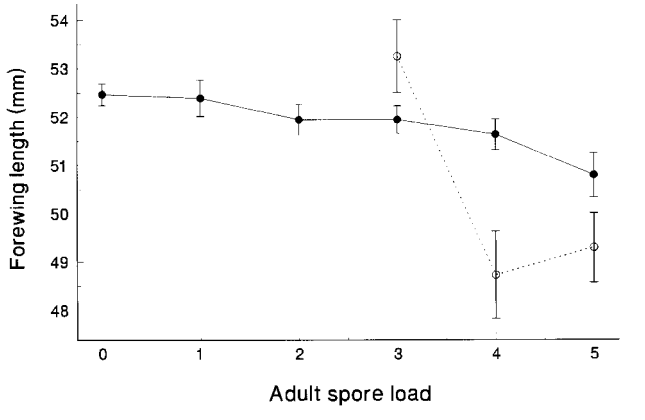

FIG. 6. Wingspans of wild monarchs in relation to parasite loads. Adults were captured breeding in western North America during July 1997 (solid line) and breeding in southern Florida during July 1996 (dashed line). Error bars represent standard errors. Sample sizes are shown in Table 1.

($\chi^2 = 29.1$, $df = 5$, $P < 0.001$). A similar trend was observed among monarchs overwintering in Mexico, but this association was not significant ($\chi^2 = 7.93$, $df = 5$, $P = 0.16$).

Among monarchs overwintering in California and Mexico, an association was observed between spore class and activity (Fig. 9). In Mexico, this association was marginally significant, with mating adults having higher frequencies of intermediate and high spore loads than roosting adults ($\chi^2 = 9.38$, $df = 5$, $P = 0.07$; Fig. 9). A more significant trend was observed in California, with mating adults associated with higher proportions of intermediate and high spore loads ($\chi^2 = 15.24$, $df = 5$, $P = 0.01$; Fig. 9). Because previous work has demonstrated an association between male condition and mating behavior at overwintering colonies (Van Hook, 1993; Oberhauser and Frey, in press), we also contrasted spore loads of males captured mating versus roosting separately. In California, significantly more mating males had high spore loads (classes 4 and 5), and fewer than expected roosting males had high spore loads ($\chi^2 = 17.39$, $df = 5$, $P = 0.004$). Although not significant, a similar pattern was observed among males captured in Mexico ($\chi^2 = 7.81$, $df = 5$, $P = 0.16$).

We found no other significant associations between parasite load and adult condition. Among overwintering monarchs in California, we found no relationship between spore loads and wingspan, sex, wing damage, or wing wear. No significant associations between spore class and any measurements of individual condition were detected among eastern breeding monarchs in Minnesota and Wisconsin. This is expected, because the frequency of infected adults captured in this area is, on average, less than 1%, and large sample sizes are required to obtain infected monarchs (Altizer *et al.*, in

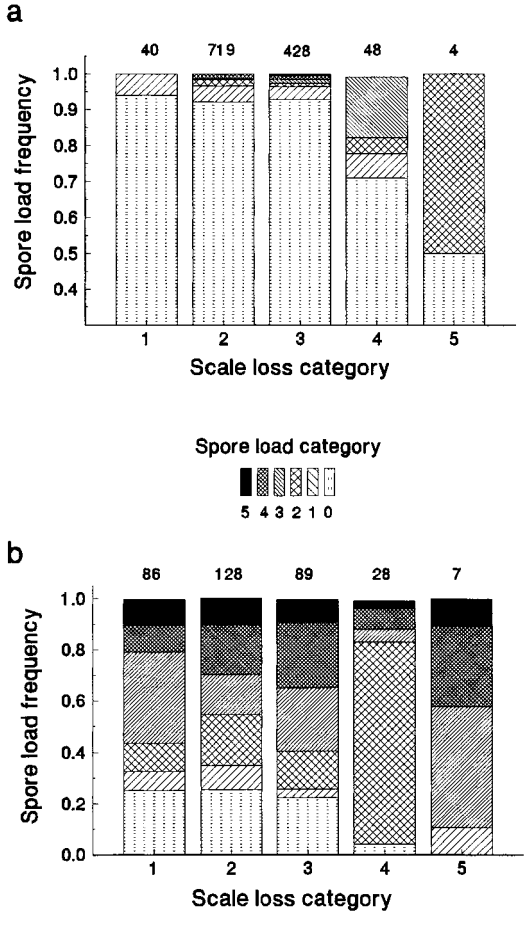

FIG. 7. Association between categories of wing scale loss and parasite loads for (a) monarchs captured overwintering in Mexico in March 1997 and (b) monarchs captured breeding in western North America in July 1997. Each bar shows the frequency of parasite loads (indicated by different hatching patterns) within each scale loss category. Sample sizes are shown at the top of each bar. See text for descriptions of scale loss and spore load categories.

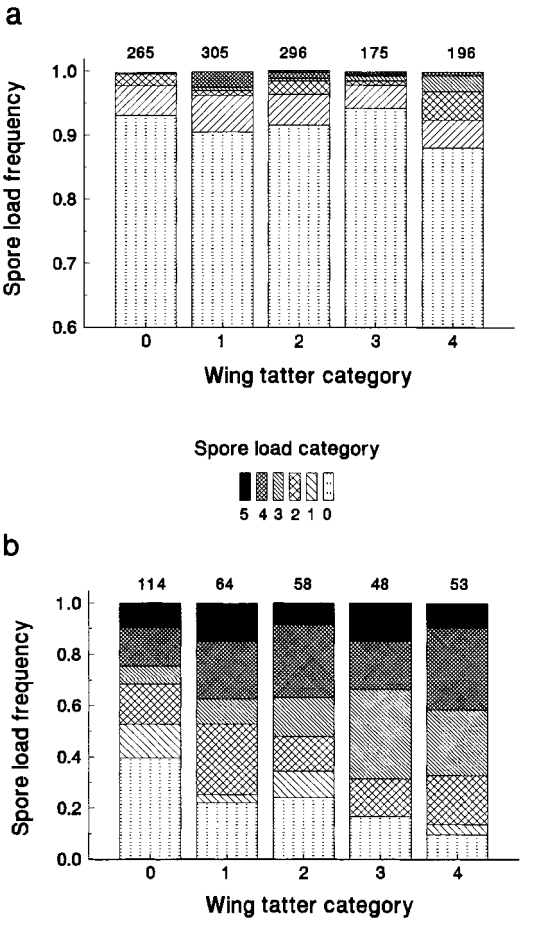

FIG. 8. Associations between categories of wing tatter and parasite loads for (a) monarchs captured overwintering in Mexico in March 1997 and (b) monarchs captured breeding in western North America in July 1997. Each bar shows the frequency of parasite loads (indicated by different hatching patterns) within each wing tatter category. Sample sizes are shown at the top of each bar. See text for descriptions of wing tatter and spore load categories.

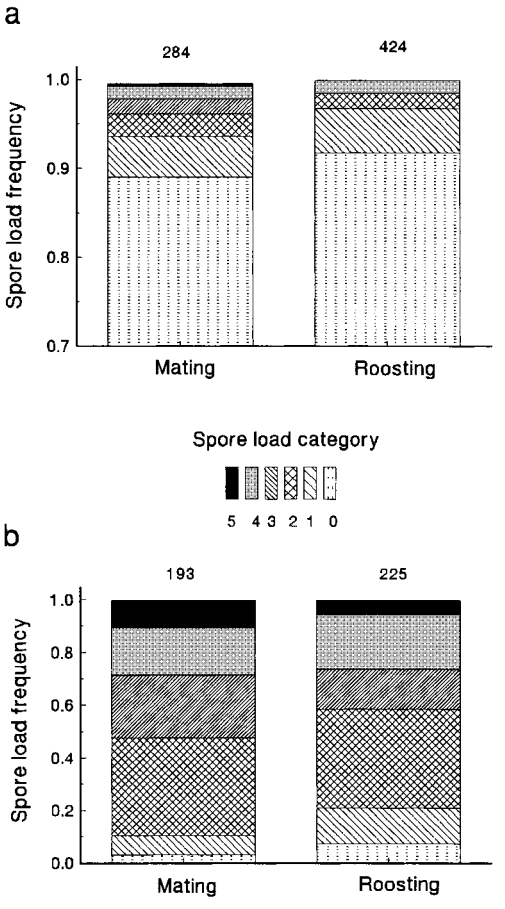

FIG. 9. Association between activity of overwintering monarchs at the time of capture and parasite loads for (a) monarchs captured in Mexico in March 1997 and (b) monarchs captured in California in February 1997. Data are shown for males and females combined. Each bar represents the frequency distribution of parasite loads (indicated by different hatching patterns) within each activity class. Sample sizes are shown at the top of each bar. See text for description of spore load categories.

press). Across all samples, we found no relationship between infection class and the presence or size of spermatophores in females.

DISCUSSION

Effects of Parasite Dose on Spore Loads and Survival to Eclosion

O. elektroscirrha compromises the fitness of monarch butterflies, affecting survival to eclosion, adult size and lifespan, and male reproductive success. These effects are dose dependent, with the highest parasite dose treatment leading to larger parasite loads and the most severe fitness consequences. The relationship between spore dose and parasite load for neogregarine parasites such as *O. elektroscirrha* may result from the mechanism of within-host replication. These parasites undergo a predetermined number of vegetative reproductive cycles within host tissues, whereby single trophozoite cells bud into several hundred daughter cells (Tanada and Kaya, 1993). Because *O. elektroscirrha* has only two such schizogonic cycles (McLaughlin and Myers, 1970), infection with higher numbers of spores is expected to lead to larger parasite loads in emerging adults. In our disease assessment scale, all adults from which we removed more than 1000 spores were categorized in the highest parasite load class. Because individual tape samples may contain up to 50 times this number of spores (S. M. Altizer, unpublished data), our scale probably underestimates the variation among heavily infected monarchs. Thus, the saturating effect of high dose treatments on spore loads in experiment 2 (Fig. 2b) may result in part from the inability of our parasite load scale to capture differences among samples with more than 1000 spores.

The survival of larvae inoculated with *O. elektroscirrha* was influenced by dose and larval instar at the time of infection. Larvae inoculated with low doses (10 or 100 spores per individual) did not have lower survival than noninoculated larvae. However, larvae inoculated with the highest dose were more likely to die before reaching eclosion, and this effect depended on the instar at the time of inoculation. In fact, most first-instar larvae inoculated with the highest dose of spores died before pupation, and none survived to eclosion (Fig. 1b). This significant interaction between dose and larval instar may result from the mechanism through which *O. elektroscirrha* invades host tissues. Following ingestion, spores lyse in the larval gut, and sporozoites penetrate the gut wall to migrate to the hypoderm (McLaughlin and Myers, 1970). High spore densities may cause more damage to the guts of early-instar larvae, leading to secondary infections and bacterial septicemia (McLaughlin and Myers, 1970; Brewer and Thomas, 1966). We observed that larvae inoculated with high spore numbers stopped feeding several days postinfection, turned pale and lethargic, and failed to molt. Inspection of tissue smears from dead and dying larvae (stained with Giemsa stain) revealed high densities of bacteria in gut and hypodermal tissues.

Of the monarchs in the highest dose treatment that matured to eclosion, many had difficulty emerging from their pupal cases. Their abdomens appeared wet, and large patches of abdominal scales were missing. In some instances, heavily infected adults fell to the floor of the plastic containers and died within 24 h. Leong *et al.* (1997b) and McLaughlin and Myers (1970) noted that heavily infected monarchs had difficulty expanding their wings and exhibited shriveled abdomens. These effects of *O. elektroscirrha* probably result from large numbers of parasites in host hypodermal tissues disrupting the development of host integument (McLaughlin and Myers, 1970).

Effects of Parasite Dose on Adult Fitness

Infection with *O. elektroscirrha* resulted in smaller, shorter-lived adults (Figs. 3 and 4). These size differences between infected and uninfected monarchs may have consequences for reproductive success. For example, wingspan has been shown to be positively correlated with female egg-laying lifespan (Oberhauser, 1997). Migrating monarchs with larger wingspans or greater lipid reserves may also be more successful in migrating to and from overwintering sites (Alonso-Mejia *et al.*, 1997; Arango, 1996; Van Hook, 1996; Masters *et al.*, 1988).

The effects of *O. elektroscirrha* on adult lifespan were relatively small. However, the highest spore dose had a negative effect on male lifespan, and females showed a similar, but insignificant trend (Fig. 4a). The average difference in adult male lifespan between the control (noninoculated) monarchs and those in the highest dose treatment was 4 days (or a 16% decline). This shorter lifespan may be responsible for the lower number of lifetime matings for infected males (Fig. 4b), as we observed no significant effect of parasite dose on the daily rate at which males mated.

Our analysis of female lifetime fecundity indicates that *O. elektroscirrha* does not affect female lifespan or egg development in a way that reduces total egg production. We observed no measurable effect of parasite dose on the number of eggs laid by females in outdoor enclosures (Fig. 5). The fact that we combined four females from the same treatment into cages (and that oviposition was highly variable among cages and over time) limited the statistical power of the experiment. However, despite the prediction that negative effects of parasites on adult size (or lifespan) should reduce reproductive success of infected females, the number of eggs laid by females in the highest dose treatment was not lower than that of noninoculated females.

The change in mass of adult monarchs held in outdoor enclosures contradicted our prior expectations of greater weight loss for higher dose treatments. Because *O. elektroscirrha* spores form in the cuticle of developing monarchs, we expected disruption of the adult integument to cause higher rates of water loss in infected adults. Leong *et al.* (1992) showed that under dry conditions, infected monarchs from a California overwintering site lost water at a faster rate than uninfected monarchs. However, our results showed no higher weight loss for infected males, and females inoculated with higher doses actually gained weight during the experiment (Fig. 4c). This effect of infection on female weight gain probably resulted from an interaction between activity level and parasite load. In fact, we observed that heavily infected females were less active and spent prolonged periods of time sitting at feeding stations or on the sides of cages.

It is worth noting that the significant block effect in experiment 1 was such that monarchs inoculated on the third day (block 3) emerged with lower spore loads, greater mass, and larger wingspans than hosts inoculated in blocks 1 and 2. In addition, the significant dose $\times$ block effect on survival was such that monarchs inoculated in block 3 did not experience increased mortality when inoculated with higher spore doses. Because we refrigerated spore suspensions between inoculation blocks, the spores used in block 3 may have lost viability across the 3-day inoculation period. In fact, Leong *et al.* (1997b) showed that spores refrigerated at 5°C for 1 year were less than 1/5 as infectious as spores recovered from newly captured infected hosts.

Association Between Infection and Condition of Wild-Captured Monarchs

Our observation of smaller wingspans among infected adults breeding in southern Florida and western North America (Fig. 6) suggests that high parasite loads carry negative fitness consequences for monarchs in natural populations. Although we saw no association among spore loads and the wingspans of overwintering adults in western North America, this may reflect an association between infection, wingspan, and migratory ability. If adults with the smallest wingspans are less likely to migrate successfully, we would predict the association between parasite load and wingspan to be stronger among monarchs sampled pre- versus post-fall migration. However, potential effects of disease on host migratory ability should be explicitly tested by measuring the flight endurance of monarchs with known spore loads.

Parasite loads were associated with wing condition in monarchs overwintering in Mexico and breeding in western North America, with greater degrees of both scale loss and wing tatter being associated with a higher likelihood of samples containing one or more parasite spores (Figs. 7 and 8). If scale loss or wing tatter is a measure of age or activity, then these results suggest two possible explanations. First, older or more active monarchs may be more likely to acquire low numbers of spores through contact with infected adults. Alternatively, monarchs infected with intermediate or high parasite loads may be physiologically stressed and therefore more likely to acquire wing damage via foraging for nectar, water, or mates.

Among overwintering monarchs, parasite loads were associated with behavior at the time of capture (Fig. 9). Monarchs captured mating were more likely to be infected with spores, whereas monarchs captured roosting were more likely to be spore-free. This may result from mating activity increasing the transfer of spores among adults. In addition, mating males showed a higher prevalence of intermediate and high spore loads than roosting males. In overwintering colonies, males

in poorer condition have been observed mating earlier and with higher frequency (Van Hook, 1993; Frey *et al.*, 1998); this finding suggests that parasite load could be a factor that leads to poorer male condition and increased mating probability.

Consequences for Transmission and Virulence in Natural Populations

We have shown that with the exception of high parasite doses, *O. elektroscirrha* has small effects on the survival and reproduction of individual monarchs. These results are consistent with the expectation that vertically transmitted parasites should have minor fitness consequences compared with horizontally transmitted parasites (Fine, 1975). In the absence of horizontal transmission, new infections of *O. elektroscirrha* depend on infected females scattering spores on eggs and milkweed during oviposition, and the net reproductive rate of this parasite is correlated with the survival and reproduction of infected hosts. However, because *O. elektroscirrha* is also transmitted paternally and horizontally, parasites that maintain measurable virulence may still reach high prevalence in host populations (Lipsitch *et al.*, 1995; Altizer and Augustine, 1997).

Parasite doses in monarch populations are likely to vary widely due to variation in adult spore loads, modes of transmission, and the background prevalence of disease in a population. For example, direct vertical transmission to larvae (via heavily parasitized females) will lead to higher spore doses than indirect paternal transmission (via infected males mating with uninfected females). In addition, larvae in nonmigratory populations are likely to be exposed to large numbers of spores that accumulate on milkweed plants through the activity of infected adults, whereas larvae in migratory populations (with low disease prevalence) are unlikely to encounter spores via this route (S. M. Altizer, unpublished data). We have shown that higher spore doses are associated with more severe effects on monarch survival and reproduction. Because the offspring of infected females that ingest high parasite doses will experience negative fitness effects, the actual contribution of maternal transmission to parasite fitness may be lower than that suggested by estimating the proportion of infected offspring (if most of these offspring die before transmitting the parasite; Kover *et al.*, 1997). However, at this point, we do not know the frequency of larvae that receive doses large enough to compromise their fitness in wild populations. Adults sampled from wild populations have passed the stage during which *O. elektroscirrha* has the greatest effects, and we therefore can only estimate the potential of this parasite to regulate monarch populations. Knowledge of parasite transmission routes and fitness effects on individual monarchs will be used in future theoretical studies to predict the population-level impact of *O. elektroscirrha*.

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REFERENCES

Alonso-Mejia, A., Rendon-Salinas, E., Montesiños-Patino, E., and Brower, L. P. 1997. Use of lipid reserves by monarch butterflies overwintering in Mexico: Implications for conservation. *Ecol. Appl.* **7,** 934–947.

Altizer, S. M., and Augustine, O. J. 1997. Interactions between frequency-dependent and vertical transmission in host–parasite systems. *Proc. R. Soc. Lond. Ser. B.* **264,** 807–814.

Altizer, S. M., Oberhauser, K. S., and Brower, L. P. 1999. Associations between host migration and the prevalence of a protozoan parasite in natural populations of adult monarch butterflies. *Ecol. Entomol.* In press.

Anderson, R. M. 1979. Parasite pathogenicity and the depression of host population equilibria. *Nature* **279,** 150–152.

Anderson, R. M., and May, R. M. 1991. "Infectious Disease of Humans: Dynamics and Control." Oxford Univ. Press, Oxford.

Arango, N. V. 1996. "Stabilizing Selection in Migratory Butterflies: A Comparative Study of Queen and Monarch Butterflies." Masters thesis, Univ. of Florida (Gainesville).

Cowley, J. M. 1989. Effect of disease caused by a neogregarine protozoan, *Mattesia* sp., on the population dynamics of a hill country sod webworm, *Eudonia sabulosella* (Pyralidae: Scopariinae). *J. Invertebr. Pathol.* **53,** 159–163.

Dobson, A. P., and Hudson, P. J. 1995. Microparasites: Observed patterns in wild animal populations. *In* "Ecology of Infectious Diseases in Natural Populations" (A. P. Dobson and B. T. Grenfell, Eds.), pp. 52–89. Cambridge Univ. Press, Cambridge, UK.

Dobson, A. P., and McCallum, H. 1995. Detecting disease and parasite threats to endangered species and ecosystems. *TREE* **10,** 190–193.

Dwyer, G. 1994. Density dependence and spatial structure in the dynamics of insect populations. *Am. Nat.* **143,** 533–562.

Ewald, P. W. 1983. Host–parasite relations, vectors, and the evolution of disease severity. *Annu. Rev. Ecol. Syst.* **14,** 465–485.

Fenner, F., and Myers, K. 1978. Myxoma virus and myxomatosis in retrospect: The first quarter century of a new disease. *In* "Viruses and the Environment." (E. Kurstak and P. Maramorosch, Eds.), pp. 539–570. Academic Press, New York.

Fine, P. E. 1975. Vectors and vertical transmission: An epidemiologic perspective. *Ann. N. Y. Acad. Sci.* **266,** 173–194.

Frey, D., Leong, K. L. H., Peffer, E., Smidt, R. K., and Oberhauser, K. 1998. Mate pairing patterns of monarch butterflies at a California overwintering site. *J. Lepidopt. Soc.* **52,** 84–97.

Gulland, F. M. D. 1995. The impact of infectious diseases on wild animal populations—A review. *In* "Ecology of Infectious Diseases in Natural Populations" (A. P. Dobson and B. T. Eds.), pp. 20–51. Cambridge Univ. Press, Cambridge, UK.

Herre, E. A. 1993. Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science* **259,** 1442–1445.

Jaenike, J. 1998. On the capacity of macroparasites to control insect populations. *Am. Nat.* **151,** 84–96.

Jouvenaz, D. P., and Anthony, D. W. 1979. *Mattesia germinata* sp. n. (Neogregarinida: Ophryocystidae), a parasite of the tropical fire ant, *Solenopsis germinata* (Fabricius). *J. Protozool.* **26,** 354–356.

Kover, P. X., Dolan, T. E., and Clay, K. 1997. Potential versus actual contribution of vertical transmission to pathogen fitness. *Proc. R. Soc. Lond. Ser. B.* **264,** 903–909.

Lenski, R. E., and May, R. M. 1994. The evolution of virulence in parasites and pathogens: Reconciliation between two competing hypotheses. *J. Theor. Biol.* **169,** 253–265.

Leong, K. L. H., Kaya, H. K., Yoshimura, M. A., and Frey, D. F. 1992. The occurrence and effect of a protozoan parasite, *Ophryocystis elektroscirrha* (Neogregarinida: Ophryocystidae) on overwintering monarch butterflies, *Danaus plexippus* (Lepidoptera: Danaidae) from two California winter sites. *Ecol. Entomol.* **17,** 338–342.

Leong, K. L. H., Yoshimura, M. A., and Kaya, H. K. 1997a. Occurrence of a neogregarine protozoan parasite, *Ophryocystis elektroscirrha* (McLaughlin and Myers), in a population of monarch and queen butterflies. *Pan Pac. Entomol.* **73,** 49–51.

Leong, K. L. H., Yoshimura, M. A., Kaya, H. K., and Williams, H. 1997b. Instar susceptibility of the monarch butterfly (*Danaus plexippus*) to the neogregarine parasite, *Ophryocystis elektroscirrha*. *J. Invertebr. Pathol.* **69,** 79–83.

Levin, B. R. 1996. The evolution and maintenance of virulence in microparasites. *Emerg. Inf. Dis.* **2,** 93–102.

Lipsitch, M., Nowak, M., Ebert, D., and May, R. M. 1995. The population dynamics of vertically and horizontally transmitted parasites. *Proc. R. Soc. Lond. Ser. B.* **260,** 321–327.

Masters, A. R., Malcolm, S. B., and Brower, L. P. 1988. Monarch butterfly (*Danaus plexippus*) thermoregulation behavior and adaptations for overwintering in Mexico. *Ecology* **69,** 458–467.

McCallum, H. 1994. Quantifying the impact of disease on threatened species. *Pac. Conserv. Biol.* **1,** 107–111.

McLaughlin, R. E., and Myers, J. 1970. *Ophryocystis elektroscirrha* sp. n. a neogregarine pathogen of the monarch butterfly *Danaus plexippus* (L.) and the Florida queen butterfly *Danaus gilippus berenice* Cramer. *J. Protozool.* **17,** 300–305.

Munster, S. M. 1991. The effect of sublethal neogregarine infections in the spruce needleminer *Epinotia tedella* (Lepidoptera: Tortricidae). *Ecol. Entomol.* **16,** 211–220.

Myers, J. H. 1993. Population outbreaks in forest lepidoptera. *Am. Sci.* **81,** 240–251.

Oberhauser, K. S. 1997. Fecundity, lifespan and egg mass in butterflies: Effects of male-derived nutrients and female size. *Funct. Ecol.* **11,** 166–175.

Oberhauser, K. S., and Frey, D. F. 1999. Coercive mating by overwintering male monarch butterflies. *In* "Proceedings of the North American Conference on the Monarch Butterfly." (J. Hoth, L. Merino, K. Oberhauser, I. Pisanty, S. Price, and T. Wilkinson, Eds.) In press. Commission for Environmental Cooperation, Montreal, Canada.

Onstad, D. W., and Maddox, J. V. 1989. Modeling the effects of the microsporidium, *Nosema pyrausta*, on the population dynamics of the insect, *Ostrinia nubilalis*. *J. Invertebr. Pathol.* **53,** 410–421.

Tanada, Y., and Kaya, H. K. 1993. "Insect Pathology."Academic Press, San Diego.

Van Hook, T. 1993. Non-random mating behavior in monarch butterflies overwintering in Mexico. *In* "Biology and Conservation of the Monarch Butterfly." (S. Malcolm and M. Zalucki, Eds.) pp. 49–60. Natural History Museum of Los Angeles County, Los Angeles.

Van Hook, T. 1996. "Monarch Butterfly Mating Ecology at a Mexican Overwintering Site: Proximate Causes of Non-Random Mating." Ph.D. thesis, Univ. of Florida (Gainesville).

Van Hook, T. 1999. The use of bursa copulatrix dissection and abdominal palpation to assess female monarch butterfly mating status. *In* "Proceedings of the North American Conference on the Monarch Butterfly." (J. Hoth, L. Merino, K. Oberhauser, I. Pisanty, S. Price, and T. Wilkinson, Eds.) In press. Commission for Environmental Cooperation, Montreal, Canada.

Weisner, J. 1963. Sporozoan infections. *In* "Insect Pathology: An Advanced Treatise" (E. A. Steinhaus, Ed.), Vol. 2, pp. 291–334. Academic Press, New York.