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Life history of a malaria parasite (*Plasmodium mexicanum*): independent traits and basis for variation

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Plasmodium mexicanum, a malaria parasite of lizards, exhibits substantial variation among infections in the life-history traits which define its blood-dwelling stages. Such variation in life histories among infections is common in *Plasmodium* and may influence the ecology and evolution of the parasite's transmission success and virulence. Insight into these issues requires identification of independent traits (some traits may be bound by developmental trade-offs) and the importance of genetic versus host effects producing the variation. We studied 11 life-history traits in 120 induced infections of *P. mexicanum* in its natural lizard host (20 each from six donor infections). The traits varied among infections and fell into three clusters: rate/peak (rate of increase and peak parasitaemia of asexuals and gametocytes), time (duration of pre-patent period and the infection's growth) and maturity (timing of first gametocytes). Thus, few life-history traits define an infection in the lizard's blood. Donor effects were significant for ten traits and two trait clusters (maturity was the exception) suggesting genetic differences among infections may influence the rate of increase and peak parasitaemia, but not the timing of the first production of gametocytes.

Keywords: *Plasmodium*; life history; gametocytes; *Sceloporus*

1. INTRODUCTION

An organism's life history includes its partitioning of assimilated resources towards growth and reproduction, as well as its schedule of developmental and reproductive events. Our current understanding of life-history biology is based mainly on free-living, multicellular organisms (Roff 1992; Stearns 1992). Despite their potential importance for human health and economics, much less attention has been given to understanding the life histories of microparasites, such as the malaria organisms (*Plasmodium*). Within its vertebrate host, the infection cycle of a malaria parasite mirrors the life history of multicellular organisms. For example, asexual proliferation of parasite cells in the host's blood represents the infection's somatic growth, its peak asexual density (peak asexual parasitaemia) is the maximum body size and the first production of sex cells (gametocytes) would be the infection's maturity. The analogy between the life history of a large organism and a malaria infection is imperfect because each malaria infection could consist of one to several genetically distinct clones rather than a single genotype of cells found in multicelluar organisms. However, clones of parasite cells within a host could coordinate life-history traits to reach some infection-wide optimum; as an example, clones can interact to evade the host's immune system (Gilbert *et al*. 1998).

Malaria parasites are a diverse group; more than 170 described species of *Plasmodium* exploit birds, mammals and, in particular, reptiles as vertebrate hosts (Schall 1996). Their life histories differ substantially between species (Garnham 1966), perhaps in part because natural selection has shaped the biology of each *Plasmodium* species in order to meet unique ecological challenges. In addition to between-species differences, investigators have long noted substantial variation in life-history traits among infections for each species of *Plasmodium* (Thomson 1911; Earle *et al*. 1939; Bruce-Chwatt 1963). What accounts for this intriguing between-host variation in the course of malaria infection? Life-history traits strongly influence an organism's fitness, so selection should erode any genetically based variation (Roff 1992); differences in the behaviour of malaria infections are therefore of intrinsic biological interest. The issue is also of great medical and public health significance because the life histories of parasites play a central role in their transmission biology (Taylor & Read 1998) and virulence (Bull 1994; Ewald 1994; Ebert & Mangin 1997).

One view is that the observed variation is non-adaptive for the parasite and only a reflection of host quality or variation in the number of parasite cells which initiated the infection (examples in Glynn 1994; Gravenor *et al*. 1995; Williams 1999). However, other evidence points to genetic differences between infections leading to life-history variation (Gilks *et al*. 1990; Taylor *et al*. 1997*a*, 1998; Mackinnon & Read 1999*a*,*b*) which could be maintained in the parasite metapopulation by selection (McKinnon & Read 1999*b*). Alternatively, clonal diversity in infections could itself alter the behaviour of each clone (Taylor *et al*. 1998). A minority view, although a venerable one (Thomson 1911; Boyd 1939), holds that variation between infections reflects adaptive phenotypic plasticity, such as a switch to gametocyte production when the host environment deteriorates (Buckling *et al*. 1997, 1999).

We approach the issue of variation in life-history traits of malaria parasites with a study of *Plasmodium mexicanum*, a parasite of western fence lizards in California, USA. As observed for other malaria parasites, the course of *P. mexicanum* infection in the lizard's blood varies substantially among hosts (Bromwich & Schall 1986; R. J. Eisen, unpublished data). We asked two questions. First, what is

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the relative importance of genetic differences between infections (genetic diversity and/or differing genotypes which directly control life-history traits) versus other factors (phenotypic plasticity of the parasite, host effects or random accidents during the parasite's life cycle)? Second, how many independent traits define an infection in the lizard's blood? Although many life-history traits can be described (we measured 11 for *P. mexicanum* infections), some of those traits could be biologically linked by some developmental constraint. Any study of the variation in life histories of malaria infections must begin with some understanding of the number of independent traits.

2. MATERIAL AND METHODS

(a) *Experimental infections*

Fence lizards are commonly infected (*ca.* 25%) at the University of California Hopland Research and Extension Center (HREC) in Mendocino County, California, USA. This parasite–host system has been under study for many years (reviewed in Schall 1996).

Six naturally infected adult male lizards (snout to vent length (SVL) > 64 mm) were collected from sites 0.5–2.75 km apart on the 2169 ha field station during late April; all six infections consisted primarily of asexual stages. We could not determine the number of genetically distinct clones present among the six donors. However, it is highly unlikely that the parasite could be uniclonal over the distances between the collection sites for the donors. Studies on *Plasmodium falciparum* in humans have shown clonal diversity in infections at sites with a high malaria prevalence, comparable to what is seen at the HREC site (Babiker & Walliker 1997). Further, the sex ratio of gametocytes in infections at the site is not strongly female biased (Schall 1989), suggesting that substantial genetic variation exists within and between infections (Read *et al*. 1992). To maximize the likelihood that infections were genetically dissimilar, we chose donors differing in an important life-history trait, asexual parasitaemia, which ranged from 1.2 to 10.2% infected red blood cells (RBCs).

Each of these six donors served to initiate infections in 20 adult male recipient lizards (a total of 120 induced infections). The recipients were collected from sites where malaria has been absent in the lizards over the past 20 years (Schall & Marghoob 1995). Examination of blood smears from these animals confirmed they were not already infected (extremely weak infections which are not detectable by such microscopic examination are rare at the HREC) (Perkins *et al*. 1998). Asexual parasitaemia was determined in the donor infections by counting 1000 RBCs at ×1000 magnification (Giemsa-stained thin blood smears) (Schall & Bromwich 1994). The density of the RBCs was determined using a counting chamber. A quantity of blood containing 200 asexual stages of *P. mexicanum* was mixed in vertebrate saline to a total volume of 20 µl of the blood–saline cocktail. This inoculum was injected into the recipient's intraperitoneal cavity.

We assumed that any donor effect seen in the recipient infections was caused by different genetic backgrounds for each donor infection. However, a spurious donor effect could result from some systematic bias in the way the recipients were infected from each infected lizard. To reduce or even eliminate this source of error, a preliminary experiment was conducted the previous summer in which 25 lizards were experimentally infected. The protocol was practised and perfected and was stringently followed during the present experiment. The recipients in each donor group were inoculated from the master mix of the blood–saline cocktail on the same day. During the preliminary study, a standard procedure was devised for keeping the cocktail uniformly mixed after repeated counts of cells using the counting chamber. All groups of lizards were inoculated over a six-day period (two groups on day 1 and one group on subsequent days, with one day during which no inoculations were performed); entering the day of inoculation into the ANOVA analysis reported here showed no day effect (data not given).

Following inoculation, the recipients were kept in six large (2.44 m × 2.44 m × 1.83 m) vector-proof outdoor cages outfitted with materials which simulated their natural habitat and placed in an area where infected lizards are common. Lizards from each donor group were evenly distributed among the cages. Each day lizards were fed to satiation with mealworms. From early May to mid-August, a drop of blood was taken weekly from a toe clip and used to prepare thin smears for staining with Giemsa (pH 7.0 for 50 min). The slides were first scanned until parasites were seen or for 6 min at ×1000 magnification during which around 10^4 RBCs were examined. One thousand RBCs were counted for all infected smears and the numbers of asexuals (trophozoites and schizonts) and gametocytes were noted.

(b) *Life-history traits*

The asexual stages and gametocytes of *P. mexicanum* occur within the erythrocytes and are similar to other species of *Plasmodium* in appearance and development (colour pictures of these stages are seen in Ayala (1970)). We measured 11 life-history traits for each recipient infection which are presented schematically in figure 1. First patency was determined as the number of weeks between inoculation and detection of the parasites in the blood. For the average growth rate of the infection (three measures, i.e. asexuals, gametocytes and total parasites), we fitted a least-squares regression line through the rising points using the number of parasites versus weeks after first patency in the blood. The growth rate is the slope of this line. To measure peak parasitaemia (again, three measures of asexuals, gametocytes and total parasites), we selected the maximum parasitaemia determined from the weekly blood smears. The timing of each measure of peak parasitaemia and first production of gametocytes (maturity of the infection) were calculated as weeks after the parasites were first seen in the blood. This method was used because the induced infections varied in their duration of the pre-patent period. Inoculated parasites were almost certainly taken up via the lymphatic system very quickly and efficiently (Ottaviani & Tazzi 1977), so the delay must have resulted from the initial replication rate of the parasites once in the blood.

(c) *Statistical analysis*

Principal components analysis was used to detect correlated traits and to reduce the original 11 traits to those that were independent. First, product-moment correlation values were calculated (some data transformed), then used to calculate loading values for each trait on the first three principal components. Varimax rotation (Kaiser 1958) allowed the detection of clusters of traits which were highly correlated. Traits with rotated factors > ± 0.6 were included together to define each component. The value for each principal component (the three new composite traits) was calculated for each of the 120 infections. We used ANOVA on the transformed values to test for significant

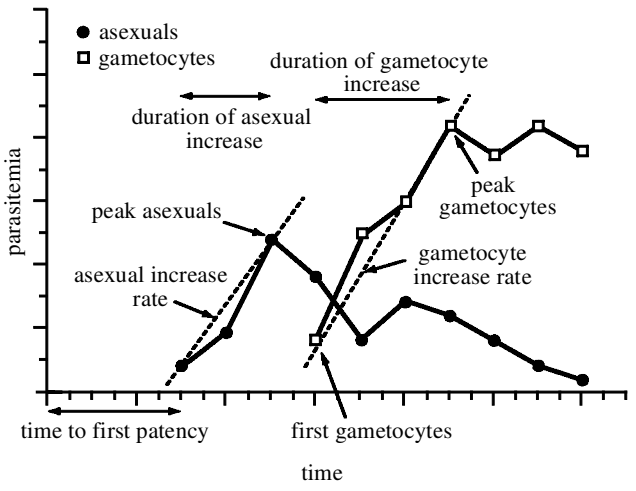

Figure 1. Schematic representation of life-history traits in the blood stages of the malaria parasite *P. mexicanum* in its fence lizard host. A description of the life-history traits is given in § 2(b). Dotted lines show regressions fitted to the data which allow calculation of the rates of increase. Total parasitaemia is not represented because it is simply the sum of the gametocyte and asexual parasite numbers. Values for the parasitaemia and time axes are not provided because these vary greatly between actual infections (table 1).

donor effects for the 11 original traits and the three principal components. A significant donor effect was assumed to represent the genetic component of the variation in life-history traits.

3. RESULTS

(a) *Variation in traits*

All experimental lizards became infected. Each of the 11 life-history traits varied substantially among the 120 induced infections (table 1). In some infections gametocytes were present as soon as the infection became patent in the blood, whereas in others gametocytes emerged as late as six weeks after patency and in one lizard gametocytes were never observed in the blood during the period of observation. The peak parasitaemia for both asexuals and gametocytes had already been reached in some infections at first patency and in others as long as 12 weeks later. For infections which ceased growing at first patency, we scored the rate of increase as zero. Other infections grew rapidly. The peak asexual parasitaemia varied from 0 to 400 parasites per 1000 erythrocytes.

(b) *Correlation between traits*

Table 2 presents the correlations between the measured life-history traits. Infections taking longer to patency tended to be those which ceased growth sooner and showed lower growth rates and peak parasitaemia, although those correlations were weak. Rapid asexual growth predicted higher asexual and gametocyte peak parasitaemia. The growth rates for asexuals and gametocytes were positively correlated, as was the peak parasitaemia for asexuals and gametocytes and the timing of these peaks. However, the timing of first production of gametocytes was either not correlated with other traits (eight traits) or only weakly so (two traits). The time until maturity was thus not dependent on the rate of increase of asexual cells nor the peak asexual parasitaemia.

(c) *Trait clusters*

The correlation between most traits (all but the time to maturity) suggests that only a few independent traits explain the life history of *P. mexicanum* in its blood stages. This was confirmed by the principal components analysis. The analysis revealed that the 11 life-history traits load into three clusters; these explained 83% of the total variation and included non-overlapping sets of traits. The first principal component, rate/peak, explained 59% of the variation and included the traits of growth rate for both the asexual stages and gametocytes (and the sum of these, the growth of overall parasitaemia) and the peak parasitaemia for both the asexual stages and gametocytes (and the sum of these, the total peak parasitaemia). The second principal component, time, explained an additional 16% of the total variation and included traits dealing with the timing of first patency and peak parasitaemia (asexual, gametocytes and total). The third principal component, maturity, explained 8% of the variation and included only the timing of the first appearance of gametocytes (the sexual maturity of the infection).

(d) *Origin of variation*

Ten life-history traits differed by donor (table 1); only the time to first production of gametocytes (maturity of infection) was independent of the donor. The composite traits rate/peak and time also differed by donor, but maturity did not. Tukey–Kramer post-hoc tests (for $p < 0.05$) compared the means for the composite traits (principal component values) between groups to determine whether recipient infections from only one or two donors were substantially different from the others and, thus, produce the significant donor effect. This was not the case. For peak/rate, the groups from donors 3 and 5 were significantly different from donors 1 and 4, for time, donors 1 and 4 differed from donor 3 and for maturity, donors 5 and 6 differed from donors 1–3. Other comparisons did not differ significantly. Thus, the significant donor effect originates from substantial complexity among the recipient groups in the three composite traits.

4. DISCUSSION

A complete life cycle of a malaria parasite includes the exoerythrocytic forms in the vertebrate host's liver and other tissues, the blood forms (asexual replication and production of gametocytes) and the complex sequence of events in the insect vector (Bruce-Chwatt 1985). We explored only the segment of the cycle which occurs within the blood, yet even that abbreviated picture can be deconstructed into a series of life-history traits. All these traits varied substantially among the induced infections of *P. mexicanum* and the variation was similar to that seen in natural infections followed in free-ranging lizards during mark–recapture studies (Bromwich & Schall 1986; R. J. Eisen, unpublished data).

We sought to determine how many of the measured traits are independent. Our results suggest that blood-dwelling infections of *P. mexicanum* are defined by only three traits: how fast they grow (and, thus, usually their final parasitaemia), how long they grow and when gametocytes first emerge in the blood. The independence of

Table 1. *Comparisons of life-history traits between groups of induced infections of the malaria parasite* P. mexicanum *in its natural host, the western fence lizard* Sceloporus occidentalis

(Each group of 20 lizards was inoculated with infected blood from one of six naturally infected lizards. The 11 life-history traits are described in the text. For each trait, the grand mean and range are given (non-transformed values). The single infection which never produced gametocytes is not included in the maximum gametocytaemia measure. Composite traits are those extracted by principal components analysis.)

	mean (range)	ANOVA test statistic	proportion of variation due to donor (r^2)	p-value
timing traits (weeks)				
first patency	6.9 (2–14)	$F_{(5,105)} = 21.45$	0.50	< 0.0001
first production of gametocytes	1.2 (0–6)	$F_{(5,105)} = 0.647$	0.03	0.6648
peak parasitaemia	5.0 (0–12)	$F_{(5,105)} = 8.43$	0.28	< 0.0001
peak asexual parasitaemia	4.3 (0–10)	$F_{(5,105)} = 3.10$	0.13	0.0119
peak gametocytaemia	5.6 (0–12)	$F_{(5,104)} = 7.43$	0.26	< 0.0001
peak traits (per 1000 RBCs)				
maximum parasitaemia[b]	101.5 (1–668)	$F_{(5,105)} = 6.64$	0.24	< 0.0001
maximum asexual parasitaemia[a]	52.0 (1–400)	$F_{(5,105)} = 5.85$	0.22	< 0.0001
maximum gametocytaemia[a]	58.4 (0–386)	$F_{(5,105)} = 5.31$	0.20	0.0002
rate traits (r)				
total parasites[c]	1.72 (0–8.6)	$F_{(5,105)} = 3.83$	0.15	0.0032
asexuals[c]	0.75 (0–5.2)	$F_{(5,105)} = 3.42$	0.14	0.0066
gametocytes[c]	0.95 (0–6.9)	$F_{(5,105)} = 4.16$	0.17	0.0017
composite traits				
rate/peak	—	$F_{(5,105)} = 7.58$	0.27	< 0.0001
time	—	$F_{(5,105)} = 2.09$	0.11	0.0367
maturity	—	$F_{(5,105)} = 0.79$	0.04	0.5594

[a] $\log_{10}$ transformations.
[b] ln transformation.
[c] $\ln(n + 1)$.

Table 2. *Product-moment correlation coefficients comparing pairs of life-history traits of* P. mexicanum

(Variables which were not normally distributed were subjected to an appropriate transformation. The life-history traits shown here are described in the text. Correlations of similar traits are followed by the same letter. Correlations which were not significant at $p < 0.05$ are shown as n.s. (non-significant).)

	rate traits		peak traits			time traits				maturity
	asexual r	gametocyte r	peak total	peak asexual	peak gametocyte	week patency	week peak total	week peak asexual	week peak gametocyte	week first gametocyte
total	0.812a	0.891a	0.917b	0.890b	0.877b	− 0.255c	0.458c	0.478c	0.402c	n.s.
asexual r	—	0.632a	0.734b	0.758b	0.652b	− 0.293c	0.375c	0.366c	0.351c	n.s.
gametocyte r	—	—	0.863b	0.807b	0.87b	− 0.267c	0.473c	0.428c	0.43c	n.s.
peak total	—	—	—	0.952d	0.947d	− 0.431e	0.636e	0.569e	0.599e	n.s.
peak asexual	—	—	—	—	0.833d	− 0.407e	0.533e	0.525e	0.545e	n.s.
peak gametocyte	—	—	—	—	—	− 0.374e	0.621e	0.479e	0.602e	n.s.
week patency	—	—	—	—	—	—	− 0.560f	− 0.398f	− 0.674f	− 0.197
week peak total	—	—	—	—	—	—	—	0.774f	0.799f	n.s.
week peak asexuals	—	—	—	—	—	—	—	—	0.623f	n.s.
week peak gametocytes	—	—	—	—	—	—	—	—	—	0.214

rate/peak from time presents a minor conundrum; if peak parasitaemia is the product of the rate of increase and the duration of the increase, how can the rate/peak principal component be independent of time? This is explained by the lower variation seen in the duration of the increase in parasitaemia compared to the variation in the rate of increase of the parasites. Information on the time traits therefore provides little additional information in explaining the variation in the peak traits and so time emerges from the analysis as an independent trait. A similar situation may exist for the timing of the first gametocytes or maturity of the infection. However, the variation in maturity of infections (zero to six weeks) was not much different for that of the duration of the overall parasite growth (zero to 12 weeks) or the duration of asexual growth (zero to ten weeks). The two growth measures were correlated with other traits, whereas maturity was only weakly correlated with two out of ten

other traits. It is possible that the entire suite of 11 life-history traits studied here can be collapsed into a single overall strategy by the parasite. However, we believe the substantial variation seen in the traits suggests that the principal component analysis reveals real biological clusters of traits.

To summarize these results, a sample infection will illustrate the interaction between the clusters of life-history traits. In this example, a rapidly growing infection quickly becomes patent in the blood and, once patent, continues to increase rapidly. Although infections vary in their duration of growth, this variation is substantially less than the variation in growth rate, so the growth rate of the infection is a good predictor of final asexual parasitaemia (which for this example should be high). The rapid growth rate and high parasitaemia of asexuals also predicts that the infection will produce a large number of gametocytes. The first production of gametocytes cannot be predicted because the maturity of the infection is independent of the growth rate or final parasitaemia. However, once gametocytes appear, their production comes at the expense of additional asexual replication. Asexual proliferation maintains a source for future gametocytes, so a trade-off must exist between present and future transmission.

Although most *P. mexicanum* infections follow these rules, a residual variance remains for all correlations. For example, some unusual infections grew rapidly, but ceased growth early, leading to an unusually low maximal parasitaemia and some slowly growing infections continued on to a higher parasitaemia because of a long duration of increase. If physiological trade-offs lead to the correlations between traits, the intriguing outlier infections indicate that violations of these trade-offs are possible.

These results reflect on a central tenet in discussions of the evolution of parasite virulence, that is rapidly growing parasite strains will reach higher parasitaemia and be more readily transmitted (Ewald 1994; Mackinnon & Read 1999*a*). This is generally true for *P. mexicanum*, yet some unusual infections violate this rule. More striking is the fact that the variation in the timing of the production of first gametocytes in an infection of *P. mexicanum* is low relative to other timing traits and is not correlated with the rate of increase of either asexuals or gametocytes after they are produced and the infection's maturity stands as a distinct trait. Therefore, it is possible that some rapidly growing infections could have relatively low transmission success if they produce gametocytes later in the infection. The density of gametocytes is positively correlated with transmission success for *P. mexicanum* (Schall 1996), but the total transmission success of an infection will also depend on the duration of the presence of gametocytes in the lizard host's blood. The vectors *Lutzomyia vexator* and *Lutzomyia stewartii* are active throughout the warm season (Chaniotis & Anderson 1968), but are most abundant in late summer (Schall & Marghoob 1995). Infections which become established late in the season do not grow faster than early starters (Bromwich & Schall 1986). Perhaps this is why maturity is not linked to the growth rate or parasitaemia; gametocytes can even emerge very early in an infection when asexual parasitaemia is low. In fact, some of the induced infections had produced gametocytes by the first patency of the infection (thus, the maturity was scored as zero weeks). This would allow transmission in infections which start late in the season when vectors are common but when the winter dormancy period looms.

We found a significant donor effect for all traits measured except the timing of the first production of gametocytes (maturity). This donor effect could be the result of several factors. First, different parasite genotypes could produce different life-history phenotypes. The donor effect could be a result of different genotypes for life-history traits which existed among the original six infections. Genetic control over life-history traits has been observed for some other species of *Plasmodium*. For example, Mackinnon & Read (1999*a*,*b*) isolated clonal lines of *Plasmodium chabaudi* and found fixed differences in the maximal asexual and gametocyte parasitaemia among clones. Second, differences in the number of clones per infection could themselves result in variation in life histories even if each genotype when solitary were to yield the same overall life history. Taylor *et al*. (1997*a*,*b*) demonstrated that the gametocyte density and asexual parasitaemia of *P. chabaudi* infections is significantly higher in mixed-clone infections relative to single-clone infections of the genotypes comprising the mixed-clone infections. Therefore, if donor infections differed in the number of clones per infection, strain interaction could lead to variation between donor groups. Third, concurrent infections with other species of parasites in donor lizards could lead to between-group variation (reviewed in Mackinnon & Read 1999*b*). We did not detect any other species of blood parasite in the donor lizards, such as *Trypanosoma* or viruses which form clusters within erythrocytes, which are visible under the light microscope (Telford 1984). Therefore, we believe this effect is less likely than the explanations presented above. Finally, between-group variation could be driven by non-genetic differences among the maternal infections which were carried into the recipient hosts. However, we know of no similar examples in *Plasmodium* infections.

No data describing the genetic structure of *P. mexicanum* populations are available. However, based on empirical data indicating that genetic variation is substantial both within and between infections in other *Plasmodium* species (Babiker & Walliker 1997; Paul *et al*. 1998; Konate *et al*. 1999; Smith *et al*. 1999), we favour a genetic explanation for variation between donor groups. However, we were unable to distinguish between genotype differences between donor infections or differences due to competition between clones. If the former scenario explains between-donor group variation, this demonstrates that genetic diversity for life-history traits remains in the parasite metapopulation. If the latter scenario is true, this also suggests that there are multiple genotypes in the population (although these genotypes may not differ in their life-history traits when solitary in an infection) and there must be substantial phenotypic plasticity between strains such that, in different competitive environments, the strain displays variation in any given trait (Roff 1992; Stearns 1992). In any case, it is intriguing that the proportion of the variation in life-history traits which was accounted for here by the donor effect (and, thus, perhaps genetic variation) was comparable to that seen in

many studies on the heritability of life-history traits in large multicellular organisms (often *ca.* 20%) (Roff 1992).

Although this discussion has centred on the significant donor effect observed, no donor effect was seen for maturity and a large residual variation remained for peak/rate and time. We have no way of distinguishing between host effects on the parasite (such as different immune competency of the 120 recipient lizards) which would disrupt any 'optimal' life history for the parasite and possible adaptive phenotypic plasticity which allows the parasite to mould its life history to prevailing host conditions. A companion experiment (R. J. Eisen and D. F. DeNardo, unpublished data) altered the testosterone levels of recipient lizards and, thus, caused major physiological changes (Nelson 1995), yet no treatment effect was seen on the rate, time or peak traits of induced *P. mexicanum* infections. This experimentally induced variation in host quality caused no predictable changes in the induced infections save for maturity, the only trait found here to be independent of donor infection.

Thus, one life-history trait deserves special attention; the first production of gametocytes was an independent trait and no donor effect was observed. Although the ability to produce gametocytes and how many are produced appears to have a strong genetic component in *Plasmodium* (Vanderberg & Gwadz 1980; Graves *et al*. 1984; Carter & Graves 1988), the timing of the production of gametocytes has long been suspected to be driven by deterioration in the host environment (Thomson 1911; Carter & Graves 1988). As conditions become unsuitable for further asexual growth, the parasite switches to the only stages which can leave the host, the gametocytes. The proximate cues used by the parasite can include the host's immune response (Cornelissen & Walliker 1985), the production of stress hormone by the host (Maswoswe *et al*. 1985) and even novel chemical stressors presented by various anti-malarial drugs (Buckling *et al*. 1997, 1999; Butcher 1997). The mechanism used by the parasite in detecting such a broad array of signals and then altering its development to produce gametocytes is unknown (Sinden 1983; Carter & Graves 1988), but the very fact that *Plasmodium* species have evolved a suite of transduction pathways illustrates the importance of plasticity in this life-history trait. Although all of our recipient lizards were adult males and they were kept under similar conditions, there must have been substantial differences in host quality to provoke the range of the first appearance of the gametocytes (immediately upon patency to six weeks after that time and one infection which never produced gametocytes).

We conclude that the three independent life-history traits, rate/peak, time and maturity, vary substantially between infections of *P. mexicanum*. It is likely that this variation has a genetic element, but also a great deal of residual variation remains. Boyd's (1939) view that such variation, as is seen in *Plasmodium*, has an adaptive function, i.e. to mould infections to local (host) conditions, may have been prescient. If so, understanding variation in life history traits will be critical, not just for general studies on the life-history biology of parasites, but in efforts to control human malaria's dreadful toll on human populations.

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