Demodex brevis and D. folliculorum are mites that live in the human pilosebaceous unit. Their tube-shaped bodies are approximately 0.2-0.4 mm long, with four pairs of legs on the anterior region (Nutting, 1976). Until very recently, it was unclear whether the two should be recognized as separate species, the main difference between them being their size, but recent, unpublished work by an honors student in the Palopoli lab suggests they should, indeed, be classified as distinct species. It turns out the two mites are very divergent at the molecular level, more so, even, than the mitochondrial genome of a fly is from that of a mosquito (Endrizzi, 2009). It is estimated that the two species diverged more than 200 millions years ago.

The widespread distribution of follicle mites, found on every mammal tested to date, suggests an ancient, coevolutionary relationship between these Demodex species with their mammal hosts (Minot, 2008). It is yet uncertain whether or not this relationship can be classified as parasitic or benign. There is no evidence that these mites do substantial harm to their hosts, but they have been implicated as the cause of certain skin disorders, such as human rosacea and blepharitis (Jansen et al., 2001). We’ve begun looking at mite species on different mammals, and sequencing their mitochondrial DNA (mtDNA) to determine how closely related they are to the two species living on humans. Starting with Demodex kutzeri, found on elks, we attempted to amplify their mtDNA through a long range polymerase chain reaction (PCR) in order to make enough product to be digested and cloned into pBlueScript plasmid for sequencing (Figure 1.). Once successful, we will compare D. kutzeri’s mtDNA sequence with that of D. folliculorum and D. brevis to begin constructing a phylogeny for the mites. The phylogeny will help answer the question of how two mite species that appear to have diverged from one another millions of years ago can manage to coexist on the same host.

One hypothesis, dubbed the “skin partition” hypothesis, is that the two Demodex species occupy distinct niches and utilize different resources, and are therefore able to coexist. A second hypothesis, the “host switch” hypothesis, is that the one or both of the two species recently migrated onto humans from a different host, and not enough time has passed for one species to outcompete the other.

From a proposed phylogeny based on an ~300 base pair sequence of D. kutzeri’s mitochondrial genome (recently obtained from Mike Palopoli’s bio 216 class) (Figure 2.), it appears that D. kutzeri and D. folliculorum are more closely related to each other than D. folliculorum is to D. brevis. We looked to sequence the rest of D. kutzeri’s mitochondrial genome to either refute or support these findings. We successfully re-amplified D. kutzeri mitochondrial genome through long range PCR using amplified mtDNA obtained by a previous student who used the initial 300 base pairs sequence acquired from Bio 216 as template. To determine whether we successfully re-amplified our putative D. kutzeri mitochondrial genome, we ran the PCR product through gel electrophoresis. Re-amplification was considered a success if a ~14kb band was visible, as this is the approximated size of D. kutzeri full mitochondrial sequence (Figure 3.). If product was obtained, we purified and concentrated the DNA using QIAgen kits.

Unfortunately, a significant portion of our time this summer was devoted to improving the results of this first step in our cloning process, the long range PCR. Our PCR often had a lot of smearing, potentially due to the formation of primer dimers (Figure 4.). This forced us to repeatedly alter our PCR conditions to attempt to get cleaner product bands. Some of the conditions we changed were decreasing the extension time, increasing the annealing temperature, and varying the amounts of
magnesium, template, enzyme, and primers. Yet, we discovered the alterations we made to our PCR conditions did not consistently improve our gel.

Though we didn’t get good PCR, we continued through the full cloning process three times. We used restriction enzymes such as EcoRI and HindIII to cut the re-amplified mtDNA and clone it into a plasmid that had been cut with the same enzymes. Afterwards, we grew up the bacterial colonies using blue/white selection, and picked white colonies the following day that were assumed to contain plasmids with putative PCR insertions. The colonies chosen were grown up overnight and mini-prepped the next morning to isolate the DNA from the overnight cultures. The purified DNA was then sent for sequencing. Had we been successful and received clean sequence back, we could have used those segments to design more primers and add to our existing *D. kutzeri* mitochondrial genome sequence. Unfortunately, we never seemed to get a section of mite DNA inserted into our plasmid. We received interpretable sequence back on two occasions, but it appeared to be sequence from *Escherichia coli* and a human adenovirus (likely resulting from contamination during the cloning process) (Figure 5.). Hopefully, additional attempts to purify the PCR product and ligate digested segments into a plasmid will yield our desired results (sequence from elk mite mtDNA) in the near future.
Graphs/images/figures

Figure 1. *Demodex kutzeri*

Figure 2. Proposed phylogeny showing *D. folliculorum* and *D. kutzeri* are more closely related to one another than *D. folliculorum* is to *D. brevis*.

Figure 3. Approximate 14kb band we expected to see if PCR was successful.
Figure 4. Example of a gel with significant smearing near the top and bottom.

Figure 5. Three bottom bands are plasmid DNA that has not been cut, four middle bands are cut plasmids with nothing inserted, and the circled band is cut plasmid with an insert. When sent for sequencing, it appeared to contain part of a genome but that was the result with human adenovirus.

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References


• Minot, SS. 2008. Evolutionary history and population genetics of the human follicle mite, Demodex folliculorum. An Honors Project for the Department of Biology, Bowdoin College.