The geographic mosaic model of coevolution posits that geographically widespread interacting species, such as parasites and their hosts, may exist as a patchwork of genetically distinct local populations, provided gene flow is restricted. This population structure could allow evolution of local adaptations, such as shifts in a parasite’s life history traits based on local transmission ecology and host density (Gandon et al., 1996; Tucker and Stevens, 2003; Thompson, 2005). High levels of gene flow, however, would not only preclude local adaptation, but might yield maladapted populations of both parasite and host (Thompson, 2005). Thus, understanding the geographic genetic structure of parasites is critical for ecological, epidemiological, and genetic studies, and for control efforts of the pathogens of humans, domestic animals, and endangered wildlife species.

Investigators have long suspected that malaria parasites of humans vary geographically in important life history traits, including virulence (Hackett, 1937; Rogerson and Carter, 2008), which suggests that each Plasmodium species represents a genetic mosaic over space. Molecular techniques now permit detailed studies on genetic differentiation of malaria parasites at several spatial scales, and give some indication of the likely degree of gene flow among sites. Surveys reveal substantial genetic differentiation among continental regions (Creasey et al., 1990; Walliker et al., 1998; Anderson et al., 2000; Conway et al., 2001; Li et al., 2001; Conway, 2007) and among isolated Pacific islands (Lum et al., 2004; Conway, 2007). Within countries, the results are more variable, with some studies identifying little or no variation among sites 30–100 km apart (Zhong et al., 2007; Prugnolle et al., 2008; Bonizzoni et al., 2009), and others finding significant within-country differentiation (Creasey et al., 1990; Machado et al., 2004; Zhong et al., 2007). Most striking are findings of genetic differentiation of Plasmodium falciparum for a fine spatial scale, over only 7 km in Borneo (Anthony et al., 2005; Conway, 2007), and villages in Papua New Guinea only 2–5 km apart (Forsyth et al., 1989). These results are significant for understanding the dispersal of malaria parasites via their vertebrate and insect hosts, and must be considered for planning any public health program (Walliker et al., 1998).

We approached this issue with a study of a malaria parasite of a nonhuman vertebrate host, Plasmodium mexicanum, a parasite of fence lizards (Sceloporus occidentalis) in northern California. We surveyed a series of sites from 0.23 to 43 km apart, and determined the degree of genetic differentiation of both parasite and its lizard host with the use of microsatellite genetic markers. The vectors, 2 species of psychodid sand fly, Lutzomyia vexator and Lutzomyia stewardi (Fialho and Schall, 1995), are weak fliers (at least in laboratory cages) and emerge at night only when there is little or no air movement (Schall and Marghoob, 1995). Thus, their dispersal is predicted to be slight. Gene flow for fence lizards among sites is also likely to be low because the lizards are strongly territorial, and individuals remain within a small patch of the habitat for 1–4 yr (their lifetime) (Bromwich and Schall, 1986; Sheldahl and Martins, 2000; Eisen, 2001). Previous studies have found variation in parasite prevalence among sites even a few hundred meters apart (Schall and Marghoob, 1995; Eisen and Wright, 2001), and differences in genetic diversity for sites with chronically high versus low prevalence (Vardo and Schall, 2007). Thus, the overall natural history of this system suggests that P. mexicanum could be genetically distinct among sites.

**MATERIALS AND METHODS**

The base for field studies was the University of California Hopland Research and Extension Center (HREC), located near the town of Hopland in Mendocino County, California, approximately 160 km north of San Francisco. The HREC has been the site of long-term studies on the biology of P. mexicanum since 1978 (Schall, 1996; Vardo-Zalik and Schall, 2008). Four sites were surveyed on the field station; these sites were from 0.23 to 0.97 km apart (Fig. 1). Four sites were chosen off the station, with overall distance from 6.4 to 42.6 km from one another and HREC (Fig. 2). Site data, including latitude and longitude, are provided in Table 1. The parasite was not present in 2 of the lizard populations off station (Boonville and Keiffer Ranch), but genetic data on the lizards were included in the study. All sites were in the valleys of the coastal ranges, and had similar habitats of rolling grassy hills dominated by oaks (Quercus spp.) and Pacific madrone (Arbutus menziesii). Although sites on the HREC property were closely positioned, the steep terrain and patchy distribution of suitable lizard habitat (fallen trees, rock piles) would make movement between some sites difficult for lizards and perhaps for the sand flies.
Lizards were sampled by noosing with a fishing pole and a loop of nylon fishing line. The lizards were brought into the laboratory, and in the evening a toe clip allowed sampling of a few drops of blood to make a thin blood smear and dried blood dots on filter paper. The smears were stained (Giemsa stain) to identify infected lizards (scanning the smear at 3,000), and the dried blood drops were stored frozen until processing. All lizards were returned to their point of capture the next morning. Sampling of lizards followed an approved animal care protocol of the University of Vermont and collecting permits from the California Department of Fish and Game.

Microsatellite markers were used to determine genetic differentiation among sites for both *P. mexicanum* and its fence lizard host. These markers are short tandem repeat regions of 1 to many nucleotides in an organism’s genome, and are very common in most species, including *Plasmodium* spp. parasites and vertebrates (Selkoe and Toonen, 2006; Schall and Vardo, 2007). The number of repeats represents a length allele. Microsatellite markers are presumed to be neutral and thus allow determination of geographic patterns in population structure (Anderson et al., 2000; Selkoe and Toonen, 2006). The 5 *P. mexicanum* microsatellite markers used for the analyses (Pmx306, Pmx710, Pmx732, Pmx747, and Pmx839) contain a tribase repeat (ATT) and were characterized by Schall and Vardo (2007). Mixed-clone infections make determination of linkage among these markers problematic, but measures of linkage disequilibrium suggest that the 5 markers are not linked (Schall and Vardo, 2007). The 3 lizard microsatellite markers used in this study were identified from a genome digest library created in the search for *P. mexicanum* microsatellites (Schall and Vardo, 2007). A linkage disequilibrium analysis performed with the use of FSTAT found no evidence of linkage among any of the lizard markers (*P* > 0.05).

DNA was extracted for both infected and noninfected lizards from the dried blood with the use of the DNeasy kit (Qiagen, Valencia, California) and the supplier’s protocol. All microsatellite markers were amplified in a 25-μl PCR using Ready-to-Go beads (GE Healthcare, Piscataway, New Jersey) that contained DNA polymerase, dNTPs, and buffers; 1 μl of each 10 μM primer and 3 μl of DNA template were added to each reaction mixture.

The *P. mexicanum* microsatellite markers were amplified with the use of primers and PCR programs given in Schall and Vardo (2007), with 1
Table I. GPS coordinates (in decimal degrees), elevation, and parasite prevalence (proportion of lizard host, *Sceloporus occidentalis*, infected with malaria parasite *Plasmodium mexicanum*) at sites surveyed in study. Sites in bold are those on the Hopland REC field station, and are all within 1 km pair-wise distance. Code abbreviation used in other tables is given for each site.

<table>
<thead>
<tr>
<th>Site name (code abbreviation)</th>
<th>Elevation (m)</th>
<th>Coordinates</th>
<th>Parasite prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boonville (Boon)</td>
<td>175</td>
<td>38.9882N, 123.3653W</td>
<td>0/103 = 0</td>
</tr>
<tr>
<td>Bradford Ranch (BR)</td>
<td>223</td>
<td>38.9085N, 123.0848W</td>
<td>11/143 = 0.077</td>
</tr>
<tr>
<td>Kiefer Ranch (KR)</td>
<td>396</td>
<td>39.2876N, 123.0484W</td>
<td>0/55 = 0</td>
</tr>
<tr>
<td>Wheeler Ranch (WR)</td>
<td>272</td>
<td>38.9957N, 123.1601W</td>
<td>46/245 = 0.187</td>
</tr>
<tr>
<td>Greenhouse (GH)</td>
<td>256</td>
<td>39.0044N, 123.0763W</td>
<td>12/59 = 0.203</td>
</tr>
<tr>
<td>Lower Horse (LH)</td>
<td>383</td>
<td>39.0044N, 123.0760W</td>
<td>35/195 = 0.179</td>
</tr>
<tr>
<td>Parson’s Creek (PC)</td>
<td>260</td>
<td>39.0010N, 123.0771W</td>
<td>31/216 = 0.144</td>
</tr>
<tr>
<td>Water Tank (WT)</td>
<td>262</td>
<td>39.0063N, 123.0869W</td>
<td>36/165 = 0.218</td>
</tr>
<tr>
<td>Bradford Ranch (BR)</td>
<td>223</td>
<td>38.9085N, 123.0848W</td>
<td>11/143 = 0.077</td>
</tr>
<tr>
<td>Kiefer Ranch (KR)</td>
<td>396</td>
<td>39.2876N, 123.0484W</td>
<td>0/55 = 0</td>
</tr>
<tr>
<td>Wheeler Ranch (WR)</td>
<td>272</td>
<td>38.9957N, 123.1601W</td>
<td>46/245 = 0.187</td>
</tr>
<tr>
<td>Greenhouse (GH)</td>
<td>256</td>
<td>39.0044N, 123.0763W</td>
<td>12/59 = 0.203</td>
</tr>
<tr>
<td>Lower Horse (LH)</td>
<td>383</td>
<td>39.0044N, 123.0760W</td>
<td>35/195 = 0.179</td>
</tr>
<tr>
<td>Parson’s Creek (PC)</td>
<td>260</td>
<td>39.0010N, 123.0771W</td>
<td>31/216 = 0.144</td>
</tr>
<tr>
<td>Water Tank (WT)</td>
<td>262</td>
<td>39.0063N, 123.0869W</td>
<td>36/165 = 0.218</td>
</tr>
</tbody>
</table>

For each microsatellite marker from the lizard genome, 1 peak represented a single haploid clone in the infection. For a marker, 1 peak represented a single clone present in the infection; 2 or more peaks resulted if the infection included multiple clones. All alleles (clones) were included if the peak had a characteristic shape (stutter pattern), with a minimum of 50 fluorescent units on the pherogram. A previous study verified that this method detects clones at low density in the lizard’s blood (Vardo-Zalik et al., 2009), and studies on the effect of multicle clones on the lizard genome of *P. mexicanum* also demonstrated that these smaller peaks represented distinct clones (Vardo-Zalik and Schall, 2008, 2009). Mixed-clone infections are very common at the study area (50–80% of infections over sites) (Vardo and Schall, 2007), so it was not possible to define reliably multicle genotypes of parasites. Therefore, each marker was analyzed separately. Details on the distribution of clone numbers among infections, changes in genetic diversity over time, etc., are presented in Vardo and Schall (2007).

For each microsatellite marker from the lizard genome, 1 peak represented a homoygote for that allele, and 2 peaks indicated the lizard was a heterozygote. For uniformity in method of analysis, the lizard data were also examined by marker.

Diversity of microsatellite length alleles was determined in 2 ways—by the number of alleles detected in the sample, and by expected heterozygosity (He = \[n(n−1) \times (1 − p_i^2)\], where n is the number of infections and \(p_i\) is the frequency of the i-th allele; this is a measure of genetic diversity that includes number of alleles and their relative proportions (Vardo and Schall, 2007). Sample sizes were number of lizards for (lizard data) and number of clones (total number of peaks seen for each locus) for all infections for the parasite.

Genetic differentiation of *P. mexicanum* and *S. occidentalis* was measured through pairwise \(F_{st}\) estimates (Weir and Cockerham, 1984; Cockerham and Weir, 1993), calculated with the program FSTAT (Goudet, 1995). Balloux and Lugon-Moulin (2002) compared the \(F_{st}\) with other methods to analyze microsatellite data, and found that \(F_{st}\) produced unbiased results and appears to be the best way to identify differentiation among sites. Defining a cut-off value for \(F_{st}\), that represents biologically important genetic differentiation between sites remains subjective. We use cutoffs that are commonly used (Balloux and Lugon-Moulin, 2002): \(F_{st} \geq 0.05\) means no, or very little, differentiation, and we regard this as “no differentiation”; \(0.05 \leq F_{st} < 0.25\) means “moderate differentiation”; \(0.25 \leq F_{st} < 0.5\) is “great differentiation”; and \(F_{st} \geq 0.5\) is “very great differentiation.” Although samples were available for most sites from 1997 to 2008, a preliminary analysis revealed that microsatellite allele frequencies for *P. mexicanum* have changed over that time, so only the samples from 2006 to 2008 were used for this analysis among sites. No differences in allele frequency were found among these samples (pairwise by year, FSTAT analysis; data not shown).

**RESULTS**

All microsatellite markers used here for both parasite and lizard revealed substantial variation, both in number of alleles and genetic diversity measured by expected heterozygosity (Table II) and, therefore, are useful in measuring degree of genetic differentiation among sites. *Plasmodium mexicanum* was found infecting lizards at all Hopland REC sites and at 2 of the more distant sites; no infected lizards were identified from Boonville or Kiefer Ranch (Table I).

For *P. mexicanum*, of 75 comparisons of \(F_{st}\) values for all sites and all markers (total cells for *P. mexicanum* results in Table III), 34 (45%) were genetically differentiated, with \(F_{st} > 0.05\). For sites >6 km apart, 27/45 (60%) were genetically differentiated, with \(F_{st} > 0.05\).

**Table II.** Sample sizes among sites (range), and number of alleles detected and expected heterozygosity (He), a measure of genetic diversity for each microsatellite marker used in the study (median and ranges). The sample sizes for the parasite are based on the total number of peaks seen on the pherograms for each marker at each site; for the lizard, sample sizes are for the number of individual lizards sampled.

<table>
<thead>
<tr>
<th>Lizard</th>
<th>N</th>
<th>N alleles</th>
<th>He</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pmx306</td>
<td>14–54</td>
<td>10 (5–18)</td>
<td>0.867 (0.696–0.912)</td>
</tr>
<tr>
<td>Pmx710</td>
<td>7–49</td>
<td>10 (4–14)</td>
<td>0.708 (0.696–0.912)</td>
</tr>
<tr>
<td>Pmx732</td>
<td>9–52</td>
<td>9.5 (3–16)</td>
<td>0.598 (0.189–0.767)</td>
</tr>
<tr>
<td>Pmx747</td>
<td>11–47</td>
<td>7 (4–10)</td>
<td>0.576 (0.396–0.792)</td>
</tr>
<tr>
<td>Pmx839</td>
<td>11–49</td>
<td>10 (6–16)</td>
<td>0.723 (0.667–0.828)</td>
</tr>
<tr>
<td>Lizard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soc700</td>
<td>28–34</td>
<td>8.5 (7–10)</td>
<td>0.826 (0.797–0.849)</td>
</tr>
<tr>
<td>Soc840</td>
<td>23–34</td>
<td>10 (7–11)</td>
<td>0.824 (0.783–0.878)</td>
</tr>
<tr>
<td>Soc857</td>
<td>28–35</td>
<td>9 (8–12)</td>
<td>0.825 (0.769–0.871)</td>
</tr>
</tbody>
</table>
values ranging from 0.0562 to 0.4316. For Bradford Ranch, of 25 comparisons with other sites, 13 showed moderate differentiation from other sites, 5 with great differentiation, and 3 with very great differentiation. Even on the Hopland station itself, there was an indication of differentiation between sites <1 km apart (Table III; Fig. 1). The topography between these sites is complex and steep, and suitable areas for lizards are separated by steep, grassy slopes.

Results for the lizard host revealed a different pattern, with little evidence for differentiation among sites, except for those furthest apart (Table IV). Of 84 comparisons for all sites and markers, only 4 were scored as differentiated (5%), and all of these were for a single marker (Soc857). These measures of moderate differentiation were for sites most distant, >6 km apart (4 of 63 such comparisons, or 6%).

**DISCUSSION**

Genetic differentiation among sites was determined with the use of variable microsatellite markers for a malaria parasite *P. mexicanum* and its lizard host *S. occidentalis*. With the use of pairwise $F_{ST}$ measures, the genetic diversity of both *P. mexicanum* and the lizard for sites from very nearby (200 m) to 40+ km was examined. The goal of this project was to test the hypothesis that low movement of the small territorial vertebrate host and its sand-fly vector should result in genetic differentiation of both lizard and parasite among sites (even those fairly nearby). Low levels of gene flow would thus allow the potential for local adaptation by both host and parasite to coincide with already observed differences in prevalence patterns (and thus likely transmission intensity) among sites (Schall and Marghoo, 1995; Eisen and Wright, 2001). The results, though, were unexpected, with the parasite found differentiated even for sites <1 km apart, but little, or no, differentiation for its vertebrate host. Thus, gene flow for the lizard is substantial, even over distances as great as 40+ km, but, despite this mobile host, the parasite remains locally distinct. The results suggest there is little opportunity for local adaptation to the parasite for fence lizards, but the parasite could well be selected for locally adaptive characters such as life history traits to match prevailing transmission intensity and lizard densities.

Our results resemble those for several studies of human malaria parasites. Anthony et al. (2005) examined the genetic structure (using microsatellite markers) of *P. falciparum* in villages in Borneo separated from <4 to 7 km, $F_{ST}$ values were associated with distance, rising from moderate differentiation for villages <4 km to very great differentiation at 7 km (for the standards used here). Forsyth et al. (1989) examined variation in the s-antigen serotypes of *P. falciparum* over even smaller distances,
villages only 2–5 km apart in Papua New Guinea. They found substantial differentiation among villages. These results contrast sharply with other studies that find little variation for sites even 100 km apart (Zhong et al., 2007; Prugnolle et al., 2008; Bonizzoni et al., 2009). The latter case is expected because even if the vectors move little, people are mobile and would transport the parasite long distances. What would account for the very local differentiation of *P. mexicanum* when its vertebrate host experiences substantial gene flow, and could the same process that drives local differentiation in *P. mexicanum* account for the finding of local differentiation of *P. falciparum* in some situations?

When infected with *P. mexicanum*, fence lizards suffer a broad range of hematological, physiological, reproductive, and behavior deficits (Schall et al., 1982; Schall, 1990; Dunlap and Schall, 1995; Schall, 1996). Of particular relevance here are the behavioral changes in infected lizards. Infected lizards are less socially active (Schall and Sarni, 1987), and less active in their territory (Schall and Houle, 1992), and males are poor competitors for access to females (Schall and Deering, 1987). Thus, infected animals are less likely to move any substantial distance, but their close relatives, carrying the same microsatellite alleles, may move to new sites. In mark–recapture studies (Bromwich and Schall, 1986), we occasionally found a marked animal as far as 1 km from its original home range only 24 hr after sighting the animal. Thus, gene flow can be substantial for the lizard, whereas the parasite would remain behind in its much less mobile host. The same could be true for *P. falciparum* infecting humans in Borneo and Papua New Guinea. Travel in these locations may be difficult and health care facilities not generally available, so infected people may remain at their home villages, especially when parasites are present in the blood and able to be transmitted.

The local differentiation of parasite, but not host, could derive from changing prevalence of the parasite over time rather than lack of movement of infected lizards. The prevalence of *P. mexicanum* at the HREC sites has changed substantially over the past 3 decades, with consequences for its overall genetic diversity (Vardo and Schall, 2007); allele frequencies may also have changed (see Materials and Methods section). Although not measured, the population density of the lizard appears to have remained high at all sites and constant over the past decades (our subjective judgment). Thus, shifts in the prevalence of the parasite could have led to stochastic genetic changes and the resulting differences among sites.

Studies on the genetic differentiation and geographic differentiation of malaria parasites infecting wildlife hosts have lagged behind similar studies on the parasites of humans. Such studies are possible only after variable genetic markers are characterized for the parasite. Full-genome sequencing of the human malaria parasites has revealed many useful genetic markers (Anderson et al., 2000). Methods are now available for discovery of microsatellite markers of the broader diversity of malaria parasites that infect primarily birds and lizards (Schall and Vardo, 2007). The study of genetic structure of a broader range of malaria parasites will open windows into their general biology and evolution.

**Acknowledgments**

We thank the staff of the Hopland Research and Extension Center for their continuing logistical and moral support of the long-term studies of lizard malaria; this project would not be possible without their help. Allison Neal assisted in the lab, and she and Alice Ford helped with lab duties. We thank the Bradford, Keiffer, and Wheeler families for access to their properties. C. W. Kilpatrick taught us analytical methods and was the best critic throughout the study. Reviewers offered creative suggestions on the analysis and discussion. Funding was provided by NSF (to J.S.J.) and the URECAI, Helix, Honors College, and APLE programs of the University of Vermont (to J.M.F.).

**Literature Cited**


