Evol Ecol (2014) 28:89–102
DOI 10.1007/s10682-013-9654-y

ORIGINAL PAPER

Life history focus on a malaria parasite: linked traits and variation among genetic clones

Allison T. Neal · Jos. J. Schall

Received: 14 January 2013 / Accepted: 7 May 2013 / Published online: 16 May 2013

Abstract Life history theory has long been a major campaign in evolutionary ecology, but has typically focused only on animals and plants. Life history research on single-celled eukaryotic protists such as malaria parasites (*Plasmodium*) will offer new insights into the theory's general utility as well as the parasite's basic biology. For example, parasitologists have described the *Plasmodium* life cycle and cell types in exquisite detail, with little discussion of evolutionary issues such as developmental links between traits. We measured 10 life history traits of replicate single-genotype experimental *Plasmodium mexicanum* infections in its natural lizard host to identify groups of linked traits. These 10 traits formed 4 trait groups: "Rate/Peak" merges measures of growth rate and maximum parasitemia of infections; "Timing" combines time to patency and maximum parasitemia; "Growth Shape" describes the fit to an exponential growth curve; and "Sex Ratio" includes only the gametocyte sex ratio. Parasite genotype (clone) showed no effect on the life history trait groups, with the exception of gametocyte sex ratio. Therefore, variation in most life history traits among infections appears to be driven by environmental (individual host) effects. The findings support the model that life history traits are often linked by developmental constraints. Understanding why life history traits of *Plasmodium* are linked in this way would offer a new window into the evolution of the parasites, and also should inform public health efforts to control infection prevalence.

Keywords Protists · *Plasmodium* · Principal components · Clonal infections · Sex ratio

Introduction

The partitioning of assimilated resources into growth, maintenance, and reproduction, and the timing of those developmental decisions, define an organism's life history. Important

Electronic supplementary material The online version of this article (doi:10.1007/s10682-013-9654-y) contains supplementary material, which is available to authorized users.

A. T. Neal (✉) · Jos. J. Schall
Department of Biology, University of Vermont, Burlington, VT 05405, USA
e-mail: aneal@uvm.edu

life history traits include age at first reproduction, the number and size of offspring, the frequency of reproductive episodes, and for sexually reproductive species, the allocation of effort to male and female offspring. Theoretical and empirical study of life histories has led to insights on how natural selection shapes complex trade-offs resulting from conflicts in resource and time allocation as well as sometimes obscure developmental constraints (Reznick and Endler 1982; Eisen and Schall 2000; Stearns 1992; Charnov 1993; Gotelli 2008). This research approach has been profoundly successful for a great variety of large multi-cellular species, but many life history traits studied in such organisms also have close analogues for single-celled protists such as malaria parasites (*Plasmodium* and related genera, Martinsen et al. 2008). For more than a century, malaria researchers have focused on the parasite's life cycle, describing each cell type in this cycle in detail, but with remarkably little emphasis on evolutionary questions. A life history approach, however, leads to unexpected insights in the study of malaria parasites. For example, the parasite's life history traits intersect with competition among clonal lines within mixed-genotype infections (Taylor et al. 1997; Vardo-Zalik and Schall 2009; Pollitt et al. 2011), resource availability and environmental (host) variation (Graves et al. 1984; Pollitt et al. 2011), potential vaccines (Buckling et al. 1997, 1999; Barclay et al. 2012), and virulence (Mackinnon and Read 1999a, b).

Malaria parasites have a two-host life cycle, alternating between a vertebrate host and an insect vector. Within both hosts, an infection may harbor a single genetic clone of asexually reproducing cells, or two to several such clones (Read and Day 1992; Paul and Day 1998; Vardo and Schall 2007). Asexually replicating cells (schizonts) undergo cycles of replication in the vertebrate host in which each schizont produces several to many new cells, which then develop into a feeding stage (trophozoite). Trophozoites either continue asexual replication as schizonts or develop into non-reproducing dimorphic male and female sex cells, or gametocytes. Production of gametocytes therefore necessarily reduces asexual growth (Fig. 1). Only the gametocytes survive transmission to the insect host where they initiate the sexual cycle, followed by asexual production of the transmission stages (sporozoites). All parasites stages are haploid with the exception of the ephemeral zygotes that undergo meiosis to yield haploid sporozoites. The parasites therefore reproduce as clones of cells that could well coordinate their efforts to maximize reproductive success of the group (Eisen and Schall 2000; Reece et al. 2009). Indeed, clones within both hosts have characteristics that would be expected of individual organisms such as apoptosis (Al-Olayan et al. 2002) and, potentially, self-sacrifice to trigger down-regulation of the immune response (Guilbride et al. 2012), so each clone appears to function as a multi-cellular organism.

Many events of *Plasmodium* biology in the vertebrate host mirror life history traits of multi-cellular organisms: asexual proliferation corresponds to somatic growth and maintenance of future reproductive ability, maximum asexual parasitemia is the maximum body size, timing of first production of gametocytes is the age of maturity, and number of gametocytes produced is the investment in male and female offspring. Although measuring the precise allocation of resources to growth versus reproduction in multi-cellular organisms is difficult, counts of asexual stage parasites and gametocytes make evaluating investment in these two traits feasible for malaria parasites (Reece et al. 2009).

Understanding how life history traits are linked for malaria parasites is important for public health efforts. For example, the rate of increase in the asexually reproducing cells in the vertebrate's blood is associated with virulence (Mackinnon and Read 1999a), so identifying links between asexual replication rate and other life history traits would help predict how different treatment or prevention strategies select for increased or decreased

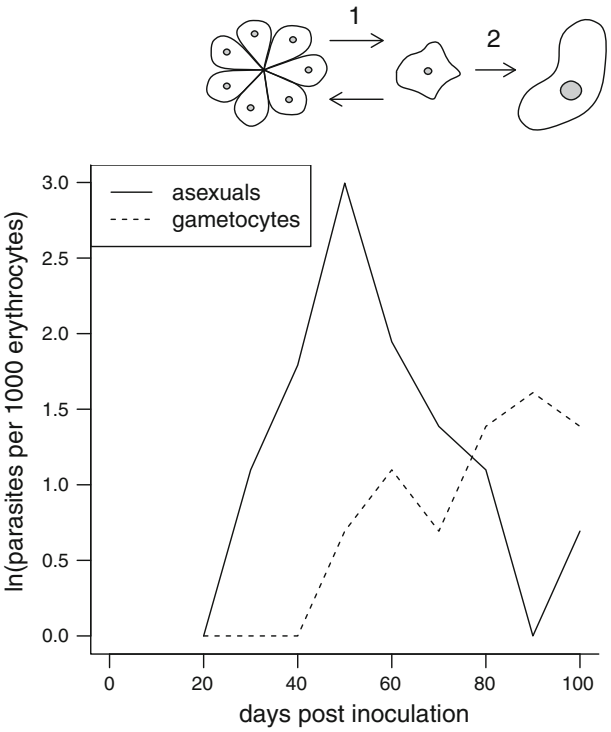

Fig. 1 Blood-stage life cycle and life history of the malaria parasite *P. mexicanum*. At the *top* of the figure is the life cycle: the parasite reproduces asexually within the blood (*arrows at 1*), alternating between the single-nucleated trophozoite stage (*middle drawing*), which develops into the multi-nucleated schizont stage (*left*, number of nuclei in illustration not meaningful), and schizonts, which divide to produce multiple new trophozoites. Some trophozoites develop instead into sexual cells (gametocytes, *right drawing*, *arrows at 2*). The *lower portion* of the figure shows parasite counts from one infection in this study. The life cycle is aligned with the *plotted data* to show which type of cell (1-asexual vs. 2-sexual) is more prevalent in the blood at that time. Shown in the plot are the log transformed number of parasites including asexually replicating stages (schizonts and trophozoites) and gametocytes over 100 days, the normal period for reproduction in the seasonal environment of this parasite-host system. 1 was added to all counts before transformation to avoid undefined values. Counts are from an infection in this study that was experimentally initiated with blood from donor 3

virulence (e.g. Barclay et al. 2012). Also, gametocyte sex ratio influences transmission success, and sex ratio theory posits that male gametocyte fecundity (production of gametes) drives equilibrium sex ratio in single-genotype infections (Neal 2011; Hamilton 1967). The rate of gametocyte production could alter the number of male gametes that can be produced, thus linking to gametocyte sex ratio and transmission success.

To pursue these issues, we examined the relationships among life history traits for *Plasmodium mexicanum* Thompson and Huff 1944, a malaria parasite of lizards in northern California. We induced experimental infections containing only a single clone of parasite cells and followed the infections for 100 days, roughly the period available for the full course of an infection of this parasite in its seasonal environment (Bromwich and Schall 1986). A previous study on *P. mexicanum* found life history traits were linked and varied among experimental infections initiated with blood from different donor lizards (Eisen and Schall 2000). However, the number of clones in the experimental infections was not

assessed, so any patterns could have been a result of clonal interactions rather than genetic variation for the traits. The life histories of malaria parasites are influenced by clonal interactions in the vertebrate blood (Taylor et al. 1997; de Roode et al. 2005; Reece et al. 2009; Vardo-Zalik and Schall 2009), so our experiment eliminated the confounding factor of clonal interactions such as competition. Therefore, using replicate single-genotype infections we sought to determine (1) which life history traits were closely associated for *P. mexicanum* and (2) whether groups of associated traits showed evidence of genetic variation.

Materials and methods

Infected and not infected western fence lizards (*Sceloporus occidentalis* Baird and Girard 1852) used for this study were collected at the University of California Hopland Research and Extension Center (HREC, Hopland, Mendocino County, CA; Schall 1996; Schall and St. Denis 2013) in late May to early June 2010, and each lizard was marked with a unique toe clip combination. A few drops of blood from a toe clip were stored frozen on filter paper for genetic analysis and a drop was used to make a thin smear for processing with Giemsa stain. Infected lizards were captured at sites where *P. mexicanum* has long been most common at the HREC (5–18 % of lizards infected in 2010), and thin smears were scanned for 3 min to identify infected individuals. DNA was extracted from the stored blood dots of infected lizards using the Qiagen DNeasy Kit (Qiagen Sciences, Germantown, Maryland, USA) and subjected to PCR for 4 microsatellite loci using primers and conditions developed by Schall and Vardo (2007). We measured the density of erythrocytes using a hemocytometer and counted the number of asexual stage parasites (trophozoites and schizonts) per 1,000 erythrocytes to estimate the density of asexual stage parasites per μL of blood. Eight infections were selected that had high asexual parasite density (8–105 asexuals/1,000 erythrocytes; 535,000–1,580,000 cells/μL; 9,900–81,885 asexuals/μL blood) and a single allele at all 4 microsatellite loci (scored therefore as single-clone infections; Anderson et al. (2010) showed that 4 microsatellites were sufficient to differentiate approximately 90 % of *P. falciparum* clones). These infected lizards served as blood donors to initiate replicate infections in previously not infected lizards.

Not infected lizards were collected from sites where *P. mexicanum* has been very rare or absent over many years at HREC (0–2 % of lizards infected in 2010). All lizards in which new infections were initiated were males at least 60 mm in length (snout to vent, excluding tail), and DNA was extracted from their blood and subjected to PCR to amplify a short segment of the *Plasmodium* cytochrome *b* gene to confirm they were not already infected. This protocol can identify infections that are not patent upon microscopic examination (Perkins et al. 1998). Infected blood from each donor was diluted with PBS, and each of 8 recipient lizards was injected intra-peritoneally with 20 μL of the mix containing 1×10^5 asexual parasites. Recipient lizards were housed outdoors in vector-proof cages for the first 60–70 days of the experiment and were moved indoors for the remaining 30–40 days for logistical reasons. Lizards were fed each day with crickets and mealworms to satiation and blood samples were used to make thin smears every 10 days for 100 days.

This sampling regime was based on previous experience. Naturally infected lizards have been studied at the field site for more than 3 decades (Schall and St. Denis 2013), including mark-recapture studies over the entire lifespan of infected lizards (Bromwich and Schall 1986). Sand fly vectors become active by June, new infections go through their annual cycle during the next ~100 days, and the blood parasite density (parasitemia) drops during

the host's winter brumation and then rebounds the next spring (Bromwich and Schall 1986; Schall and Marghoob 1995). Events in *P. mexicanum* infections, such as rate of increase in parasitemia and production of gametocytes, take far longer than for the malaria parasites of mammals. Validation studies early in the project sampled infections from every hour to 10 days and determined that a 7–10 days sampling program provided clear data on the course of infection. This sample program has been successful for prior studies, including determining infection growth rate, first patency, and gametocyte sex ratio (Eisen and Schall 2000; Eisen 2000; Osgood et al. 2003; Osgood and Schall 2004; Vardo-Zalik and Schall 2008, Vardo-Zalik and Schall 2009; Neal and Schall 2010; Ford and Schall 2011). Therefore, we followed the experimental infections for 100 days, sampling the infection every 10 days.

Ten life history traits measured were: Day post inoculation for patency of (1) asexuals (schizonts and trophozoites) and (2) gametocytes in the blood while counting 1,000 erythrocytes. Peak (3) asexual and (4) gametocyte parasitemia per 1,000 erythrocytes for any sample period. The number of days since inoculation for maximum (5) asexual and (6) gametocyte parasitemia. Growth rate of (7) asexual and (8) gametocyte parasitemia in the blood. Growth rates were measured as (a) maximum parasitemia/number of days from inoculation to day of that maximum; (b) maximum parasitemia/number of days since the infection became patent to maximum; (c) maximum growth in parasitemia over a single 10 day period between samples. These measures were tightly correlated ($r^2 > 0.84$), so only the third (c) is included the analysis. (9) The degree to which growth was exponential. The most simple growth would be exponential, with a constant rate of replication of schizonts, and deviations from this pattern would indicate more complex forces at work, such as interaction with the immune system, production of gametocytes, and even apoptosis to regulate somatic growth. To quantify this, a linear model was fit to *ln* transformed parasitemia for the days from patency to maximum parasitemia (provided at least 3 sample points were available). The r^2 value of this relationship was then used as a measure of the fit to an exponential growth model. (10) Gametocyte sex ratio. Mature gametocytes (based on size and shape; Schall 1989) were scored as male or female until 100 were counted or for 1 h to calculate sex ratio as proportion male gametocytes. Studies on natural infections (Bromwich and Schall 1986; Schall 1989) found that gametocyte sex ratio was constant in most infections (but varied among infections). For experimental infections, gametocyte sex ratio appears to stabilize by day 80 (Osgood et al. 2002, 2003; Osgood and Schall 2004; Neal and Schall 2010), therefore gametocyte sex ratios were determined at day 80 post inoculation.

Principal components analysis was used to cluster these 10 traits. For 9 infections, the lizard host died early or parasites did not become patent in the blood until late in the infection, resulting in insufficient data for estimation of certain life history traits (e.g. gametocyte patency, exponential growth fit). Sex ratios were only included in the analysis if at least 50 mature gametocytes were counted. Because this further limited the sample size (55/64 infections had complete data without sex ratio, 37/64 did with sex ratio, Table 1), the analysis was run with and without sex ratio included. The number of principal components analyzed was determined based on examination of a scree plot (Joliffe 2002; Gotelli and Ellison 2004). Variables with eigenvector coefficients (loadings) at least 70 % of the maximum coefficient for that principal component are reported as contributing to the component, whereas those at least 50 % of the maximum are reported as marginally contributing (Jolliffe 2002). Opposite signs of the loadings can be used to highlight tradeoffs between the variables to which they correspond (Gotelli and Ellison 2004). Most of the traits measured were not normally distributed, and in some cases, no transformation

was found that could correct the variable to a normal distribution. As a result, the principal components were also not normally distributed, so we used a permutation test written in R (R Foundation for Statistical Computing, Vienna, Austria) to determine if the first principal components (number based on scree plot, with and without sex ratio) varied significantly among donor groups or genotypes (see results for explanation of genotypes). The permutation test extracted F from ANOVA for the observed data, then randomly shuffled the principal components 1,000 times (10,000 times if the resulting P was <0.1), storing F for each permutation to create a randomized F distribution. The observed F was then compared with the randomly generated F distribution to obtain P. The principal components obtained using the data from before the lizards were moved inside or all together gave similar results. Only the results from the full study are reported here.

Results

Of the 8 donor infections used in this study, there were 7 unique genotypes. Two infections had identical alleles at all 4 microsatellite loci, and the two lizards with matching alleles at all loci were collected from 2 adjacent (and in contact) sites. Based on the frequencies of these 4 alleles at the 2 sites during 2010 (0.138, 0.121, 0.517, 0.097), the probability of having this genotype and not sharing a recent ancestor is very low (product = 0.00084). It is therefore likely that these 2 infections derive from the same clonal lineage. Subsequent analysis was performed separately with both donor (N = 8) and genotype (N = 7) as independent variables.

Table 1 shows pairwise correlations among the 10 traits measured. Many of these traits are tightly correlated, so the Principal Component analysis was appropriate to cluster non-independent traits. Based on the scree plots (Supplemental Fig. 1), we analyzed 4 principal components for the data including gametocyte sex ratio, which explain 81 % of the overall variation, and 3 principle components for the data that excluded gametocyte sex ratio, which explain 87 % of the variation.

The first principal component encompasses predominantly rate of increase and peak parasitemia of infections, and will be referred to as "Rate/Peak" (Table 2). The loadings for traits associated with rate and peak were all positive (Table 2). The second principal component, referred to as "Timing", describes the time to reach peak parasitemia and, to a lesser extent, to become patent, and relevant loadings are again all positive (Table 2). The traits contributing to the first and second principal components are not greatly affected by inclusion of sex ratio in the analysis (Table 2). The third principal component mainly describes sex ratio if sex ratio is included in the analysis ("Sex Ratio", Table 2). The third principal component if sex ratio is excluded and the fourth principal component if sex ratio is included both largely describe how well an exponential curve fits the data on the growth phase of the infection ("Growth Shape", Table 2).

Neither Rate/Peak nor Growth Shape varied among donors or genotypes, whether or not sex ratio was included in the analysis (Fig. 2a, d, $F < 1.1$, $P > 0.4$, permutation tests). Timing varied among donors if sex ratio was excluded from the analysis (Fig. 2b, $F = 2.98$, $P = 0.0096$, $N = 55$, permutation), but not if sex ratio was included ($F = 0.649$, $P = 0.722$, $N = 37$, permutation), and did not vary among genotypes ($F < 1.3$, $P > 0.25$, permutation). Sex Ratio showed significant variation among both donors (Fig. 2c, $F = 2.56$, $P = 0.0329$, permutation) and genotypes ($F = 3.10$, $P = 0.0203$, permutation). Sex ratio itself (not the Sex Ratio principal component) also showed significant variation among donors

Table 1 Correlations among traits

	aPeak	gPeak	aRate	gRate	aMaxDay	gMaxDay	aPatency	gPatency	SexRatio	ExpFit
aPeak	1	**0.77**	**0.95**	**0.66**	0.17	0.11	−0.24	**−0.40**	−0.04	**0.34**
gPeak		1	**0.74**	**0.96**	0.11	0.15	**−0.30**	**−0.44**	0.062	**0.30**
aRate			1	**0.62**	0.13	0.07	−0.20	**−0.32**	−0.14	**0.32**
gRate				1	0.11	0.14	**−0.32**	**−0.41**	0.11	**0.31**
aMaxDay					1	**0.73**	**0.37**	**0.28**	0.06	−0.10
gMaxDay						1	0.23	0.14	0.10	0.22
aPatency							1	**0.58**	−0.12	−0.15
gPatency								1	0.14	−0.22
SexRatio									1	0.11
ExpFit										1

Values in bold indicate that the 95 % confidence interval does not include 0

Table 2 Summary of traits measured for experimental infections with average, maximum and minimum values and contribution to principal components

Trait	Mean (median)	Min	Max	N	PC1	PC1*	PC2	PC2*	PC3	PC3*	PC4	PC4*
Asexual peak parasitemia (asex/1,000 RBC)	137 (56)	2	1144	61	+	+						na
Gametocyte peak parasitemia (gam/1,000 RBC)	18.2 (11)	1	89	58	+	+						na
Asexual max rate of increase (asex/day)	7.63 (3.2)	0.2	80.9	61	+	+						na
Gametocyte max rate of increase (gam/day)	1.09 (0.65)	0.1	5.6	58	+	+						na
Asexual max parasitemia day (days)	77.9 (80)	30	100	61			+	+				na
Gametocyte max parasitemia day (days)	80 (80)	40	100	58			+	+				na
Asexual patency (days)	33.8 (30)	10	60	61			(+)	+			(+)	na
Gametocyte patency (days)	51.9 (50)	20	80	58	(−)	(−)	(+)	(+)				na
Sex ratio (proportion male)	0.47 (0.48)	0.36	0.58	37		na		na	+	na		na
r^2 log transformed parasitemia	0.86 (0.903)	0.314	0.989	55					(+)	−	+	na

Starred principal components indicate results from analysis without sex ratio data. "+" and "−" signs without parenthesis indicate the coefficient in the eigenvector was at least 70 % the maximum coefficient for that principal component. Parentheses indicate the coefficient was 50–70 % of the maximum

($\chi^2 = 15.96$, $P = 0.0255$, GLM with binomial errors) and genotypes ($\chi^2 = 15.90$, $P = 0.0143$, GLM).

Discussion

We take a life history approach for a malaria parasite, and break down events during the course of an infection in the vertebrate host's blood into traits analogous to those for the life history of multi-cellular organisms, such as growth rate, maximum somatic size, and investment into male and female offspring. We asked if suites of correlated traits fall into clusters defined by the Principal Components, and examined the loadings of traits included in each cluster for evidence of trade-offs that would result from developmental or energetic

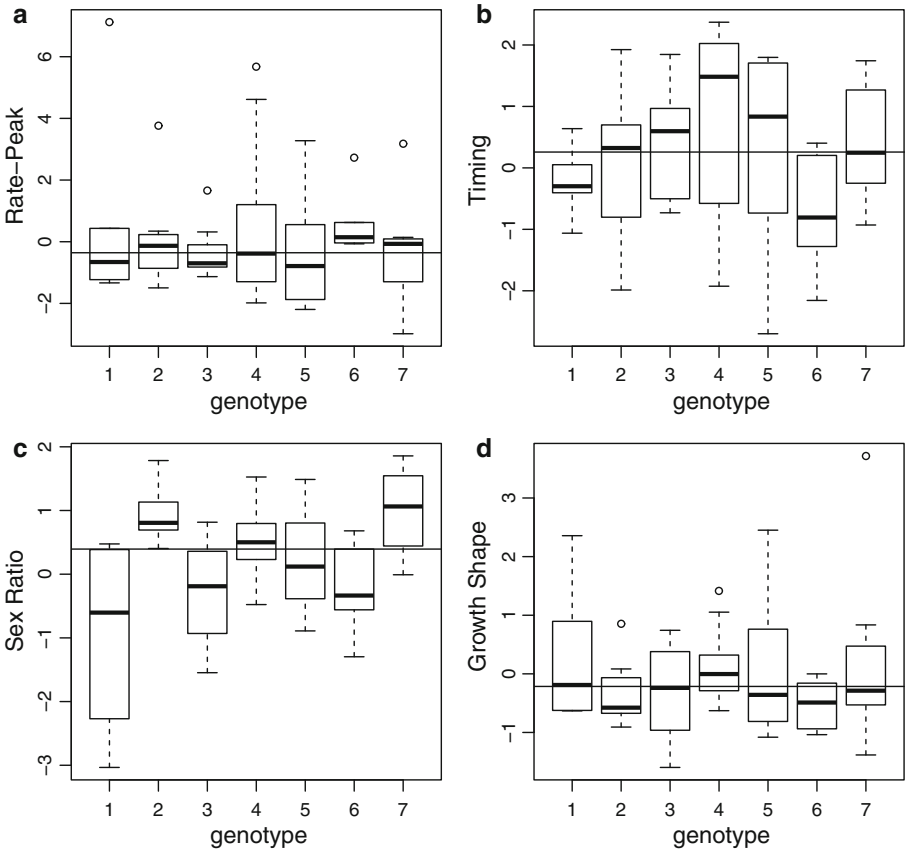

Fig. 2 Influence of donor infection (each with a single parasite genetic clone) on combined life history traits of *P. mexicanum* in its natural lizard host. Shown are distribution of **a** Rate/Peak, **b** Timing, **c** Sex ratio and **d** Growth Shape principal components for each donor. Data presented are from analysis excluding sex ratio with the exception of plot **c**. Donors 4 and 5 have the same microsatellite genotype at 4 scored loci and are therefore assumed to be the same clone. The *horizontal line* is the overall median. Shown are standard information for a *box plot*: the *middle* (*bold*) *bar* is the median, the *box* shows the interquartile range (IQR, range that includes middle 50 % of data), the whiskers extend to the highest and lowest data point within 1.5 × IQR of the *box*, and points are outliers

constraints. Further, we asked if variation for these clusters is based on genetic variation among parasite clones. Our results show that the 10 life history traits we measured are grouped into 4 independent clusters: Rate/Peak, Timing, Growth Shape, and Sex Ratio. These trait groups did not vary among parasite genotypes with the exception of Sex Ratio. We now discuss the biological relevance of the trait clusters and comment on the lack of clonal variation for these clusters except for Sex Ratio.

Rate/Peak

The Rate/Peak Principal Component indicates that the growth rate and maximal density (parasitemia) of asexual and gametocyte stages are linked into a single life history trait. It appears that faster growing infections are more capable of reaching a higher parasitemia during some fixed time. This confirms the pattern seen previously for this and other *Plasmodium* species (e.g. Mackinnon and Read 1999a; Eisen and Schall 2000), and supports the idea that rapid growth (and probable higher virulence) likely increases transmission opportunities through higher gametocytemia.

An oddity of the life history of mammalian malaria parasites is their apparent reproductive restraint; that is, although the gametocytes are the only cells that make the transition to the insect vector, few are produced in the blood (Taylor and Read 1997; Mideo and Day 2008; Pollitt et al. 2011). However, *P. mexicanum* (and other lizard malaria parasites we have examined) typically produces large numbers of gametocytes with no clear restraint, and here we again observed that infections with high asexual growth rate and peak parasitemia also had high gametocyte rate and peak. The fact that there is a positive rather than negative association between asexual and gametocyte numbers suggests a difference among infections of total energy investment rather than a difference in how that energy is allocated. Though cells that differentiate into gametocytes are no longer available to continue asexual proliferation (the classic trade-off between reproduction and somatic growth of life history theory, Gotelli 2008) the observed positive association between gametocyte and asexual parasite numbers may reflect variation in host suitability (resource availability, strength of immune response). Indeed, there is often a positive correlation between size and reproductive capacity among individuals (rather than species or clades) due to variation in their ability to obtain and assimilate resources, meaning that some individuals will have more energy to invest in both growth and reproduction (Van Noordwijk and De Jong 1986). Variation in host suitability would not be surprising because we performed our experiment using wild-caught lizards.

Timing

The Timing Principal Component includes the timing of asexual and gametocyte peak parasitemia and, to a lesser extent, patency in the blood after the day of inoculation. The relationship between asexual and gametocyte timing in reaching maximum parasitemia could indicate that asexual replication occurs for some fixed time, then switches to production of gametocytes, with the peak of gametocytes following the peak in asexual parasites on a fixed schedule. However, because most infections were still growing on the last date sampled (45/64 had maximum asexual count on last day sampled, 36/64 for gametocytes), the pattern may in part be indicative of variation in the number of days different hosts lived. Buckling et al. (1997, 1999) also found that gametocytes of *P. chabaudi* are produced on schedule in infections, but heavy mortality of asexual stage

parasites driven by introduction of several antimalarial drugs shifts gametocyte production earlier, exactly what life history theory predicts (Reznick and Endler 1982).

Growth Shape

The Growth Shape Principal Component describes the degree to which the growth trajectory of the infection fits an exponential curve. An exponentially growing infection results when there is a constant rate of growth (r in the exponential growth equation of $dN/dt = rN$). Deviations from exponential growth due to variation in r would occur when resources are shunted toward production of gametocytes, if the parasite experiences interference from the immune system, if resource availability varies over time, or if growth rate is adjusted for other reasons (such as gradual leveling off when a maximum parasitemia is reached). Most infections in our study grew close to exponentially (median $R^2 = 0.86$), but despite substantial differences among infections in shape (R^2 0.31–0.99) and rate of increase (400-fold for asexuals, 56-fold for gametocytes), Growth Shape was independent from other life history traits, including how rapidly the infection grew.

Sex Ratio

Gametocyte sex ratio was the only trait that showed significant variation among parasite genotypes. This result agrees with a previous study on replicate single-clone infections of *P. mexicanum* (Neal and Schall 2010) as well as data for two other *Plasmodium* species (*P. falciparum* in humans and *P. chabaudi* in rodents) (Burkot et al. 1984; Reece et al. 2008). The relative proportion of male and female gametocytes transmitted to the insect vector should play an important role in the number of mated parasites and thus ultimate transmission success into the next vertebrate host. That is, a bias toward females would result in a larger number of zygotes produced. Sex ratio theory predicts female-biased sex ratios for single-clone infections, but posits that mixed-clone infections should produce a more balanced gametocyte sex ratio when males of different clones compete for relative local success (Düsing 1884 (translation in Edwards 2000); Fisher 1930; Hamilton 1967; Schall 2009; West 2009).

Why should gametocyte sex ratio differ among single-clone infections and why should this variation have a genetic basis? We first suspected that other life history traits might constrain allocation of cells to males and females, but this proved incorrect. It is possible that sex ratio is linked to male gametocyte fecundity (Neal 2011), but this would beg the question of why there is genetic variation for such an important life history trait as male fecundity.

Variation among genotypes and environmental influences

Life history traits are assumed to be under strong selective pressure, yet heritable variation typically remains substantial for multicellular species (Mousseau and Roff 1987; Stearns 1992). Data for malaria parasites are still surprisingly scant, but genetic variation for life history traits has been reported for species including *P. falciparum*, the most important human malaria parasite, and *P. chabaudi* in laboratory experimental infections (reviewed by Reece et al. 2009). A previous study on *P. mexicanum* found that donor lizard whose blood was used to initiate replicate experimental infections accounted in part for variation in life history traits, but a confounding factor was lack of information on the clonal

diversity of these infections (Eisen and Schall 2000). Using only single-clone infections, we found no evidence of genetic variation for the life history traits summarized as Rate/Peak, Timing, and Growth Shape, which argues that the effect reported by Eisen and Schall (2000) was indeed driven by interaction among clones in mixed-genotype infections. Vardo-Zalik and Schall (2009) found that clonal diversity was associated with shifts in life history traits of *P. mexicanum*.

It is possible that the lizards used as the source of blood to initiate single-clone infections may have harbored closely related parasites with little genetic variation, and this led to the lack of detected genetic variation for life history traits. Conflicting with this notion, Sex Ratio shows a clone effect despite rather little variation in sex ratio among infections (36–58 % males). Additionally, these infections were taken from 5 unconnected sites, and a previous study showed that two of these sites' parasite populations are genetically differentiated from one another (Fricke et al. 2010).

What, then, accounts for the substantial variation observed in life history traits (Table 2)? Although we attempted to reduce possible environmental (host) variation by selecting only adult male lizards from a very restricted area at the field site, the hosts most likely differ physiologically (erythrocyte density, blood haemoglobin concentration, serum glucose levels, and immune competency). A high degree of environmental variation could make detection of a genotype effect less feasible. Previous studies have found variation in many physiological traits in these lizards (Schall et al. 1982; Schall 1990; Dunlap and Schall 1995), which may account for the variation in parasite life history traits observed. An intriguing question remains: are life history differences seen among hosts an adaptive response by the parasite to different environmental conditions, an adaptive interaction by both parasite and host, or simply an imperfect response of the parasite to environmental challenges?

Acknowledgments N. Hicks and J. Grauer assisted with the field experiments, and J. Grauer, K. St. Denis, N. Hicks, P. Teixeira, W. C. Stevens, D. Golschneider, and M. Lind assisted with the lab duties. We also thank the staff of the Hopland Research and Extension Center for all their help and support. The research was supported by funding from the US National Science Foundation (grant number DEB-0813832 to JJS) and ATN was supported by a US National Science Foundation Graduate Research Fellowship.

References

Al-Olayan EM, Williams GT, Hurd H (2002) Apoptosis in the malaria protozoan, *Plasmodium berghei*: a possible mechanism for limiting intensity of infection in the mosquito. Int J Parasitol 32:1133–1143

Anderson TJC, Williams JT, Nair S et al (2010) Inferred relatedness and heritability in malaria parasites. Proc R Soc Lond B 277:2531–2540

Barclay VC, Sim D, Chan BHK et al (2012) The evolutionary consequences of blood-stage vaccination on the rodent malaria *Plasmodium chabaudi*. PLoS Biol 10:e1001368

Bromwich CR, Schall JJ (1986) Infection dynamics of *Plasmodium mexicanum*, a malarial parasite of lizards. Ecology 67:1227–1235

Buckling AG, Taylor LH, Carlton JM et al (1997) Adaptive changes in *Plasmodium* transmission strategies following chloroquine chemotherapy. Proc R Soc Lond B 264:553–559

Buckling AG, Crooks L, Read AF (1999) *Plasmodium chabaudi*: effect of antimalarial drugs on gametocytogenesis. Exp Parasitol 93:45–54

Burkot TR, Williams JL, Schneider I (1984) Infectivity to mosquitoes of *Plasmodium falciparum* clones grown in vitro from the same isolate. Trans R Soc Trop Med Hyg 78:339–341

Charnov EL (1993) Life history invariants: some explorations of symmetry in evolutionary ecology. Oxford University Press, New York

de Roode JC, Helinski MEH, Anwar MA et al (2005) Dynamics of multiple infection and within-host competition in genetically diverse malaria infections. Am Nat 166:531–542
Dunlap KD, Schall JJ (1995) Hormonal alterations and reproductive inhibition in male fence lizards (*Sceloporus occidentalis*) infected with the malaria parasite *Plasmodium mexicanum*. Physiol Zool 68:608–621
Edwards AWF (2000) Carl Düsing (1884) on the regulation of the sex-ratio. Theor Pop Biol 58:255–257
Eisen RJ (2000) Variation in life-history traits of *Plasmodium mexicanum*, a malaria parasite infecting western fence lizards: a longitudinal study. Can J Zool 78:1230–1237
Eisen RJ, Schall JJ (2000) Life history of a malaria parasite (*Plasmodium mexicanum*): independent traits and basis for variation. Proc R Soc Lond B 267:793–799
Fisher RA (1930) The genetical theory of natural selection. Dover Publications, New York
Ford AM, Schall JJ (2011) Relative clonal proportions over time in mixed-genotype infections of the lizard malaria parasite *Plasmodium mexicanum*. Int J Parasitol 41:731–738
Fricke JM, Vardo-Zalik AM, Schall JJ (2010) Geographic genetic differentiation of a malaria parasite, *Plasmodium mexicanum*, and its lizard host, *Sceloporus occidentalis*. J Parasitol 96:308–313
Gotelli NJ (2008) A primer of ecology. Sinauer Associates, Sunderland
Gotelli NJ, Ellison AM (2004) A primer of ecological statistics. Sinauer Associates, Sunderland
Graves PM, Carter R, McNeill KM (1984) Gametocyte production in cloned lines of *Plasmodium falciparum*. Am J Trop Med Hyg 33:1045–1050
Guilbride DL, Guilbride PDL, Gawlinski P (2012) Malaria's deadly secret: a skin stage. Trends Parasitol 4:142–150
Hamilton WD (1967) Extraordinary sex ratios. Science 156:477–488
Joliffe IT (2002) Principal component analysis, 2nd edn. Springer, New York
Mackinnon MJ, Read AF (1999a) Genetic relationships between parasite virulence and transmission in the rodent malaria *Plasmodium chabaudi*. Evolution 53:689–703
Mackinnon MJ, Read AF (1999b) Selection for high and low virulence in the malaria parasite *Plasmodium chabaudi*. Proc R Soc Lond B 266:741–748
Martinsen ES, Perkins SL, Schall JJ (2008) A three-genome phylogeny of malaria parasites (*Plasmodium* and closely related genera): evolution of life history traits and host switches. Mol Phylogenet Evol 47:261–273
Mideo N, Day T (2008) On the evolution of reproductive restraint in malaria. Proc R Soc Lond B 275:1217–1224
Mousseau TA, Roff DA (1987) Natural selection and the heritability of fitness components. Heredity 59:181–197
Neal AT (2011) Male gametocyte fecundity and sex ratio of a malaria parasite, *Plasmodium mexicanum*. Parasitology 138:1203–1210
Neal AT, Schall JJ (2010) Gametocyte sex ratio in single-clone infections of the malaria parasite *Plasmodium mexicanum*. Parasitology 137:1851–1859
Osgood SM, Schall JJ (2004) Gametocyte sex ratio of a malaria parasite: response to experimental manipulation of parasite clonal diversity. Parasitology 128:23–29
Osgood SM, Eisen RJ, Schall JJ (2002) Gametocyte sex ratio of a malaria parasite: experimental test of heritability. J Parasitol 88:494–498
Osgood SM, Wargo A, Eisen RJ et al (2003) Manipulation of host's testosterone does not affect gametocyte sex ratio of a malaria parasite. J Parasitol 89:190–192
Paul RE, Day KP (1998) Mating patterns of *Plasmodium falciparum*. Parasitol Today 14:197–202
Perkins SL, Osgood SM, Schall JJ (1998) Use of PCR for detection of subpatent infections of lizard malaria: implications for epizootiology. Mol Ecol 7:1587–1590
Pollitt LC, Mideo N, Drew DR et al (2011) Competition and evolution of reproductive restraint in malaria parasites. Am Nat 177:358–367
Read AF, Day KP (1992) The genetic structure of malaria parasite populations. Parasitol Today 8:239–242
Reece SE, Drew DR, Gardner A (2008) Sex ratio adjustment and kin discrimination in malaria parasites. Nature 453:609–614
Reece SE, Ramiro RS, Nussey DH (2009) Plastic parasites: sophisticated strategies for survival and reproduction. Evol Appl 1:11–23
Reznick D, Endler JA (1982) The impact of predation on life history evolution in the Trinidadian guppies (*Poecilia reticulata*). Evolution 36:160–177
Schall JJ (1989) The sex ratio of *Plasmodium* gametocytes. Parasitology 98:343–350
Schall JJ (1990) Virulence of lizard malaria: the evolutionary ecology of an ancient parasite-host association. Parasitology 100:S35–S52
Schall JJ (1996) Malaria parasites of lizards: diversity and ecology. Adv Parasitol 37:255–333

Schall JJ (2009) Do malaria parasites follow the algebra of sex ratio theory? Trends Parasitol 25:120–123
Schall JJ, Marghoob AB (1995) Prevalence of a malarial parasite over time and space: *Plasmodium mexicanum* in its vertebrate host, the western fence lizard, *Sceloporus occidentalis*. J Anim Ecol 64:177–185
Schall JJ, St. Denis KM (2013) Microsatellite loci over a thirty-three year period for a malaria parasite (*Plasmodium mexicanum*): bottlenecks in effective population size and effect on allele frequencies. Parasitology 140:21–28
Schall JJ, Vardo AM (2007) Identification of microsatellite markers in *Plasmodium mexicanum*, a lizard malaria parasite that infects nucleated erythrocytes. Mol Ecol Notes 7:227–229
Schall JJ, Bennett AF, Putnam RW (1982) Lizards infected with malaria: physiological and behavioral consequences. Science 217:1057–1058
Stearns SC (1992) The evolution of life histories. Oxford University Press, New York
Taylor LH, Read AF (1997) Why so few transmission stages? Reproductive restraint by malaria parasites. Parasitol Today 13:135–140
Taylor LH, Walliker D, Read AF (1997) Mixed-genotype infections of malaria parasites: within-host dynamics and transmission success of competing clones. Proc R Soc Lond B 264:927–935
Van Noordwijk AJ, De Jong G (1986) Acquisition and allocation of resources: their influence on variation in life history tactics. Am Nat 128:137–142
Vardo AM, Schall JJ (2007) Clonal diversity of a lizard malaria parasite, *Plasmodium mexicanum*, in its vertebrate host, the western fence lizard: role of variation in transmission intensity over time and space. Mol Ecol 16:2712–2720
Vardo-Zalik AM, Schall JJ (2008) Clonal diversity within infections and the virulence of a malaria parasite, *Plasmodium mexicanum*. Parasitology 135:1363–1372
Vardo-Zalik AM, Schall JJ (2009) Clonal diversity alters the infection dynamics of a malaria parasite (*Plasmodium mexicanum*) in its vertebrate host. Ecology 90:529–536
West SA (2009) Sex allocation. Princeton University Press, Princeton