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LIFE HISTORY OF A MALARIA PARASITE (*PLASMODIUM MEXICANUM*) IN ITS HOST, THE WESTERN FENCE LIZARD (*SCELOPORUS OCCIDENTALIS*): HOST TESTOSTERONE AS A SOURCE OF SEASONAL AND AMONG-HOST VARIATION?

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ABSTRACT: The course of infection of a malaria parasite (*Plasmodium mexicanum)* is highly variable in its host, the fence lizard (*Sceloporus occidentalis*). However, a seasonal trend is superimposed on this variation such that gametocyte production is intensified during mid- to late summer. Host testosterone levels follow a similar seasonal fluctuation and are variable among individual lizards. We sought to determine if testosterone levels affect seasonal and among-host variation in 11 *P. mexicanum* life history traits: rate of increase in level of infection (3 measures), peak parasitemia (3 measures), duration of increase (3 measures), time to detectable infection, and timing of production of gametocytes. We followed the course of infection in 125 male *S. occidentalis,* each randomly assigned to 1 of 4 treatment groups: castrated, castrated and implanted with exogenous testosterone, sham implanted, and unmanipulated controls. Median values for the 11 life history traits did not differ among treatment groups, and variances were homogeneous among the treatment groups for 10/11 traits. However, elevated testosterone significantly reduced the variation in timing of the onset of gametocyte production. Therefore, testosterone does not appear to be a primary regulator of *P. mexicanum* life history, yet testosterone may have some effect on when gametocytes first become detectable.

Life history traits vary substantially among species of *Plasmodium,* among strains within species, and among infections of a single parasite species in a local area (Graves et al., 1984; Bruce-Chwatt, 1985; Bromwich and Schall, 1986; Taylor et al., 1997a). For example, the number of gametocytes produced in the blood by an infection (a life history trait of major significance for transmission to the vector) ranges from scarcely detectable in some species to massive in others (Taylor and Read, 1997) and even differs among infections for a single species (Bromwich and Schall, 1986). The evolutionary and ecological significance of this variation in life history traits remains elusive (Day et al., 1992; Buckling et al., 1997, 1999; Taylor et al., 1997a, 1997b). Our study centers on the variation seen among infections within a population of lizards infected with a malaria parasite. Some of this life history variation appears to be of genetic origin, including sex ratio, asexual replication rate, and peak parasitemia (Taylor et al., 1997b; Mackinnon and Read, 1999).

Environmental factors also contribute to life history variation. For instance, host genetics can affect the life history of parasites in general and malaria parasites in particular (Molineaux, 1988; Grosholtz, 1994; Sorci et al., 1997). In addition, changing host conditions can act as cues for the parasite to time events during the infection (Maswoswe et al., 1985; Lingnau et al., 1993; Cornelissen and Walliker, 1995; Gautret et al., 1996), including rapid production of gametocytes when conditions for the parasite deteriorate in the host (Buckling et al., 1997, 1999). Thus, variation in the parasite's life history traits could reveal adaptive changes that maximize the reproductive success of the parasite.

Malaria parasites are widespread in lizard hosts; 77 species of *Plasmodium* have been found in lizards on all the warm continents except Europe (Schall, 1996). Among the best known ecologically is *P. mexicanum,* a parasite of western fence lizards (*Sceloporus occidentalis*) in California (Schall, 1996). Both newly established infections and infections that have overwintered follow a general seasonal trend in which asexual forms predominate in early spring and density of the gametocytes is highest in late summer (Ayala, 1970; Bromwich and Schall, 1986). The timing of gametocyte production and its peak density are critical to transmission success because the abundance and flying activity of vectors, 2 species of psychodid sandflies (*Lutzomyia vexator* and *L. stewartii*) peak in late summer (Chantiotis and Anderson, 1968; Ayala, 1977; Schall and Marghoob, 1995; Schall, 1996). In addition, weak evidence suggests that parasites that initiate infections early in the summer have slower asexual replication rates than those beginning later (Bromwich and Schall, 1986). Although these seasonal trends are apparent from examination of many infections, the course of infection of *P. mexicanum* is also highly variable among individual infected lizards (Thompson and Huff, 1944a, 1944b; Ayala, 1977; Schall, 1996). Such variable parasite life history traits include replication rate, peak parasitemia, timing of first gametocyte production, and time to peak parasitemia (Bromwich and Schall, 1986; Schall, 1989).

We examined the possible role of host testosterone as a cue for *P. mexicanum* to seasonally modify their life histories. Reproductive hormones in the blood of both male and female fence lizards change seasonally but also vary among lizards at any given time (Moore and Marler, 1987; Saad et al., 1990; Dunlap and Schall, 1995). For example, testosterone levels in males peak in the spring with the beginning of the activity period and breeding season. Thus, if reproductive hormones such as testosterone are used by the parasite as a seasonal cue, both the general pattern seen in *P. mexicanum* infections and variation among lizards could be explained by differences in hormone titers. We therefore examined the role of host testosterone as a cue for parasites to follow seasonal adaptive changes in their life history traits. Host testosterone could affect the infection in 2 nonexclusive ways. First, the parasite cells could detect testosterone directly and use it as an indication of season. Second, testosterone is known to be immunosuppressive in vertebrates, including lizards (Saad et al., 1990; el Masri et al., 1995; Salvador et al., 1995; Veiga et al., 1998). Results of many

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theoretical and empirical studies have suggested that differences in the host's immune defenses may explain the variation in the course of infection of malaria parasites (Day et al., 1992; Gravenor et al., 1995; Hetzel and Anderson, 1996; McKenzie and Bossert, 1997), as may be true for *P. mexicanum*. In general, malaria is more common in males of many lizard host species (Ayala and Spain, 1976; Schall and Vogt, 1993; Schall, 1996), suggesting that immune suppression by testosterone may be involved.

Our goal was to experimentally manipulate testosterone levels of male lizards to determine if the hormone acts as a cue to alter 11 life history traits of experimentally induced *P. mexicanum* infections. We sought to determine if males with experimentally reduced testosterone levels hosted infections that would mimic those seen in late summer, with reduced asexual replication and an increase in gametocyte production. In addition, we examined the effects of testosterone on among-host variation in the same life history traits. Our experiment was not designed to differentiate between the parasite cueing directly on testosterone levels or on testosterone-induced changes in the host.

MATERIALS AND METHODS

The research was conducted at the University of California Hopland Research and Extension Center, an approximately 2,145-ha track of oak woodland located near the town of Hopland in southeastern Mendocino County, California. Adult male *Sceloporus occidentalis* (snout–vent length $>$ 64 mm) were collected from 16 to 30 April 1999 using a slip-noose affixed to the end of a pole or, occasionally, by hand. Recipient lizards were collected at sites where malaria infection prevalence was known to be low ($<$5%), whereas potential donor lizards were collected in high-prevalence areas, based on data collected over the past 20 yr (Schall and Marghoob, 1995). Lizards were maintained in cloth sacks until late afternoon when they were brought into the laboratory, toe-clipped for permanent identification, and given an easily visible unique mark on their dorsal surface with nontoxic Liquid Paper®. A drop of blood was obtained from a toe clip to prepare a thin blood smear. Each smear was stained with Giemsa and examined at $\times$1,000 for 6 min to determine *P. mexicanum* infection status. In a 6-min scan, approximately 10,000 red blood cells (RBCs) can be observed (Bromwich and Schall, 1986; Schall, 1996). If no malaria parasite was detected, the lizard was considered to be uninfected. Comparisons of weak infections using microscopy and the polymerase chain reaction indicate that failure to detect weak infections ($<$1/10,000 RBCs) by microscopy is rare at the study site (Perkins et al., 1998).

To demonstrate the effects of removal of testosterone on parasite life history traits, we randomly assigned uninfected lizards (n = 125) to 1 of 4 groups. A prospective power analysis showed that the total sample size used in this study was adequate to detect effect sizes greater than or equal to that observed in a previous experiment (Eisen and Schall, 2000) using blood-induced infections to compare differences in life history traits among blood donor groups. To minimize testosterone levels, a group of lizards (n = 33) was castrated through bilateral flank incisions while under isoflorane anesthesia (DeNardo and Licht, 1993). Other lizards (castrate + testosterone group; n = 22) were castrated and also implanted intraperitoneally with testosterone (T1500, Sigma Chemicals, St. Louis, Missouri) in a 3-mm silastic tube (0.078 mm inside diameter, 0.125 mm outside diameter, Dow Corning, Midland, Michigan) sealed at both ends with silicon glue (DeNardo and Licht, 1993). These lizards thus would have had high levels of testosterone and served as controls for any effect of castration other than minimizing testosterone levels. An unmanipulated control group (n = 41) did not undergo any surgical procedures, and therefore would have had moderate levels of testosterone. A sham-implant group (n = 29) was left gonadally intact but was implanted subcutaneously with 3-mm saline-filled silastic implants to serve as a surgical controls. These lizards would have had testosterone levels similar to those of the controls.

We could not compare testosterone levels among groups at the time of parasite inoculation because blood sampling would have removed large numbers of RBCs and could have altered the course of infection. Similarly, we did not sample testosterone at the end of the experiment because these lizards were used in an additional study in which blood loss for radioimmunoassays would have been detrimental. However, previous studies have demonstrated that castration of lizards significantly reduces plasma testosterone levels, whereas testosterone implants significantly elevate plasma testosterone levels relative to controls (Moore, 1987; DeNardo and Licht, 1993). Although not quantified, observations made after the manipulations supported the effectiveness of the manipulations. The ventral scales of castrated lizards were pale in comparison to the bright blue color displayed by the other groups. In addition, relative to the castrated group, a dominance hierarchy appeared to be better established in the intact, intact + saline, and castrated + testosterone groups. When lizards were fed, more displaying (push ups and head bobbing) was observed in the castrated + testosterone group, and some lizards in that group consistently ate before the others, chasing off any that challenged the dominant lizard.

All lizards were housed by treatment (23–30 lizards per enclosure) in 1.82- $\times$ 1.82- $\times$ 2.40-m vector-proof outdoor enclosures to eliminate the possibility of *P. mexicanum* transmission by the natural sandfly vectors. Based on other castration studies using congeneric lizards (Moore, 1987; Moore and Marler, 1987), lizards were given 2 wk to recover from surgery and to respond to induced changes in hormone levels.

Each uninfected lizard was inoculated intraperitoneally with approximately 200 asexual parasites (trophozoites and schizonts) in a total volume of 20 μl of blood–saline cocktail. The parasites for the inoculation of all lizards originated from blood taken from the retroorbital sinus of a single naturally infected lizard, this minimizing the variation in the inoculum among lizards. The density of infected cells in the donor was determined using microscopy and a cell counting chamber.

Each week for 18 wk, a drop of blood was obtained from each lizard by clipping a toe, and a thin smear was made for Giemsa staining. The thin smears were examined at $\times$1,000 for 6 min or until an infected host cell was observed. For infected smears, 1,000 RBCs were counted, with an effort to sample the entire smear randomly, and numbers of asexual stages and gametocytes per 1,000 RBCs were determined. A preliminary experiment showed no significant differences in the estimated intensity of infection when 1,000, 2,000, and 3,000 RBCs were counted (unpubl. data).

Based on these cell counts, we measured 11 life history traits for each recipient infection. Timing of the appearance of the first parasite was determined as the number of weeks between inoculation and detection of the parasites in the blood. For average rate of increase of the level of infection (3 measures of numbers of asexual forms, gametocytes, and total parasites), we fit a least-squares regression line through the rising points using number of parasites versus week after first parasite was detected in the blood. Rate of increase is the slope of this line. To measure peak parasitemia (3 measures of numbers of asexual forms, gametocytes, and total parasites), we selected the maximum parasitemia observed from the weekly blood smears. Timing of each measure of peak parasitemia and first production of gametocytes were calculated as the week after parasites were first seen in the blood.

None of the measurements for the 11 traits was normally distributed, and many distributions could not be rendered normal via transformations; therefore, we compared all 11 traits among experimental groups using Kruskal–Wallis and Mann–Whitney *U*-tests (Sokal and Rohlf, 1995). Homogeneity of variance among treatment groups was tested using O'Brien tests (O'Brien, 1979).

RESULTS

For all 11 life history traits measured, no differences were detected in median values among the 4 treatment groups (Tables I, II). Pooling the sham and control groups had no effect on the results (Kruskal–Wallis tests with χ^2 approximations; $P > 0.05$ for all traits compared). That is, lizards with lower than normal testosterone levels (the castrated group) did not differ in any life history trait from lizards with normal (unmanipulated con-

TABLE I. Kruskal–Wallis tests with chi-square approximations were used to compare 11 life history traits of *P. mexicanum* among fence lizards in 4 different treatment groups: castration, castration plus testosterone implant, unmanipulated control, and sham-implanted group gonadally intact with saline implant.

Trait	χ^2, df = 3	*P*
First parasite seen on blood smear	3.61	0.307
Timing (weeks after first positive smear)		
First gametocyte	1.56	0.661
Peak parasitemia	3.92	0.270
Peak asexual parasitemia	2.33	0.507
Peak gametocytemia	2.13	0.547
Abundance (number of parasites/1,000 RBCs)		
Maximum parasitemia	1.24	0.743
Maximum asexual parasitemia	1.58	0.665
Maximum gametocytemia	1.54	0.673
Growth rates (parasites/day)		
Average total	3.82	0.282
Average asexual	1.51	0.681
Average gametocyte	5.12	0.163

trol and sham group) or unnaturally elevated (castrated + testosterone groups) testosterone levels. Surgical manipulation itself did not significantly alter the life history of *P. mexicanum* infections (combining the 3 surgery groups to compare with the single unmanipulated group, Mann–Whitney *U*-test; $P > 0.05$).

Variance for all life history traits did not differ detectably for the group with lowest testosterone (castrated) compared with the group with normal testosterone (unmanipulated control) (O'Brien test, $P > 0.05$). The groups with lowest testosterone (castrated) and highest testosterone (castrated + testosterone) showed homogeneity of variance for all traits except for when gametocytes were first detectable (O'Brien test; $P = 0.02$). The variation in timing of first production of gametoctyes was significantly reduced in the lizards with the highest testosterone levels.

DISCUSSION

The traits defining the course of infection of a malaria parasite vary substantially among hosts (Jordan, 1957; Bruce-Chwatt, 1985; Taylor et al., 1997b). Much of this variation has been attributed to differences in the number of parasites that start the infection (Glynn, 1994), parasite genetics (Taylor et al.. 1997b; Mackinnon and Read, 1999), host genetics (Molineaux, 1988), or various host conditions (Sinden, 1983). This variation is found in *P. mexicanum*, both in natural infections in free-ranging lizards (Bromwich and Schall, 1986) and in experimentally induced infections (Eisen and Schall, 2000). Depending on the trait, from 13% to 50% of the observed variation can be accounted for by genetic differences among infections, with only the time of detection of gametocytes being entirely phenotypically plastic (Eisen and Schall, 2000). Our experimental infections were initiated with equal volumes of blood from a single donor and were thus likely to have similar initial parasite population sizes and genetic composition; therefore, the variation in parasite life history traits should be driven entirely by differing host environments.

Part of the variation seen in life history traits of *P. mexicanum* may be adaptive. As an example of how infections may conditionally match the host environment, there is a seasonal trend in the course of infection of *P. mexicanum* in *S. occidentalis*. We sought to determine whether testosterone, which itself follows a seasonal cycle in male fence lizards, acts as the primary timing cue for the parasite. Additionally, because testosterone levels among male lizards differ at any given time (Saad et al., 1990; Dunlap and Schall, 1995; el Masri et al., 1995), we sought to determine whether this variation accounts for the residual variation observed in *P. mexicanum* life history traits.

We found little evidence that lizard testosterone regulates life history traits of *P. mexicanum.* Measures of population growth of asexual cells and gametocytes, peak parasitemia of both types of cells, and the timing of these peaks all were similar in their distributions (median values or variance). Therefore, we concluded that testosterone levels do not act as the primary cue

TABLE II. Median value (range) for 11 life history traits of *P. mexicanum* in fence lizards exposed to 4 different treatments: castration, castration plus testosterone implant, unmanipulated control, and sham-implanted gonadally intact with saline implant.

Trait	Castrated	Castrated + testosterone	Control	Sham implanted
First parasite seen on blood smear (wk postinoculation)	8.0 (5.0–12.0)	7.0 (6.0–11.0)	8.0 (5.0–11.0)	8.0 (4.0–11.0)
Timing (weeks after first positive smear)				
First gametocyte	2.0 (0.0–4.0)	1.0 (0.0–2.0)	1.0 (0.0–4.0)	0.5 (0.0–4.0)
Peak parasitemia	7.0 (1.0–12.0)	5.0 (0.0–8.0)	6.0 (0.0–10.0)	8.0 (0.0–11.0)
Peak asexual parasitemia	5.00 (0.0–12.0)	5.0 (0.0–8.0)	5.0 (0.0–11.0)	6.0 (0.0–11.0)
Peak gametocytemia	5.0 (1.0–12.0)	5.0 (0.0–8.0)	6.0 (0.0–10.0)	6.0 (2.0–10.0)
Abundance (number of parasites/1,000 RBCs)				
Maximum parasitemia	51.0 (13.0–803.0)	201.0 (21.0–652.0)	62.5 (1.0–968.0)	78.0 (14.0–558.0)
Maximum asexual parasitemia	38.0 (7.0–552.0)	99.0 (11.0–475.0)	46.5 (1.0–823.0)	50.0 (11.0–338.0)
Maximum gametocytemia	30.0 (1.0–320.0)	82.0 (7.0–177.0)	33.5 (0.0–279.0)	30.0 (2.0–220.0)
Growth rates (parasites/day)				
Average total	0.9 (0.0–17.3)	3.5 (0.5–12.1)	0.9 (0–18.1)	1.7 (0.0–9.5)
Average asexual	1.7 (0.0–14.7)	2.6 (0.0–8.6)	0.9 (0.0–14.7)	0.9 (0.0–8.6)
Average gametocyte	0.9 (0.0–6.0)	1.7 (0.1–4.3)	0.6 (0.0–4.3)	0.55 (0.0–4.32)

for seasonal trends in the life history of *P. mexicanum.* There may not be a single cue that alone regulates the course of infection of a malaria parasite. The timing of each event during an infection, such as the trade-off in asexual replication and production of gametocytes, is critical to the success of parasite transmission. Therefore, the integration of multiple cues would be likely. However, the near absence of any effect of major changes in testosterone levels is surprising because changes in other environmental conditions such as light and temperature (Stauber, 1939), suitable host cell density (Trager et al., 1999; Williams, 1999), hormonal titers (Masoswe et al., 1985; Lingnau, 1993), and administration of antimalaria compounds (Buckling et al., 1997, 1999) do alter the course of infection for other malaria parasites. It is possible that the correlation between lizard testosterone levels and changes in the parasite life history traits is a result of the lizard and the parasite responding to the same environmental cues (such as light or temperature). The lizards in our experiment were exposed to natural light and thermal conditions. The parasite may be able to discard cues that are scored as unreliable when they conflict with other environmental information.

One trait examined in the present study deserves additional scrutiny because it was the only life history trait affected by host testosterone levels. When differences in testosterone levels were intensified (comparing castrated and castrated + testosterone groups), the lizards with unnaturally elevated testosterone showed a significant reduction in variance for one trait (timing of first production of gametocytes). This finding could simply be due to a type I error. However, we find it curious that the one trait that was not controlled by parasite genetics was the only trait to show a response to variation in host testosterone levels. In a previous study, Eisen and Schall (2000) found that parasite genetic variation among infections explained a significant amount of phenotypic variation for 10 of the 11 life history traits used in this study, but timing of gametocyte production was phenotypically plastic. To determine whether these findings are spurious or real, more detailed study of the effect of testosterone on the timing of gametocyte production is needed.

Regardless of the possibility that testosterone might regulate gametocyte production (Lingnau, 1993), the primary regulator of *P. mexicanum* infections and the source(s) of the extreme variation in infections remains unknown. Variations in environmental conditions (such as light and temperature) at both the macro- and microhabitat levels could explain both the seasonal regulation of the parasite cycle and the variation seen among individual infections. Alternatively, host characteristics independent of testosterone could be primarily responsible for differences among infections. Because of the medical and conservation importance of understanding the natural regulation of malarial infection, the relationship between the host's condition and parasite infection warrants further study.

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