Variation in life-history traits of *Plasmodium mexicanum*, a malaria parasite infecting western fence lizards: a longitudinal study

Rebecca J. Eisen

Abstract: The life history of malaria parasites (*Plasmodium* spp.) is directly related to their transmission, virulence, and population dynamics. I followed the life history of *Plasmodium mexicanum* in naturally infected western fence lizards (*Sceloporus occidentalis*) over a 4-year period, using a mark–recapture technique. The life-history traits measured included peak parasitemias and population growth rates of asexual forms, gametocytes, and total parasites. Among malaria infections, variation was high for all measured traits. Growth rates varied up to 11-fold, and among stable infections, average asexual parasitemias ranged from 0.2 to 13.2 and gametocytemias from 0.5 to 66.2 parasites per 1000 erythrocytes. This variation was not related to infection prevalences, which were similar among years and between male and female hosts. Host age and gender were not related to peak parasitemia or average growth rate of asexual forms. However, the growth rate of gametoctyes was higher in older lizards. Gametocytemia and parasitemia were significantly higher late in the warm season, when sand-fly vectors are active. These data reveal that life-history traits of *P. mexicanum* are highly variable within an infected host population, and that the variation is partially related to the age of the infected host or the time of year the host was examined.

Résumé : Le cycle des parasites (*Plasmodium* spp.) qui transmettent la malaria est directement relié à la virulence du parasite et à la dynamique de la population. J'ai étudié le cycle de *Plasmodium mexicanum* par marquage–recapture chez des lézards *Sceloporus occidentalis* porteurs d'infections naturelles au cours d'une période de 4 ans. J'ai mesuré les parasitémies maximales et les taux de croissance au sein de la population chez les individus asexués, les gamétocytes et chez l'ensemble des parasites. La variation s'est avérée élevée pour toutes les variables. Le taux de croissance variait par un facteur de 11 et, dans le cas des infections stables, les parasitémies moyennes chez les individus asexués se situaient entre 0,2 et 13,2 et les gamétocytémies, entre 0,5 et 66,2 parasites par 1000 érythrocytes. La variation n'était pas reliée à la prévalence des infections qui s'est révélée semblable d'année en année chez les mâles et les femelles. L'âge et le genre de l'hôte n'étaient pas reliés aux parasitémies maximales ou au taux de croissance moyen des formes asexuées. Cependant, le taux de croissance des gamétocytes était plus rapide chez les lézards âgés. Les gamétocytémies et les parasitémies étaient significativement plus graves vers la fin de la saison chaude au moment où les cératopogons vecteurs de la malaria devenaient actifs. Ces données révèlent que la dynamique de population de *P. mexicanum* est très variable au sein d'une population infectée et qu'une partie de la variation est reliée à l'âge de l'hôte infecté ou au moment de l'année où l'hôte est examiné.

[Traduit par la Rédaction]

Introduction

Life-history traits are related directly to an organism's reproductive success and thus should have a greater influence on fitness than other traits, such as morphological components (Stearns 1992). Roff and Mousseau (1987) therefore predicted that selection would reduce the heritability of life-history traits. Comparative data reveal that heritability of these traits is lower than for many other phenotypic characteristics, yet substantial genetic and phenotypic diversity remains (Mousseau and Roff 1987; Roff 1992). This suggests that selection maintains a diversity of genotypes associated with life-history phenotypes in a population, as well as adaptive phenotypic plasticity (Roff 1992; Stearns 1992; Poulin 1996). Although most life-history studies have focused on multicellular free-living organisms, the same reasoning can apply to single-celled parasites such as those causing malaria. Such studies have additional importance for applied parasitology because parasite life histories are directly related to transmission biology, parasite virulence, and parasite population dynamics (Aaron and May 1982; Bull 1994; Ewald 1994; Read et al. 1999).

The life cycle of malaria parasites (*Plasmodium* spp.) consists of a series of traits that are intimately associated with the parasites' reproductive success. For example, an infection is initiated in the vertebrate host when an infected vector (mosquito or sand fly) takes a blood meal and injects sporozoites into the bloodstream. The parasite reproduces asexually in exo-erythrocytic tissue before invading erythrocytes (RBCs). Within the RBC the parasite (trophozoite) feeds and divides asexually to become a schizont. When the schizont ruptures, it releases merozoites that either repeat

Received November 23, 1999. Accepted March 9, 2000.

R.J. Eisen.[1] University of Vermont, Department of Biology, Marsh Life Sciences, Burlington, VT 05405, U.S.A.

[1]Present address: University of California, Hopland Research and Extension Center, 4070 University Road, Hopland, CA 95449, U.S.A. (e-mail: reisen@zoo.uvm.edu).

the erythrocytic cycle or differentiate into the transmission stage (male and female gametocytes). New vectors become infected when they consume the gametocytes in a blood meal, and development continues in the vector until the parasite is again infective to new hosts (Garnham 1966).

Several life-history traits, such as how quickly infections grow, how many cells they infect, when they produce gametocytes (maturity), and how many gametocytes are produced vary among infections of any species of *Plasmodium* (Garnham 1966; Bruce-Chwatt 1985; Schall 1996). Variation in most of these traits (maturity being the exception) has a genetic component (Taylor et al. 1997*a*, 1997*b*; Mackinnon and Read 1999*a*, 1999*b*), with heritabilities similar to those for life-history traits of free-living species (Roff 1992; Eisen and Schall 2000). The remaining phenotypic variation may represent an adaptive response to heterogeneous environments, such as diversity in the quality of hosts (Carter and Graves 1988; Williams 1999), the presence of concomitant infections (Taylor et al. 1997*a*), temporal variation in vector behaviour (Chaniotis and Anderson 1968), or nonadaptive variation due to host effects.

Any attempt to understand the ecological and evolutionary significance of differences in life histories among infections of *Plasmodium* spp. requires information on the naturally existing variation in life-history traits. Such understanding is essential for studies of malaria transmission (Taylor et al. 1997*b*) or virulence (Ewald 1984; Mackinnon and Read 1999*a*, 1999*b*).

Here I report on a 4-year study of *Plasmodium mexicanum*, a malaria parasite of western fence lizards (*Sceloporus occidentalis*). My primary goals were to examine the variation in life-history traits within single warm seasons (May–September) as well as among years, and to determine possible sources of such variation. For example, are life-history traits related to infection prevalence of *P. mexicanum*, and do infections behave differently in male versus female hosts, juveniles versus adults, and early versus late in the warm season?

Materials and methods

Western fence lizards were collected daily over a 21-month period covering 4 warm seasons, from June to August 1996, April to September 1997, March to September 1998, and April to August 1999, at the University of California Hopland Research and Extension Center (HREC) located in southeastern Mendocino County, California. The study site was a 4.5-ha oak savannah woodland area, 310–350 m in elevation, with numerous rocky outcrops, logs, and oak (*Quercus* sp.), California bay (*Umbellularia californica*), and California buckeye (*Aesculus californica*) trees. The climate is mediterranean with hot, dry summers and cool, wet winters (mean seasonal maximum temperatures and mean seasonal precipitation measured at HREC from 1990 to 1999: June–September, 31.8°C, 5.1 mm; December–February, 13.1°C, 193 mm (C.E. Vaughn, University of California at Davis, unpublished data)). Lizards can be active at this location from March to October (Bromwich and Schall 1986).

Lizards were captured by hand or slip noose between 08:00 and 14:00. Upon first collection, each lizard was marked on its dorsal surface with a number, using nontoxic Liquid Paper®, and permanently marked with a unique toe-clip combination. The exact sites of capture were recorded and flagged with surveyor's tape to ensure release at the point of capture. Recaptures of the same individual were spaced at least 3 days apart.

All animals were transported in cloth bags to a field laboratory, where sex and snout–vent length (SVL) were recorded. Age and SVL are correlated in western fence lizards (Schall 1996). Therefore, for age-group comparisons, lizards with SVL >64 mm were considered old (approximately 2 years or older), whereas those with SVL <65 mm were grouped as young (younger than 2 years old). A drop of blood was drawn from a clipped toe of each lizard and used to make thin blood smears that were later stained with Giemsa (pH 7.0, 50 min). Lizards were returned to the exact site of capture within 24 h. These procedures followed a protocol approved by the University of Vermont Institutional Animal Care and Use Committee and are in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Each slide was examined at 1000× for 6 min or until a malaria parasite was detected. A 6-min scan allows examination of approximately 10 000 RBCs. Infections with parasitemia <1 per 10 000 RBCs, and thus not detected by this protocol, are rare at HREC (Perkins et al. 1998). If an infection was detected, 1000 RBCs were counted and the numbers of uninucleate trophozoites, schizonts, and gametocytes were noted. A preliminary experiment was conducted to verify that cell counts were accurate and repeatable when this number of RBCs were counted (unpublished data).

For each infection, I measured 6 life-history traits. Peak parasitemia (3 measures: asexual forms, gametocytes, and total parasites) was the observed maximum number of parasites per 1000 RBCs. In the case of stable infections (infection growth rate is equal to zero), the arithmetic mean value of parasitemia was calculated and is referred to as average parasitemia. To calculate the average growth rate of the infection (again 3 measures: asexual forms, gametocytes, and total parasites), I fit a least-squares regression line through the rising (or declining) points using number of parasites versus days of observation. The growth rate is the slope of this line.

Growth rates were calculated only for lizards captured at least four times in one season. Four data points were used because the test was less sensitive to any rare cell-counting errors than if fewer points were used; restricting the analysis to more than four captures greatly reduced the sample size. To eliminate among-season variation in growth rates, I restricted the estimate of growth rates to a single season.

To ascertain that the time of day when blood was drawn was not a source of variation in measured life-history traits, eight naturally infected lizards were monitored over a 6-h period that coincided with the time of day when blood was usually sampled from lizards captured in the field (08:00–14:00). The lizards were housed outdoors in plastic containers (41 × 27 × 17 cm) with a metal screen top and leaf litter on the bottom. Sampling was conducted during September, a month when parasites are detected in the peripheral blood of lizards and sand-fly vectors (*Lutzomyia vexator* and *Lutzomyia stewartii*) of the malaria parasites are still active. Commencing at 08:00, a drop of blood was taken from a clipped toe of each animal every 2 h, producing a total of 4 samples per lizard. Thin smears were prepared, stained, and examined as previously described.

Statistical analysis

Contingency-table analyses were used to compare infection prevalences among male and female lizards and among years and months. An ANOVA with repeated measures was used to compare parasitemias every 2 h over a 6-h period. Mann–Whitney *U* tests were used to compare growth rates and peak parasitemias by host size class and gender. For these analyses, peak parasitemia was estimated on the basis of the observed maximum parasitemia of infections seen at least four times throughout the study. Mann–Whitney *U* tests were also used to compare parasitemias early (April–June) and late (July–August) in the warm season. Estimates of parasitemia for this comparison were based on the first capture

Table 1. *Plasmodium mexicanum* infection status of western fence lizards (*Sceloporus occidentalis*) captured once or multiple times within or between years from 1996 to 1999.

No. of years	Observations	Infection status	1996	1997	1998	1999	Total
1	Single	Uninfected	73	60	37	94	264
		Infected	29	12	11	13	65
1	Multiple	Remained uninfected	83	141	36	34	294
		Remained infected	23	22	4	15	64
		Gained infection	4	8	1	2	15
		Lost infection	3	0	0	0	3
2	Multiple	Remained uninfected	48	17	13	na	78
		Remained infected	21	5	2	na	28
		Gained infection	20	4	4	na	28
		Lost infection	5	0	0	na	5
3	Multiple	Remained uninfected	1	7	na	na	8
		Remained infected	1	0	na	na	1
		Gained infection in year 3	1	1	na	na	2
		Gained infection in year 2	1	2	na	na	3
		Lost infection in year 3	1	0	na	na	1
		Lost infection in year 2	0	1	na	na	1
Total			314	280	108	158	860

Note: na, not applicable.

per infected lizard each season. Wilcoxon's signed-rank tests were used to compare parasitemias among years in infected lizards observed over multiple years.

Pairwise Spearman's correlations were used to determine if the 6 measured life-history traits were correlated. To adjust for multiple comparisons, a sequential Bonferroni test was used (Rice 1989). These analyses were restricted to infections observed at least four times in a single season (N = 40 infections) for which infection growth rates could be accurately estimated. The observed maximum parasitemia for the same group of infected lizards was used for all comparisons of peak parasitemias in these analyses.

Results

Description of life history and variation in traits

During four warm seasons, from 1996 to 1999, 860 lizards were collected and marked; these initial samples, plus recaptures, produced 2425 blood smears. A total of 329 lizards (38%) were captured once, 376 (44%) were captured multiple times during a single season, and 155 (18%) were captured over 2–3 seasons. No lizard was captured during all 4 seasons (Table 1). For the majority of lizards that were observed more than once (N = 531), infection status did not change throughout the observation period (89%). Some lizards acquired infections during the study (9%), and, rarely, infections were lost or became undetectable on blood smears (2%) (Table 1).

For each of the 6 measured life-history traits, there was considerable variation among infections (as exemplified in Fig. 1). Among all infections at their observed peak per year (N = 132 infections), peak parasitemias ranged from 1 to 846, peak asexual parasitemias ranged from 0 to 346, and peak gametocytemias ranged from 0 to 501 parasites per 1000 RBCs. However, in the majority of infections, parasite loads were low to moderate (median values of peak parasitemia, asexual parasitemia, and gametocytemia were 22, 8, and 15 parasites per 1000 RBCs, respectively).

A previous study (Bromwich and Schall 1986) demonstrated that longitudinal data provide more accurate estimates of peak values. Therefore, to obtain a more conservative estimate of peak values, I used only infections observed at least four times during the course of the study. For these infections (N = 70), the ranges of peak asexual parasitemia and gametocytemia were identical with those presented above, but peak total parasitemias ranged from 4 to 846. In addition, based on median values, the majority of infections consisted of low to moderate parasite loads (median values of peak parasitemia, peak asexual parasitemia, and peak gametocytemia were 28, 7.25, and 20 parasites per 1000 RBCs, respectively).

Forty infections were observed at least four times within a single season and were studied in more detail than those with less than four captures per season. Of these, most infections were stable (75% with a growth rate equal to 0, 82.5% with an asexual growth rate equal to 0, and 72.5% with a gametocyte growth rate equal to zero) (Table 2). Infections with stable parasitemia (N = 30) fell into three categories (Figs. 1*a*–1*c*): 40% (N = 12 infections) expressed low average parasitemia (<1% of cells infected), 50% (N = 15 infections) had moderate average parasitemia (1–5% infected cells), and 10% (N = 3 infections) showed heavy average parasitemia (5–11% infected cells). The majority of stable infections were dominated by gametocytes (median maximum gametocytemia relative to median maximum number of parasites was 0.725, range 0.16–0.86).

Changing infections (N = 9) also varied greatly (Figs. 1*d*–1*f*). Some infections tended to grow slowly and reach low peak levels of parasitemia, while fewer infections grew quickly and reached high levels (Table 2). One infection declined throughout a single observation season, and the lizard was found to be uninfected the following season (Fig. 1*d*). None of the heavy infections were observed to decline through an entire observation period.

Among infections observed over 2 consecutive years (N = 37), peak total and asexual parasitemia and peak gametocytemia were higher in the first year than in the second (Wilcoxon's signed-rank test, $P < 0.01$ in all cases).

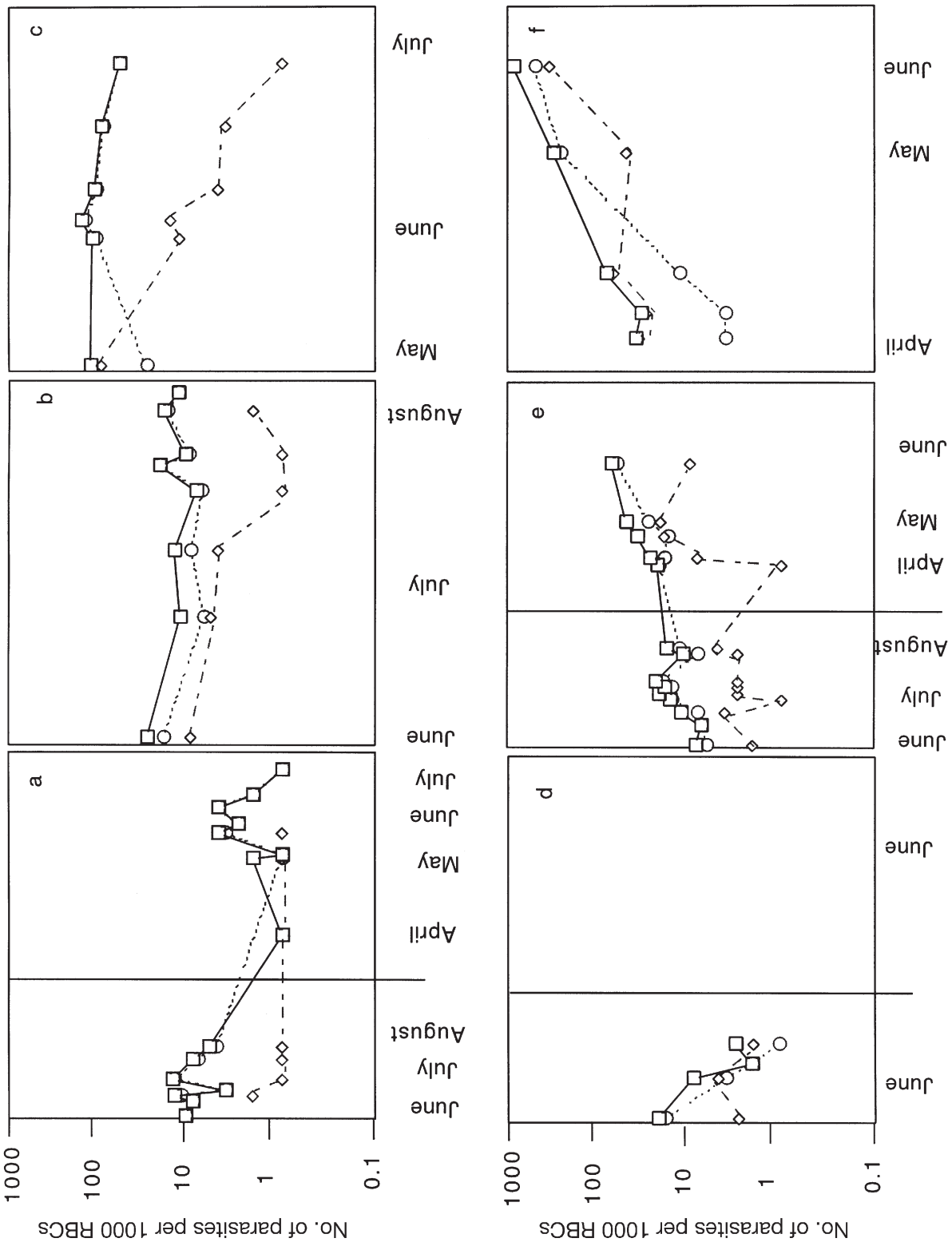

Fig. 1. Natural *P. mexicanum* infections in free-ranging lizards. A vertical line indicates that lizards were recaptured following a period of winter dormancy. Representative infections are presented for stable infections with low (*a*), moderate (*b*), and high parasitemia (*c*), as well as declining (*d*) and slowly (*e*) and rapidly growing infections (*f*). Total parasitemia is indicated by squares and solid lines, asexual parasitemia by diamonds and broken lines, and gametocytemia by circles and dotted lines.

Table 2. Parasitemias and growth rates of stable and changing *Plasmodium mexicanum* infections.

	Average parasitemia (stable infections)*			Average growth rate (changing infections)†		
Infection type	Median	Range	*N*	Median	Range	*N*
Total parasitemia	11.07	0.73–109.96	30	0.74	–0.86–11.23	10
Asexual parasitemia	2.40	0.20–13.20	33	–0.18	–1.73–3.26	7
Gametocytemia	9.28	0.48–88.16	29	0.47	–0.84–6.9	11

*In stable infections, the growth rate equals 0. Average parasitemia is measured as parasites per 1000 RBCs.
†In changing infections, the growth rate is not equal to 0. The average growth rate is measured in parasites per day.

Table 3. Correlation coefficients of peak parasitemias and growth rates of *Plasmodium mexicanum*.

			Growth rate		
	Peak asexual parasitemia	Peak gametocytemia	Total	Asexual	Gametocyte
Peak parasitemia	0.87*	0.99*	0.48*	–0.15	0.49*
Peak asexual parasitemia	—	0.82*	0.36	–0.16	0.51*
Peak gametocytemia		—	0.50*	0.50	0.51*
Growth rate					
Total			—	0.09	0.83*
Asexual					–0.08

*Significant Spearman's rank correlation at the tablewide significance level $\alpha = 0.05$.

The growth rate of asexual forms was not significantly correlated with any of the other measured traits. In addition, average growth rate and peak asexual parasitemia were not significantly correlated (Table 3). Peak asexual parasitemia was strongly correlated with peak gametocytemia, and rapidly growing infections tended to reach higher peaks of parasitemia.

Infection prevalence

From 1996 to 1999, 22.5% of captured lizards were infected with *P. mexicanum* (range among years, 20.3–26.4% infected). Infection prevalences were similar both among years (*G* test, $P = 0.24$) and among months (*G* test, $P = 0.92$). Also, male and female lizards were equally likely to be infected (Fisher's exact test, $P = 0.17$). In contrast, approximately 26% of older lizards were infected, while only 15% of younger ones were infected (Fisher's exact test, $P < 0.01$). Therefore, the variation in life-history traits cannot be accounted for by differences in infection prevalence among years or months.

Impact of host gender and age on life-history traits

Neither host gender nor age affected variation in most life-history traits. Peak parasitemias were similar among male and female lizards and among size classes (<65 and >64 mm) (Mann–Whitney *U* test, $N = 70$, $P > 0.08$ in all cases). Likewise, median values of infection growth rates (3 measures) were similar for male and female lizards (Mann–Whitney *U* test, $N = 40$, $P > 0.36$ in all cases), and asexual growth rates were similar among age-classes (Mann–Whitney *U* test, $N = 40$, $P = 0.61$). However, the rates of growth of the total populations and the gametocyte populations within hosts was more rapid in the larger size class (Mann–Whitney *U* test, $N = 40$, $P = 0.02$ and $P = 0.04$, respectively).

Temporal trends

The time of day when blood was sampled did not contribute to the observed variation in life-history traits. I found no evidence of variation in parasite density throughout a 6-h period (ANOVA with repeated measures, $P > 0.38$ for all 3 measures of parasitemia).

Asexual parasitemias were similar early (March–May) and late (June–August) in the warm season (Mann–Whitney *U* test, $P = 0.81$). However, total parasitemia and gametocytemia were both significantly elevated late in the warm season (Mann–Whitney *U* test, $P < 0.01$). Growth rates among infections that became detectable before mid-June did not differ significantly from those that became detectable after that date ($P > 0.19$ in all three cases).

Discussion

Variation in life-history traits

Life-history traits are intimately associated with the fitness of an organism. Therefore, selection should act strongly on these traits and erode genetic variation (Fisher 1930; Falconer 1981). However, concordant with other studies of lizard malaria (Thompson and Huff 1944; Goodwin and Stapleton 1952; Bromwich and Schall 1986), I found substantial variation in life-history traits of *P. mexicanum* infecting fence lizards. For example, infection growth rates ranged from 0.10 to 11.23 parasites per 1000 RBCs per day, and among stable infections there was a 150-fold difference in average parasitemia. In addition, some infections (<3%) deviated from this general trend by decreasing to undetectable levels.

Several general explanations for the maintenance of such variation in life-history traits within populations have been proposed, including mutation-selection balance, selection in patchy environments, a gene-by-environment interaction, negative genetic correlations, phenotypic plasticity, and flat fitness profiles (Roff 1992; Stearns 1992). Other possible explanations proposed for *Plasmodium* spp. include variations in host quality (Sinden 1983), the number of parasites initi-

ating an infection (Glynn 1994), the strain infecting the host (Graves et al. 1984), and competition between strains within the host (Taylor et al. 1997*a*, 1997*b*).

The existence of such variations raises several questions. For example, because the gametocytes are the only stage transmitted from lizard to sand fly, increasing their abundance could increase the likelihood of transmission (Taylor and Read 1997). If infections have the potential to infect approximately 50% (maximum gametocytemia reported in this study) of host cells with gametocytes, why do most infections produce far fewer (the median value of gametocyte-infected cells in this study was 0.9%)? Also, if infections can remain patent, why do some fall to undetectable levels? A common explanation is that the variation is nonadaptive and is simply due to variation in host immune defenses against the parasite (Day et al. 1992; Hetzel and Anderson 1996; McKenzie and Bossert 1998). Life-history variation could also result from genetic constraints unique to each strain (Mackinnon and Read 1999*b*). Alternatively, reproductive restraint could be a prudent strategy (Taylor and Read 1997). For example, increasing the density of infected cells could lead to a decrease in host survival and thus in parasite fitness (Bull 1994; Ewald 1994).

From 14 to 24% of the variation in *P. mexicanum* life-history traits studied here could be explained by genetic differences among infections (genetic diversity and (or) differing genotypes that directly control life-history traits) (Eisen and Schall 2000). The remaining variation is believed to be due to phenotypic plasticity of the parasite in response to variable host/environmental conditions, random events during the parasite's life cycle, or environmental effects. Here I discuss possible sources of variation.

Sources of variation

Infection prevalence

Infection prevalence is dependent on the number of infected hosts in the population, the abundance and feeding behaviour of biting vectors, and host susceptibility to infection, and is positively associated with transmission rate (MacDonald 1957; Aaron and May 1982). In turn, the transmission rate is positively associated with within-host replication rates (Read 1994; Frank 1996). The relationship between parasitemia and transmission to the vector has been established in many species (Taylor and Read 1997). The possibility that infection prevalence influences within-host replication rates and maximum parasitemia has drawn less attention. Interestingly, in areas where transmission rates are high, there tend to be more clones per infection than in areas where transmission rates are low (Babiker and Walliker 1997). Therefore, interaction between strains could lead to increased replication rates and parasitemia (Taylor et al. 1997*a*).

Because no significant differences in infection prevalence were detected over the 4-year period studied, except in older lizards that presumably had longer exposure periods, it is unlikely that the observed variation in life-history traits is related to infection prevalence. A longer term study in which variation in infection prevalence is significant, or a comparative study, is necessary to determine whether any associations exist between infection prevalence and the life-history of *P. mexicanum*.

Host age and gender

Male and female lizards, as well as young and old ones, differ with respect to physiology and behavioural patterns (Schall and Sarni 1987; Saad et al. 1990). For example, during the breeding season, male testosterone levels are elevated and males are more active than females (Davis and Ford 1983; Moore 1987). This could lead to differences in exposure to infection, with some hosts being infected multiple times. Also, differences in host immune responses mediated by testosterone could create the potential for variation between sexes with respect to life-history traits.

In this study, no significant differences were detected in any of the life-history traits when these were compared between male and female hosts; parasitemias and asexual growth rates were similar among age-classes. Interestingly, total and gametocyte growth rates were higher in older lizards. Perhaps these lizards were exposed to multiple infections throughout their lives, and the increased growth rates resulted from competition between strains. Several host attributes that were not measured here could also have contributed to the observed variation. For example, host nutrition (McGregor 1988), variation in suitable host-cell density (Trager et al. 1999; Williams 1999), and differences in hormonal titres (Maswoswe et al. 1985; Lingnau et al. 1993) can affect the life-history of malaria parasites.

Temporal trends

The seasonal trends observed in *P. mexicanum* life histories may be adaptive. The mean values of gametocytemia and total parasitemia were significantly higher late in the warm season than early, while asexual parasitemia was similar throughout. Investment in gametoycte production appears to be synchronized with the peak of adult sand fly activity in July–August (Chaniotis and Anderson 1968; Ayala 1977; Schall 1996).

Because sand-fly vectors overwinter as larvae, and transovarial transmission has not been demonstrated, the parasite must overwinter in the vertebrate host (Chaniotis and Anderson 1968; Bromwich and Schall 1986). Lizard mortality is high during the winter months (Bromwich and Schall 1986), posing a threat to the reproductive success of parasites that are not transmitted during the first season. Bromwich and Schall (1986) hypothesized that infections beginning early in the season, and thus having a potentially longer transmission season, may grow more slowly to reduce host pathology, while those initiated later in the season, which have a shorter window of transmission, might grow more rapidly. Like Bromwich and Schall (1986), I found no significant differences in growth rates according to when the infection first became patent in the blood.

In *P. mexicanum* infections, peak gametocytemia is correlated with the growth rate of gametocytes (Eisen and Schall 2000), and gametocyte density is positively associated with transmission success up to a density of 20 gametocytes per 1000 RBCs (J.J. Schall, University of Vermont, unpublished data); thus, slow-growing infections started later in the season could have reduced transmission success. However, maturity of an infection, or the time when gametocytes are first produced, is independent of peak parasitemia and growth rate (all 3 measures) (Eisen and Schall 2000). Like that of other species of *Plasmodium*, maturity of *P. mexicanum*

infections may be phenotypically plastic, with strains responding differently, based on environmental cues (Buckling et al. 1997, 1999; Taylor et al. 1997*a*, 1997*b*). Therefore, slow-growing infections that become patent late during the warm season could still produce gametocytes.

Further studies

While long-term natural studies of parasites are critical to understanding the extent of variation in life histories of parasites, manipulative experiments are needed to determine how much of the life-history variation is related to differences in the number of parasites starting an infection, parasite genetics, host condition, and host microhabitat. An understanding of these relationships is precursory to comprehending how and why so much variation in life-history traits is maintained in a population. Such knowledge is necessary if we are to predict how parasites will respond to selection pressures imposed by parasite-eradication programs or changing environmental conditions (Stearns 1999).

Acknowledgements

I thank A. Chatfield, S. Osgood, K. Gurski, and L. Talleklint-Eisen for assistance in the field. J.J. Schall, L. Talleklint-Eisen, S. Perkins, and an anonymous reviewer provided valuable comments on the manuscript. C. Vaughn, R. Timm, and R. Kieffer and the staff of the Hopland Research and Extension Center provided logistical support. Funding was provided by the U.S. National Science Foundation (NSF), from the Vermont–NSF EPSCoR program to J.J. Schall, and by the American Museum of Natural History and a graduate training grant from NSF to R.J.E.

References

Aaron, J.L., and May, R.M. 1982. The population dynamics of malaria. *In* Population dynamics of infectious diseases. *Edited by* R.M Anderson. Chapman and Hall, London. pp. 139–179.

Ayala, S.C. 1977. Plasmodia of reptiles. *In* Parasitic protozoa. *Edited by* J.P. Kreier. Academic Press, New York pp. 267–309.

Babiker, H.A., and Walliker, D. 1997. Current views on the population structure of *Plasmodium falciparum*: implications for control. Parasitol. Today, **13**: 262–267.

Bromwich, C.R., and Schall, J.J. 1986. Infection dynamics of *Plasmodium mexicanum*: a malarial parasite of lizards. Ecology, **6**: 1227–1235.

Bruce-Chwatt, L.J. 1985. Essential malariology. Heinemann, London.

Buckling, A.G.J., Taylor, L.H., Carlton, J. M.-R., and Read, A.F. 1997. Adaptive changes in *Plasmodium* transmission strategies following chloroquine chemotherapy. Proc. R. Soc. Lond. B Biol. Sci. **264**: 553–559.

Buckling, A., Ranford-Cartwright, L.C., Miles, A., and Read, A.F. 1999. Chloroquine increases *Plasmodium falciparum* gametocytogenesis in vitro. Parasitology, **118**: 339–346.

Bull, J.J. 1994. Perspective: virulence. Evolution, **48**: 1423–1435.

Carter, R., and Graves, P.M. 1988. Gametocytes. *In* Malaria: principles and practice of malariology. *Edited by* W.H. Wernsdorfer and I. McGregor. Churchill Livingstone, Edinburgh. pp. 253–305.

Chaniotis, B.N., and Anderson, J.R. 1968. Age structure, population dynamics and vector potential of *Phlebotomus* in northern California: Part II. Field population dynamics and natural flagellate infections in parous females. J. Med. Entomol. **5**: 273–292.

Davis, J., and Ford, R.G. 1983. Home range in the western fence lizard. Copeia, 1983: 933–940.

Day, K.P., Koella, J.C., Nee, S., Gupta, S., and Read, A.F. 1992. Population genetics and dynamics of *Plasmodium falciparum*: an ecological view. Parasitology, **104**: S35–S52.

Eisen, R.J., and Schall, J.J. 2000. Life history of a malaria parasite (*Plasmodium mexicanum*): Independent traits and basis for variation. Proc. R. Soc. Lond. B Biol. Sci. **267**: 793–800.

Ewald, P.W. 1994. Evolution of infectious disease. Oxford University Press, Oxford.

Falconer, D.S. 1981. Introduction to quantitative genetics. 2nd ed. Longman Press, London.

Fisher, R.A. 1930. The genetical theory of natural selection. Clarendon Press, Oxford.

Frank, S.A. 1996. Models of parasite virulence. Q. Rev. Biol. **71**: 37–78.

Garnham, P.C.C. 1966. Malaria parasites and other haemosporidia. Blackwell Scientific, Oxford.

Glynn, J.R. 1994. Infecting dose and severity of malaria: a literature review of induced malaria. J. Trop. Med. Hyg. **97**: 300–316.

Goodwin, M.H., and Stapleton, T.K. 1952. The course of natural and induced infections of *Plasmodium floridense* Thompson and Huff in *Sceloporus undulatus* (Latreille). Am. J. Trop. Med. **1**: 773–783.

Graves, P.M., Carter, R., and McNeill, K.M. 1984. Gametocyte production in cloned lines of *Plasmodium falciparum*. Am. J. Trop. Med. Hyg. **33**: 1045–1050.

Hetzel, C., and Anderson, R.M. 1996. The within-host cellular dynamics of bloodstage malaria: theoretical and experimental studies. Parasitology, **113**: 25–38.

Lingnau, A., Margos, G., Maier, W.A., and Seitz, H.M. 1993. The effects of hormones on the gametocytogenesis of *Plasmodium falciparum* in vitro. Appl. Parasitol. **34**: 153–160.

MacDonald, G. 1957. The epidemiology and control of malaria. Oxford University Press, London.

Mackinnon, M.J., and Read, A.F. 1999*a*. Genetic relationships between parasite virulence and transmission in the rodent malaria *Plasmodium chabaudi*. Evolution, **53**: 689–703.

Mackinnon, M.J., and Read, A.F. 1999*b*. Selection for high and low virulence in the malaria parasite *Plasmodium chabaudi*. Proc. R. Soc. Lond. B Biol. Sci. **266**: 741–748.

Maswoswe, S.M., Peters, W., and Warhurst, D.C. 1985. Corticosteroid stimulation of the growth of *Plasmodium falciparum* in gametocytes in vitro. Ann. Trop. Med. Parasitol. **79**: 607–616.

McGregor, I.A. 1988. Malaria and nutrition. *In* Malaria: principles and practice of malariology. Vol. 1. *Edited by* W.H. Wernsdorfer and I. McGregor. Livingstone Press, Edinburgh. pp. 753–768.

McKenzie, F.E., and Bossert, W.H. 1998. The optimal production of gametocytes by *Plasmodium falciparum*. J. Theor. Biol. **193**: 419–428.

Moore, M.C. 1987. Castration affects territorial and sexual behaviour of free-living male lizards, *Sceloporus jarrovi*. Anim. Behav. **35**: 1193–1199.

Mousseau, T.A., and Roff, D.A. 1987. Natural selection and the heritability of fitness components. Heredity, **59**: 181–198.

Perkins, S.L., Osgood, S.M., and Schall, J.J. 1998. Use of PCR for detection of subpatent infections of lizard malaria: implications for epizootiology. Mol. Ecol. **7**: 1587–1590.

Poulin, R. 1996. The evolution of life history strategies in parasitic animals. Adv. Parasitol. **37**: 107–134.

Read, A.F. 1994. The evolution of virulence. Trends Microbiol. **2**: 73–76.

Read, A.F, Aaby, P., Antia, R., Ebert, D., Ewald, P.W., Gupta, S., Holmes, E.C., Sasaki, A., Shields, D.C., Taddei, F., and Moxom, E.R. 1999. What can evolutionary biology contribute to understanding virulence? *In* Evolution in health and disease. *Edited by* S. Stearns. Oxford University Press, Oxford. pp. 205–215.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution, **43**: 223–225.

Roff, D.A. 1992. The evolution of life histories: theory and analysis. Chapman and Hall, New York.

Roff, D.A., and Mousseau, T.A. 1987. Quantitative genetics and fitness: lessons from *Drosophila*. Heredity, **58**: 103–118.

Saad, A.H, Khalek, N.A., and Ridi, R. 1990. Blood testosterone level: a season-dependent factor regulating immune reactivity in lizards. Immunobiology, **180**: 184–194.

Schall, J.J. 1996. Malarial parasites of lizards: diversity and ecology. Adv. Parasitol. **37**: 255–333.

Schall, J.J., and Sarni, G.A. 1987. Malarial parasitism and the behavior of the lizard, *Sceloporus occidentalis*. Copeia, **1**: 84–93.

Sinden, R.E. 1983. Sexual development of malarial parasites. Adv. Parasitol. **22**: 153–216.

Stearns, S.C. 1992. Evolution of life histories. Oxford University Press, Oxford.

Stearns, S.C. 1999. Evolution in health and disease. Oxford University Press, Oxford.

Taylor, L.H., and Read, A.F. 1997. Why so few transmission stages? Reproductive restraint by malaria parasites. Parasitol. Today, **13**: 135–140.

Taylor, L.H., Walliker, D., and Read, A.F. 1997*a*. Mixed-genotype infections of malaria parasites: within-host dynamics and transmission success of competing clones. Proc. R. Soc. Lond. Biol. B Biol. Sci. **264**: 927–935.

Taylor, L.H, Walliker, D., and Read, A.F. 1997*b*. Mixed-genotype infections of the rodent malaria *Plasmodium chabaudi* are more infectious to mosquitoes than single-genotype infections Parasitology, **115**: 121–132.

Telford, S.R. 1994. Plasmodia of reptiles. *In* Parasitic protozoa. Vol. 7. *Edited by* J.P. Kreier. Academic Press, San Diego. pp. 1–71.

Thompson, P.E., and Huff, C.G. 1944. A saurian malarial parasite, *Plasmodium mexicanum*, N. SP., with both elongatum- and gallinaceum-types of exoerythrocytic stages. J. Infect. Dis. **74**: 48–67.

Trager, W., Gill, G.S., Lawrence, C., and Nagel, R.L. 1999. *Plasmodium falciparum*: enhanced gametocyte formation in vitro in reticulocyte-rich blood. Exp. Parasitol. **91**: 115–118.

Williams, J.L. 1999. Stimulation of *Plasmodium falciparum* gametocytogenesis by conditioned medium from parasite cultures. Am. J. Trop. Med. Hyg. **60**: 7–13.