The Black Salt Marsh Mosquito:
The Deadly Threat to the Galapagos

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Introduction: Diseases, Mosquitoes, and the Galapagos

Throughout the world—and especially over the past century—emerging infectious diseases of wildlife have been recognized as a major threat to biodiversity. Isolated oceanic islands like the Galapagos are particularly at risk in the face of introduced pathogens and disease vectors (Wikelski et al. 2004; Kilpatrick et al. 2006). Populations are smaller on archipelagos and have been exposed to a smaller diversity of outside diseases, making them more susceptible to a vector-borne disease due to the generally inferior amount of variation amongst various species. Vector-borne diseases, or those in which the pathogenic microorganism is transmitted from an infected individual to another individual by an arthropod or other agent, sometimes with other animals serving as intermediary hosts, have and will continue to make their appearance in the Galapagos.

According to the Charles Darwin Foundation, vector-borne diseases, such as West Nile virus and avian malaria, if introduced into Galapagos ecosystems, could cause widespread mortality of native birds. We have already seen foreign diseases from invasive species come and wreak havoc in the Galapagos. Newcastle disease has been detected in the rapidly growing poultry industry of the Galapagos and has the potential to cause declines of the flightless cormorant, lava gull, and Galapagos penguin, species with population sizes of less than 1500 individuals. Domestic cats, found mainly in human-inhabited areas like San Cristobal Island, are carriers of *Toxoplasma gondii*, a protozoan parasite that may cause the deaths of many Galapagos birds and marine mammals. Domestic dogs could introduce canine distemper and leptospirosis—diseases that could cause the mortality of many Galapagos sea lions.

Figure 1: Avian Pox in Galapagos Endemic Wildlife, Courtesy of the Darwin Foundation

Disease vector introductions in recent history are closely linked with the development of global transportation links, such as the worldwide airline network (Tatem et al. 2006). It was the interconnectedness of our globalized world that made swine flu into a pandemic, as classified by the World Health Organization. Human-aided transport and human-induced environmental changes are only going to make things worse for the endemic wildlife of isolated oceanic islands like the Galapagos—they have dramatically increased the capacity of disease vectors, such as mosquitoes, to reach, establish and spread in previously inaccessible areas (Lounibos 2002). Recent outbreaks of West Nile fever in the USA (Kramer et al. 2008) and of Chikungunya disease throughout the Indian Ocean region (Chevillon et al. 2008) are striking examples of the impacts of globalization on human and animal health. The co-introduction of the southern house mosquito and avianpoxvirus in Hawaii was followed by a later introduction of avian malaria, causing dramatic declines and extinctions among Hawaiian endemic birds (Warner 1968; Van Riper et al. 1986, 2002).

Figure 2: An Example of the Transportation Systems that Spread Disease Vectors

Only 3 mosquito species have been found in the Galapagos up to this point: the southern house mosquito (*Culex quinquefasciatus*), the black salt marsh mosquito (*Aedes taeniorhynchus Wiedemann*), and the globally distributed Yellow Fever mosquito (*Aedes aegypti L.*) (Kilpatrick et al, 2006). *Culex quinquefasciatus*, an important vector of wildlife diseases (e.g., avian malaria, avian pox), was introduced into the Galapagos in 1985 and its presence is currently restricted to human settlements (Whiteman et al, 2005). However, recent publications and media, all within the last month, indicate that the southern house mosquito may be more widespread than originally indicated. *Aedes aegypti*, introduced in the 1990s, is highly anthropophilic (i.e. favors humans) and is found only in and around Puerto Ayora; therefore *C. quinquefasciatus* and *A. taeniorhynchus*are are the only 2 mosquito species which might play a significant role in the transmission of wildlife diseases in the Galapagos Islands. For the purposes of this research, the black salt marsh mosquito will be the primary focus due to its distribution throughout the archipelago.

Figure 3: The Black Salt Marsh Mosquito, Courtesy of the American Mosquito Control Association

The black salt marsh mosquito is an important nuisance to wildlife, e.g., nesting birds, across the islands. It is generally a flood-water specialist found in temperate and tropical coastal areas of the Americas, from New Hampshire to Brazil on the Atlantic coast and from California to northern Peru on the Pacific coast (Carpenter et al, 1955). The Black Salt Marsh Mosquito in the Galapagos has never been studied in depth and its potential importance as a disease vector has only recently been considered. Of the three mosquitoes on the archipelago, it is also the only one whose origins are not completely certain.

Hypotheses

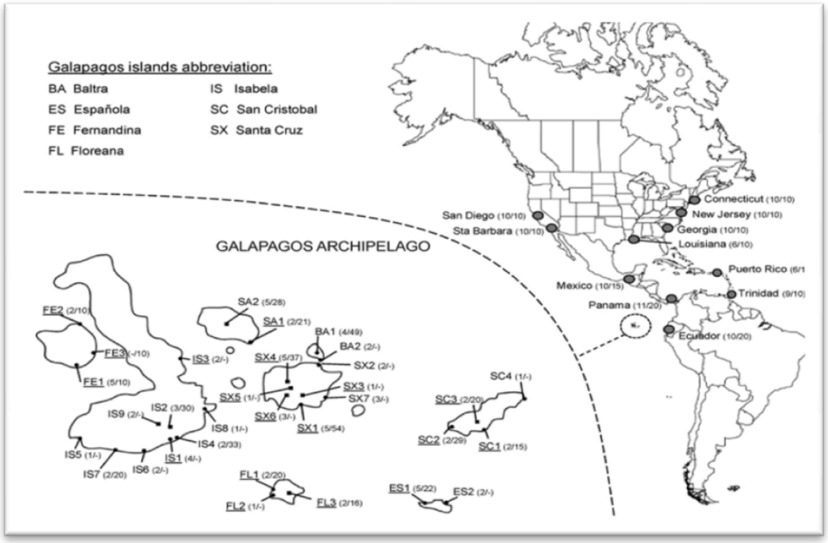
The black salt marsh mosquito's presence in the Galapagos Islands was first recorded in the late 1880s (Howard et al, 1889), but it is not known whether it arrived naturally or with man. The other two species of mosquitoes on the archipelago arrived within the last thirty years by man, either through boats or via the air. Therefore, the first hypothesis of this study is *the Black Salt Marsh Mosquito was introduced by man sometime before its first presence was recorded.*

Elsewhere, few studies have focused on the black salt-marsh mosquito, and it is usually considered only as a nuisance mosquito with respect to human health. However, research has shown that the mosquito plays a major role in the transmission of the dog heartworm (*Dirofilaria* *immitis*) in South and Central America and has been identified as a competent vector of many arthropod-borne viruses such as St. Louis encephalitis virus and West Nile virus (WNV) (Labarthe et al, 1998; Nayar et al, 1986). The black salt marsh mosquito has been considered as an important bridge-vector of WNV between birds and mammalian hosts and studies indicate that it could only be a matter of time before WNV makes its appearance in the Galapagos. Therefore, the second hypothesis of this study is that *the black salt marsh mosquito's feeding patterns in the Galapagos make it even more dangerous for the endemic wildlife on the archipelago.*

Methods – Hypothesis I and II

In a study published on June 23rd, 2009, researchers used an approach using mitochondrial DNA (mtDNA) sequence analysis and microsatellite genotyping. Because mtDNA is not highly conserved and has a rapid mutation rate, it is useful for studying the phylogeny, or evolutionary relationships, of various organisms. Comparing mtDNA sequences, biologists can build an evolutionary tree for the species examined. Because mtDNA is transmitted from mother to child (both male and female) it can be a useful genealogical tool to track the mother's line. This can help predict when the black salt marsh mosquito actually migrated to the Galapagos archipelago.

Figure 4: Map of the sites where Aedes taeniorhynchus specimens were collected on the American continents and on the Galapagos Islands (Bataille et. al. 2009)



In this particular experiment conducted by the Institute of Integrative and Comparative Biology, the black salt marsh specimens were collected on the American continents and on the Galapagos Islands. Numbers between brackets are the number of specimens collected in each site used for the mtDNA and microsatellite studies, respectively. Sites where bloodmeals from bloodfed mosquitoes were analyzed are underlined. The bloodmeal analysis was the method used to determine the feeding patterns of the black salt marsh mosquito (hypothesis II).

Seventy-eight specimens were obtained from mosquitoes collected in various islands and habitat zones of the Galapagos. Every major island of the archipelago had mosquito samples, showing the infiltration of the black salt marsh mosquitoes all over the islands. The highest haplotype diversity was found in Isabella Island whereas the mosquitoes from the mainland had the highest mtDNA diversity, as expected.

Results/Discussion – Hypothesis I – Origination of the Mosquito

The mtDNA experiment was used to find the phylogenetic relationships between each of the Galapagos and continental haplotypes by using maximum likelihood and Bayesian inference methods. A Bayesian Hypothesis is a subjective statistical inference in which evidence or observations are used to infer the degree of belief in a particular hypothesis. The experiment showed that the Galapagos haplotypes separated from continental haplotypes with a posterior probability, or the probability after all of the evidence is taken into account, of 100%, indicating that that the Galapagos population of black salt marsh mosquitoes originated from a single colonization event and now represents a distinct evolutionary unit clearly divergent from the continental populations.

However, it is interesting to note the numerical value assigned to the particular divergence. The Galapagos and continental clades didn't occur two hundred years ago, but 200,000 years ago! The 95% confidence interval, which was measured using 1.15%/million years, a rate widely used for insect mtDNA nucleotide substitution, is between 100,000 and 350,000 years. Even though this 250,000 year interval seems to be imprecise, the estimation demonstrates that the colonization of the black salt marsh mosquito was *not* human driven, unlike previously thought. Actual mutation rates would need to be between 200 times higher to push the confidence interval into the period of first human discovery (1535 AD) or 1000 times higher to push the confidence interval into the period of first human colonization.

It is important to note, however, that while the black salt marsh mosquito appeared hundreds of thousands of years ago, other endemic insects arrived soon after the Galapagos was formed, greater than 5 million years ago (Sequiera et al 2000). This relatively recent colonization of the black salt marsh mosquito compared with other Galapagos endemic fauna suggests that it arrived in a system that may not have had vector-borne disease hosts, allowing pathogens and diseases to invade. They may have also had time to adapt to the Galapagos Islands and various niches, as we will see in the subsequent discussion of the second hypothesis.

Results/Discussion – Hypothesis II – Feeding Patterns

Prior to jumping into the results of the second hypothesis, it is necessary to take a quick look at habitat patterns of the black salt marsh mosquito on the mainland and on the Galapagos. On the continent, the species is rarely found 6 kilometers from the coast and has been reported to breed inland only rarely. In the Galapagos Archipelago, the black salt marsh mosquito was regularly caught in the humid highland zone of various islands up to 20 kilometers from the coast and at 700 meter altitude. These mosquitoes are going further inland and higher up than ever before, giving them an ever greater access to species.

But the story doesn't end there. A total of 105 bloodmeals were successfully analyzed across six highland areas and twelve coastal areas on seven islands using a PCR method. Fifty-eight percent of the mosquitoes had fed on reptile blood, 47% on marine iguanas, and 11% on the Galapagos tortoises. Forty-one percent of the mosquitoes had fed on mammal blood (16% on Galapagos sea lions, 16% on human beings, and 9% on domestic animals). Only 1 bloodmeal was identified as avian (flightless cormorant). This is the first time that the black salt marsh mosquito has been found to feed on reptile blood. What's even more striking, however, is that it seems to be *preferentially* feeding on reptile blood.

Figure 5: A Galapagos land iguana, a preference for the black salt marsh mosquito

The Galapagos black salt marsh mosquito may actually be another species. The genetic distance separating the Galapagos lineage from the most closely related continental lineage is of the order used to define species designations for other taxa (Bataille et al 2009). Its feeding and habitat patterns are, from an epidemiological perspective, dangerous. These mosquitoes are now reaching further up and further inland than ever before as well as targeting almost every type of animal in the archipelago. Such diverse feeding behavior provides the potential for this mosquito to act as a bridge-vector across the majority of the Galapagos endemic wildlife, especially since WNV has a wide host range, including birds, mammals, and reptiles. If—and some scientists don't believe it is a question of 'if', but rather a question of 'when'—WNV hits the Galapagos Islands, the black salt marsh mosquito could easily become the biggest threat to the endemic wildlife of the archipelago.

Figure 6: Avians of the Galapagos - a Host for the West Nile Virus

What Do We Do?

The impact of disease introduction to Galapagos could be heightened if human-aided movement of mosquitoes between the islands occurs. It isn't helping that many cruise ships go island-hopping every single day in the Galapagos and that planes continually are landing form the mainland. Monitoring of mosquito populations and strict adherence of dis-insection protocols for both boats and planes must be implemented to reduce movement of mosquitoes to and among the Galapagos Islands to lower the risk of novel disease-spread across the archipelago. Planes are getting wiped down and luggage is getting checked, but it may be beneficial to screen luggage coming into Ecuador for mosquitoes. Colombia has already reported WNV activity—it may be only a matter of time before it crosses the border to Ecuador.

Figure 7: Countries marked in black have reported cases of the WNV - it is only a matter of time before it reaches Ecuador

Conclusions/Future Research

After various experiments conducted by a wide variety of scientists, the hypothesis that the black salt marsh mosquito was brought over by man within the last 200 years, was **rejected**. It turns out that the black salt marsh mosquito likely came over about 200,000 years ago, giving it plenty of time to fill new niches. The second hypothesis, that the black salt marsh mosquito's feeding habits in the Galapagos make it even more dangerous for the endemic wildlife on the archipelago – is **supported**. For the first time, the mosquito is feeding on reptiles as well as mammals and birds, making it an especially lethal carrier of pathogens.

Figure 8: The sea lion could be eliminated in large numbers by the WNV

It would be especially exciting to take a look at three fundamental research questions looking forward:

1) What is the difference between coastal and highland populations of the black salt marsh mosquito in the Galapagos? Is there a difference at all? Or, are these a different species compared to the continental ancestors?
2) When did the differentiation occur amongst the mosquitoes in the Galapagos?
3) When is West Nile Virus predicted to enter the islands? Is there any way to prevent its entry or, at the very least, delay it?

The black salt marsh mosquito is clearly a danger to the endemic wildlife of the Galapagos. It is rather surprising that humans were not the ones that introduced them—it was purely chance. However, it will be up to humans to try to save the wildlife (and potentially control a natural selection inducing event) from its wrath once WNV enters.

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