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Prathyusha Kandala March 19, 2021

Examining the effects of gene flow on host resistance to parasites

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Abstract

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Incoming gene flow in a population has an important effect on the rate of adaptation in a population. While in some cases the addition of genetic variation through any gene flow is enough to increase the rate of adaptation, the source population of this gene flow can affect how the rate of adaptation changes. For example, a source population possessing certain alleles can increase the focal population's rate of adaptation more than a different source population with different alleles can. Some populations may even reduce the rate of adaptation due to an influx of deleterious alleles. To understand the impact of gene flow on rapid adaptation, we used obligately outcrossing populations of *Caenorhabditis elegans* that are capable of evolving resistance to the virulent bacteria, *Serratia marcescens*, and added gene flow from different populations. We added no gene flow, gene flow from the ancestor *C. elegans* population, or gene flow from a resistant *C. elegans* population. While we observed no significant differences in average mortality rate between populations grown in the presence of live *S. marcescens* that received no gene flow and populations that received ancestor gene flow, the populations that grew with live *S. marcescens* and that received gene flow from a resistant population had a significantly lower average mortality rate, indicating that adaptation occurred more rapidly in these populations. Furthermore, we saw high average mortality rates in all populations that were grown in the presence of heat-killed *S. marcescens*, regardless of gene flow status, suggesting that the alleles for resistance are not universally beneficial, and instead only increase in frequency when live *S. marcescens* is present and able to select for those alleles. Therefore, we see that gene flow can increase the rate of adaptation, but the effects are dependent on the source of the gene flow.

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Introduction

As certain phenotypes are favorable in a given environment, individuals with favorable phenotypes are more likely to reproduce and pass down their alleles than individuals with unfavorable phenotypes (Darwin 1859). Adaptation to an environment occurs when the frequency of beneficial alleles within a population increases due to the environment selecting for individuals that possess these alleles; it is a process that is ultimately fueled by mutations that generate standing genetic variation (Barrett and Schluter 2008; Orr and Unckless 2008). While mutation can generate standing genetic variation in a population, it is a slow process that does not often allow for rapid adaptation (Patwa and Wahl 2008). One means of increasing the rate of adaptation is incoming gene flow (Yeaman and Otto 2011). As gene flow allows a population to access the standing variation present in another population, the focal population will no longer depend solely on *de novo* mutation as a source for generating standing genetic variation. Consequently, gene flow is often predicted to increase the potential for rapid adaptation in populations with limited standing genetic variation (Ralph and Coop 2015; Feder et al. 2019).

While gene flow can affect the rate of adaptation, the rate may not always increase with gene flow. Rather, gene flow may act to either accelerate or impede adaptation, depending on the nature of the alleles that enter the population (Conte et al. 2012). While some populations may become so limited in variation that the addition of any new alleles can facilitate the adaptive response, the source of the alleles may determine whether the rate of adaptation increases or decreases (Tigano and Friesen 2016). Gene flow can introduce novel or rare

beneficial alleles that could increase the population's rate of adaptation. In *Tetranychus uticae*, gene flow from a population that possessed an allele for bifenazate resistance allowed a non-resistant population to also develop resistance (Shi et al. 2019). The influx of the beneficial allele into the nonresistant population gave the population access the allele for resistance, thus increasing the rate of adaptation and allowing the population to adapt resistance to bifenazate. Conversely, the influx of deleterious alleles into a population can slow adaptation by diluting the frequency of the beneficial alleles and generally decreasing fitness. Gene flow from a population that does not possess the beneficial alleles that are present in the evolving population can slow a population's rate of adaptation. In *Rana temporaria* frog populations in Switzerland, gene flow between populations adapted for higher or lower elevations gives rise to maladaptation in the receiving population (Bachmann et al. 2020). As a result of gene flow allowing alleles that would not benefit the population to enter, the unfit migrants, along with any offspring they may have produced, were selected against by the environment. Offspring that were the result of interpopulation breeding would also be selected against, thereby reducing the strength of selection against deleterious alleles already present in the population (Comeault et al. 2015). Thus, gene flow may only be conditionally beneficial, with any benefits depending on the source of the incoming alleles. In this study, we investigate whether gene flow increases the rate of adaptation in non-*Serratia marcescens*-resistant *Caenorhabditis elegans*, and whether the source population of the gene flow determines the ability of gene flow to facilitate adaptation.

To test the effects of gene flow on rapid adaptation, and the effects of gene flow source, we used *Caenorhabditis elegans*. *C. elegans* are microscopic nematodes that consume bacteria,

typically *E. coli* in lab settings, and possess innate immune systems that are activated when they consume *Serratia marcescens,* a virulent parasite of *C. elegans* (Penley and Morran 2018). *S. marcescens* is virulent when consumed by *C. elegans,* and can create strong selective pressure under which *C. elegans* can evolve resistance. Importantly, *C. elegans* are capable of evolving defense mechanisms over several generations while in the presence of *S. marcescens* (Penley et al. 2017). Furthermore, the life span of *C. elegans* is very short, with two new generations growing every week. This allows us to examine evolution over several generations, and thus it enables us to observe adaptation to *S. marcescens* in real-time.

Here, we evolved populations of obligately outcrossing *C. elegans* under selection from *S. marcescens* and simulated gene flow into these populations from either a non-resistant ancestor population containing standing genetic variation or a resistant *C. elegans* population, both of which were generated in Morran et al. (2011). After 9 generations of selection, we performed mortality assays to find the mortality of each population. Comparison of mortality across treatments allowed us to determine how different gene flow affects the rate of adaptation to the environment.

Methods

Host-Parasite System and Gene Flow Source

The host-parasite system that we used in this experiment was *Caenorhabditis elegans* and *Serratia marcescens*. The two strains of *C. elegans* used were the CF3-30 and EF3-30 strains. CF3-30 and EF3-30 are evolved forms of the strain F3-0, which is a natural *C. elegans* strain, CB4856, that has a mutation in the *fog-2* gene. It prevents hermaphrodites with that mutation from producing sperm, thus preventing self-fertilization and resulting in obligate outcrossing (Morran et al., 2011). CF3-30 and EF3-30 were produced in the experiment detailed in Morran et al., 2011. The CF3-30 strain was exposed to the control treatment over 30 generations, and does not possess resistance to *S. marcescens* as a result, while the EF3-30 strain was exposed to the evolution treatment over 30 generations, and gained some resistance to *S. marcescens*. The Sm2170 strain of *S. marcescens* was used as the parasite in the Serratia Selection plates. This strain was chosen because it is especially virulent in *C. elegans*, with up to 80% mortality in one generation of exposure (Morran et al, 2009). The OP50 strain of *E. coli* is a strain typically used in experiments with *C. elegans* as a standard food source. It was used in the Serratia Selection Plates.

Experimental Evolution

We used six different experimental treatment groups, with each group differing in *S. marcescens* environment and gene flow treatment (Fig. 1). The two *S. marcescens* environments were heat-killed *S. marcescens,* in which *C. elegans* were evolved in the presence

of heat-killed *S. marcescens*, and live *S. marcescens,* in which *C. elegans* were evolved in the presence of live *S. marcescens*. The three gene flow treatments were no gene flow, in which no gene flow was added, ancestor gene flow, in which gene flow was from the ancestor population, and resistant gene flow, in which gene flow was from the resistant population. Each treatment had a different *S. marcescens* environment and a different gene flow treatment, for a total of six treatments. Each of the six treatments also had five replicate populations.

Our evolution process followed a seven-day schedule, with one generation of selection and two *C. elegans* generations per seven days, for 9 total generations of selection. The CF3-30 strain was used as our ancestor population due to its lack of resistance to Sm2170. Initially, 2 days before day 1 of the first generation, CF3-30 was chunked onto NGM plates seeded with OP50. The plates were kept in a 20˚C incubator for 48 hours to allow *C. elegans* to grow on the plates. On day 1 of the process, the nematodes and eggs were washed off the plates with M9 buffer solution and treated with a 4:1 mixture of M9 and bleach. This effectively killed all nematodes, leaving only the unhatched eggs. The eggs were plated onto plates seeded with OP50 and incubated at 20˚C until day 3. On day 3 of the first generation, the nematodes that hatch from these eggs are plated onto the SSPs.

This weekly process started on day 1 with inoculation of two types of bacteria: Sm2170 and the OP50 strain of *Escherichia coli*, a strain normally used as food for *C. elegans*. The inoculated bacteria were used on day 2 to create Serratia Selection Plates (SSPs), a selection plate developed to expose *C. elegans* to pathogen while also allowing for selection of surviving *C. elegans* on an *E. coli* lawn (Morran et al., 2009). These SSPs consisted of Sm2170 grown on

1/3 of the plate and OP50 grown on another 1/3. This left the middle 1/3 without bacteria, but with ampicillin (200 ug/mL) spread in a line down the center of this section. 15 plates were made with heat-killed Sm2170, as a control with no C. elegans evolution, and 15 were made with live Sm2170. On day 3, 1000 C. elegans were plated onto the side with either the heat killed or live Sm2170. In the first generation, these 1000 *C. elegans* were from the CF3-30 ancestor strain, while in subsequent generations, the *C. elegans* were from the previous generation. The plates were stored at 20˚C in an incubator until day 6, when the OP50 section of the SSPs were chunked onto antibiotic plates seeded with a strep-resistant OP50 strain, called OP50-1, and were allowed to grow until day 3 of the process.

Gene Flow into Population

After 5 generations of selection, we added *C. elegans* from different strains into our weekly passage onto SSPs along with some *C. elegans* from chunked plates from the previous generation. Rather than adding 1000 worms from the previous passage, we added 950 worms from the previous passage, and 50 worms from either the CF3-30 ancestor strain or the EF3-30 strain. Of the 15 control SSPs, 5 plates received no migrants, 5 received migrants from the ancestor strain, and 5 received migrants from the EF3-30 strain. The 15 SSPs plated with live Sm2170 received migrants in the same format. Figure 1 illustrates the pathogen used and gene flow source in each of the six treatments used.

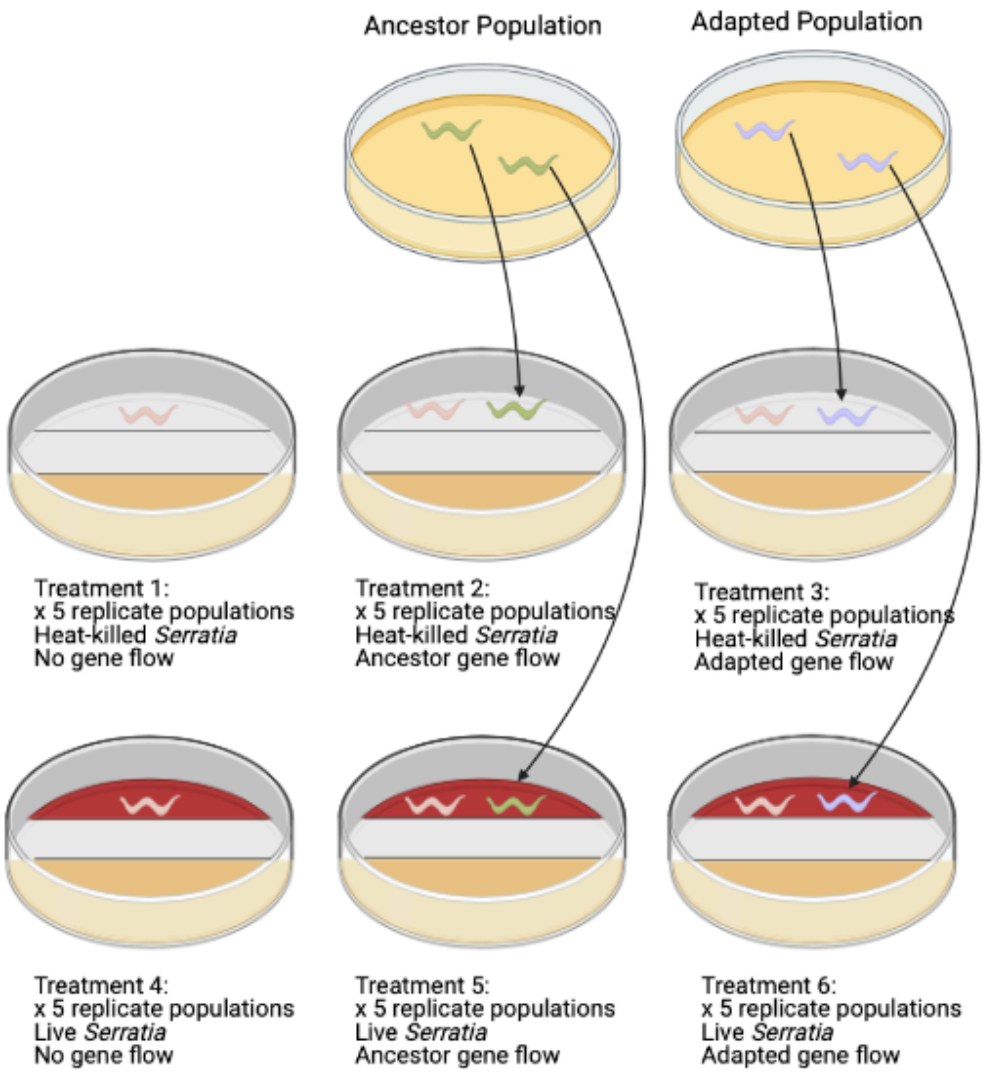

Figure 1: Overview of treatments used.

We used 15 control and 15 experimental Serratia Selection Plates (SSPs), set up as described in Morran et al., 2009, using *E. coli* and *S. marcescens*. The 30 plates were used to evolve the 30 populations of *C. elegans* added at the beginning of the experiment over 9 generations. The *C. elegans* from the beginning of the experiment are shown in peach. Of the 15 control SSPs, containing heat-killed *S. marcescens*, 5 plates received no migrants, 5 received migrants (shown in green) from the ancestor strain, and 5 received migrants (shown in purple) from a strain that possess resistance to *S. marcescens*. The 15 SSPs plated with live *S. marcescens* received migrants in the same format. Therefore, we had six total experimental treatment groups (2 *S. marcescens* environments x 3 gene flow treatments) with 5 replicate populations within each treatment group.

Assessment of Mortality Rates

We assessed each population's resistance to *S. marcescens* by using survival assays as described in Penley et al. 2017. Approximately 200 *C. elegans* from each population, with 3 replicates per population, were assayed on SSPs seeded with OP50 and live Sm2170. After 48 hours, the surviving *C. elegans* on the OP50 side were scored for mortality. The populations were assayed with this procedure twice, for a total of two trials.

We performed a linear mixed model on mean mortality values, with trial as a random effect and treatment, gene flow, and treatment by gene flow as fixed effects. We then evaluated the effects of treatment by gene flow by using Student's T post-hoc test.

Results

In order to assess how exposure to Sm2170 and gene flow affected host adaptation, we performed mortality assays to test the parasite resistance of each evolved host population. We exposed each treatment's populations to live Sm2170 on SSPs and measured each population's mortality level after 48 hours.

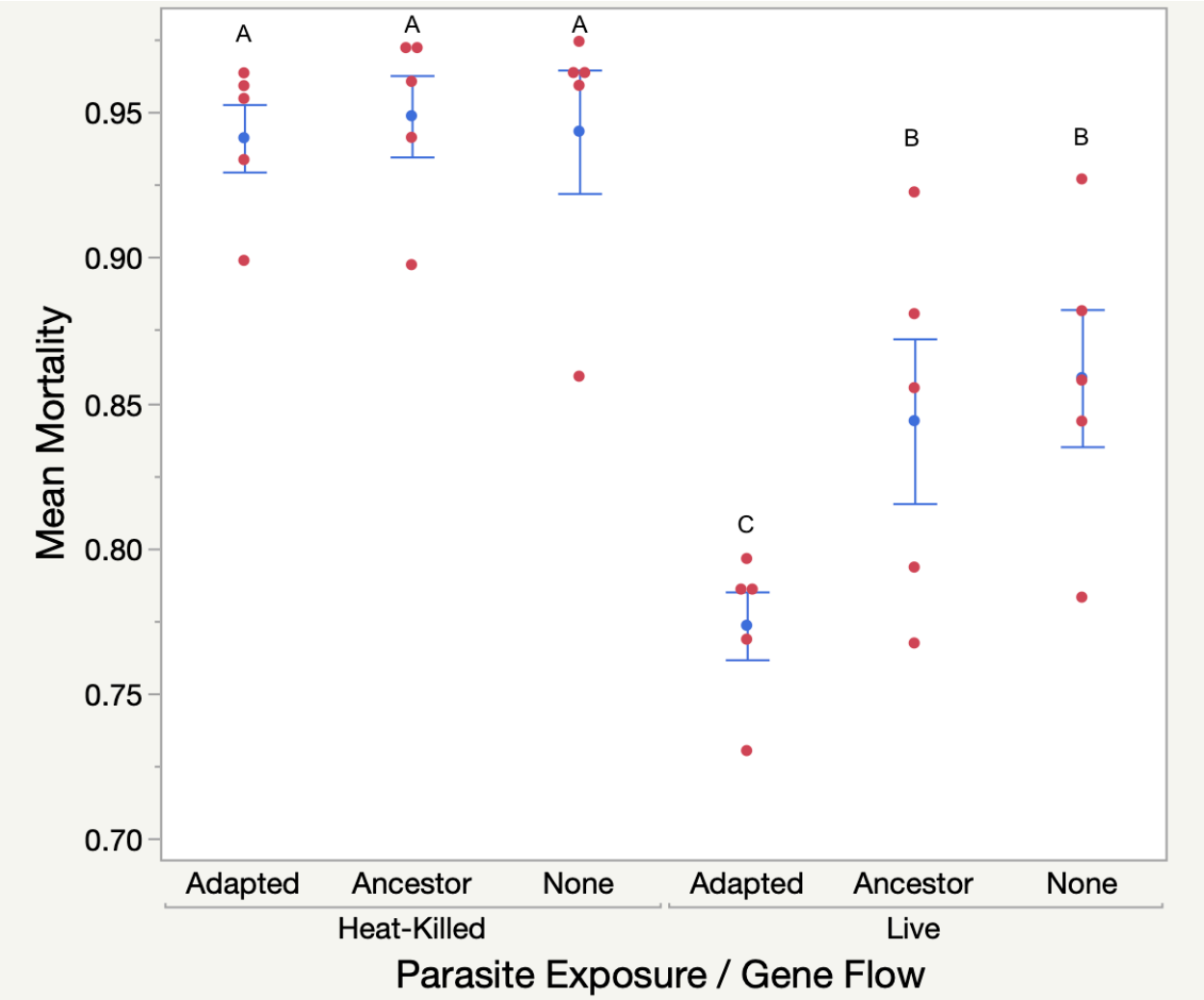

Figure 2: Mean mortality rate of each treatment.

The mean mortality of each replicate population was calculated via mortality assays, and the average mortality for each treatment was determined. The red circles indicate the mean mortality for each replicate population within each treatment, while the blue circles indicate the mean for each treatment. Error bars represent +/- 1 s.e.

We first assessed whether adaptation to the pathogen took place over the course of this experiment. As we predicted, we observed lower mortality rates in *C. elegans* populations that were evolved in the presence of live Sm2170 than in populations that were evolved in the presence of heat-killed Sm2170, regardless of gene flow ($F_{1, 29}$=108.93, $p < 0.0001$) (Fig. 2). The difference in mortality rate was significant, demonstrating that adaptation only took place in populations from the three treatments in which *C. elegans* were exposed to live Sm2170 during evolution.

We then examined mortality rates across treatments to examine the effects of gene flow. We observed no difference in resistance regardless of presence or source of gene flow in treatments that were exposed to heat-killed Sm2170 during evolution (HK adapted vs HK ancestor: t = -0.38, p = 0.7093; HK adapted vs HK none: t = -0.11, p = 0.9099; HK ancestor vs HK none: t = 0.27, p = 0.7897) (Fig. 2). In the treatments that were exposed to live Sm2170 during evolution, we observed lower mortality rates in populations that received gene flow from the resistant population than in populations that received gene flow from the ancestral population or received no gene flow (Live adapted vs Live ancestor: t = -3.56, p = 0.0013; Live adapted vs Live none: t = -4.31, p = 0.0002) (Fig. 2). Thus, gene flow from the resistant population allows more rapid adaptation to a parasite. However, it is important to note that this effect is only observed when selection via the live parasite is occurring.

Discussion

As we investigate the effects of the presence and source of gene flow on adaptation to the environment, comparison of mortality rates informs us how quickly and effectively each population was able to adapt to the environment. When comparing the mean mortality rates of the populations evolved in the presence of heat-killed *S. marcescens* to the those of the populations evolved in the presence of live *S. marcescens*, we observed that resistance did not increase in the *C. elegans* evolved in heat-killed *S. marcescens*, but increased in the *C. elegans* evolved in live *S. marcescens* (Fig. 2). This is a result of the lack of selective pressures in these populations; because there is no parasite selecting for resistance in these populations, the *C. elegans* populations do not develop resistance. We see here that adaptation did not occur without live *S. marcescens* present in the environment.

Of the populations that evolved in live *S. marcescens*, we see a significantly lower mortality rate in the populations that received gene flow from the resistant population. This suggests that the *S. marcescens*-resistant allele (or alleles) that entered the population was selected for in this environment, allowing it to increase in frequency within the population. The allele(s) enables the populations to evolve resistance by increasing the adaptation rate from the rates seen in the other populations evolved in live *S. marcescens*. It also likely enters the population at higher frequency than would be expected via spontaneous mutation, allowing selection to drive greater changes in allele frequency at a higher rate. Furthermore, it is possible that the beneficial allele(s) is not present in the focal population, so gene flow into the population may be adding novel beneficial alleles. This trend is not seen in populations that

evolved in heat-killed *S. marcescens*; rather than the populations receiving gene flow from the resistant population having a significantly lower mortality rate, all three gene flow treatments saw similar mortality rates. Once again, this is likely due to the lack of selective pressures on these populations. Furthermore, this suggests that the incoming alleles for resistance are not universally beneficial, and instead only increase in frequency when in the presence of live *S. marcescens*. In summary, the lower average mortality rate seen in populations of *C. elegans* that evolved in the presence of live *S. marcescens* and received gene flow from a *S. marcescens*-resistant population indicates that gene flow can increase the rate of adaptation to *S. marcescens*, but only from populations that contain beneficial alleles. The mere addition of standing genetic variation from other populations was not enough to significantly increase adaptation.

The findings of this experiment were surprising, given that *C. elegans* only evolved over 9 generations of selection, and that gene flow was only added once throughout the experiment. Furthermore, while we did not expect to see gene flow decrease the rate of adaptation, we expected to see a lower average mortality rate in the population that received gene flow from the ancestor population (Fig. 2). Our initial plan for this experiment was to perform 20 generations of selection, adding gene flow every 5 generations, but the experiment was cut short due to the COVID-19 pandemic. As the original experiment would have been twice as long as the experiment performed in this study, the results of that longer experiment might have been very different from the results shown here. For example, given more generations, the gene flow from the ancestor population may have also increased the rate of adaptation simply by adding alleles and preventing the populations from becoming limited in variation. In a

simulation-based study, it was found that high genetic variation can use small-effect alleles, such as those present in an ancestor population, to contribute to adaptation (Yeaman 2015). Due to the limited number of generations, we also were unable to see the long-term effects of gene flow from the resistant population on the initial population of *C. elegans*.

As we predicted at the beginning of this study, gene flow into the evolving populations does increase the rate of adaptation, but this effect is dependent on the source population of the gene flow. This suggests that in nature, an influx of beneficial alleles into a population can allow the population to better adapt to the environment, where as an influx of individuals that do not possess beneficial alleles may not increase the adaptation rate, or may even slow adaptation. These findings may prove useful for fields such as conservation biology, as gene flow from a population with beneficial alleles may allow the rate of adaptation to proceed at greater rates, thus improving fitness for the initial population overall. The introduction of individuals with beneficial alleles into a population can promote interpopulation breeding, allowing those beneficial alleles to be passed down within the focal population, thus improving fitness within that population. However, given that such an introduction would not be performed in a controlled environment, outside factors may impact fitness of these offspring.

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