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Thermal ecology of a malarial parasite and its insect vector: consequences for the parasite's transmission success

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Summary

1. We examined the transmission biology of *Plasmodium mexicanum*, a parasite of the fence lizard *Sceloporus occidentalis*, and its vector, the sandfly *Lutzomyia vexator*. Female *L. vexator* produced a clutch of eggs after each blood meal taken from a lizard. Mortality was high after oviposition, so few sandflies were likely to take two blood meals and almost none took three. Therefore, to maximize its transmission success, the parasite must complete development in its insect host before the vector lays its eggs and takes another blood meal.

2. Between 16°C and 32°C, temperature did not affect the longevity of female sandflies, but did affect the rate of parasite development in the insect, the rate of maturation of sandflies' eggs, and the probability of sandflies becoming infected.

3. The above relationships with temperature were non-linear and differed in shape among the variables such that an increase in temperature between 22°C and 32°C benefited the parasite by shortening its development while not reducing the time until the sandfly's next blood meal.

4. We measured the temperatures available to the vectors in nature (burrows of ground squirrels). Within this range, there was a window that allowed successful transmission of the parasite (based on laboratory studies).

5. In a thermal gradient, unfed female sandflies selected mean temperatures approximately 4°C below the minimum required for transmission. After a blood meal from a non-infected lizard, the insect's mean preferred temperature increased 1·6°C, presumably to aid digestion, and if a blood meal was taken from an infected lizard mean preferred body temperature increased by 3·6°C.

6. Compared with 10 other *Plasmodium* species, *P. mexicanum* has a very rapid rate of development in its vector.

7. The results suggest *P. mexicanum* enhances its transmission success through a combination of rapid development in the insect host and manipulation of the vector's thermoregulatory behaviour.

Key-words: behavioural manipulation, *Lutzomyia vexator*, malaria life cycles, parasite, *Plasmodium mexicanum*, *Sceloporus occidentalis*, transmission success.

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Introduction

Many parasites have evolved complex life cycles that often include the use of a mobile host, or vector, which facilitates the parasite's transmission between individuals of the second host species. In such systems natural selection must favour a developmental schedule of the parasite for each life stage that is timed to ensure escape from one host to another. This is because a host's immune attack can make the parasite's habitat intolerable over time, and also because each individual host eventually dies.

Malarial parasites (*Plasmodium* spp.) provide an excellent example of the challenges facing a parasite using two hosts. In *Plasmodium*, several life stages live in a vertebrate (primarily in the blood cells), and

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others in an insect vector. If an insect vector feeds on an infected vertebrate host, the parasite's gametocytes break free from infected red blood cells, yield gametes that join, and produce motile ookinetes that move to the midgut wall where they undergo development and reproduction. Sporozoites are finally released and travel to the insect's salivary glands to infect another vertebrate host when the vector feeds again. Although the vertebrate hosts of malaria are often long lived, selection should still favour any mechanism to increase transmission to the vector. An example may be the preference of mosquitoes for malaria-infected individual vertebrate hosts (reviewed in Kingsolver 1987). The situation for the malarial parasite in its insect host is far more tenuous. Daily mortality of mosquitoes in the wild is often high; even the long-lived species suffer 5–10% mortality each day, and in many others the figure is 25–80% (Boyd 1930; Bates 1949a; Macdonald 1952; Clyde 1967; Pampana 1969; Bruce-Chwatt 1985; Kettle 1990; Rodriguez *et al.* 1992). Thus, Anderson & May (1992) concluded that '... the life expectancy of mosquitoes under field conditions is often very short ... and of not dissimilar magnitude to the latent period of infection [development within the vector].' Estimates of the probability of a mosquito living long enough after feeding on blood to be a successful vector for the parasite range from 80% (Macdonald 1956) to only 10% (Boreham *et al.* 1979), and even as low as 0·001–2% (Macdonald 1956; Gillies & Wilkes 1965; Charlwood 1986; Rodriguez *et al.* 1992). Parasitologists have long suspected that some species of anopheline mosquitoes are poor vectors of malaria because of the delayed maturation of the parasite in those species and the low probability of the insect living long enough for the parasite to reach maturity (Pampana 1969; Bruce-Chwatt 1985; Knell 1991).

Thus, a major problem facing malarial parasites in the vector is to complete development before the insect takes its last blood feeding before dying. Even for those mosquito species that are competent vectors of malaria (survival is high enough for the vectors to maintain the parasite system), most individual vectors do not live long enough to take more than two blood meals, and natural selection should favour any parasite genotypes that allow more rapid development in the insect.

We have studied the lizard malarial parasite *Plasmodium mexicanum* Thompson & Huff in its insect host, a sandfly (Psychodidae: *Lutzomyia vexator* Coquillett). *Plasmodium mexicanum* exploits the Iguanid lizard *Sceloporus occidentalis* Baird & Girard as its vertebrate host. In this system, the parasite is in an even more precarious position than described above for mosquito-borne malarias. *Lutzomyia vexator* shows gonotrophic concordance in which each blood meal is used to produce a clutch of eggs with no supplementary feedings between reproductive episodes (Chaniotis & Anderson 1967, 1968). Examination of the reproductive tract of wild-caught *L. vexator* at the same site as we used for our studies showed that almost none of the sandflies produce more than one clutch of eggs (Chaniotis & Anderson 1967, 1968); that is, few of the insects survived the stressful egg-laying process (Chaniotis 1967). We found that mortality of *L. vexator* after laying a clutch of eggs was very high; only about 2% lived long enough to take a second blood meal (J. Schall, R. Fialho & J. Bliss, unpublished data). These results are similar to those reported elsewhere for both *L. vexator* and some other sandfly species (Chaniotis 1967, 1986; Chaniotis & Anderson 1968; Kellick-Kendrick, Leaney & Ready 1977; Lawyer & Young 1987; Endris, Young & Perkins 1987; Klein *et al.* 1988). Thus, to be ready for transmission during the second, and typically last, blood meal taken by *L. vexator*, *P. mexicanum* must have a developmental period that is shorter than the time needed for the insect to produce and oviposit its clutch of eggs and feed again.

In this study we focused on the thermal ecology of *P. mexicanum* in its vector. We did this because the developmental schedule and transmission success of the parasite must be closely controlled by the temperature of the insect host. Many studies have documented thermal tolerance ranges of plasmodia in their insect hosts (Boyd & Stratman-Thomas 1933; Stratman-Thomas 1940; Bates 1949b; Gillett 1972; Bruce-Chwatt 1985), but how these temperatures compare to those normally experienced, or selected, by the insect has scarcely been examined (but see Vanderberg & Yoeli 1966). The duration of the development of malaria in its vector is controlled by temperature (Garnham 1964); likewise, the rate of digestion of blood and development of eggs in the vector are also influenced by temperature (Gillett 1972; Kettle 1990). *Plasmodium mexicanum* could suffer very low transmission success if the temperature normally experienced by the vector (either because it simply matches ambient temperatures or actively selects a preferred temperature) results in egg development being completed before the parasite's sporozoites are mature. In such cases, the vector may take its second (usually last) blood meal before the parasite's infective stages can be transmitted successfully. The parasite may encounter yet another problem if the infected insect selects a body temperature outside the parasite's tolerance range, even for brief periods (Stratman-Thomas 1940; Vanderberg & Yoeli 1966). Selection, then, should favour evolution by the parasite of temperature requirements that match those of its normal host, and perhaps even a means to manipulate the host's behaviour to achieve optimal temperatures for the parasite's development. In contrast, the insect could be under selective pressure to evolve temperature preferences that harm the parasite (such as behavioural fevers) if the parasite significantly reduces its fitness.

These issues have not been adequately explored

despite the importance of malarial parasites for human welfare and the great diversity of plasmodia known to infect non-human vertebrates (over 170 species; Schall 1990b). Our goals were to determine if the parasite's optimal temperature matches that experienced or chosen by the vector, and to determine if there is any evidence of alterations in the insect's thermoregulatory behaviour when infected, either as an anti-parasite fever or as an adaptive manipulation of the host's behaviour by the parasite.

Materials and methods

Both field and laboratory studies were conducted at the University of California Hopland Field Station in south-eastern Mendocino County California, 160 km north of San Francisco, USA. Fence lizards are commonly infected there with *P. mexicanum*. Details of previous studies on this system are reviewed by Schall (1990a, 1990b). Two species of sandfly (Psychodidae) are the vectors of *P. mexicanum* at Hopland: *Lutzomyia vexator* and *L. stewarti* (Mangabeira & Galindo) (Ayala 1971; J. Schall, unpublished data). As *L. vexator* is far more common than *L. stewarti* (about 10 : 1; J. Schall, unpublished data), we studied only this species. Transmission of *P. mexicanum* from lizard to lizard takes place throughout the warm season at Hopland (Bromwich & Schall 1986).

Sandflies typically have fairly strong species–specific breeding and resting locations (Lehane 1991). In the case of *L. vexator*, gravid females lay eggs on faeces in the faecal chambers of ground squirrel *Spermophilus beecheyi* burrows (Mangabeira & Galindo 1944; Chaniotis & Anderson 1967). The larvae feed on faeces, pupate, and adults emerge from burrows to feed and mate. Adult *L. vexator* spend the daytime within squirrel burrows and begin leaving them in early evening. Only females take blood and lizards and snakes are the usual hosts (Chaniotis 1967). After feeding, the sandflies remain in the rodent burrows until they lay a clutch of eggs on the rodent faeces (Chaniotis & Anderson 1968).

The climate at Hopland is Mediterranean with mild rainy winters and hot, dry summers. Mean summer temperature (July to September) is 21 °C and July is usually the hottest month when maximums occasionally reach 45 °C (field station records for 30 years ending 1990). Sandflies cannot tolerate high temperatures and low humidity (Chaniotis 1986; Chaniotis & Anderson 1968; Lehane 1991), so at Hopland they must spend daytime hours within the cooler and more humid burrows. Little is known about the effect of temperature on *P. mexicanum* in the sandfly; Klein *et al.* (1988) reported from a few trials that the temperature range for sporogonic development in the sandfly is 20–30 °C.

Our methods in catching and housing sandflies are modifications of those reported by Chaniotis & Anderson (1968) and Modi & Tesh (1983). Wild caught female *L. vexator* were allowed to feed on heavily infected fence lizards (gametocyte-predominant infections) at room temperature. Blood-fed females, which were easily identified by the bright red and swollen abdomen, were removed from feeding cages at 12-h intervals, and kept in incubators at constant temperature and approximately 80% relative humidity. Parasite development and egg development of sandflies were examined at temperatures ranging from 16 °C to 34 °C in increments of 2 °C. Sandflies were dissected using the method of Vanderberg & Gwadz (1980) and their midguts examined under 400×. A variable number of infected midguts ($n \geqslant 3$ for time intervals near parasite maturity) was examined every 12 h after day 3 post-ingestion (PI) until parasite maturity was confirmed for at least two consecutive 12-h intervals. The parasite was considered mature when fully sporulated oocysts containing sporozoites, as described by Klein *et al.* (1988), and/or free sporozoites in the haemocoel were observed in at least 2/3 of the sandflies examined. We confirmed that these oocysts were mature by injecting an uninfected lizard with the crushed midgut of one sandfly; this lizard showed an active infection within 2 weeks.

Duration of egg development was examined at the same temperatures used above. Sandflies were maintained in individual vials and dissected each 12 h after 2 days PI. The gonotrophic cycle (period of time from ingestion of blood to the completion of egg development) was estimated as the time needed to produce mature eggs in more than 2/3 of the sandflies examined. Eggs were considered mature based on the description given by Chaniotis (1967). Those eggs judged mature when dissected from sandflies were very similar in appearance to freshly laid eggs. The day PI when the first egg was laid was recorded. To assure that eggs would be visible on the white plaster bottoms of the vials, no larval food (faeces) was put into the vial; this probably altered the oviposition behaviour of the insects (Elnaiem & Ward 1992; see the Results). Longevity of another group of female flies was determined by keeping them in individual vials, supplied with a moist plaster bottom and a few drops of 30% fructose solution on the cotton top.

An important assumption in this study is that sandflies can thermoregulate while in burrows by moving towards the opening of or retreating deeper within the burrow until they find a suitable temperature that remains fairly constant. Therefore, we determined the diversity and stability of temperatures in burrows in three ways. Temperatures within ground squirrel burrows were examined at two sites (274 and 518 m elevation) to determine the thermal diversity available to the sandflies. First, the gradient of temperatures within natural burrows was measured at the lower site by slowly pushing a thermocouple attached to a spring wire into burrows and recording the air temperature every 5 cm. The frequent presence of rattlesnakes in

the burrows precluded more detailed study of the thermal profile of natural burrows.

Secondly, eight natural burrows at each site were monitored every hour over a 24-h period once in June and once in July. A thermocouple was inserted within each natural burrow with the probes located approximately 110 cm from the entrance.

Thirdly, an artificial burrow was constructed at each of the two sites. Natural burrow systems are complex, with tunnels running parallel to the ground, but often forming chambers at a typical maximum depth of 1 m (Grinnel & Dixon 1918). Our artificial burrows had chambers (buried plastic boxes, 33 cm^3) at 50 and 100 cm deep to mimic the structure of real burrows. Hourly temperatures within each chamber of the artificial burrows were monitored from thermocouples enclosed within the chambers during the same 24-h period chosen for the study of natural burrows.

The temperature preference of sandflies was measured in a thermal gradient constructed from a glass aquarium 61·5 cm long × 31·5 cm wide × 42·4 cm high. The interior walls of the tank were lined with plaster of Paris and the mouth of the gradient, which faced the observer, was closed with fine mesh dacron cloth with three small openings to allow entry of a thermal probe and aspirator. The plaster was sprayed with 250 ml of distilled water to keep the relative humidity of the air close to the plaster at >80%. If warm air at high relative humidity moved over a cooler area of the gradient, water distilling from the air would enter the absorbant plaster, preventing any water drops from forming on the surface used by the sandflies to rest. To create a gradient of temperatures, aluminum plates (5 mm thick) were placed over the entire outside back and rear of the aquarium and ice packs and electric hot plates were placed on these plates. The gradient was equilibrated by turning on the hot plates and positioning the ice packs on the tank 1 h before release of sandflies into the apparatus. Temperatures at randomly selected points on the plaster within the apparatus showed that a wide range of conditions was available to the sandflies (16·8–37·7 °C).

Female sandflies were allowed to remain in the gradient for 1 h before data were taken. All measurements were taken at night (22·00–01·00 h) and the number of sandflies observed at one time varied but never exceeded 30. Light within the gradient was kept at a very low level (just bright enough for the sandflies to be seen on the white plaster surface) and appeared uniform, but the effect of any variation in light intensity within the gradient was minimized by turning the apparatus 180° once during each trial. *Lutzomyia vexator* is a very small insect (about 1–2 mg), so we assumed that the temperature of the plaster at the point where a sandfly was resting equalled body temperature of the insect itself. A fine thermocouple probe that had its lead wire attached to a glass tube aspirator was gently placed next to a resting sandfly and the temperature of the plaster taken. The sandfly was then aspirated into the glass tube and blown into a diluted soap solution in individually marked plastic vials. As *L. vexator*, our study subject, and the less common *L. stewarti* are very similar in external morphology, each sandfly used in this study was dissected and the spermatheca examined to differentiate the species (Young & Perkins 1984).

To determine any differences in thermal preference with respect to feeding and infection status, measurements were taken for three groups: unfed sandflies, sandflies fed on uninfected lizards, and sandflies fed on infected lizards. For the last two classes of sandflies, temperature preferences were measured 24 h and 3 days PI.

Results

THERMAL ENVIRONMENT AVAILABLE FOR THE SANDFLIES

For 15 burrows examined during the hot mid-day (13·00 h) in late August, the temperature at 30–45 cm into the burrow ranged from 23·6 °C to 32·2 °C (mean = 26·1 °C). The air temperature at 30 cm above ground was 30·1 °C at that time. For four burrows in which the temperature could be measured in the first 1 m from the opening, the temperature dropped 1 °C for every 30 cm distance. Temperature varied both among burrows and during the course of the day (Fig. 1). However, the temperatures were almost constant over time in the two deeper artificial burrows (27 °C at 0·5 m and 24 °C at 1·0 m in June within the artificial burrow at the lower elevation site). Note that the temperature in the deep artificial burrow, which approximated the deepest part of a natural burrow, was not the lowest temperature recorded for the burrow system. Temperatures in the burrows 1 m below ground were 3 °C higher in July compared to June for both sites and 1–2 °C lower each month at the higher elevation site in both months. These results show that the sandflies experience a diverse thermal environment within and among burrows, ranging from about 18 °C to 33 °C. Maintaining a constant body temperature while near the opening of the burrow would require the sandfly to move often during the course of the day; however, if temperatures at lower depths were preferred, the insects could readily select a constant temperature.

PARASITE DEVELOPMENT AND TEMPERATURE

Fully sporulated oocysts containing mature sporozoites were observed under experimental temperatures ranging from 20 °C to 32 °C. Development was most rapid between 28 °C and 32 °C, when mature oocysts were observed 4·5–5 days PI, and slowest at 20 °C when oocysts became mature only on day 14 PI (Fig. 2). A second-order polynomial regression analysis

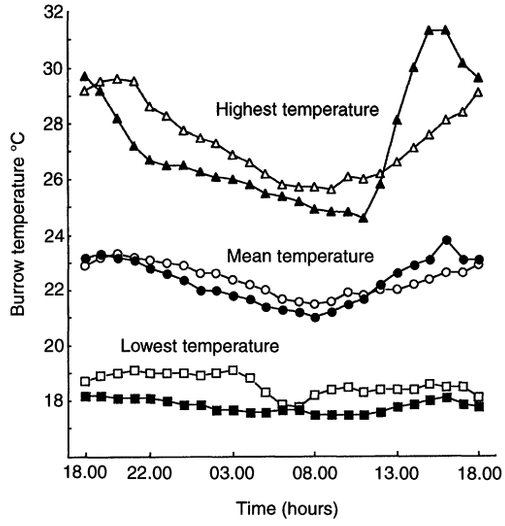

Fig. 1. Temperature within natural squirrel burrows at two sites, one at a higher elevation (518 m; closed points), one at lower (274 m; open points). Temperatures are shown during a continuous 24-h sample period in late June. Mean for the eight natural burrows at each site, plus the highest temperature and lowest temperature recorded among the eight burrows at each site, are given. Thus, the mean and range for temperatures among burrows at each site for each hour are shown. Burrows varied in their placement, some were in open sun throughout the daylight hours, whereas others were sometimes in the shade of a tree. Results show burrows were fairly constant in temperature at a sample location (approximately 110 cm from mouth of burrow), but temperature varied among burrows.

between temperature and arcsin-transformed values of 1/time to develop in days (rate of development per day) gave an estimated cessation of parasite development at approximately 17°C ($r^2 = 0{\cdot}99$; $P < 0{\cdot}0001$). In agreement with this prediction, we observed no development of oocysts in sandflies kept at 16°C and oocysts never reached maturity at 18°C (sandflies were kept at these temperatures for 20 days and dissected every 12 h). When kept at 34°C sandflies died before oocysts reached maturity. At 28°C sporozoites were seen in the salivary glands on day 6 PI, 1 day after the oocysts appeared mature on the midgut wall. Klein *et al.* (1987, 1988) reported that sporozoites of *P. mexicanum* were observed 24 h after oocysts became mature in the midgut of *L. vexator* at both 24°C and 27°C. Therefore, we assume that it takes approximately 1 more day after oocysts are mature for sporozoites to migrate to the salivary glands of the vector, and this period is not influenced by temperature from at least 24–28°C. The predicted sporogonic cycle for each temperature is thus 1 day longer than shown in Fig. 2.

Very little variation in the time needed for development of mature oocysts was observed for each temperature. The curve shown in Fig. 2 was drawn based on the 2/3 rule described in the Methods section. No infection took longer to reach maturity in the sandfly than the time shown by this curve. Only six of the 197 infections appeared to be mature earlier than indicated by the curve (these six are shown in Fig. 2 and they represent the entire variation away from the drawn curve). Four were mature 12 h early and two were mature 24 h early.

No sandflies became infected at 16°C ($n = 14$), and the percentage was low (10·5%; $n = 38$) at 18°C and intermediate at 20°C (77·8%; $n = 18$) and 32°C (56·7%; $n = 30$). The optimal temperature range for infection to be established was 22°C–30°C ($n = 15$–54). Within this range 93·3–100% of sandflies examined showed oocysts on their midguts (χ^2 test for number infected, comparing 22 to 30°C, $P > 0{\cdot}05$).

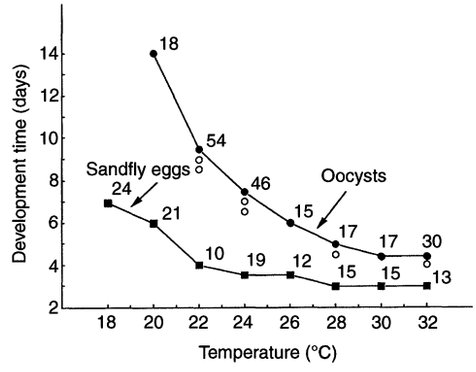

Fig. 2. Oocyst development time by temperature for the malarial parasite *P. mexicanum* in the sandfly *L. vexator*, and duration of sandfly egg development by temperature. Sample sizes are shown next to each point. The curve is based on a rule requiring that 2/3 of sandflies dissected at that temperature showed mature oocysts or mature eggs. The open points below the 'oocyst development' curve show single infections with mature oocysts earlier than predicted. These open points show all of the variation seen in the experiments. Oocysts never reached maturity at 16°C or 18°C, so these data are not presented on the figure.

SANDFLY REPRODUCTION AND LONGEVITY AND TEMPERATURE

Figure 2 shows the time needed for sandflies to develop mature eggs at different temperatures. In contrast to the strong effect of temperature on development time of parasite oocysts, maturation time of the insect's eggs was similar (3–4 days) over a broad range of temperatures (22–32°C). Little variation in time needed to develop apparently mature eggs was observed (only five of 129 sandflies produced mature eggs earlier or later than estimated in the curve shown in Fig. 2, by only 12–24 h).

Another 68 sandflies were permitted to oviposit on the vial's moist plaster bottom. When data for these sandflies were compared with those from the dissected females, we found that most sandflies delayed laying their eggs from 1 to 10 days. Some sandflies laid eggs within 0–2 days after we judged them to be mature; this argues that the criteria used in classing eggs as mature were valid. Long delays in laying eggs is probably a laboratory artifact because suitable oviposition

substrate was not available (Elnaiem & Ward 1992). We conclude that the sandflies were ready to oviposit within 1–2 days after their eggs were scored as mature, and that in nature they would locate a suitable oviposition site (chamber with rodent faeces) and be ready to take another blood meal within a day or two after the eggs reached maturity.

Longevity of the insects at different temperatures was compared using the percentage of sandflies alive on the date when eggs were judged mature (based on dissection experiments for each temperature). At 34 °C none of the insects survived. Between 16 °C and 32 °C, 82–100% survived, with no significant effect of temperature on survival ($R \times C$ G-test, $P > 0{\cdot}05$).

SANDFLY THERMAL PREFERENCE

Upon being placed into the gradient, the insects would fly in short 'hops', frequently changing position at first, then settling down at one location. Table 1 presents descriptive statistics for substrate temperatures selected by different groups of sandflies: unfed; fed on non-infected lizards (24 h and 3 days PI); and fed on infected lizards (24 h and 3 days PI). Also shown are results for temperatures of random positions in the gradient. All groups of sandflies had temperature distributions significantly different from the distribution of substrate temperatures available in the thermal gradient (Kolmogorov–Smirnov tests, $P < 0{\cdot}05$–$< 0{\cdot}001$). That is, sandflies did not choose resting locations randomly with respect to temperature, but chose a narrower range of temperatures than was available in the gradient.

A two-way ANOVA was used to evaluate the effects of infection status (infected vs. not infected) and time after a blood meal (24 h and 3 day) on substrate temperature chosen by the sandflies (Table 2). To examine the effect of taking a blood meal on temperature preferences, a one-way ANOVA of temperature by feeding status (fed or unfed) was used. Orthogonal contrasts between means of infected and uninfected sandflies, as well as between means of fed and unfed sandflies, were conducted.

There was a significant effect of feeding status on substrate temperature preference such that fed sandflies preferred temperatures higher than unfed sandflies. No significant interaction effect on substrate temperature chosen was observed between infection status and time after a blood meal, nor was there a significant effect of time after a blood meal on substrate temperature preference. However, infection status exerted a significant effect on temperatures selected by sandflies. Othogonal contrasts showed that, despite the absence of a significant effect of time after a blood meal, the temperature preferences of infected sandflies were significantly higher than those of uninfected sandflies at 24 h. No significant difference was observed 3 days after a blood meal was taken (Table 1). In summary, sandflies feeding on a non-infected lizard raised their body temperature by about 1·6 °C, and if they fed on an infected lizard their mean body temperature rose by about 3·6 °C. This increase was significant only when measured at 24 h after a blood meal was taken.

SANDFLY THERMAL PREFERENCES AND PARASITE DEVELOPMENT

As variation in the time needed to complete oocyst development was very small for each temperature, we can confidently predict duration of parasite development at a specified temperature with a regression equation ($4{\cdot}53x - 0{\cdot}066x^2 - 48{\cdot}85$; $R^2 = 0{\cdot}995$; $F = 372{\cdot}85$, $P < 0{\cdot}0001$). Complete oocyst development would take 7·8 days at a temperature of 22·9 °C for the first 24 h and 23·5 °C thereafter (temperature preference observed for uninfected fed sandflies at 24 h and 3 days, respectively). Oocyst development in the midgut would be complete by 7·2 days at 24·9 °C for the first 24 h and 23·9 °C thereafter (temperature preferences observed for infected sandflies at 24 h and 3 days, respectively). The difference between these two estimated development times was 14 h. We did not measure preferred temperature between 24 h PI and 72 h PI, but if sandflies were to prefer the mean temperatures observed at 24 h for the first 72 h PI, then switch to the mean preferred temperature observed at 3 days PI, development times would differ by 23 hours. For both of these scenarios the development time of the oocysts was reduced by >12 h. As the sandflies

Table 1. Means, SD, sample size, and ranges for substrate temperature measurements of different treatment groups of female sandflies. Also included are data for substrate temperatures available to the sandflies in the gradient. The temperature chosen by infected sandflies was higher after 24 h (orthogonal contrasts $F = 7{\cdot}58$, $P = 0{\cdot}006$), but not after 3 days ($F = 0{\cdot}336$, $P = 0{\cdot}563$)

		Fed on uninfected lizard		Fed on infected lizard		
	Unfed	24 h	3 days	24 h	3 days	Thermal gradient
Mean	21·3	22·9	23·5	24·9	23·9	26·5
SD	3·97	3·69	3·56	3·97	3·94	4·36
n	54	51	59	54	55	164
Range	14·4–29·2	16·0–30·1	17·2–31·8	17·6–33·0	16·0–32·3	16·8–37·7

Table 2. Analysis of variance table for data on sandfly thermal preferences

ANOVA	Source of variation	df	Sum of squares	*F*	*P*
1. One-way	Feeding status	1	259·04	17·37	<0·0001
	Error	271	4042·63		
2. Two-way	Time after meal	1	2·22	0·15	0·695
	Infection status	1	81·95	5·70	0·018
	Time × infection (interaction effect)	1	36·12	2·51	0·114
	Error	215	3090·12		

feed only at night, this means the parasite would be ready 1 full day (one possible feeding time) earlier because of the elevated temperature chosen by infected sandflies. Note that all of these estimates are based on the mean temperature chosen by sandflies in the thermal gradient. Approximately half of the measurements fell within 2 SD above the mean, so parasite development would be cut by at least 1 day for parasites in at least half of the vectors.

Discussion

For the *P. mexicanum–L. vexator* system, the probabilities of a second or third blood meal by the vector are so small that the parasite typically has at best only one chance to leave the sandfly and become established in a new host; that is, during the second blood meal taken by the insect. This is an extreme situation compared to many other malaria systems; however, mortality for small insect vectors is typically high and selection should favour any trait that increases the chance of the parasite leaving the vector early. We emphasize here that the issue being discussed is not the ability of the vector to maintain a malarial parasite in a population of vertebrate hosts (even a vector with high daily mortality can be a 'competent' host for *Plasmodium*), but how such high mortality would result in strong selection acting on individual parasite cells.

The lizard malaria story therefore illustrates a general point: parasites that exploit multiple hosts must regulate their development to be ready to escape their environment (host) before it no longer exists, and that this may require some elaborate adaptations to optimize the chances of transmission. We expected to find such adaptations in *P. mexicanum*. Temperature regulates the rate of both the maturation of the sandfly's eggs and the development of the malarial oocysts; therefore, the parasite could evolve an optimal temperature for development that matches that experienced by the insect, or could manipulate the thermoregulatory behaviour of infected vectors to favour the parasite's success. We believe the data suggest *P. mexicanum* has evolved a mix of both of these strategies.

Has *P. mexicanum* evolved thermal control over its development that matches the thermal biology of the insect host? To answer this question we compared the duration of the sporogonic cycle of *P. mexicanum* with other plasmodia from literature records. Data for 10 species of *Plasmodium* were found, four in natural, and six in experimental, vectors. The duration of the sporogonic cycle can depend on the vector species (Garnham 1966), so we treated the two sets of data separately (Fig. 3). *Plasmodium mexicanum* has a significantly accelerated development compared to the other species of *Plasmodium*. We assume this rapid development has evolved to match the rapid development of eggs in sandflies and their high mortality after oviposition.

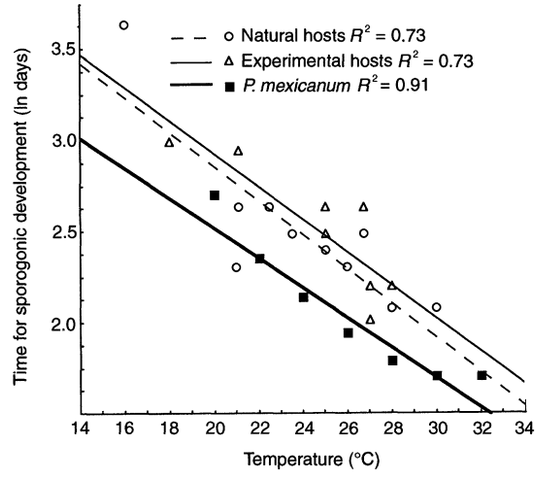

Fig. 3. Duration of sporogonic development plotted against temperature for data in this study (*P. mexicanum*) and for two sets of literature data: natural insect host for the parasite, and experimental hosts. The slopes for data on *P. mexicanum* and other species in natural and experimental vectors did not differ ($P = 0{\cdot}83$). Also, the elevations of the regressions for natural and experimental vectors did not differ, but that for *P. mexicanum* was significantly lower than for the other sets of data. Ten species are represented in addition to *P. mexicanum*. The species studied, and number of temperatures studied, were *P. berghei* (1), a parasite of rodents, *P. falciparum* (2), *P. malariae* (2), *P. vivax* (6) and *P. ovale* (1) of humans, *P. floridense* (1) of lizards, *P. cynomolgi* (1) and *P. inui* (1) of monkeys, and *P. gallinaceum* (1) and *P. relictum* (2) of birds.

Figure 4 summarizes the thermal biology of *P. mexicanum*. The curve for oocyst development time shown in Fig. 2 has been raised by 1 day, the time estimated for the sporozoites to travel to the insect's salivary glands. The new curve thus represents the total parasite development time. The egg development curve of Fig. 1 has been raised 3 days because the sandflies started ovipositing eggs 1–2 days after the time they were judged mature in dissected individuals, and the sandflies must take some time to find a suitable place to lay the eggs, recover from egg laying (if they survive), and then find another lizard for a second blood meal. Figure 4 shows that the tolerable range of temperatures for the parasite is about 25–30 °C. Below this range the parasite would not be ready for transmission when the sandfly is likely to take its next blood meal, and above this range the probability of the sandfly becoming infected drops abruptly. Although *P. mexicanum* has a rapid rate of development compared to other plasmodia, it still appears to be too slow at the normal body temperature chosen by the sandflies. This suggests that the developmental rate of *P. mexicanum* has been pushed to the upper limit possible with the basic physiological plan of malarial parasites. Very little variation in development rate was seen for *P. mexicanum* at all temperatures, suggesting that there is little or no genetic variation for this trait for selection to mould. Instead, the parasite appears to have taken another tack: to alter the temperature preference of the vector.

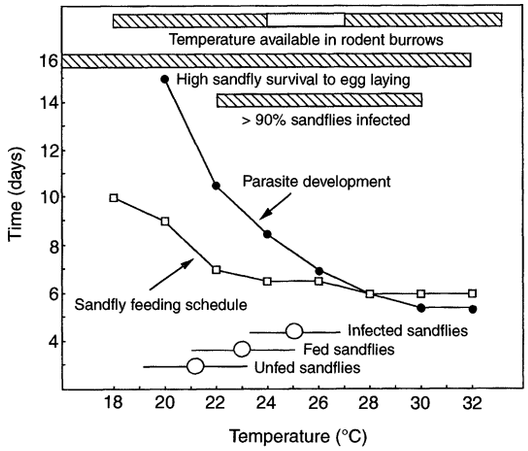

Fig. 4. Summary of thermal biology of the malarial parasite, *P. mexicanum*, in its insect host, the sandfly *L. vexator*, in northern California. Shown are curves for 'parasite development', which assumes sporozoites take 1 day to travel from a mature oocyst to the sandfly's salivary glands, and 'sandfly feeding schedule', which assumes that the sandfly finds a suitable oviposition site, lays the eggs, and is ready for another blood meal within 3 days of the time when the eggs appear to be mature, using criteria described in the Methods section. Also shown are the range of temperatures found in rodent burrows at the study site, the temperatures at which sandflies had high survival to egg laying, and the temperatures at which a high proportion of sandflies became infected with malaria. The stippled area in the bar for temperatures in rodent burrows indicates the range of very constant temperatures in artificial burrow chambers. The mean temperature chosen by sandflies in the first 1–3 days is shown by a large open point and SD by a line, for unfed sandflies, those fed on an uninfected lizard, and those fed on an infected lizard.

Lutzomyia vexator clearly is an active thermoregulator; the temperature of its selected microenvironments was narrow compared to those available in the thermal gradient. Examination of the distribution of temperatures chosen by the sandflies (mean and SD indicated on Fig. 4) showed that most unfed sandflies were well below the minimum required by the parasite for successful development. However, the sandflies increased their body temperature after feeding, presumably to facilitate digestion, and those sandflies feeding on infected blood, and becoming infected themselves, increased their body temperatures even more. Because the sandflies feed only at night, this would shift the feeding schedule by about 1 day, which would barely allow the parasite to reach full development before the vector's second blood meal. Note from Fig. 4 that the very constant temperatures recorded from deep artificial burrow chambers matched the temperature selected by infected sandflies.

The central reason why the increased temperature chosen by the sandflies works to the benefit of the parasite is that the relationship between temperature and both sandfly egg development and parasite development is non-linear and the two curves have different shapes. The rate of sandfly egg development is flat over a broad range of temperatures (22–32 °C), whereas oocyst development continues to shorten until the very upper end of this range. Thus, between 22 °C and 32 °C, an increase in temperature would not shorten the feeding schedule of the sandfly, but would reduce the time needed for the parasite to finish development and be ready for transmission.

Malaria apparently causes various changes in vector behaviour. Mosquitoes are more likely to feed on a malarious vertebrate host (Day & Edman 1983; Kingsolver 1987) and infected mosquitoes make more biting attempts before being able to take a blood meal (Ribeiro, Rossignol & Spielman 1985; Rossignol *et al.* 1986). These behavioural changes are clearly beneficial to the parasite and are likely to affect the overall transmission dynamics of the system (Kingsolver 1987; Dobson 1988). However, in these cases it is unclear if the changes in host behaviour is a 'boring byproduct' (Dawkins 1990) of infection, or a true adaptation on the part of the parasite. Parasite manipulation of host behaviour has been proposed for many parasite–host systems, but these claims have often been anecdotal and lacking rigorous tests of the adaptive significance of the changes in the host induced by infection (Moore & Gotelli 1990; Yan, Stevens & Schall 1994). In the lizard malaria system described here, the parasite may well be adaptively manipulating the thermoregulatory behaviour of *L. vexator*; that is, the change in the host's behaviour

clearly benefits the parasite. If so, we believe this is the first demonstration of an adaptive manipulation of the thermoregulation of the vector caused by a malarial parasite.

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