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# Blood parasite infection in mice from cloud forest and nearby disturbed habitats

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# Blood parasite infection in mice from cloud forest and nearby disturbed habitats

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## ABSTRACT

Conversion of habitat by humans is pervasive, increasing, and the root of serious environmental problems. This study was performed to find differences in blood parasite infections of host mice in neotropical cloud forest versus nearby disturbed habitat. In Monteverde, Costa Rica, mice were trapped and examined for blood parasite species richness and abundance in primary forest and disturbed areas near houses. Twenty-eight mice were captured: 15 from the forest and 13 from the disturbed habitats. Eighty-six percent of these mice were of the species *Peromyscus nudipes*. There was no significant difference of parasite species richness between mice from the forest habitat (mean = 2.818 ± 1.25; N = 11) and disturbed habitat (mean = 3.25 ± 0.75; N = 12)(Mann-Whitney U = 54.5; P > 0.05) or of parasite abundance between the forest habitat (mean = 8.36 ± 5.12; N = 11) and disturbed habitat (mean = 10.33 ± 5.02; N = 12) (Mann-Whitney U = 57.0; P > 0.05). Transference between the habitats may be frequent, causing similarities in parasite infection rates and types between the populations. *Peromyscus nudipes* is a "weedy" species and possibly does not discern between the different areas, resulting in this transference.

## RESUMEN

La conversión del habitat por los seres humanos sigue en aumento y es la raíz de problemas ambientales serios. Este estudio fue realizado para encontrar diferencias en las infecciones de parásitos de la sangre en ratones del bosque neotropical nuboso comprado con habitats degradados aledanos. En Monteverde, Costa Rica, atrapé ratones y examiné la riqueza de las especies de parásitos de la sangre y su abundancia en bosque primario y las áreas degradados cerca de las casas. Veintiocho ratones fueron capturados, 15 del bosque y 13 de los habitates degradados. Ochenta y seis por ciento de estos ratones pertenecieron a la especie *Peromyscus nudipes*. No hubo una diferencia significativa de la riqueza de las especies de parásitos encontrados entre los ratones del habitat del bosque (promedio = 2.818 el ± 1.25; N = 11) y habitat degradado (promedio = 3.25 ± 0.75; N = 12) (Mann-Whitney U = 54.5; P > 0.05), o de la abundancia del parásito entre el habitat del bosque (promedio = 8.36 ± 5.12; N = 11) y habitat degradado (promedio = 10.33 ± 5.02; N = 12) (Mann-Whitney U = 57.0; P > 0.05). La transferencia entre los habitates puede ser frecuente, lo que puede estar causando las semejanzas encontrados en los índices de infección de los parásitos entre las poblaciones. *Peromyscus nudipes* es una especie oportunista y posiblemente no discierne entre las diversas áreas, dando por resultado esta transferencia.

## INTRODUCTION

Transformation of land by human use represents the most significant human modification of the planet. Human impact on Earth's ecosystems includes intensive row-crop farming, urban development, and various other destructive practices resulting in a nearly 30% increase in atmospheric $CO_2$ since the Industrial Revolution (Vitousek et al. 1997). According to some estimates, approximately 50% of the potential closed-canopy forest on earth has already been cleared and converted to other uses. During the 1990s, secondary forest reclaimed just one hectare for every six or seven deforested. Therefore, we are cutting forest at a much faster rate than it is growing back (Wright 2005).

Climate change, loss of biodiversity, and changes in community composition are among the most notable effects of human land use (Vitousek et al. 1997). As a result of continuing population growth and growing consumption, the few large, undisturbed areas remaining are threatened. This is especially true in developing countries in the Tropics, where the rate of human population growth and biodiversity are the highest. Instead, many species will go extinct and a mosaic of persisting, disturbed habitats will contain the majority of remaining tropical diversity. These disturbed patches are rarely studied, yet the response of biodiversity to this

fragmentation will determine its future. According to a study of avifauna in southern Costa Rica, social interactions drastically change in open habitats, negatively affecting some species and favoring other, more generalist species (Daily 2001). These generalist species may be non-native and may only invade a habitat and increase in density if a disturbance occurs and conditions change. The species that most often suffer when environments are transformed for human use are forest specialist species, which are very sensitive to change in their habitat.

Rate and frequency of infection and transmission of pathogens, depend on several parameters of the host population, their habitat, and the nature of the pathogen. Factors of the host population that affect pathogen transmission include population density and migration. In a more dense population, susceptible individuals may be in closer contact with infected individuals. A high rate of migration in a population affects transmission because it potentially puts more individuals that are susceptible to infection into contact with infected individuals. The qualities of a pathogen that affect its transmission are things such as its virulence, its method of transmission, and its host specificity. If a pathogen is a host generalist, there may be one or more reservoir species on which the pathogen has less impact than it does on the definitive host species. In this case, the reservoir host can transfer the infection to more affected species and thus contact between species may affect the spread of disease (McCallum and Dobson 2002). One habitat factor that affects pathogen transmission is variability in the external environment of a host, such as habitat disturbance. This could stress the host population, increasing the susceptibility of a host to infection, allowing parasite transmission regardless of whether the change has any direct effects on parasite development and survival (Morgan et al. 2004).

Based on recent data, (Pederson 1998, Rothman 1999, Lippert 2001, Hayes and Laval 1989) predominantly one species of mouse, *Peromyscus mexicanus*, exists in the Monteverde area in Habitat Zone Three (Hayes and Laval 1989). The specific subspecies that exists in Montverde is the *P. mexicanus nudipes*, sometimes considered its own species as *P. nudipes*. *P. nudipes* is a small, omnivorous, "weedy" species that exists in many different habitats. This rodent community was not always dominated by one species. In a trapping study conducted to compare population ecology of *P. nudipes* from 1979 to 1981, in plots in Monteverde habitat zone three, several other species of rodents were trapped with moderate to high frequency. In fact, individuals of the species *Herteromys desmarestianus* were determined to be equal or greater in abundance than *P. nudipes* in this study (Anderson 1982). This "weedy" species has since invaded the forest habitat, increased in density, and potentially linked disturbed habitat and forest. This could have passed parasites from one habitat to the other, causing widespread effects from an unspecified change in community structure from the past.

To examine whether land changes from forest to open, human use areas have an effect on the prevalence of blood parasites in rodents, I compared the species richness and abundance of parasites found in blood samples from rodents captured in forest to rodents captured in close proximity to forest. In a past experiment by Roelands and Taft (1999), several identified parasites were found to occur in the mouse populations of Monteverde. *Trypanosoma*, *Plasmodium*, and Microfilarial stages of parasites were found in varying abundances in several rodent species in secondary and primary forests. The study suggests that both the abundance and species richness of endo-parasites in rodents are higher in hosts found in secondary forest than in primary forest sampled in this study (Roelands and Taft 1999). I hypothesized that my study would demonstrate similar trends between parasite levels and population habitats when comparing primary forest and habitats that have been deforested for many years. This comparison of forest habitat to transformed areas should show a higher abundance and frequency

of infected individuals in disturbed habitats because the conditions in a disturbed habitat should increase the rate of parasite infection according to pathogen transmission parameters.

I also compared host weight and length in each habitat to parasite abundance and species richness to determine whether size is related to parasite infection rate. If small mice were observed to have a higher parasite richness or abundance, then it could be assumed that the young were more susceptible to parasite infection and that highly infected individuals were not healthy enough to spend energy on growing to full size.

# METHODS

### Study Sites

This study was conducted around the Estación Biológica de Monteverde, in Monteverde, Costa Rica. I trapped rodents over seven nights in two habitats: forest and in close proximity to houses. Both habitats were in Lower Montane Wet Forest (Holdridge 1967), in the Monteverde habitat zone three (Hayes and Laval 1989), and between 1520 m and 1600 m. The forest study site was along the Sendero Principal trail, beginning approximately 150m into the forest with traps placed along the sides of the trail every ten meters. The house study traps were placed surrounding the Estación Biológica de Monteverde, nearby cabins, and a house approximately 150m down the road, all within ten meters of a human habitation.

### Rodent Trapping

Seventeen Sherman live traps were used to capture rodents in each of the two habitat types. The traps were baited with one tablespoon of a mixture of peanut butter and oatmeal before each trapping night. The following morning each trap was checked. Captured rodents were identified to species and sex and measurements of weight, head to vent length, and hind foot length were taken. Rodents were marked to prevent repetition of data through recaptures by cutting a small amount of hair off the upper part of the back. A blood sample was collected from each individual by lancing the bottom of the back foot behind the pad. The resulting droplet of blood was pressed against a glass slide.

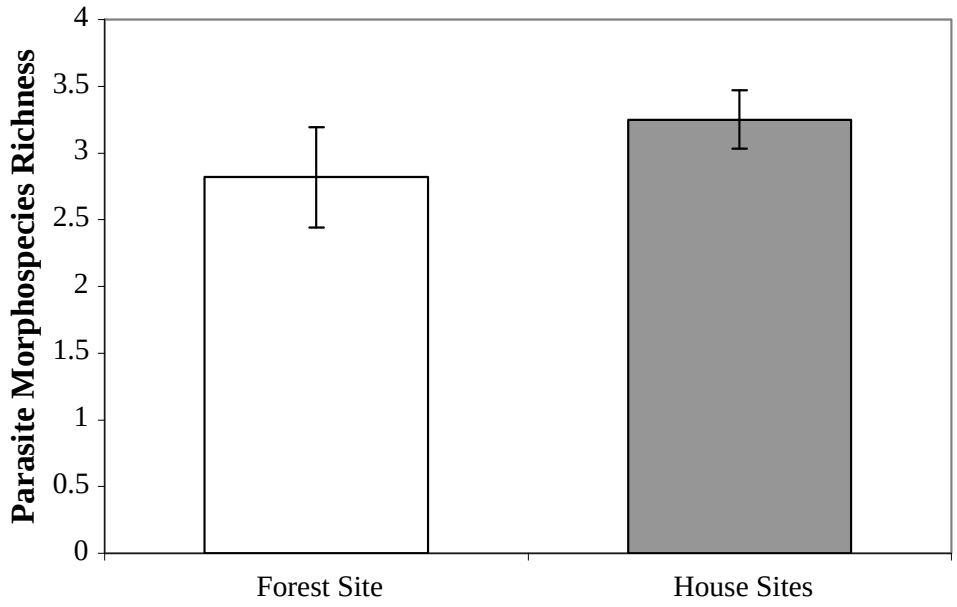
### Identifying and Counting Blood Parasites

At the end of each collection day, the slides with blood were fixed with 100% methanol (MeOH) for 2-5 minutes (Gardner 1996). They were then stained with a solution of 5% Geimsa stain diluted with Wright buffer for 20 to 30 minutes, face down on a shallow plate, propped up on other slides. Twelve of the collected samples were initially observed with a microscope under oil immersion 1000x magnification to identify several categories of morphospecies. The nine morphospecies identified (Appendix) were then used in the analysis of each of the blood samples under oil immersion 1000x magnification. If a new morphospecies was found during sample analysis, it was added to the list of possible morphospecies. Ten random fields of view were observed in each slide for the presence and abundance of the nine morphospecies.

# RESULTS

Over seven nights of trapping, 30 mice were captured. Two of these were recaptures. Twenty-four of the mice were *P. nudipes*, and four individuals trapped were *Scotinomys teguina*. Sixteen captures were made in the forest site, with one known recapture. Out of these 16 captures from

(a)




(b)

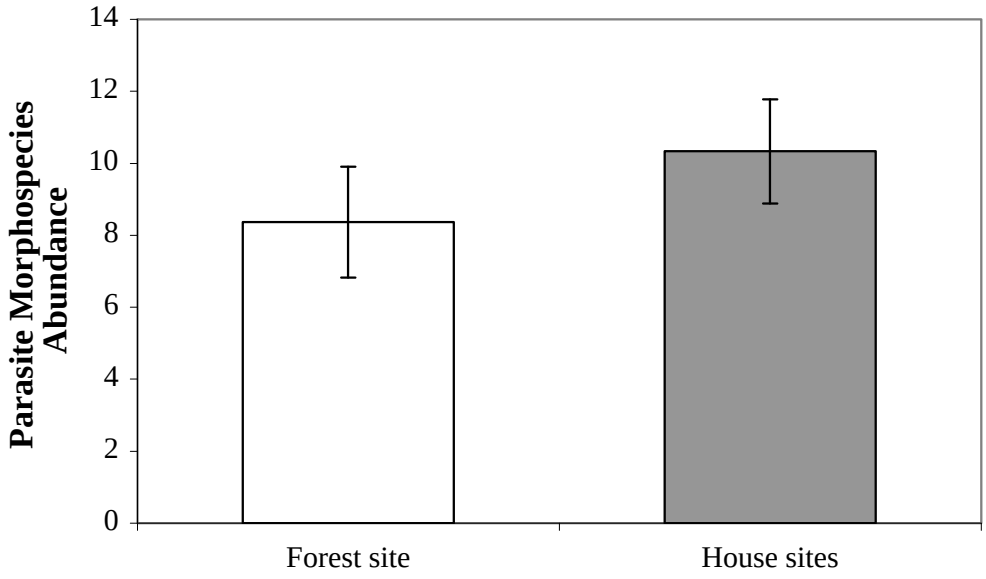



Figure 1. Comparisons of means between samples from a forest study site and multiple house study sites of (a) Parasite Morphospecies Richness and (b) Parasite Morphospecies Abundance in mouse hosts. In both comparisons, the average value from the house sites was higher than the average value from the forest site. However, this difference was not significant in (a) the comparison of the means of parasite morphospecies richness (Mann-Whitney $U = 54.5$; $P > 0.05$), or in (b) the comparison of the means of parasite morphospecies abundance (Mann-Whitney $U = 57.0$; $P > 0.05$) (SE bars included).

the forest site, 13 were *P. nudipes* and three were *S. teguina*. In the four different house sites, 14 mice were trapped, with one known recapture. Thirteen of the house site captures were *P. nudipes* while one was *S. teguina*. No captured rodents showed any outwardly apparent signs of illness or infection.

No significant difference was observed between the mean parasite morphospecies richness in blood samples from the forest habitat ($2.818 \pm 1.25$; $N = 11$) and the mean parasite morphospecies richness in the blood samples from the house habitats ($3.25 \pm 0.75$; $N = 12$) (Figure 1a) (Mann-Whitney $U = 54.5$; $P > 0.05$). No significant difference was seen between the mean parasite abundance in blood samples from the forest habitat ($8.36 \pm 5.12$; $N = 11$) and the mean parasite abundance in the blood samples from the house habitats ($10.33 \pm 5.02$; $N = 12$) (Figure 1b) (Mann-Whitney $U = 57.0$; $P > 0.05$). There were no clear trends regarding which parasites were found in each habitat or in each host species (Appendix B).

Regressions comparing morphospecies richness in blood samples to host weight showed no significant relationships in the forest site ($R^2 = 0.003$; p-value = 0.87; n = 11) or in the house sites ($R^2 = 0.001$; p-value = 0.92; n = 12). Regressions comparing morphospecies richness in blood samples to host head to vent length showed no significant relationships in the forest site ($R^2 = 0.0001$; p-value = 0.98; n = 11) or in the house sites ($R^2 = 0.002$; p-value = 0.90; n = 12). Regressions comparing parasite abundance in blood samples to host weight showed no significant relationships in the forest site ($R^2 = 0.094$; p-value = 0.36; n = 11) or in the house sites ($R^2 = 0.008$; p-value = 0.79; n = 12). Regressions comparing parasite abundance in blood samples to host head to vent length showed no significant differences in the forest site ($R^2 = 0.059$; p-value = 0.47; n = 11) or in the house sites ($R^2 = 0.028$; p-value = 0.60; n = 12).

## DISCUSSION

The lack of relationship between parasite infection (as morphospecies richness or as morphospecies abundance) to host size (as weight or as head to vent length) leads to several conclusions. As smaller mice were not observed to have a higher parasite morphospecies richness or abundance, it cannot be assumed that the young were more susceptible to parasite infection. Instead, all individuals are apparently being exposed to parasites. Another conclusion that can be drawn from these results is that infection does not seem to be keeping mice from growing to full size. My data on mouse length and weight are very similar to average literature values for adult size (Reid 1997). All mice also showed no outwardly apparent signs of infection, yet all were infected with at least one morphospecies of parasite. Parasite infection, therefore, may not have significant effects on mouse health.

Individuals in both habitats are equally infected by parasites. This could result from three different mechanisms. First, there may be contact between some individuals living in the two different habitats. Many of the parasites that infect mice, including many of those parasites infecting my samples (suggested by morphospecies appearance), are vector-borne. Another possibility is that vectors of the parasites may be able to travel between habitats, infecting individuals in both areas (Morgan et al. 2004). The final way that individuals in both habitats could have equal exposure to parasite infection, is that the mice may see both habitat types as one and range over both disturbed areas and forest without discern. The species has a relatively small average home range of approximately 0.10 hectares. However, if the species has a high

density, as it has been shown to have in this location, then it is possible that an infection by a parasite could easily spread through a continuous population spanning the transition from a forest area to a disturbed area (Anderson 1982).

There is further evidence that *P. nudipes* does not discern between habitats and has actually benefited from the destruction of forest habitat. The population ecology study of *P. nudipes* conducted from 1979 to 1981 demonstrated a significantly diverse community of small rodents (Anderson 1982). In the past six years, however, several studies conducted in the same areas have found *P. nudipes* to make up a much larger subset of the small rodent population (Pederson 1998, Rothman 1999, Lippert 2001). One study collected 69 mice, of which, 95.7% were *P. nudipes* (Pederson 1998).

It seems apparent that something has happened in the recent past facilitating a change in the rodent species richness of the area and allowing *P. nudipes* to become the dominant species. A possible explanation is that a threshold has been reached where sufficient habitat clearing in the past created a community change where other species that were dominant could no longer survive and the "weedy" *P. nudipes* species took over. This could be an example of what Nee and May (1992) found in their study on habitat destruction and competition. As habitat loss increases, a formerly inferior competitor may increase in numbers relative to a superior competitor. This will occur if the inferior species is a "weedy" generalist-type species and has a higher rate of colonization than the competitor (Nee and May 1992).

Another study suggested that the competitive force could be a pathogen, preventing multiple host species to coexist in the same patch (McCallum and Dobson 2002). Therefore, a possible explanation of the recent dominance of *P. nudipes* could be that the species may not be as affected by the parasites as other species are. It is possible that when the forest was cleared, the habitat change caused the other small rodent species' fitness to be more negatively affected than the fitness of *P. nudipes*, causing them to be outcompeted. The loss of competing species, since 1982, may have resulted in easier parasite transmission between the dense, monodominat host community. This hypothesis, however, must be tested by future studies. One such study could test the idea that currently, *P. nudipes* does not discern between the habitats, by trapping along a continuous stretch from one habitat to the other. Individuals could be marked specifically to observe movement between the habitats. If available, genetic data could be collected from collected mice to further analyze similarity between individuals the habitats.

With current deforestation claiming two percent of forests worldwide each year, problems associated with the loss of biodiversity and changing community dynamics as a result of habitat destruction will likely worsen (Terbourgh 1992). The microscopic world is often less studied, yet it can have immense impacts on an ecosystem. Microorganisms often act as an obscure keystone species (Dobson 1997), potentially determining the community structure. Future conservation efforts must take into account the possibility of microorganisms affecting the outcome of altering land use. Processes, such as destruction of habitat can lead to the degradation of biodiversity and, perhaps a more selfish concern, effects on human health.

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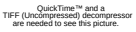
## Appendix A

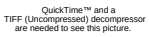
Photo examples of nine parasite morphospecies

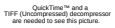

Cluster
(Ruptured Schizont stage of *Plamodium*)



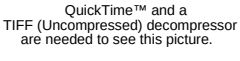

Trypanosome

Possible *Hepatozoon*

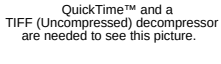





Rods




Microfilarial parasite

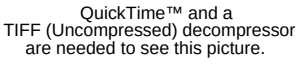



Gametophyte stage
(Possible *Plasmodium*)

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Ribbons

Infected Red Blood Cell
(Possibly *Plasmodium, Babesia, or Grahamella*)




Flagellate