BIOL 104: Ecology & Evolutionary Biology
Professor Leo Buss

By submitting this essay, I attest that it is my own work, completed in accordance with University regulations. —Laura Goetz

The Sooner, The Better: Modeling Evolutionary Recovery Following Isolated Incidents of Environmental Pollution

by Laura Goetz

Abstract:

Accidental release of mutagenic pollutants is a major problem associated with industrial production. Numerous studies have demonstrated the stark effects of oil spills and nuclear power plant accidents on mutation rates in populations, but the long-term recovery of populations following the cleanup of these messes has not been well characterized. In this study we explored the relationship between the length of a period of pollution and the extent of population normalization following three different length periods of recovery utilizing the simulation software Aipotu. The period of pollution was modeled with an increased mutation rate and the recovery period was modeled by a return to the baseline, lower mutation rate, representing the normal mutation rate in a population without environmental pollution. We found that more members of the population carried phenotypic mutations following longer periods of simulated pollution, and the extent of recovery was not as complete for the experiments with longer pollution times as for scenarios with shorter pollution times. This suggests that speed of cleanup is of the essence when responding to an incident of environmental pollution.

Introduction:

Environmental pollution is a problematic side effect of industrialization. Waste products occasionally elude protective measures and are released into the environment. Ideally, these spills and leaks are quickly cleaned up and the affected area can return to normal. The exact results of these instances of isolated pollution however are not fully understood. It is not definitively clear if the duration of the polluting event changes how the organisms in the area respond to the cleanup. In this study we aim to explore the potential long-term evolutionary implications of an isolated instance of environmental pollution.

In order for a pollution event to impact evolution, it would have to change the mutation rate of the organismal populations in the surrounding area. Research studies overwhelmingly support that assertion. Chemical waste has been shown to increase population mutation rates in fish[1] and planktonic crustaceans.[2] Data regarding nuclear waste is even more widespread. In Belarus, for instance, studies have tracked mutation rates in the germ lines of people in the region affected by the catastrophic 1986 Chernobyl nuclear power plant accident and found that there were much higher mutation rates in children conceived after the incident than their siblings born before.[3] Comparing families affected by the disaster to a control group found that the offspring from the polluted area had double the mutation rate of the unaffected children and germ line mutations were apparent years after the nuclear radiation disaster.[4,5]

To investigate the long-term effects of pollution and cleanup efforts on a population, we used the simulation software Aipotu to model the evolution of color in a diploid plant species after a finite period of pollution. The period of pollution was modeled with an increased mutation rate and the recovery period was modeled by a return to the baseline, lower mutation rate, representing the normal mutation rate in a population without environmental pollution. We examined how the length of a pollution event affects long-term prevalence of phenotypic mutations in an organismal population. Our experimental method involved nine scenarios, each of which offered a different combination of time of pollution and time of recovery (with a return to pre-pollution conditions after the period of pollution). By comparing the apparent mutations in the population after the various pairings, we were able to consider potential for debilitating mutations' disappearance from a population after successful cleanup efforts. We predicted that the phenotypic variation in a population and the time needed for a population to stabilize after a return to normal conditions is directly correlated to how long the mutation rate is raised.

Materials and Methods:

We explored this question using Aipotu software to model the evolution of a population. We decided to model an isolated instance of environmental pollution by manually imposing a higher mutation rate. This is based on the assumption that the pollution increases mutation rate. The experimental trials tracked a population of 100 organisms from "normal" conditions, through a period of increased mutation rate, and then through a recovery period with the original "normal" conditions. The number of organisms was selected to have enough variation to track mutation changes, while still running quickly enough to be experimentally useful. These stages are analogous to before a radioactive waste spill, during the crisis, and after the cleanup effort. (This assumes pollution cleanup was 100% successful and the pollution has no residual effects after the cleanup.)

Aipotu was selected as a platform for this investigation for a variety of reasons. Aipotu is software that simulates color production in a simple diploid flower species. The "color" of a given digital organism depends on the sequence and structure of a certain "protein." Mutation rate can be set manually and mutations then occur in the coloration protein randomly at that specified frequency in the two alleles separately. Certain mutations can cause the color of the virtual flower to change. Since each flower carries two coloration protein alleles, the color that results is a combination of both. For example, a green plant could have two green alleles, a green allele and a colorless allele, or a blue and a yellow allele. This aspect of the simulation makes it more realistic because a small mutation doesn't necessarily change the organism's phenotype, but a buildup of mutations does cause distinct changes. Another benefit of Aipotu is non-random selection, which is based on the ability to change the fitness of different colors. This is advantageous because in nature, certain colorings are better for survival than others.[6,7,8] By having the starting color at a higher fitness than other colors (arising from mutations), Aipotu can be used to model evolution of a trait in a population in a fairly realistic manner.

For this experiment, in order to address the question of interest, we ran a series of evolutionary scenarios with three circumstantial stages: establishing the "normal" world, during the period of pollution, and after the cleanup (restored to normal conditions). There were nine different experimental scenarios created by changing the duration of pollution and restoration. These scenarios are shown and explained below in Figure 1.

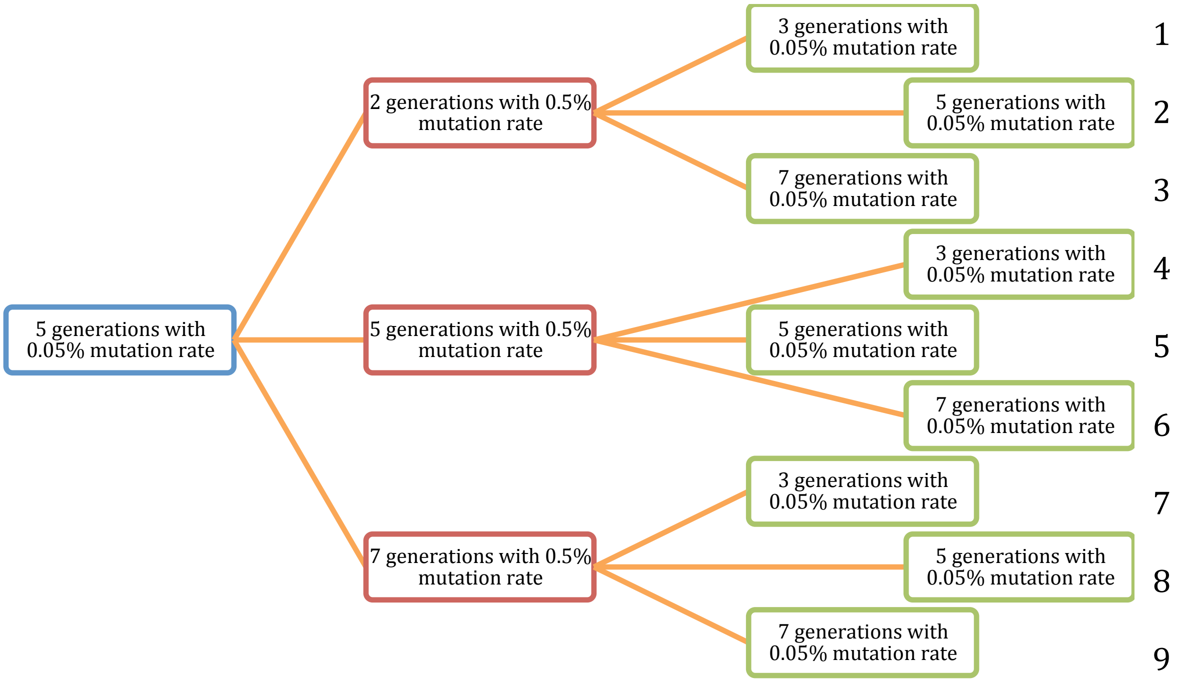


Figure 1

Flow chart showing the different experimental scenarios:
Stage 1: Blue, Stage 2: Red, Stage 3: Green
For each of the 9 experimental scenarios, the simulator was run for 3 stages. The first stage was 5 generations with a 0.05% mutations rate (modeling normal mutation in a population), which is represented by the single box in the left column. After those 5 generations, the second stage included 3 different options for durations of the polluted generations (modeled by mutation rate being increased to 0.5%): 2, 5, or 7. The 3 boxes in the middle column

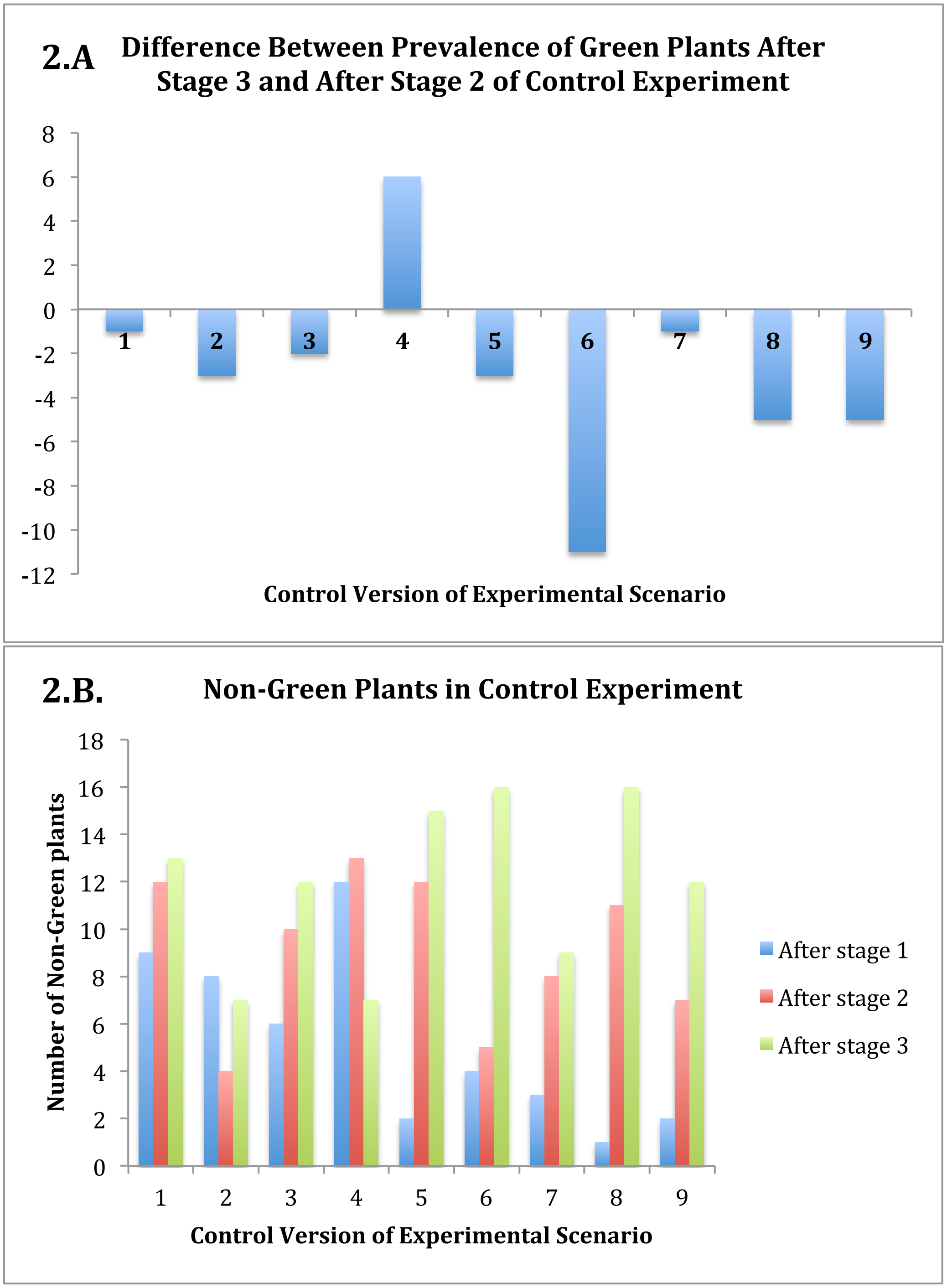
represent this choice of how long higher mutation rate lasts. After the polluted generations, for the third stage the mutation rate was restored to the normal rate of 0.5% for 3, 5, or 7 generations. The choice in duration of the restoration period is shown in the right column, where there are 9 options, each the end of a unique path representing 1 of the 9 experimental scenarios. The numbers next to each of the stage 3 boxes correspond to the scenario number of following that path.

For each scenario, the world was first loaded with a starting model organism. For this study, we selected the pre-loaded organism "Green-1," which is homozygous green and has a green phenotype. Green was an arbitrary selection (many plants are green so it was a reasonable dominant phenotype). Homozygous was selected to start because we wanted the different alleles to occur due to random mutations alone, to limit confounding that could occur if we started with a blue/yellow heterozygote, for example. Starting with a heterozygous yellow/blue plant (Green-2) typically results in less than 1/3 green plants after 1 generation of experimental conditions, so the increased mutation rate would not have an apparent effect. Green was given a relative fitness of 8 and the other colors were each given a relative fitness of 2. We assumed that the mutations hurt the evolutionary viability of the organism because research studies have shown that this often happens and because we are interested in how a population recovers from a period of injurious mutations. For this study we will refer to plants exhibiting a green color as "green" and plants displaying any other color as "non-green." Green plus non-green always added up to 100 organisms in the population.

The methods were first tested and verified with a set of control experiments, and then the experimental scenarios were tested. The populations were characterized following each study and each experimental scenario was simulated five times to verify results and the data were averaged. The results were analyzed to consider the speed and extent of recovery for conditions modeling slower and faster cleanup responses to a pollution event.

Results:

First, we executed a control experiment to verify our methods and give a baseline for comparing the experimental results. The details of the control experiment execution and the results are expressed below in Figure 2.



2.A Difference Between Prevalence of Green Plants After Stage 3 and After Stage 2 of Control Experiment

2.B. Non-Green Plants in Control Experiment

Figure 2

For the control experiment, the procedure was followed for each previously explained experimental scenario, except instead of raising the mutation rate for the second stage of the simulation (from 0.05% to 0.5%) the mutation rate was held constant (at 0.05%). This served to model the expected outcome of the normal mutation of a population, imagining that no polluting event occurred. Experimental scenarios correspond to the numbers presented in Figure 1.

As Figure 2.A. shows, there were generally fewer green plants after stage 3 than after stage 2. Additionally, the scenarios with more generations generally had a greater decrease in the number of green plants between stages 2 and 3. These observations are both as expected because with each additional generation more mutations would occur and with that a higher chance of a mutation occurring that would result in a color change. Figure 2.B. reinforces the assertion that when the mutation rate is kept constant, the number of non-green plants increases over time, as that occurred all but one trial. Figure 2.B. also shows that at a low mutation rate, relatively few plants develop different colors. Even when taken through as many as 19 generations, the number of non-green plants never got higher than 1/7 of the population. This is also to be expected, as in a population, mutations that reduce fitness generally do not become widespread quickly.

After confirming the strength of the experimental procedure by running the controls, we proceeded with our experiment as outlined above. Selected results are displayed below in Figures 3 and 4.

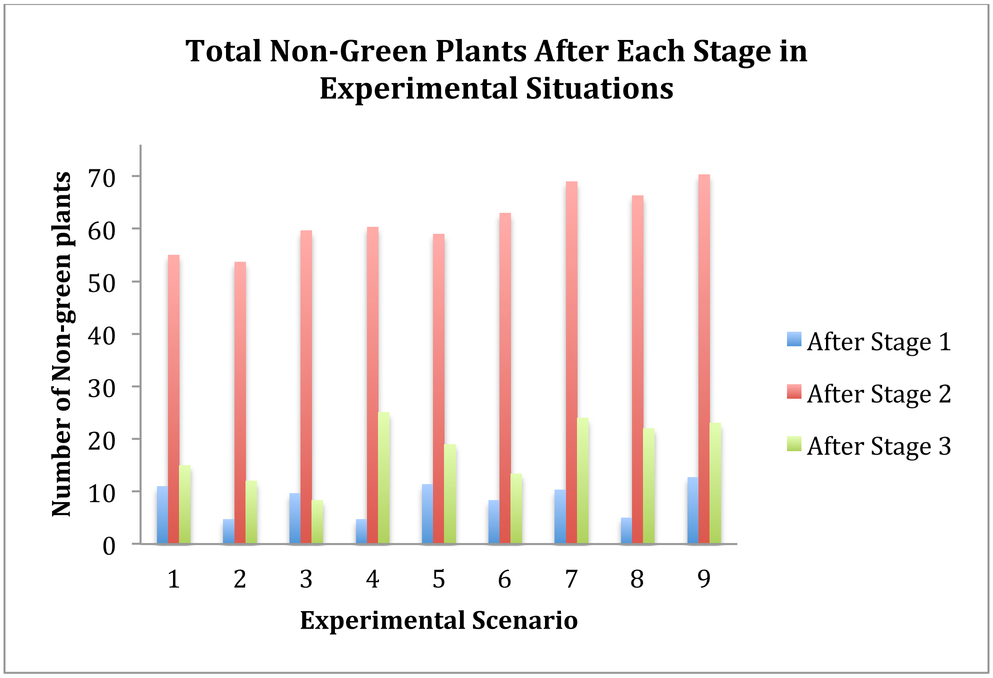


Figure 3

This chart expresses the number of non-green plants in the population after stage 1 (normal generations), stage 2 (polluted generations), and stage 3 (restored generations). Experimental scenarios correspond to the numbers presented in Figure 1.

Figure 3 suggests numerous trends in the results. The overarching observation is that after the “normal” mutation condition generations (stage 1), in all trials there are very few non-green plants in the population. After the “polluted” generations (stage 2), the population has a much higher number of non-green plants. Then, at the end of the experiment (after the “restored” generations, stage 3), the population has fewer non-green plants than it did right after the pollution. Specific trends also related to the duration of the pollution. Generally, the more generations with higher mutation rates that occurred, the higher the number of non-green plants. For the scenarios with two and five generations with a higher mutation rate, the number of non-green plants after stage 3 decreased substantially. However, for the experiments with the longest pollution times, the number of non-green plants did not reduce as definitively with an increased recovery time as it did for scenarios with shorter pollution times.

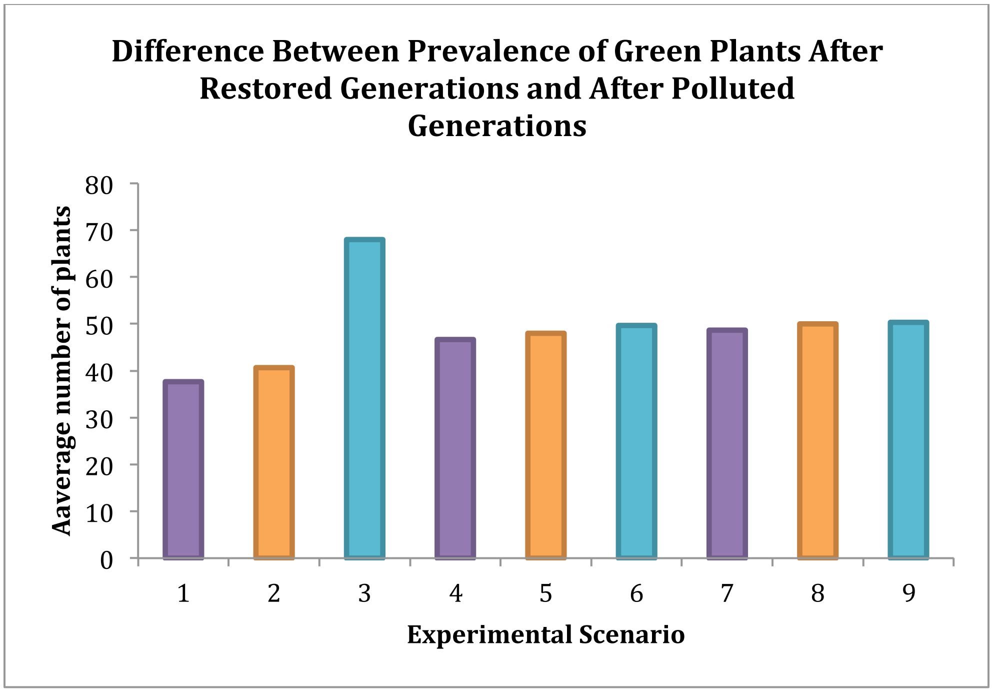


Figure 4

This chart expresses how many more average green plants were in the simulation after the restored generations than were in the simulation after the polluted generation for a given experimental scenario. The average values were derived from taking the mean of 5 trials for each experimental scenario. The purple bars (1, 4, 7) were scenarios with 3 recovered generations. The orange bars (2, 5, 8) were scenarios with 5 recovered generations. The blue bars (3, 6, 9) were scenarios with 7 recovered generations.

Figure 4 shows the difference between the average number of green plants after the completion of stage 3 (the restored generations) and the average number of green plants after completion of stage 2 (the polluted generations) for each experimental scenario. This figure suggests a few important ideas. First, unlike for the control, the experimental scenarios had much higher numbers of green plants after stage 3 than after stage 2. This suggests that despite having more generations during which mutations can occur, returning to a lower mutation rate does have a significant restorative effect on the population. Another key observation is the clear winner for most restored population is scenario 3, which had the shortest pollution time and the longest recovery time. For the other experimental scenarios, the length of the recovery time did not have nearly as great of an impact.

Discussion

While the immediate effects of pollution are unambiguous, the long-term implications are less clear. Can cleanup efforts ever reverse the results? Does the length of the pollution event change the environment's ability to recover? These questions were addressed in an evolutionary simulation study. We predicted that the variation in a population and the time needed for a population to stabilize after a return to normal conditions are directly correlated to how long the mutation rate is raised. The results of this study have important implications for the optimal design strategies for responding to isolated pollution events.

First, they suggest that the longer a population is exposed to a mutagenic pollutant, the more mutations its members will develop (even when causing loss of fitness). That is as predicted. The data also indicate that longer recovery times restore more of the population to the normal phenotype than shorter recovery times. This means that if cleaned up properly, pollutants effects can diminish over time.

The most important results though, are the data that suggest that while populations do recover over time, significant recovery happens by far the most quickly and completely following shorter periods of pollution. This means that if an environmental pollution is going to be effectively cleaned up without long-term consequences, the faster it is dealt with, the better. This finding is extremely significant for strategizing how to confront accidents like the Chernobyl power plant incident or industrial chemical spills in water sources. While organizing to respond to a crisis is difficult, this study shows that time truly is of the essence. Responding quickly could mean a huge difference in when and if the organisms in the area stop being affected, which is an important discovery. This conclusion is directly supported by previous real-world studies. Research charting ecosystem recovery following oil spill cleanups supports our claim that organismal populations are able to recover much more quickly following shorter periods of pollution.[9] Studies on long-term recovery are difficult to generalize, as different organisms have varying recovery times, but flora and fauna both seem to recovery more quickly from quick cleanups.[10]

Additionally there is the question of, how applicable is data gained from simulation of digital organisms? These methods, though relatively strong, were not without fault. Using a simulation instead of studying actual pollution events means that the "organisms" were simplified, and only one trait was mutated. In nonvirtual organisms, all genes would have the potential for mutation. While using a simpler system allowed us to avoid gene interactions, it also is not an entirely accurate representation of nature and consequently the results are less applicable. However, the Aipotu software, while not a perfect replica of nature, is a fairly strong model system. The assumptions made for the basis of this study are supported by real-world research. Following the Chernobyl accident, non-human organisms were affected as well. Much higher rates of partial albinism were reported in barn swallows mating in the area in the years following the spill – a coloring mutation that significantly reduced the fitness of the birds.[11] This suggests that mutations stemming from pollution that affect the aesthetic appearance of organisms can have negative impacts on their evolutionary viability. That finding supports the reduced fitness of non-green plants in the simulation.

There is the possibility that some of these results were simply due to chance. As mutations are random in the simulation, some trials could have had unusual outcomes. We attempted to minimize this by running and averaging numerous trials, but even more trials would have strengthened these results. Additionally, potential pitfalls of the design of this experiment include the basic assumptions that an instance of environmental pollution translates to an increased mutation rate and that through cleanup efforts that mutation rate can be reduced completely to the original frequency, which is not necessarily true in nature.

For future studies, we would like to pursue more extended recovery times and see if more complete recovery is possible following the longer pollution periods. This would reveal whether a slower response to pollution simply delays ecosystem recovery or whether the mutagenic pollutant's effects become permanent after a certain threshold. Those results in turn would contribute to informing environmental cleanup efforts. A possibly more realistic version of this experiment would be having the stage 3 mutation level be lower than stage 2, but not fully reduced to the initial "normal" mutation rate. That set-up would model an incomplete cleanup effort, which is what actually happens in real world scenarios. Alternatively, a fourth stage could be added, so the recovery happened in gradations, with a third stage at an above normal mutation rate (partial recovery) and a fourth stage of total recovery. Charting population mutation through that could have more applicable results than this simpler study.

Acknowledgements

This study would not have been possible without the help of Jagannath Padmanabhan, Danée Fitzgerald, and Katherine Garvey. Additional gratitude to the makers of Aipotu for free access to their evolutionary modeling software.

---

[1] Dauba F1, Lek S, Mastrorillo S, Copp GH. (1997) Long-term recovery of macrobenthos and fish assemblages after water pollution abatement measures in the River Petite Baïse (France). Arch Environ Contam Toxicol. 33(3):277-85.

[2] Ribeiro R, Lopes I (2013) Contaminant driven genetic erosion and associated hypotheses on alleles loss, reduced population growth rate and increased susceptibility to future stressors – an essay. Ecotoxicology 22: 889-899.

[3] Weinberg HS, Korol AB, Kirzhner VM, Avivi A, Fahima T, Nevo E, et al. (2001) Very high mutation rate in offspring of Chernobyl accident liquidators. Proc R Soc Lond Ser B-Biol Sci. 268:1001–1005.

[4] Dubrova YE, Nesterov VN, Krouchinsky NG, Ostapenko VA, Neumann R, Neil DL, Jeffreys AJ. (1996) Human minisatellite mutation rate after the Chernobyl accident. Nature. 380(6576):683-6.

---

[5] Dubrova YE *et al.* (1997) Further evidence for elevated human minisatellite mutation rate in Belarus eight years after Chernobyl accident. Mutant. 281, 267–278

[6] Endler, J.A. (1978) A predator's view of animal color patterns. Evol Biol 11:319-364.

[7] Endler, J.A. (1980) Natural selection on color patterns in Poecilia reticulata. Evolution 34:76-91.

[8] Sobel J.M., Stresifeld M.A. (2013) Flower color as a model system for studies of plant evo-devo. Frontiers in Plant Science 4:321.

[9] Evans, S.M., Hutton, A., Kendall, M.A. & Samsoir, A.M. (1991) Marine Pollution Bulletin 22, 331-333.

[10] *Effects Of Oil Pollution On The Marine Environment*. Tech. no. 13. The International Tanker Owners Pollution Federation, n.d. Web. 27 Apr. 2014.

[11] Ellegren H, Lindgren G, Primmer CR, Moller AP (1997) Fitness loss and germline mutations in barn swallows breeding in Chernobyl. Nature 389: 593–596.