Pigeonetics: A Study of the Genomes of Pigeons in New York City

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Abstract

Our experiment attempted to pinpoint where certain breeds of pigeon live throughout New York City. This will be done by collecting DNA from ten pigeons and comparing the DNA to purebred species. These purebred species include the Indian Fantail, Racing Homer and Capuchin Red. This will allow us to see what breeds mixed to form the pigeons we see today. Our main goal is to record the area from which they were brought into the Wild Bird Fund, to create a map of various breeds in NYC. In order to extract the DNA, we will use cheek swab samples from the birds. We will then extract the DNA to PCR the samples, run the samples through gel electrophoresis and then finally, we will sequence the DNA to identify it. We were only able to get PCR results from pigeon 2, which prevented us from creating a map. This may be because of problems with primers and an insufficient amount of DNA.

Introduction

Everyone knows about Darwin's finches, but do they know about his pigeons? When Darwin visited the Galapagos Islands, one of the birds he studied in depth were the common rock pigeons. He theorized that through evolution, the traits that best allowed for an organism to survive and reproduce would be passed on while less advantageous traits would die out.

Today, pigeons are commonly found across urban areas and are notorious for their dirtiness, acting as vectors for certain species of bacteria. Scientifically known as *Columba livia*, or the rock pigeon, these birds were introduced into the United States and many parts of North and South America by settlers in the 1600s. Although it is difficult to distinguish between breeds of pigeon, various breeds of pigeons do exist and coexist.

Our experiment attempted to pinpoint where certain breeds of pigeon live throughout New York City. We collected DNA samples from cheek cells of pigeons with approval from the Wild Bird Fund, as one of our members, Louise Wagenseil, is a volunteer at this rehabilitation center. Our intention was to examine hotspots for specific breeds, mating habits, and how far pigeons travel from their origins. We hypothesized that most rock pigeons present in New York City will have a mixture of breeds because it is rare to find pure breed pigeons.

Materials

1. Pigeon Samples (11 samples)
2. Chicken Sample (control)
3. Distilled water (350 µL)
4. Lysis solution [6 M GuHCl] (700 µL)
5. Silica resin(10 µL)
6. Wash buffer (2.5 mL)
7. Ice Pack
8. Microcentrifuge
9. Microcentrifuge tube rack
10. Microcentrifuge tubes (1.5 mL)
11. Micropipettes and tips (1-1000 µL)
12. Plastic Pestles
13. Permanent Marker
14. Vortexer
15. Water bath at 65°C and 57°C
16. Agarose gel powder
17. Volumetric flask
18. Electronic scale
19. Microwave
20. Refrigerator
21. miniPCR Machine
22. DNA Subway
23. Primer
24. Pre-packaged sterile Q-tips
25. Sterile test tubes
26. Towels
27. Gloves

Methods

Team member, Louis Wagenseil, volunteers at the Wild Bird Fund and has 2 years of experience treating and handling pigeons, including but not limited to washing, hand-feeding, bandaging, sub-qing, and giving medication to birds.

For each pigeon, a picture was first taken without flash(so that the bird would not be scared). Then, the pigeon is wrapped in a clean towel with their wings inside the towel to prevent them from lashing out or escaping. A Q-tip is then unwrapped and placed in the pigeon's mouth and swirled around gently for 30 seconds. Make sure the pigeon is not in pain. After 30 seconds, place the Q-tip in the designated container and close.

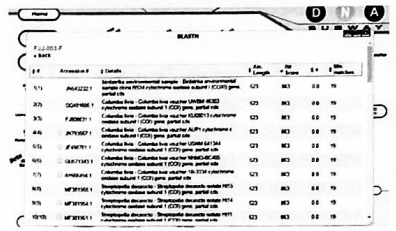
First we labeled four different tubes (A-D) for each sample, for example, 1A, 1B, 1C and 1D. We then added the lysis solution to Tube A and swirled a Q-tip with the sample DNA quickly for 15 seconds and ground the mixture with a pestle. We placed it in the hot water bath at 65°C for 10 minutes. Afterwards, we centrifuged the tube for 1 minute. We transferred the supernatant into Tube B and mixed in silica resin by pipetting in and out. Tube B was incubated in a 57°C hot bath for five minutes, then centrifuged for 30 seconds. The supernatant was removed and placed into Tube C. We then added wash buffer to the remaining pellet in Tube B. The tube was vortexed for a minute, centrifuged for 30 seconds, and the supernatant was removed again. This wash step was then repeated one more time. After the supernatant was removed, we let Tube B to dry overnight to evaporate the excess wash buffer. A paper towel was placed above the open tube to reduce contamination. After, we added dH_2O and pipetted in and out until the distilled water was fully mixed. We incubated the solution for five minutes in a 57°C hot bath and centrifuged for 30 seconds. The supernatant was then transferred into Tube D and stored at -20°C.

We placed some of the supernatant from Tube D into tubes with PCR beads and put them in the mini-PCR machine for 34 cycles at 94°C for initial denaturation and denaturation. It was 54°C for annealing. It was 72°C for extension and the final extension.

We prepared the 2% agarose gel for gel electrophoresis by using agarose powder. We placed the samples after PCR in each well and set the gel. We also included a ladder in the gel. We set the gel for forty-minutes at 93 volts.

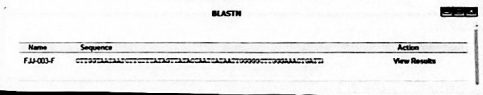
After we got results, we sent the DNA to be sequenced by the DNA Subway. We used the program to get rid of the primer and strings of N's so that we would be able to only compare the DNA that was helpful. This shorted the sequence. We then blasted the DNA which matches the nucleotide sequences to something identifiable.

Identifying Sample DNA as Columbia livia



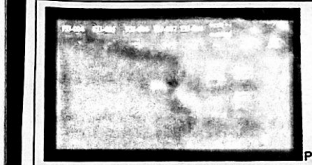
The sequencing that worked successfully was for sample 3 (FJJ-003) instead of sample 2 (FJJ-002). Sample 2 (FJJ-002) was the only sample that had successful PCR results. This may have been because of the sequencing center misreading the label on the tube.

DNA Sequence After Trimming

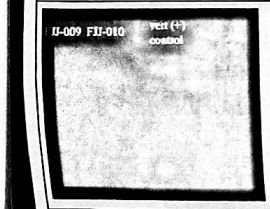


Photos of PCR Results

PCR results of pigeon samples 1-3



PCR results of pigeon samples 4-8



PCR results of pigeon samples 9-10

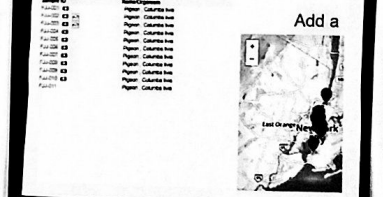
PCR products were run on 2% agarose gels at 93V for 40 minutes

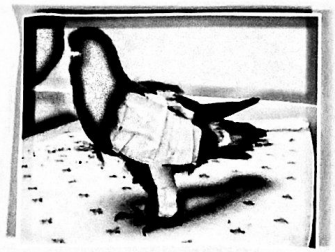
Results

Collection Data for the Pigeon Samples

Types of Birds (all collected from the Wild Bird Fund)	Location Bird was Found	Gene Sequence of Bird
Bird Sample 1	5th Ave, Brooklyn	N/A
Bird Sample 2	187th Street and Ft Washington	N/A
Bird Sample 3	50th and 2nd Ave	BLASTN Sequence CTTGGTAATAACTTTTTTATAGTTATACAATCATAATTGGGGGTTTGGAAACTGATT
Bird Sample 4	58th and 8th Ave	N/A
Bird Sample 5	42nd between 9th	N/A
Bird Sample 6	182 Harerreyer Street	N/A
Bird Sample 7	Unknown	N/A
Bird Sample 8	200 Central Park West	
Bird Sample 9	75th and Broadway	N/A
Bird Sample 10	8th Ave between 33rd and 32nd street	N/A

Samples of DNA Sequenced





Discussion

- Our hypothesis is rejected because in the sample of pigeon DNA that we were able to sequence, it is identified as Columbia livia, or the rock dove. There are no mixtures of breeds in our results.
- We had many mistakes and most of the samples did not give us successful PCR results, thus not being able to be sequenced. We were only able to get PCR results for pigeon 2.
- Mistakes include
  - We were unable to get results for PCR from the other pigeons may be that the primer that we used in PCR is a vertebrate primer that would work the best for mammals. The primer may not be efficient in binding to the barcode region for pigeons.
  - We observed that after we centrifuged the DNA, it was hard for us to see the cell pellets. The cell pellets were light in color and small. This may indicate an insufficient amount of DNA for PCR.
  - In the future if this happened again and we had enough time we would be able to measure the amount of DNA with a spectrophotometer. With more time, we could have also concentrated the DNA, used more DNA for PCR or had more PCR cycles.
  - Another reason why the PCR reaction did not work may be because of possible impurities in the extracted DNA which would have blocked the PCR from happening. We are certain that the impurity is not alcohol but other molecules involved in the procedure.
- In the future, we can have more experiments and more samples for the experiments since there has to be more samples for reliable information. There was also contamination so in the future we should have redone that part of the experiment. Since we only had one sample that worked, our experiment is unreliable. Future research can also include collecting and identifying DNA from pigeons in different states in the United States and different countries to learn more about pigeons in general. This can also teach us the variations of pigeons in different locations and the differences in their mating habits and lifestyles.

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