Guppy Longevity, Inbreeding and Outcrossing
by Diana Walstad (March 2021)

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1 Acknowledgements: Christophe Pélabon, Jonathan P. Evans and David N. Reznick all provided valuable input.

This article is a continuation of two shorter articles: ‘Small-Scale Guppy Breeding’ and ‘Breeding Guppies: Genetic Pitfalls and Successes’. Both are available on my website: http://dianawalstad.com

Abbreviations: ~ = about; > = greater than; ≥ = greater than or equal to; F = inbreeding coefficient; Fig = figure; hr = hour(s); ID = inbreeding depression; mg = milligrams; min = minute(s); mos. = months; N = population size; Ne = effective population size; n = sample size; OBD = outbreeding depression; P = probability; s.d. = standard deviation; S.E. = standard error
**Introduction**

If the guppies you bought died soon after purchase, you are not alone. Too often, the blame for premature guppy death is put onto the hobbyist, the store, shipping stress, disease, high temperature, over-feeding, etc. However, the cause of a guppy’s death could just as well be genetic weakness.

Guppies are no longer the hardy fish they once were. Too often they die of disease or what-not within a few weeks of purchase. Where are the “child-proof” guppies of yesteryear that could survive without pampering? 1950’s guppies reportedly had a 2-5 year life span [1].

Before I began writing this article, I blamed it on the seismic shift from “basement bred” American guppies to mass-produced guppies from Southeast Asia. Beginning in the 1950s, Southeast Asian farmers realized that producing guppies was more profitable than growing vegetables. Thus, began a flood of inexpensive imported guppies.

However, when I learned that many old lab strains in research facilities were not doing that well, I realized that the cause might lie elsewhere.

Granted, environmental factors (temperature, food, water quality, etc) will affect lifespan. However, I want to keep the focus here on the genetic causes. Many common guppy breeding practices inadvertently select for an inherited short lifespan.

Disease resistance and longevity are intertwined. The simple fact is that disease-susceptible fish do not live as long as those that are disease resistant. In turn, short-lived fish are more likely to contract disease as they enter a prematurely early senescence and overall body decline. Yes, the genes for disease resistance and longevity differ. However, for all practical purposes, breeding fish for longevity invariably increases disease resistance. Thus, I have lumped disease resistance with longevity.

Longevity is the sum of miscellaneous behavioral, physiological, and immune factors that contribute to a fish’s enhanced survival with age. These fitness traits are influenced by many genes with small effects and low heritability [2, 3]. The genes may be on different chromosomes. They may be switched on or off by modifying genes (i.e., ‘epistatic effects’). All this makes the genetics of longevity difficult to quantify, interpret, and explain.

To make a complex issue more understandable, I describe experiences with my own guppies. Later, I will present scientific information that backs up my concluding recommendations on increasing guppy longevity.

**Q&A**

I’m intrigued by your longevity project. I’ve never been successful with guppies in spite of every aquarium article saying they’re easy, so I finally gave up my serial killer ways and stopped buying the poor things. I’m now wondering if it wasn’t me at all........

**Answer**

Please don’t blame yourself. It’s hard to keep modern fancy guppies alive. Some people get lucky, but they are the exception.

Most “designer” strains are genetically weak. They have little disease resistance and are physiologically unfit. They may thrive in the original breeder’s tanks or ponds, but the fish do not have the genetic capacity to adapt to new situations such as a hobbyist's tank. No matter how good their care, they often don’t last long.

Many guppy breeders keep progeny only from very young adults (“seasonal breeding”). They select for fast growth over slower maturity, longevity, and hardiness. This selection bias gradually weakens guppy strains, resulting in disease susceptibility and a short lifespan.
Short Life in a Blue Grass Strain

Longevity in guppies became an issue with my BG (Blue Grass) strain of guppies (Fig 1). I had purchased 2 pairs off the Internet in 2017. Big, beautiful, prolific, and relatively disease-resistant, it took about a year for me to realize that these fish were just not living that long. The odd death here and there had begun to add up.

In July 2018, a prized BG female produced a large batch of healthy fry. Raised outside in summer tubs and exposed to the elements, the offspring were uniformly vigorous. When they were 3 mos. old, I culled inferiors, retained 2 males and 7 females for breeding. I then documented the lifespan of the 9 select individuals.

Like my earlier BG breeder males, the two July males did not live long—about 4-5 mos. Irreversible decline began when they rested on the bottom with swollen bellies and lost all interest in food and females.

As to the females… From my 7 select females, I had expected to obtain several good breeders (Fig 2). Alas, I ended up with only one—Female #3. At 5.5 mos. of age, her superiority became evident (Fig 3). Problems with the other 6 females: #1 developed a weird, black coloration on her head; #2 was culled because of a small dorsal; #5 developed abdominal swelling; #4, #6, and #7 grew slowly and declined rapidly after giving birth.

I believe that the short lifespan of my BG strain was caused by genetic “plumbing problems.” The males suffered from intestinal blockage; the females, reproductive problems.

Apparently, BG strains have a reputation for a short lifespan. One advanced breeder reported that his BG males rarely lived longer than 9 mos.; attempts to improve their longevity by outcrossing to 3 other strains were unsuccessful [4]. The original breeder of my BG guppies reported that the strain’s lifespan in his tanks was ~8 mos. Their even shorter lifespan in my tanks could have been due to the challenges of a new environment. Moreover, since their purchase, the offspring were the product of full-sibling matings. Inbreeding probably further shortened their lifespan.
Natural Lifespan of Guppies

In 1961, the British scientist Comfort [1] officially reported a potential lifespan of 5 years for the guppy *Poecilia reticulata*.

For his comprehensive longevity study [5], he used 4 domestically bred strains. He maintained fish by themselves in small jars and tanks until their death. Young guppies were sexed and placed into single-sex containers with gravel, snails, and floating plants.

Apparently, he provided minimal care, ”Aquaria looked most unsatisfactory, but the fish in them lived for 4-5 years, as against a normal aquarium life of 2 years or less.” No filters or aerators. They were fed live Tubifex worms once a week and a cereal supplement. Water was changed only when the fish showed “discomfort.”

Fig 4 shows 100% survival at the start when all fish were 100 days old.2 At 2 years (See graph’s vertical bar), survival of both males and females was equal to or greater than 55%. At this timepoint, males began dying out slightly sooner than females. At 1500 days (4.1 years), all males were dead, but ~12% of females were still alive. Presumably, the last females died around 5 years of age. The investigator noted that disease (e.g., ‘Fish TB’) and other pathologies increased with aging, ultimately causing the fishes’ death.

The survival curve applies to virgin guppies, which have a longer lifespan than normal mating and reproducing guppies. Nevertheless, the 5 year potential lifespan assigned by Comfort to the species [1] is amazing compared to the longevity of today’s fancy guppies. Apparently, the domestic guppies of the 1950s were much more fit than today’s guppies.

Comfort’s guppies had a lifespan more in keeping with wild guppies. Ditto for feral guppies… Given enough time, domestic guppies that are introduced into the wild often regain the longevity of their wild ancestors. For example, domestic guppies were somehow introduced around 1970 into the warm springs of New Mexico’s Jemez Mountains. Thirty years later, the feral descendants were found to have an appreciable lifespan. Females lived for 18-24 mos. before they began dying off [6].

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2 I show here only his tank results, but survival was similar for guppies kept in jars.
Reproductive Lifespan v. Total Lifespan

Selection for traits that fuel evolution cannot occur after reproduction, so most biologists consider life’s post-reproductive period as but a random add-on. For the purposes of evolution, once a guppy ends its reproductive life, it is essentially dead. Thus, knowing the reproductive lifespan may be just as important as knowing the total lifespan.

For male guppies, the answer is simple. Sperm production continues throughout their adult life. Gasparini [7] showed that older males were just as fertile as younger males.3 Working with descendants of wild Trinidadian guppies, the investigators compared 43 older males (~14 mos.) with 43 younger males (~5 mos.). Age difference was 8-9 mos. The study involved manually “stripping” males of their sperm. Results showed that older males contained more sperm (average 10 million) than younger males (average 7 million) (P=0.022).4 While the sperm of older males had a lower velocity (swimming speed), there was no difference in sperm viability. Females presented with a choice in mating trials showed no preference for younger males. Most importantly, 34 virgin females that were artificially inseminated with a 1:1 mix of sperm from old and young males produced broods with no significant paternity bias (P=0.77). Out of the 542 total fry produced, 252 (or 46%) were sired by younger males [7].

In Drosophila, male fruit flies are fertile throughout their lives. Female flies, however, may live long after they have stopped reproducing. The post-reproductive portion of the female fly’s lifespan averages ~40% [9].

Female guppies along with other female animals (e.g., platy fish, birds, rodents, and primates) have been found to have a similarly long post-reproductive period—up to one-third of their maximum total lifespan [10]. Guppies stop reproducing when their ovaries stop producing eggs, not when they deplete an original stash of eggs (as in birds and mammals).5 Thus, we can infer that guppy reproduction ceases with old age and the accompanying senescence of the ovaries.

Reznick [10] found that reproduction was highly variable in females. Some females produced a new litter every month for years and then died promptly after their last litter; others skipped litters for months before getting back on a steady reproduction cycle; some lived many months after their last litter. He calculated that 60% of individuals lived at least one month past their last litter and that the average post-reproductive portion of a female’s total lifespan was 14% (range 0% to 76%). He concluded that guppies have a significant post-reproductive lifespan, similar to birds and mammals.

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3 The question arises because sexually reproducing animals lose some fertility as they age. The deterioration of egg and sperm in older humans (i.e., the “Lansing effect”) is well known in in vitro fertility clinics [8].

4 P (probability) quantifies the statistical significance of differences and gives it meaning. The P=0.022 here means that the probability that the sperm quantity difference between young and old males could be due to chance is only 0.022 (or 2.2%). Said another way, it means that there is a 97.8% certainty that the difference is “real” (100% - 2.2% = 97.8%). Probabilities equal to or greater than 0.05 (P ≥ 0.05) are generally considered to be statistically non-significant; the difference could be due to chance and random variation.

5 In teleosts (i.e., bony fishes) such as guppies, dividing oogonia persist in the adult ovary and continue throughout reproductive life to enter meiosis to become oocytes [11]. In contrast, human females at birth have a finite reservoir of 1-2 million oocytes with which they draw on for adult reproduction [12].
Reznick [13] studied the total and reproductive lifespan of females in great detail. He used 2nd generation descendants of wild guppies. Each female was isolated at 25 days of age and then maintained in a 7.8 liter (2 gal) container until the end of her life. A male was added every week until the female gave birth and then again after each parturition. (Unlike the females in Comfort’s study [1], they were not virgins, so they would not be expected to live as long.)

**Fig 5** shows that the lifespan of 4 different wild populations varied between 700 and 1,000 days. The graph’s range bars (representing individual variation) show that some guppies survived over 1,200 days (>3 years).

The reproductive lifespan varied from 880 (Population 1) to 550 days (Population 4). Range bars show that some females in all populations dramatically beat the averages. For example, Population 3 shows an average reproductive lifespan of ~700 days, but at least one female in that group was still reproducing at over 1,200 days.

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6 Rearing wild fish in the lab for two generations is standard practice when studying genetics in wild guppies. This brief captive rearing acclimates the guppies and eliminates environmental and maternal factors.

7 Guppies represented 4 different natural habitats in Trinidad. Populations 1 and 2 were from the Oropuche drainage; populations 3 and 4, the Yarra drainage. Populations 1 and 3 were derived from guppies captured in HP (high predation) habitats; 2 and 4, from LP (low predation) habitats. *(More about wild guppies in a later section)*
Another investigator (Evans) [14] conducted an in-depth analysis of fecundity (or fertility) in Australian feral guppies. His study provides us with indirect but vital information on female longevity.

Evans started with 84 virgin females (age 3 mos.) that were mated to 1-2 males during their brood cycles. Average lifespan of females was 478 days (range 275-911). Three of the 84 females produced no offspring.

**Fig 6** shows the fecundity of the study’s 81 reproducing females. First broods averaged only ~5 fry. By their 3rd brood, the average # of offspring had tripled to over 15. As the females grew older and larger in size, their fecundity predictably increased [15].

By the 7th brood cycle, the number of broods had declined from the starting 81 to 38 due to female mortality.

The big dip in fecundity at the 9th brood cycle, where the average number of offspring was only ~8, could be due to the increasing senescence of the population’s weaker females (i.e., the ones that were dying off).

Interestingly, a huge surge in fecundity followed the 9th brood cycle. I think that the weaker females had died off and the remaining, more robust females had come into their own.

They were older, bigger, and thus, had bigger litters. By the 15th cycle, graph shows that the 10 remaining females produced broods averaging 31 offspring.

At the final 18th brood cycle, the 3 still-reproducing females produced an average of only 5 offspring. The sharply reduced fecundity within the remaining females could be due to deterioration within the aging ovary, resulting in reduced egg production and dead embryos.
Among the study’s 81 reproducing females, I selected the 8 least and the 8 most fecund females for a closer look. All 16 females produced at least 4 broods, but the 8 weaker females had stopped producing after their 4th brood cycle, while the 8 fittest females produced offspring for ≥16 cycles. Assuming a typical one month interval between broods and knowing that all females were 3 mos. old when first mated, my 8 fittest females would have lived at least 19 mos., while the 8 weakest females would have lived only 7-8 mos.

Did the 8 weak females signal their weakness early in life, such that they could be selected out early on? The results in Table 1 say no. Their initial fecundity was very similar to the fittest females. Indeed, for their fourth brood, they actually produced more offspring than the fittest females (18 v. 13). Thus, there was no way to predict which females would survive and continue reproducing.

Because the fittest females continued reproducing after 4 litters, their lifetime output was over 4 times greater than that of the weakest females (267 v. 55 offspring). One particularly fecund female produced 18 broods and a record 349 offspring over her lifetime.

These results jive with my own observations. Many young females in a batch look equally healthy and reproduce well at 4-7 mos. of age. Only by waiting does the inherent weakness of some individuals—and the superiority of others—become apparent.

### Effect of Reproduction on Longevity

Mating and reproduction often decrease longevity. Virgins live longer than reproducing guppies [16]. Indeed, I have seen many female guppies die shortly after giving birth [Fig 7]. Investigator Comfort [5] kept many of his study guppies in single sex tanks. The “half-life” of Comfort’s virgin females was estimated to be ~40 mos., but only ~14 mos. for reproducing females descended from wild guppies [16].

Drosophila studies have shown consistently that mating reduces the fruit fly’s lifespan, particularly in females [9]. Zwaan [17] reported 28% and 44% mating-related decreases in longevity for males and females, respectively. Reduced longevity due to sexual activity was highly significant (P<0.001).

### Table 1. Fecundity Extremes in a Population [14]

<table>
<thead>
<tr>
<th>Number of Offspring</th>
<th>Weakest Females</th>
<th>Fittest Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Brood</td>
<td>6.6 (±3.9)</td>
<td>5.1 (±3.6)</td>
</tr>
<tr>
<td>Second Brood</td>
<td>13 (±3.6)</td>
<td>12 (±5.2)</td>
</tr>
<tr>
<td>Third Brood</td>
<td>17 (±3.2)</td>
<td>18 (±5.1)</td>
</tr>
<tr>
<td>Fourth Brood</td>
<td>18 (±5.6)</td>
<td>13 (±10)</td>
</tr>
<tr>
<td>Lifetime Production</td>
<td>55 (±10)</td>
<td>267 (±40)</td>
</tr>
</tbody>
</table>

Fig 7. Reproduction is Stressful

Female #3 (pure BG) had out-lived all her siblings. But she became morbid at 9 mos., a few days after parturition. Note swelling at vent area.
Luckinbill [18] also measured longevity in Drosophila as a consequence of mating. Males and females were raised alone as virgins or paired with an individual of the opposite sex. Fig 8 shows that female longevity decreased from 62 days for virgins to 47 days for paired females (a 24% decline). For males, longevity decreased from 64 days to 56 days (a 12% decline). Longevity decreases due to mating were significant for both males and females ($P<0.02$).

Theories abound for why reproduction exacts such a heavy toll on female longevity. However, multiple Drosophila studies have uncovered one cause—Acps (“accessory gland proteins”) within the male ejaculate. Investigators [19] showed that sperm and the mating act itself were not the problem as much as Acps. (Females mated to genetically modified males without these proteins exhibited the same survival as non-mated females [22].)

Acps increase a male’s reproductive success. One of them, Acp70A or the “sex peptide,” stimulates the female to produce more eggs while decreasing her desire for remating. Unfortunately, some Acps have negative and unintended side-effects on the female’s physiology that reduce her longevity. This is a classic example of evolutionary sexual conflict [23]. Female survival is pitted against the male goal of increasing his reproductive success.

Mating and reproduction are essential traits in guppies. Virgins may live longer, but that longevity is meaningless if they quickly fall apart upon mating. For that reason, when I select for longevity, it is always from within a population of reproducing guppies.

Fig 8. Effect of Mating on Fly Longevity [18]
Columns represent the average lifespan of male and female flies, both virgin (solid columns) and mated (checkered columns). Bars are the s.d.
(I drew graph from data in Luckinbill’s Table 1.)
Breeding Practices that Shorten Longevity

Many common guppy breeding practices contribute to a shortened guppy lifespan. Breeders select for color, fin shape, strain uniformity, etc. Longevity is rarely considered. Indeed, when my male Blue Grass guppies kept dying at 4-5 mos., I simply replaced them with their sons. Only later, did I begin to contemplate the long-term consequences of this practice.

Many breeders use only the first few batches from young brood stock (“seasonal breeding”). Seasonal breeding begins with young virgins. After mating, these females are allowed to produce a few broods and then discarded or no longer used for breeding. Apparently, this is a common practice for commercial breeders, show breeders from the IFGA (International Fancy Guppy Assoc.), and scientists maintaining lab guppies.

Seasonal breeding with its typical generation cutoff at the 3rd or 4th brood cycle (or at ~7-8 mos. of age) cuts short the guppy’s natural life cycle and leads to long-term, detrimental genetic consequences. Weak females get the same chance to pass on their faulty genes as superior females. With seasonal breeding, weak females that die at 8 mos. add just as many progeny to the next generation as robust females that could live to 18-24 mos. There is no selection for longevity.

Seasonal breeding is not just restricted to guppies. Indeed, many Drosophila labs maintain their strains on a strict 14-day generation cycle, despite the fact that wild flies can live ~80 days [9]. Fourteen days represents only a fraction of the fly’s potential lifespan. Young flies are barely given time to lay their eggs before they are replaced by the next generation. Only eggs laid within 36-48 hr of sexual maturity contribute to the next generation. Some labs have maintained their flies for years and hundreds of generations on a 14-day cycle [24, 25].

Promislow [25] suggests that the standard “14-day” maintenance practice could easily lead to spurious results in longevity studies. Under this culture regimen, flies older than 4 days have no reproductive value. Genes for adult fitness traits expressed after 4 days of age are not exposed to evolutionary selection and removal from the population. The resulting accumulation of late-acting, age-specific mutations reduce fitness traits (e.g., longevity, fecundity, etc.). Given a mutation accumulation rate of 0.1 to 1% per generation, Promislow [25] estimated a ~50% reduction in late-life fitness after ≥100 generations. When these compromised strains are used for longevity selection studies, the selection may be more about purging spurious mutations than selecting for longevity genes [25].

Unsurprisingly, Drosophila investigators have shown that lab-cultured strains have a much shorter lifespan than the 70-80 days of wild flies. One study [26] demonstrated a life expectancy for wild male flies of 83 days (± 0.3 S.E.) compared to only 60 days (± 0.3 S.E.) for lab strain males. (Both cohorts in the comparison were virgins.)

Another study [27] showed that maintaining fly cultures with the 14-day generation time could shorten the lifespan of wild flies within 1-2 years. For the study, the investigator compared two “fresh” wild-derived populations with three 14-day cultured populations under the same conditions, the standard “common garden” experimental design. Mean lifespan (for reproducing females) decreased within one year from 44 days (± 0.1 S.E) for the parental wild population to 38 days (± 0.1 S.E) for the 14-day flies. A year later, the 38 day lifespan of the 14-day flies had further declined to 31 days (± 0.1 S.E). Both 1 and 2 year longevity decreases due to lab culturing were highly significant (P<0.001).

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9 Drosophila female flies have a ~23 day reproductive lifespan and a ~40 day total lifespan; males have ~50 days for both [9]. [These adult fly stages do not include the larval stage (egg to eclosion) of about 10-11 days. (Eclosion is the sudden metamorphosis of the larva into a fly.)]
For guppies, tail or caudal size seems to be another factor affecting longevity, with swordtail (short-tail) strains living longer than delta tail strains. The consensus among most guppy breeders is that delta strains have a lifespan of 1-1.5 years; swordtail strains, 1.5-2 years [4]. Because of their large heavy tails, older delta males are purportedly less able to inseminate females. In 1961 master guppy breeder Paul Hahnel [28] reported, “One albino male with an uncommonly long tail was useless until the last half of his tail fell off, when he again became a father.” He noted that delta males could impregnate females at 9 mos., but not at 12 mos. Thus, he recommended using younger males as breeders. No doubt, this 1960’s recommendation has become entrenched in maintaining many delta strains.

Using younger guppies for reproduction is common for other reasons. When a unique guppy with an eye-catching phenotype appears in a batch, hobbyists often want to quickly “fix” the new phenotype into a new strain. This requires backcrossing and a rapid generation turnover. No one wants to wait several months before collecting fry from an amazing new male or female.

Current guppy breeders maintain their valuable stock under conditions more benign than those described by Comfort for his 1950’s guppies [5]. They feed their guppies optimally and change water frequently. To prevent diseases, some breeders use UV sterilizing filters, salt and antibiotics. Over multiple generations, this “coddling” gradually erodes genes coding for fitness. Domestication and optimal tank conditions inevitably result in lowered disease resistance. Thus, one investigator [29] showed that wild guppies lost many alleles for disease resistance after they were maintained in a scientific lab for 50-100 generations.10

**Diseases of Older Guppies**

Longevity is not just a matter of senescence—the age-related, general decay of the body’s organs and tissues. Longevity is invariably tied to genes for immunity. During a disease outbreak, guppies lacking genes for strong immunity would be the first to die. Conversely, guppies with genes for early senescence would be the first to succumb to disease. Reznick [15] monitored pathologies found in older adult guppies that had died or been euthanized. (All guppies were from the same lab strain and had no apparent health issues.) Table 2 shows the six pathologies that would be most related to aging.

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Description</th>
<th>Guppies with Pathology (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Young</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oophoritis</td>
<td>Inflammation of the ovaries</td>
<td>0</td>
</tr>
<tr>
<td>Enteritis</td>
<td>Inflammation of the small intestine</td>
<td>8.3</td>
</tr>
<tr>
<td>Pleuroperitonitis</td>
<td>Inflammation of membranes that line the upper body cavity</td>
<td>0</td>
</tr>
<tr>
<td>Granulomas</td>
<td>Small, contained inflammation sites</td>
<td>17</td>
</tr>
<tr>
<td>Pigment Deposition</td>
<td>Accumulation of pigments from cellular degeneration</td>
<td>8.3</td>
</tr>
<tr>
<td>Myocardial vacuolation</td>
<td>Degeneration of heart muscle cells</td>
<td>0</td>
</tr>
<tr>
<td><strong>Old</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>64</td>
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<td></td>
<td></td>
<td>71</td>
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<td></td>
<td></td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>57</td>
</tr>
</tbody>
</table>

Table 2. Pathology of Aging [15] Differences between old (n = 14) and young guppies (n = 12) for each pathology were statistically significant (P<0.05).

{I extracted data from Reznick’s Table 1.}

10 In a separate study [30], the same investigator (van Oosterhout) found that wild guppies had 15-16 key MHC alleles, whereas 14 domestic strains had only 1-3 alleles. These particular MHC alleles are genetic markers for acquired immunity.
The first four pathologies shown in the table involve bacterial diseases; the last two would be due to normal, physiological aging.

Oophoritis was found in 43% of older females but none in younger ones. Oophoritis, a bacterial infection of the ovaries, is often triggered by an abnormal reproductive event (e.g., ovulation failure, resorption of eggs and embryos, etc). This localized infection can later spread into the visceral organs. I believe that the reduced lifespan in my first BG females often resulted from oophoritis. So many of the females died a few days after giving birth.

Evolution in Wild Guppies

For decades, investigators have been studying wild guppies in their native habitat—streams in the mountain forests (Fig 9) of Venezuela, Tobago, and Trinidad in South America [31]. These studies provide some insight into the origin of our domestic strains. They also give us some idea of how long it might take for longevity to evolve in guppies under natural conditions.

Predation was found to be a major factor in guppy evolution. Investigators have divided wild populations into two groups based on predation. Guppies from HP (high predation) sites are preyed on by the large pike cichlid (Crenicichla alta) (Fig 10). These large predators continuously pick off the most conspicuous and flamboyant male guppies, leaving their more drab brethren to mate with the females. Thus, predation in HP sites selects for—via evolution—drab-colored males.

Nearby in upstream sites separated by waterfall barriers are small shaded pools. Here in these LP (low-predation) sites, a small killifish is the only guppy predator. Because of this predator’s small size, it can only eat small, immature guppies. LP male guppies have evolved to be larger and more colorful than those from HP sites.

11 Other factors (guppy population density, food availability, gravel composition, etc) differ between HP and LP sites, but their effect was shown to be minor compared to the pike cichlid predator [13, 31, 32].
Endler [31] documented predation’s effect on the guppy’s color spots. He captured Trinidadian guppies from an HP site containing the pike cichlid. In 1976, he introduced ~200 of these guppies into 3 LP sites. He scored them for number and color of spots at the introduction time and then again two years later in 1978 (Fig 11). Spots, especially those that are bright blue and iridescent, make the guppy more visible and vulnerable to predators. Endler predicted that male guppies would develop brighter colors in the absence of the pike cichlid. And indeed, he was correct. The number and size of black, red, blue, and iridescent spots increased significantly. Endler confirmed the results of his field study with experimental ponds in a greenhouse.

Endler [31] hypothesized that male coloration reflected a balance between the opposing forces of sexual selection and predator selection. In the LP sites with no large predators picking off brightly colored males, females mated preferentially with more colorful males (i.e., sexual selection), resulting in the rapid evolution of males with numerous, brightly colored spots. At HP sites, in contrast, cichlid predators negated the effect of female preference by selectively removing brightly colored males. The result was the evolution of drab colored males.

Subsequent studies by Reznick [35] documented that cichlid predation also affected vital fitness traits of both sexes.

Table 3 sums up various predator-influenced traits involving evolution in wild guppies. At HP sites, both males and females matured faster sexually. Females reproduced sooner in life (82 days v. 72 days) and at a smaller body size (270 mg. v. 218 mg). They had larger litters consisting of smaller fry and they reproduced more frequently (i.e., had shorter gestation intervals).

Reproductive differences between HP and LP females added up. Total offspring averaged 545 for HP females versus only 218 for LP females.

Surprisingly, female longevity was 35% greater for HP guppies than for LP guppies (i.e., 1,007 days v. 746 days). (Guppies in another river system [13] followed a similar pattern.) Results were

<table>
<thead>
<tr>
<th>Phenotypic Trait</th>
<th>HP Sites</th>
<th>LP Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male age at maturity (days)</td>
<td>52 (±1.1)</td>
<td>59 (±1.0)</td>
</tr>
<tr>
<td>Male size at maturity (mg-wet)</td>
<td>88 (±2.8)</td>
<td>100 (±2.5)</td>
</tr>
<tr>
<td>Female age at maturity (days)</td>
<td>72 (±2.0)</td>
<td>82 (±1.9)</td>
</tr>
<tr>
<td>Female size at maturity (mg-wet)</td>
<td>218 (±8.4)</td>
<td>270 (±8.2)</td>
</tr>
<tr>
<td>Brood size (first litter)</td>
<td>4.5 (±0.4)</td>
<td>3.3 (±0.4)</td>
</tr>
<tr>
<td>Offspring Size (mg-dry)</td>
<td>0.84 (±0.02)</td>
<td>0.99 (±0.03)</td>
</tr>
<tr>
<td>Gestation Interval (days)</td>
<td>23 (±0.3)</td>
<td>25 (±0.03)</td>
</tr>
<tr>
<td>Litters/female</td>
<td>28 (14-43)</td>
<td>17 (5-32)</td>
</tr>
<tr>
<td>Offspring/female (lifetime total)</td>
<td>545 (204-1,114)</td>
<td>218 (18-568)</td>
</tr>
<tr>
<td>Female Longevity (days)</td>
<td>1,007 (636-1,264)</td>
<td>746 (338-1,136)</td>
</tr>
<tr>
<td>Male body color</td>
<td>Drab</td>
<td>Bright</td>
</tr>
</tbody>
</table>

Table 3. Effect of Cichlid Predation on Guppy Traits

Comparison of wild guppy descendants captured at various HP (high predation) and LP (low predation) sites in Trinidad. First seven rows show averages and the S.E. (P<0.01) “Maturity” refers to sexual maturity. Next 3 rows show averages and ranges.

{For table, I compiled data from multiple studies by Reznick [13, 31, 36, 37].}
unexpected. They ran counter to the evolutionary theory that there is a trade-off between early reproduction and increased longevity in virtually all animals.\textsuperscript{12}

Natural selection in HP sites would favor guppies that could reproduce quickly, before they were eaten. To evade the pike cichlid, nature would select for strong swimmers. And indeed, HP guppies were found to have faster acceleration speeds than LP guppies ($P=0.024$) [13].

Reznick [35] also tracked the natural evolution of guppy traits in the fish’s native habitat. For this field study, he introduced guppies captured at HP sites into new sites where there were no guppies or major predators. Years later, he captured guppies from those sites and compared their 2nd generation descendants to control guppies. (Control guppies, also 2\textsuperscript{nd} generation from the wild, were captured simultaneously at the study’s original HP sites.)

Results showed that males evolved faster than females. After 4 years in the absence of the pike cichlid, males showed significant increases in size and age at maturity compared to HP control guppies ($P<0.01$). In contrast, it took females 7.5 years to show significant changes [35, 39]. After 11 years, females continued their evolution; they showed significant reproductive changes (i.e., increased brood size and smaller offspring) [37].

Reznick attributed the sex difference to the greater heritability of male development than female development [16]. The speed of a trait’s evolution increases with both its heritability and the selection pressure. The Trinidadian guppy studies demonstrate a rapid rate for evolution. It is much faster than people would predict based on the general Darwinian concept of evolution. Darwin (1859) described a slow gradual process that would not be discernible within a person’s lifetime. Yet, both Reznick and Endler have shown evolution of various guppy traits within 2-11 years.

This relatively rapid evolution has real world implications, such as in commercial over-fishing of marine species. One could equate the pike cichlid predator with the human predator as an evolutionary selection force. For guppies, Reznick has shown that the pike cichlid reduced the size and age at sexual maturity by 5-15\% after 4-7.5 years (~7-12 guppy generations). If the guppy model is applied to marine fish, Reznick [35] calculated that a similar 5-15\% change would occur within 10 years for anchovy, 20 years for pink salmon, and 120 years for Atlantic cod. (Rate of change is based on the generation time of each species.) Removal of the larger individuals within a population inevitably selects for fish with faster growth and a smaller mature body size.

**Inbreeding Depression (ID)**

Inbreeding’s harmful effects [i.e., inbreeding depression (ID)] have been known for centuries and officially confirmed by Darwin’s plant experiments in the mid-19\textsuperscript{th} century [40].

ID particularly targets vital fitness traits such as survival, growth and fertility as opposed to morphological traits (e.g., adult body size and shape). An analysis [41] of different animal species showed that inbreeding reduced fitness traits by 12\% versus only 2.2\% for morphological traits. Investigators [40] ranked traits in the following order of susceptibility to ID:

\[
\text{survival} > \text{fecundity} > \text{growth rate} > \text{adult body size}.
\]

\textsuperscript{12} Reznick [13] discussed possible reasons for the unexpected longevity results. Indeed, there are valid and accepted exceptions to the classic theory of senescence evolution [38]. One exception is caused by the fact that female guppies become more fecund as they grow older and bigger [15].
In guppies, examples of ID include reduced survival [63, 42], reduced saltwater tolerance (strongly correlated with lower survival) [43, 44], and reduced fertility [45, 46, 47].

However, to say that inbreeding is invariably bad belies the fact that many successful guppy breeders and scientists have inbred their brood stock for hundreds of generations. Inbreeding is used to “fix” desired phenotypes. Some guppy breeders maintain 2-3 replicate populations for each strain. Should ID appear, they can cross the replicates. This time-honored livestock breeding practice, sometimes called “line breeding,” counteracts ID while maintaining strain attributes.

To parse differences between “mild” and “severe” inbreeding, I have quantitated inbreeding using the $F$ coefficient. As shown in Table 4, a mating between first cousins produces offspring with an $F = 0.063$. In contrast, 3 generations of matings between full siblings will produce progeny with an $F = 0.500$, representing severe inbreeding.

Fig 12 shows how fast $F$ can increase in guppy populations. The investigators [42] started with wild guppies from Trinidad and segregated them into two breeding regimens. Each year for 10 years, a new generation was started with several replicate ‘families’. One regimen (‘inbred guppies’) involved the random selection of 5 pairs to start each new generation. Because of the small population size (10N), this resulted in some sibling matings. The other regimen (‘control guppies’) had less inbreeding, because each generation was started with 10 pairs (20N), plus the pairs were deliberately chosen to be as unrelated as possible.

By the 10th generation, the graph shows that inbred guppies averaged $F = 0.41$, while control guppies averaged $F = 0.12$. It also shows the large variation between similarly bred, replicate families at each generation.

<table>
<thead>
<tr>
<th>Pedigree Examples</th>
<th>$F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 from mating second cousins</td>
<td>0.016</td>
</tr>
<tr>
<td>F1 from mating first cousins</td>
<td>0.063</td>
</tr>
<tr>
<td>F1 from mating half-siblings</td>
<td>0.125</td>
</tr>
<tr>
<td>F1 from mating full siblings</td>
<td>0.250</td>
</tr>
<tr>
<td>F2 from mating siblings of the F1 above</td>
<td>0.375</td>
</tr>
<tr>
<td>F3 from mating siblings of the F2 above</td>
<td>0.500</td>
</tr>
<tr>
<td>F1 from mating a parent to its progeny</td>
<td>0.250</td>
</tr>
</tbody>
</table>

Figure 12. Inbreeding Coefficient ($F$) for Inbred v. Control Guppies [42]
Each circle represents the calculated $F$ value of a breeding ‘family’. Solid line connects the average $F$ for each generation.

{Figure used with kind permission of the authors and publisher.}

$F$ = probability that two alleles in an individual are identical by descent. $F$ is calculated by pedigree analysis.
Wild guppies isolated in small natural pools will inbreed to some degree. For example, one small wild population averaged an \( F = 0.05 \), with a full 16% of individuals inbred to \( F = 0.13 \), the level of half-siblings [48]. \{The smaller the population size (N), the more inbred it will become [84, 49].\}

In general, large natural fish populations are genetically heterozygous. They can carry many deleterious recessive alleles with few negative consequences. Recessive genes that could produce lethal effects are rendered harmless by their dominant allelic partner. If the fish become inbred, though, ID can be severe. Thus, when investigators inbred wild-caught killifish and zebrafish, many of the litters contained progeny with lethal deformities (e.g., no swim bladder, cranial deformities, etc) [50].

Ironically, inbred “designer” guppy strains may be less susceptible to ID than wild guppies. With their frequent inbreeding, the random pairing of recessive deleterious alleles speeds up. Offspring carrying defective allelic pairs are either culled or unlikely to survive, so they and their bad genes are removed or “purged” from the strain. Theoretically, if ID was due solely to deleterious recessive alleles,\(^{14}\) one could produce an inbred strain equal in fitness to the original, non-inbred population [52].

Compared to wild guppy populations, designer guppy strains are inbred [53, 54]. Many do fine in the tanks of the original breeder. However, after purchase and/or transfer to a new tank, they may have trouble adapting to new food, water conditions, and strange microorganisms. Inbred animals—because of their reduced genetic heterozygosity—are often less able to adapt to a new environment [55].

Thus, environmental stress often reveals and exacerbates the harmful effects of inbreeding. For example, inbred and non-inbred populations of hatchery-raised salmon showed no difference in survival in the hatchery [56]. However, when fish from the two populations were released each year into the wild, a much more stressful environment, inbred fish \((F = 0.25)\) showed a consistent drop in survival compared to non-inbred controls; average reduction across 5 years was a whopping 71%.

van Oosterhout [29] found that inbred guppies survived less upon introduction into the wild than non-inbred guppies. Starting the study with \(~100\) wild guppies from two rivers in Trinidad, he crossed siblings for 3 generations to get an inbred F4 generation \((F = 0.5)\). Non-inbred controls had an \( F = 0 \). After 60 days in the wild, survival was 76% for 29 non-inbred guppies but only 44% for 71 inbred guppies.\(^{15}\) Most death was attributed to flukes \((Gyrodactylus\) species), a parasite endemic to the natural habitats where the guppies were released.

Nakadate [63] studied inbreeding’s effect on guppy survival. The investigators mated siblings from recent descendants of wild guppies captured from a Japanese river. Only 71% of the inbred offspring survived to 120 days compared to 88% of the non-inbred guppies from the parental population \((P<0.05)\).

In comparing domestic guppy strains with wild populations, Japanese investigators [54] showed that the longer guppies were maintained in captivity, the more they became inbred. They analyzed the guppies’ DNA for polymorphisms (small genetic alterations) coding for 16 different enzymes. From this data, the investigators determined the average genetic heterozygosity (or \( H_S \)) of the strains. \((H_S\) is a marker for inbreeding; the lower the \( H_S \) number, the more inbred the strain or population.)

---

\(^{14}\) A small portion of ID is due to the “heterozygous advantage,” sometimes called “overdominance.” It occurs when an allele encodes more fitness in the heterozygous state (one bad gene + one good gene) than the homozygous state (two good genes). Deleterious recessive genes at loci with the heterozygous advantage cannot be purged from a population [51].

\(^{15}\) I combined the investigator’s Table 2 data [29] for both rivers and sexes to calculate my survival percentages.
Table 5 shows the results of their genetic analysis. The 0.028 $H_S$ for all 13 lab strains was 42% lower than the 0.048 $H_S$ of Trinidadian wild guppies. The Table also shows that the longer the domestic strains were maintained in the lab, the more they became inbred (i.e., the 0.020 $H_S$ for old lab strains is 44% lower than the 0.036 $H_S$ for new lab strains). The 0.041 $H_S$ of feral guppies suggests that after release into the wild, domestic guppies may regain the genetic variation of their wild ancestors.\(^\text{16}\)

Inbreeding is not always harmful. Many conservationists cite 6 wild fox populations on 6 separate Channel Islands that have been inbreeding for over 9,000 years. Despite severe inbreeding due to their isolation, small numbers, and former predation by the golden eagle, these foxes [Fig 13] are currently healthy and without the congenital defects that many scientists had expected [57]. With the assistance of conservationists, the 6 small inbred fox populations have bounced back from their near extinction.

Allendorf [58] observed that whether inbreeding caused problems depended on the population’s founder individuals. When inbred, some founder pairs produced offspring with ID; other produced perfectly normal offspring. This suggests that a few bad alleles cause the harmful effects of inbreeding. Which individuals carry those alleles is largely a matter of chance.

Inbreeding’s harmful effects can be reduced by selection, purging, and outcrossing.

### Purging and Selection

Inbreeding can remove bad genes by “purging.” In a 10-generation study, Larsen [42] showed that ID—in the form of reduced survival—could be purged out of a guppy population by inbreeding. They first quantified the inbreeding level of their two experimental populations, one more inbred than the other ($F$ levels are shown in Fig 12, p. 15).

Each generation’s progeny was monitored for survival. Inbreeding took its expected toll. Five inbred families and one control family went extinct during Generations 1 through 5 because the females were barren. In Generations 3 and 5, average litter size of the female’s first batch decreased ~40% in inbred females.

\(^{16}\) The 13 lab strains originally came from petshops, hobbyist aquaria, etc. The 6 “Old” lab strains had been maintained in the lab for more than 10 years; the 6 “New” lab strains, less than 10 years. “Feral” guppies were captured at 10 different natural sites in Japan; “Wild” guppies, captured at 11 different natural sites in Trinidad.
Fig 14 shows the average survival of the two populations. At Generation 4, survival to one year was 77% for inbred fish; 95%, for control fish ($P<0.001$). At Generation 5, this difference lessened, but it was still significant ($P<0.04$). In other generations, there was no real difference in survival. The investigators hypothesized that after Generation 5, lethal alleles were purged from the population, such that inbred guppies survived as well as control fish.

Japanese investigators [59] provide an example of rapid purging in an old lab strain. For 5 generations, they mated siblings. Fig 15 shows that after a single mating of siblings, fry mortality jumped from 2.4% to over 25%. However, by Generation 5 when the inbreeding was even higher ($F = 0.594$), fry mortality had declined to that of the starting population.

With severe inbreeding like this, genes responsible for fry mortality were purged within 5 generations. The investigators [59] calculated that
some mortality resulted from major recessive lethal alleles, but that most was caused by many genes with small, sub-lethal effects. Interestingly, they estimated that it would have required 75 generations of random mating—no inbreeding—to purge the fry of those same mortality genes.

The severe inbreeding shown in Fig 15 is considered very risky. So many inbred progeny die or cannot reproduce that the population can go extinct or suffer permanent genetic damage.

Pekkata [49] reported vast losses (~96% death) due to severe inbreeding in Drosophila. He showed that slower inbreeding to the same F level produced more fitness with less loss of life.

I also think one can lose good genes from a population as well as purge bad ones by inbreeding. For example, two populations of wild guppies lost significant orange color after 8 generations of experimental inbreeding [60]. When they were crossed together, the hybrid progeny did not—despite the increased heterozygosity—regain the original orange coloration.

Successful livestock breeding involves selecting individuals with desired traits for breeding. However, selection always reduces population size and increases the risk of ID. Thus, there is an optimal balance between selection and inbreeding. Norwegian aquaculture managers select rainbow trout brood stock for fast growth and against early sexual maturation. Ironically, this selection led to some ID—manifested as decreased fish weight at harvest. The reduced growth occurred despite the fact that the managers avoided mating sibling and half-siblings. This conundrum inspired investigators [61] to measure inbreeding’s overall effect. Their analysis showed that ID problems were minor compared to the major gain in growth that could be achieved by selective breeding.

### Outcrossing and Heterosis

In general, inbreeding reduces fitness; outcrossing increases it. The progeny of an outcross frequently gains heterosis or hybrid vigor.

Outcrossing increases genetic heterozygosity, which has multiple benefits: (1) it masks deleterious recessive alleles with beneficial dominant alleles; (2) it increases alleles with the heterozygote advantage; and (3) it generates phenotypic variation allowing better adaptation to environmental changes [62].

Investigators [63] increased longevity after crossing two inbred lab strains of guppies (‘S’ and ‘F’). **Fig 16** shows that average survival (to 120 days) of the S strain was only 72%, but when S males were outcrossed to F females (S X F), mean survival of the F1 progeny increased to 90%. In the reverse cross, survival

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17 To create a gradient of inbred fly populations, Pekkata [49] used population size (N). He started each new fly generation with either 1, 5, or 20 pairs (i.e., N2, N10, and N40) selected from the previous generation’s offspring. (The N2, in effect, was the mating of full siblings at each generation.) Compared to the N10 and N40 populations, the N2 showed sharply reduced survival and fecundity.
also increased dramatically—from 67% for the F purebreds to 96% for the ‘F X S’ F1 progeny. The difference between both F1s and the two parental strains was statistically significant (P<0.01).

The greatly improved guppy survival due to heterosis would not apply to all outcrosses. The S and F were old lab strains known to be isogenic (i.e., genetically homozygous due to long-term culture in captivity). They benefited from outcrossing more than would be expected in “younger,” less isogenic strains.

Indeed, the more inbred the parental strains, the more heterosis in the offspring. One investigator [49] showed that crossing severely inbred Drosophila strains produced much fitter offspring than crossing similar, but less inbred strains. Indeed, crosses between their least inbred strains showed no heterosis at all in terms of offspring survival, fecundity and total fitness.

Moreover, heterosis can be transitory. Improvements due to heterosis in the F1 is often lost in subsequent generations [65]. Fig 17 shows that a key genetic trait associated with guppy longevity (i.e., survival in saltwater) was gained in the F1, but lost in the F2.

Investigators [67] started with two isogenic lab strains F and S, both with saltwater survival times of ~3.6 hr. S males were mated to F females to produce the ‘F1 (S X F)’. As expected, the F1 progeny’s survival time increased (to 5.6 hr) due to heterosis. However, survival time of the F2s [progeny of random matings of the ‘F1 (S X F)’] returned to the low survival time (< 4 hr) of the F and S strains.

Thus, one cannot assume that longevity can be gained simply by outcrossing and increasing heterozygosity. If neither strain involved in the outcross contains genes for longevity, outcrossing is not going to “create something from nothing.”

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18 Drosophila investigators actually use heterosis as a tool to detect unwanted, unintended inbreeding in their experiments [64]. They hybridize replicates of a strain and look for heterosis in the progeny. Any increased fitness in the hybrids indicates ID in the pure strain.

19 Guppy tolerance of 3.5% saltwater (seawater concentration) is genetically based and—unlike traits of adult body size, dwarfism, and heat tolerance—was found to correlate strongly with guppy survival to 120 days [63].
Outcrossing My BG Strain

In 2018, I began outcrossing my pure BG females with male swordtail guppies. An outcross would bring some genetic heterozygosity into my highly inbred BG strain. Swordtail guppies have a reputation for longevity, reportedly living 1.5-2 years versus 1-1.5 years for delta tail strains [4]. Moreover, many “short-tail” strains have sparkling iridescent colors sometimes missing in even the fanciest delta strains.

I nixed the idea of crossing my BG strain with other fancy delta strains. I did not know where to get ones with a known long lifespan. Simply outcrossing to whatever was available might increase longevity in the first generation due to heterosis, but it might not pass on to the second generation.

The first successful outcross came serendipitously while shopping in a pet store. I spotted some lyretail males, inconspicuous except they had the Japan Blue body color (Fig 18). On a whim, I purchased two males and mated them to a BG female. The resulting progeny were beautiful deltas with the Japan Blue color, plus they had increased longevity.

At about the same time, I purchased lower swordtail guppies (Fig 19) from an authoritative guppy breeder [66]. These swordtails were loaded with exotic color and pattern genes. I had no idea what their progeny would be like. Much to my delight, I got a rainbow of colors and patterns along with greater longevity.

Crossing my BG females to swordtail guppies increased the male longevity of my brood stock from <6 mos. to >12 mos. Males were no longer dying at 5-6 mos. The increased longevity continued into the F3 generation, so it was not just a matter of heterosis.

As to the females, there was some improvement, but not as much. By 2019, none of my females had survived to 12 mos.

Fig 18a. Japan Blue Sire (purchased from PetSmart)

Fig 18b. F1 Male (Japan Blue lyretail X BG) that I selected and used for breeding

Fig 19a. Lower Swordtail Male that I used for outcrossing was bred by Alan Bias. His blue body color masks a variety of color and patterns that came out in the F1.

Fig 19b. F1 Offspring (Ls Bias Swordtail x BG female) show a delightful variety of iridescent colors and patterns.
Outbreeding Depression (OBD)

Outcrossing has a downside that I only became aware of in 2020. Indeed, outbreeding depression (OBD) is less recognized in animal and guppy breeding than in plant breeding. (Farmers are keenly aware that seed kernels from hybridized corn produce inferior crops.)

Some scientists suggest that the problems of OBD are on par with ID (inbreeding depression) [52]. Outcrossing introduces novel alleles into a parent’s stable genetic architecture, breaking apart co-adapted gene complexes. The new allelic recombination may be genetically incompatible, resulting in reduced fitness and increased disease susceptibility. Not all traits are impacted the same way.

Often, symptoms of OBD do not appear until the second generation (F2), because they are masked in the first generation (F1) by heterosis.20 Thus, the F1 offspring of native brook trout hybridized with rainbow trout showed no decline in fecundity. However, in subsequent generations, brook trout hybrids showed a steady decline in fecundity with the increased infusion of rainbow trout genes. A 20% infusion reduced fecundity by a whopping 50% [68].

The more genetically different the parents, the greater the risk of OBD. An extreme example is an inter-species cross (e.g., donkey stallion X mare) producing the sterile mule.

With outcrossing, usually the further the geographical separation between the parent populations, the greater the genetic differences and the greater the OBD. One classic study [69] involved crossing marine copepods from various California tidepools. Three populations were near Los Angeles and one was 120 miles (193 km) south in San Diego. Crossing these populations indicated heterosis by their offspring’s faster development. In contrast, the F2s showed retarded or no development. As expected, OBD was greatest in all outcrosses involving the most geographical separation [i.e., between the ‘SD’ (San Diego) and any of the three Los Angeles populations].

Fig 20 shows outcross results involving the SD and one of the Los Angeles populations, the ‘FR’. The F1 offspring—both the SD X FR and the reverse cross (RC)—matured at ~16 days. In contrast, when the F1s were mated together, their F2 progeny took considerably longer. For example, F2 offspring from mating F1s of the FR X SD cross (fifth column in graph) took 26 days to develop (P<0.001).

20 In a meta-analysis [52] of 29 fitness traits in 25 different hybridized animal and invertebrate species, 13 traits (45%) showed OBD in the F1, while 16 (55%) showed OBD in the F2.
Prime OBD candidates are populations that have been isolated from each other for over 50-100 generations [70]. Should individuals from the two separated populations mate, this prolonged isolation theoretically produces enough genetic differences to generate OBD in their offspring.

Guppies from the Caroni and the Oropuche drainages in Trinidad (Fig 21) have been geographically isolated from each other for possibly 330,000 to 500,000 years [71]. They are still the same species (will mate and produce fertile offspring), but they are considered ‘genetically divergent’.

Investigators [72] crossed guppies from the two drainages and documented problems with sexual behavior and fecundity. The ‘T’ population was from the Tacarigua River (Caroni drainage); the ‘O’ population was from the Oropuche River (Oropuche drainage). Compared to the two parent populations (T and O), F1 males exhibited reduced courting behavior (e.g., less time following females, less sigmoid displays, etc). Sigmoid displays, a critical male behavior, were about one-fourth (P<0.001) [72]. This dramatic reduction suggested OBD.

Unsurprisingly, Russell [72] found OBD symptoms in the F2s and various backcrosses of the two populations. Average sperm counts (recovered from “stripped” males) declined from over 1.6 million in the F0s and F1s to 1 million in the F2s (P<0.05). In the first litter of females, brood size declined in the F0s and F1s from 8-11 fry/female to 4-6 fry in the F2s and various backcrosses (P<0.001).

Fig 21. Trinidad, an island off the northeastern tip of Venezuela, is a native habitat for wild guppies. Mountains separate the Caroni drainage on the western side of the island from the Oropuche drainage on the eastern side. Guppies from the two drainages differ genetically because they have been separated for a vast geological time. {Redrawn from Zandona [73]}
Russell [72] also examined the effect of various crosses on guppy survival. **Fig 22** shows that purebred offspring of the T and O parent populations had a median survival of 100%. Ditto for the ‘TXO’ F1. However, the reverse cross (‘OXT’), where the dam was the ‘O’ and ‘T’ the sire, showed some reduced survival. Moving down the graph, crosses between the F1s were fine. However, among backcrosses (graph’s 3 bottom bars), two showed symptoms of OBD, especially the ‘(TxO)XT’. Its median survival was 86%, and it was accompanied by a low IQR (52-100%).

Finally, Russell [72] autopsied females for dead embryos. Consistent with the lower survival of backcross offspring shown in the graph, there were more dead embryos in females producing backcross offspring than other groups.

Readers should note that the study documents population OBD in outcross descendants and that only a minority of offspring were affected. In all outcrosses, most juveniles in a brood did survive to maturity (i.e., all medians in the graph were above 86%).

There is a delicate balance between the hazards of OBD and the long-term benefits of outcrossing. Russell [72] crossed populations from drainages (Caroni and Oropuche) that were genetically divergent. Unsurprisingly, some of their descendants showed evidence of OBD (i.e., reduced survival).

**Nuances of Outcrossing**

In a more recent study, investigators [74] crossed strains within the same drainage (Caroni). It showed that outcrossing greatly benefited wild guppies. Seventy five pairs of HP guppies were introduced just upstream from each of two study sites containing LP guppy populations. The

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21 Interestingly, outcrosses with T as the sire were more problematic than T as the dam. The authors [72] cite Haldane’s Rule as a possible cause. OBD occurred in the T-sired offspring, because T males—like all males—have only one X chromosome. In contrast, T females have two X chromosomes, and a dominant normal X chromosome could compensate for a defective X chromosome.
small populations (~100 individuals) in each LP site were naturally inbred. The investigators monitored what happened after the introduced HP guppies immigrated into and intermingled with the LP guppies. Within two years, the LP and HP guppies had successfully interbred. Population size boomed 10 fold (from ~100 to ~1,000 guppies). Heterozygosity increased from 0.4 to >0.8. At one study site, immigrant guppies and their hybrid offspring (F1s, F2s, and backcrosses) had replaced the native LP population. At the other site, descendant guppies were a 1:1 mixture of native LP guppies and various hybrids, with F2s multiplying rapidly.

The above “introduction” study [74] was designed as a model for the genetic rescue of endangered species. It also provides an example of where the benefits of outcrossing outweigh OBD. The fact that the F2s and backcrosses were doing well, means that the improvement was not just due to F1 heterosis.

In a lab study, Monson [75] monitored how outcrossing affected reproduction in zebrafish (Fig 23). Goal was to maximize egg production from 4 inbred lab strains. For the study’s 480 total pairings, investigators isolated—for 60 min in a 1 liter mating chamber—a pair of young zebrafish.

Table 6 shows the results. OBD is evident in comparing egg-laying results from the ‘Inbred 2’ and ‘Outcross 2a’ matings. Females from the T strain mated to T strain males produced almost twice as many eggs per clutch (172 v. 91) as T females mated to hybrid males (‘L x A’).

Notably, hybrid females outshined hybrid males. Both ‘Mating Success’ and ‘#Eggs/Clutch’ were greater when the dam—not the sire—was the hybrid in outcross mating. For example, in comparing ‘Outcross 1a’ and ‘Outcross 1b’, the U x A females produced 120 eggs versus 101 eggs for A females mated to U x A males (P<0.05). In Expt. #2, the difference was even more dramatic. When females were the hybrid (‘Outcross 2b’), they produced 186 eggs, but in the reverse cross where males were the hybrid (‘Outcross 2a’), females produced only 91 eggs (P<0.01).

Interestingly, egg viability was virtually identical for all 8 mating types (data now shown); it was clutch size that differed.

<table>
<thead>
<tr>
<th>EXPT. GROUP</th>
<th>TYPE MATING</th>
<th>Dam X Sire</th>
<th>Mating Success</th>
<th># Eggs/Clutch</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>Inbred 1</td>
<td>A X A</td>
<td>54%</td>
<td>90 A</td>
</tr>
<tr>
<td></td>
<td>Outcross 1a</td>
<td>A X (U x A)</td>
<td>51%</td>
<td>101 A</td>
</tr>
<tr>
<td></td>
<td>Outcross 1b</td>
<td>(U x A) X A</td>
<td>69%</td>
<td>120 B</td>
</tr>
<tr>
<td></td>
<td>Hybrid 1</td>
<td>(U x A) X (U x A)</td>
<td>60%</td>
<td>126 B</td>
</tr>
<tr>
<td>#2</td>
<td>Inbred 2</td>
<td>T X T</td>
<td>23%</td>
<td>172 C</td>
</tr>
<tr>
<td></td>
<td>Outcross 2a</td>
<td>T X (L x A)</td>
<td>29%</td>
<td>91 D</td>
</tr>
<tr>
<td></td>
<td>Outcross 2b</td>
<td>(L x A) X T</td>
<td>35%</td>
<td>186 C</td>
</tr>
<tr>
<td></td>
<td>Hybrid 2</td>
<td>(L x A) X (L x A)</td>
<td>58%</td>
<td>195 C</td>
</tr>
</tbody>
</table>

Table 6. Strain Outcrossing and Fecundity in Zebrafish [75]

‘Mating Success’ is the percentage of mating opportunities that resulted in eggs. ‘#Eggs/Clutch is the group average. Identical bold letters mean the difference in #Eggs/Clutch was not significant, while differing letters mean the difference was significant (P<0.05). For example, there was no significant difference between ‘Inbred 1’ and ‘Outcross 1a’, but there was between ‘Inbred 1’ and ‘Outcross 1b’.

{I used data from Monson’s Table 2. For greater clarity, I abbreviated his strain names: ‘A’ = AB, U = Tu, ‘T’ = Tab 14, ‘L’ = TL.}
My Experience with OBD

I noted no problems in descendants from outcrossing male swordtails with BG females. The results were colorful males with major improvements in longevity. My only complaint was that only ~20% of the females had the high dorsal of the original BG strain. I attempted to rectify this by crossing my homebred guppies with an ATFG BG strain. This time I would be outcrossing to both sexes of the new strain.

The pure ATFG and their progeny were uniformly beautiful with most females having the desired high dorsal. In my opinion, the females were outstanding. However, outcrossing them to my homebred guppies produced problems that I had never seen before. I believe they were due to OBD.

One F1 batch sired by a homebred male contained progeny with abnormal “spiking” of fins (Fig 24). Also, several female offspring from this batch died unexpectedly and prematurely at 2-3 mos.

Another batch (Fig 25) with an ATFG dam showed abnormalities in the females. Males were normal, vigorous, and uniformly attractive. In contrast, many of the 16 females looked fine at 2 mos., but by 3-4 mos. they were small, weak and extremely divergent in morphology. Because the difference between the males and females was so dramatic, I suspected that the genes causing the female problems were sex-linked. OBD had disrupted genes responsible for normal female maturation.

One batch from a backcross [ATFG sire X F1 dam (ATFG X Homebred female)] contained males and females with sexual abnormalities. Some females contained no gravid spots and—upon autopsy—were found to contain no eggs; they were barren (Fig 26a). Half of the males had swollen bellies and exhibited homosexual behavior (Fig 26b).

OBD had affected sexual development, and the effect sometimes differed between males and females.

22 ATFG (All Thailand Fancy Guppies) exports a large variety of high-quality strains from Thailand. Before purchase, I informed the seller that I was particularly interested in a top-quality female with a high dorsal. I was not disappointed.
Male v. Female Longevity

By 2019, I had increased male longevity to over a year by crossing swordtail males with BG females. However, not one of my females had lived longer than 11 mos. Most died much sooner. Perhaps longevity in my guppies was sex-linked?

Nakajima’s 2002 paper [76] provided me with some insight into a possible sex linkage and also why OBD sometimes did not manifest itself in affected females until they were ~3 mos. old. The investigators were studying the genetic control of guppy growth. Using a “sib-analysis” of two old lab strains, they determined that different genes on the sex chromosomes controlled various growth stages of males and females.

For male progeny (Fig 27a), the dam’s genes affected body size at birth and then early growth. At 60 days of age, however, the sire’s genes began influencing male growth until about 90 days. At this time, a maternal gene from the dam inhibited further male growth.

In contrast, female development was dictated exclusively by maternal genes (Fig 27b) and occurred in two stages, one before reaching age 30 days and one after reaching age 90 days. Unlike males, females continued growing until at least 180 days.

Genes controlling adult male size were on the Y chromosome and highly heritable. Maternal genes controlling the two stages of female development were much less heritable. The number of DNA loci controlling adult body size was surprisingly small. They ranged from 1.7 or less for males and 3.5 to 8.0 for females [76].

The problems that I saw in my young females could have been due to OBD disruption of specific maternal genes controlling later development (>90 days age).

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23 Heritability ($h^2$) of male body size at sexually maturity for two lab strains was ~0.9 [77]. A study of wild guppies [78] also showed high heritability for male body size; heritability was less for females.
Other fitness-related genes have been linked to the guppy’s sex chromosomes (X and Y). For example, Fujio [79] showed that female guppies are more tolerant of cold than males and that this phenotype was inherited from genes on the X-chromosome. Similar results were found in an investigation of heat tolerance in guppies [80]; females tolerated heat better than males, and the genes for that enhanced tolerance were on the X-chromosome.

**Longevity Decreases with Captive Breeding**

The lifespan of wild guppies tends to shorten with time (i.e., increasing number of generations) in captivity. Some investigators suggest that environment and inbreeding cause these declines in fitness following captive breeding [67]. I would add “seasonal breeding” (i.e., a short and premature generational turnover) to the list.

Larsen’s 2010 study [42] introduced earlier (See Fig 14) showed a gradual shortening of survival of wild guppies after 10 generations in captivity. The control (non-inbred) population started out with 97% of guppies surviving to 12 mos. After 10 generations in captivity, survival declined to 87%. This small decrease is apparently not uncommon in scientific labs [81].

Larsen [42] started each of the 10 generations with progeny from a female’s first 1-3 broods. (If the female did not produce at least 5 fry in her first litter, she was given two more chances.) Thus, there was no selection for females that could produce more than 3 broods. A short generation cycle like this selects against longevity. Females that died after producing the requisite 5 fry in their first 1-3 broods were given the same opportunity to pass on their genes as superior females that—like some of their wild ancestors—were capable of surviving to produce 20-30 broods.

One German investigator [82] described 3 mos. as a “conservative” generation time. He estimated that his 50 year old strains had undergone 200 generations of captive breeding, meaning a 4 mos. generation time (200 ÷ 50 = 4). Unsurprisingly, he reported that many of his females died before they produced their third brood.

Australian investigators [83] worked with lab descendants of a feral strain captured in 2006. Many years later (in 2018), they commented on what must have been a huge loss of longevity. Only 55% of females (24 out of 44)—first mated at 6 mos.—produced a third brood. The majority of females either died or stopped reproducing at 9-10 mos. [83].

Japanese investigators [84] maintained their lab strains using a 6 month generational turnover. Despite keeping large populations sizes (>300 guppies) for each strain, they detected—via allozyme analysis—a 200-fold increase in genetic homozygosity after 16 years in two tested strains. Unsurprisingly, their old lab strains did not have much longevity; average survival to 4 mos. was only 72% and 67% for strains S3HR and F22, respectively [63].

Nature is not so biased against longevity. Female guppies like most fish—as opposed to birds and mammals—have indeterminate growth [85]. They keep growing many months after sexual maturity and their first litter. And bigger, older females produce larger litters [15]. In nature, robust older females are given a chance to contribute their numerous and often superior progeny to the population.

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24 Males and females from ten strains were tested for cold tolerance [survival after 24 hr at 12°C (53.6°F)]. Average survival of females ranged—depending on the strain—from 81% to 30%, while survival in males ranged from 58% to 21%. Females in all 10 strains survived longer than males. By crossing various survivors, Fujio proved that cold tolerance was inherited and on the X-chromosome [79].

25 Male guppies attain sexual maturity at < 2 mos. Females give birth to their first litter at < 3 mos.

26 The increased genetic homozygosity (calculated as a ~97% drop in Ne) was attributed [84] to “genetic drift,” which is a random increase in homozygosity that occurs in small, closed populations over multiple generations.
Selecting For Longevity in Drosophila

Drosophila investigator Rose [86] created a long-lived ‘O’ strain by gradually increasing the typical 14-day egg-collection time for one of his lab strains. Instead of collecting eggs from adult cultures at 14 days, he collected eggs at 28 days for 4 generations. Then, he collected eggs at 35 days for 2 generations. He continued this gradual, step-wise increase in the egg collection time. After 15 generations, the flies were living long enough that eggs could be collected at 70 days.

Using this procedure, Rose [86] obtained ‘O’ strain flies with greater longevity than the control strain (‘B’) where eggs were still collected every 14 days ($P<0.01$). Female longevity (average) was 33 days for the B strain and 43 days for the O strain; for males, it was 39 days for the B strain and 44 days for the O strain [86].

Since their initial selection in the 1980s [86], the O and B strains—and their replicates—have been maintained under the same 70 day v. 14 day egg-collection regimens. A decade later, the strains had gone through many additional generations (i.e., 90 for the ‘O’, 390 for the ‘B’); longevity was now 54 days for the O strain and 28 days for the B strain [87].

Other investigators found that creating long-lived fly strains was not so straightforward. Luckinbill [88] applied a selection procedure—similar to Rose’s—but on a different fly population. He gradually increased the time of egg collection from 14 days to 70 days.

Fig 28 shows that Luckinbill succeeded in increasing fly longevity. At “Generation 21,” the “Old” flies had a longevity of 58 days versus 41 days for the “Young” control flies.

However, there was a key variable—stress. The investigators [88] repeated longevity selection on cultures that they maintained at a low population density. These fly cultures were much less stressed as indicated by their lower mortalities and faster larval development times than high density populations. After 21 generations of selection, the uncrowded (i.e., unstressed) flies failed to show any substantial increase in longevity relative to controls. Average longevity varied randomly with no discernable pattern between generations [88].

---

27 Rose [86] made 5 replicates of the long-lived strain (O₁, O₂,...,O₅) and 5 replicates of the short-lived B strain (B₁, B₂,...,B₅). This precaution helped detect any confounding effects of genetic drift, inbreeding, etc.

28 Rose [86] maintained his Drosophila cultures under high density, stressful conditions during his longevity selection process. Stearns [89] confirmed that stress was required for longevity selection to work effectively.
Some investigators [9, 25] suggest that “longevity selection” may really mean correcting genetic problems brought on by the 14-day generation turnover practice used by so many Drosophila labs. Quite possibly, selecting for increased longevity in old lab strains succeeds only in regaining the potential ~80 day lifespan of wild flies, but nothing further.

Factors Correlated with Increased Longevity

Selection for longevity in Drosophila revealed hundreds of genetic changes [90, 91]. Thus, we would expect other traits to be expressed along with increased longevity. Table 7 summarizes the results of 30 selection studies from 12 different laboratories.

Labs agreed on a negative correlation between longevity and early fecundity (i.e., shorter-lived females reproduced earlier in life than longer-lived females). In the reverse selection, table results (‘0/-’) indicate that selecting for early fecundity had either no effect on longevity or that it decreased longevity.

Service [92] showed that there was a similar trade-off between longevity and reproduction in males. Males from the long-lived O strain were compared with males from the short-lived B strain in competitive mating trials. Two males, one male from each strain, were housed with a virgin female with only one male copulating. (Copulation by one male prevented copulation by the other male.) When mating males were one day old, those from the B strain averaged 71% of copulations; O males, only 29% ($P<0.001$). At 3 weeks of age, O males moved ahead, garnering 69% of copulations; B males, only 31% ($P<0.05$). Supplemental paternity experiments found the same pattern of longer-lived males having greater fertility later in life; shorter-lived males having greater fertility early in life.

Indeed, most scientists agree that there is a trade-off between early reproduction and longevity (i.e., a long lifespan means less early fecundity). It supports a widely accepted theory (‘antagonistic pleiotropy’) for the evolution of aging. Individuals that invest more in early reproduction do so at the expense of their body maintenance, resulting in a shorter lifespan.

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Table 7. Summary of Drosophila Selection Studies [9]

<table>
<thead>
<tr>
<th>Selected Trait</th>
<th>Correlated Response to Selection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Longevity</td>
</tr>
<tr>
<td>Longevity</td>
<td>0/-</td>
</tr>
<tr>
<td>Adult Size</td>
<td>+/-</td>
</tr>
<tr>
<td>Early Fecundity</td>
<td>0/-</td>
</tr>
<tr>
<td>Late Fecundity</td>
<td>+/-</td>
</tr>
<tr>
<td>Starvation Resistance</td>
<td>0/+</td>
</tr>
</tbody>
</table>

Table 7. Summary of Drosophila Selection Studies [9]

Signs 0, + and - indicate the direction of the correlated response. ‘0’ = no correlation, ‘+’ = positive correlation, ‘-’ = negative correlation. Mixed signs (e.g., 0/-, 0/+, or +/-) represent inconsistent and/or opposing results across different laboratories. Blank boxes mean no reported results.

29 Antagonistic pleiotropy (AP) results from genes that simultaneously control both beneficial traits and detrimental traits. In terms of longevity, AP genes exert beneficial effects in early developmental stages, but bad effects later in life leading to early senescence. All sexually reproducing animals senesce (gradually deteriorate with age) and have finite lifespans. The AP theory of Williams [93] explains why senescence, a deleterious condition, is not selected out during evolution.
Reproduction aside, one would expect that individuals capable of living longer would be more fit overall. Indeed, that seems to be the case. Many studies with Drosophila show a positive relationship between longevity and fitness.

Rose [86] showed that longer living strains had greater survival at all ages. He calculated age-specific mortality rates for flies at 10 day intervals from age 1-50 days. The longer living O strain showed less mortality for all 5 age groups than the shorter living B strain ($P<0.05$).

A later study [94] showed that O strain flies survived longer than B strain flies when starved. Males from the O strains survived for 28 hr, while those from the apparently B strain survived only 22 hr ($P<0.01$). Females from the O strain survived 61 hr while those from the B strain survived only 47 hr ($P<0.02$).

Mating reduces survival.\(^{30}\) Luckinbill [95] showed that mating decreased survival of short-lived strains, but not long-lived strains. He suggested that longer living flies could tolerate reproduction’s deleterious effects better than shorter living flies.

Luckinbill [95] compared his SL (lifespan ~50 days) and LL (lifespan ~80 days) strains for physiological fitness by quantitating their flying ability. For measuring flying fitness, investigators glued a small piece of fishing line to the fly’s thorax. They then put the tethered fly into a wind tunnel in which the fly flew until reaching a point of exhaustion. It was a “stress test” for physiological function and endurance. For females, average flight duration was 37 and 164 min for SL and LL strains, respectively ($P<0.0001$); for males, it was 36 and 117 min ($P<0.0001$). Flies of the LL strain were dramatically more fit than those of the SL strain. Moreover, hybrids of the LL and SL strains had flight ability that was intermediate between the parent strains suggesting that this longevity-associated trait was also an inherited trait.

Flying ability requires good heart function, and indeed, a more recent study [96], showed that the long-living O strain had much greater heart function than the short-living B strain. (The O and B strains used were descendants of Rose’s original 1980’s strains [86].) Test consisted of subjecting flies to a 30 second electrical shock and then monitoring them 2 min afterwards for heart failure (fibrillation or cardiac arrest). Female flies of the O strain averaged ~23% heart failure, while the B strain averaged ~40%. Differences between O and B strains (both males and females) was consistent and highly significant ($P<10^{-6}$).

In summary, Drosophila studies show a positive correlation between fitness and longevity. Flies selected for greater longevity had greater survival at all ages. They were more resistant to mating stress, had stronger hearts, had more endurance, and were more resistant to starvation. Studies showed a negative correlation between early reproduction and longevity. Flies with a short lifespan had earlier fecundity (i.e., matured earlier and reproduced sooner) than flies selected for longevity.

As for guppies, there are no comparable experimental studies where investigators took a large guppy population and bred half (with 5 replicates) for a long lifespan and the other half (with 5 replicates) for a short-lifespan and then later compared traits between replicates of the two strains under identical environmental conditions.

Longevity in each guppy breeder’s setup is subject to multiple environmental factors (feeding levels, water quality, temperature, stocking densities, potential pathogens, etc). Thus, I would urge caution in correlating traits (e.g., early reproduction, growth rate, etc) with the longevity of ones own guppies.

Theoretically, rapid growth and early sexual development is associated with a shorter lifespan. However, slow growth and delayed sexual development could just as well reflect individuals “stunted” by reduced physiological fitness. At this time, I favor large, fast-growing males and females and later select from them the longer-living individuals as breeders.

\(^{30}\) I discussed this in an earlier section ‘Effect of Reproduction on Longevity’.
My Results from Breeding Older Fish

Older males are valuable as sires simply because they have proven their ability to survive. Ditto for females….Moreover, older females produce much larger broods. Female guppies keep growing after 6 mos. and litter size will increase accordingly. A female’s first brood might number less than 10 fry, but a large older female (7-10 mos.) can produce 50 -80. That gives the guppy breeder a larger number of progeny to choose from.

Using a longer generation time instead of the typical 4-6 mos. slows the accumulation of deleterious mutations. Many genetic problems only become apparent with increased age. For example, two male siblings with what I call the “Christmas” phenotype looked fine at 6 mos. (Fig 29). At 8 mos., however, one male lost his fins within a matter of days. The other male kept his fins just fine. By simply waiting a couple months before using them for breeding, I was able to eliminate a potential genetic problem.31

Every population has genetic outliers (Fig 30), the ones that beat the averages for survival. They are the ones that carry genes for longevity, disease resistance, and superior physiology. They are the ones I keep progeny from to start the next generation.

At this time (March 2021), my older females do seem less fertile. They are not producing litters every 25-30 days. Litters are large but produced sporadically. This may be due to ID, OBD, or the known trade-off between longevity and reproduction. Or it may be just the fact that I am now working with older females. Should reduced fecundity be the price for greater longevity, that’s fine with me. (The females already produce more than enough babies for my purposes.)

31 This sudden fin loss may have been a function of swordtail genetics where genes “signal” which cells to grow and which cells to die (apoptosis). Apoptosis is intrinsic to the sculpturing process to form the desired swordtail shape (lower sword, double sword, etc.) [97].
Selecting Guppies for Longevity

Granted, breeding older guppies for longevity is slow and tedious. It means keeping records of birth and death dates of potential breeders. It slows down the entire production process, for one must wait to see which female and male lives the longest. Because males and females must be reproducing for them to qualify, it means discarding lots of unwanted offspring.

What I do is select ~6-10 big, attractive females from a mixed sex litter at around 4 mos. of age. If at 7-8 mos. some females start looking weaker than others, I may cull the group down to the best 2 or 3 individuals.

When the select females are 6-7 mos. old, I start keeping them with my best males, because sometimes it takes 1-2 mos. for new males to gain paternity. Around 8 mos., I start saving litters. The process is not always predictable, so there are no hard and fast rules. My present goal (2021) is to have breeding stock that routinely lives over 12 mos.

The main thing is just setting a threshold for generation time. Currently, mine is 8 mos. Breeders must live at least 8 mos. before I will keep their progeny. I hope that I can gradually extend the threshold, just as the Drosophila breeders did in selecting for longevity in their fly cultures.

In 2020, I had two good females that made the cut. One died at 11.5 mos., two weeks short of her first birthday (Fig 31). When she made my tentative 8-month survival threshold, I kept her progeny. After her death, I was down to one single female who—even as a young female—was noticeably bigger and more vigorous than her sisters. When she was 7 mos., I started saving her progeny in hopes she would make my 8 month threshold. She did and then some (Fig 32). She represented the 3rd generation of females that I had selected for longevity.

Fortunately, I managed to get three big litters from her, all sired by different males. Because each litter was sired by a different male, the offspring were not full siblings. They were half-siblings. I could mate males from one litter with females from another litter to concentrate genes from their superior dam and yet avoid severe inbreeding.

This one female was a genetic treasure, the relatively long-lived female that—in my particular setup—beat the averages. Although breeding for longevity is a time-consuming process, I believe it is worth the trouble.

I timed matings with a new male to include the female’s only fertile period (0-4 days post-parturition). I arbitrarily gauged paternity success in these planned matings by the fact that all the male progeny had the color phenotype of my chosen sire(s). Males were from my homebred stock, but all had different and distinguishing phenotypes: Red Grass, Japan Blue, and Christmas.

Fig 31. Female that lived to 11.5 mos. is photographed here at 10 mos. She shows the lengthwise “droop” due to muscle degeneration associated with aging.

Fig 32. One Year Plus Survival
This big beauty is my first female to survive past one year. At 15 mos., she showed signs of senescence and became incapacitated. She reached the impressive size of 4.5 cm (distance from tip of snout to base of the caudal fin, which fish scientists call the “Standard Length.”).
Young males from a batch may all look beautiful and promising as breeders (Fig 33). Only time will tell which ones have longevity. Correlations between longevity and other traits have been shown to be tenuous and unpredictable for both Drosophila and guppies. I believe that the surest method is simply to select for longevity directly. That is, save progeny from older guppies.

Rather than discard high-quality older males with large delta tails, I may trim their tails just to improve their chances of inseminating females. However, I am not sure that trimming is necessary when a healthy male (Fig 34) is kept long enough (1-2 mos.) with receptive older females.

One may get rapid improvement by tackling ‘low-hanging fruit’, that is, selecting out a major genetic problem from the population. I believe that is what happened with my male BG guppies. I am almost sure that the purebred males of 2017 and 2018 died young, because they got easily constipated with the generous feeding I provide. By outcrossing my original BG strain to swordtail guppies and then using only long-living males for reproduction, I was able to “unblock” this genetic defect.

In other instances, changes may not be so rapid and one must patiently settle for a gradual increase by selecting only longer-lived individuals over several generations.

And breeding stock should be selected from reproducing guppies. Virgin guppies have not been stressed by mating and reproduction, so naturally they will live longer. A female or male guppy that lives to 24 mos. as a virgin is meaningless in terms of longevity. If mated, the fish might have died at 5 mos.

Selecting older adults for breeding may not always produce the desired result—a genetic increase in longevity. In the typical captive environment where guppies are well-fed and

Fig 33. Choosing Males  Young males from an outcross batch born in the Fall of 2019. A few survived over one year. Of the 6 blue males in this batch, only one (Fig 34) survived to 15 mos. The rest died long beforehand.

Fig 34. 2021 Star Breeder  This old male with the BG phenotype was born in the Fall of 2019. Photo shows him at 15 mos. of age. I have been using him as a breeder for the past few months and hope to get some precious babies from him.
protected from predators and disease, there may be little selection for fit, longer-living individuals. Moreover, most domestic guppies with their entrenched strain uniformity are highly inbred and isogenic. Selecting individuals from within a population that has little genetic variation may not work very well.

In keeping older stock until they run out the clock, one caution. With senescence, older fish lose their immunity. As they age, they become increasingly vulnerable to endemic pathogens and can develop contagious diseases. If an older fish—or any fish for that matter—becomes a disease reservoir, it will eventually release huge numbers of pathogens that could endanger tankmates. Thus, I euthanize older fish when they show advanced disease symptoms or become severely incapacitated.

Readers should understand that my experience with guppy longevity to date (2021) is limited. I can only draw on scientific studies and my own recent experience with the Blue Grass strain. However, I must say that I am satisfied with the improved longevity of my guppies. Less disease, less death, longer lives….

**Recommendations for Increasing Longevity**

For those who want to improve—or just maintain—longevity in their guppies, here are my recommendations.

❖ Save progeny from older breeders. They have proved their superiority.
❖ Consider outcrossing to strains that are recognized for greater longevity. While some outcrosses might generate problems (i.e., outbreeding depression), they will also produce superior individuals that can tremendously improve the brood stock.
❖ Avoid mating full-siblings. Instead, mate less related guppies (cousins, half-siblings, etc).
❖ Consider line breeding, test-breeding and other strategies used by successful, large-scale guppy breeders [66].
❖ Let breeder females have a choice of males to give the most vigorous males paternity.
❖ Select from reproducing guppies, not virgins.33
❖ Practice rigorous culling for deformities, small size, weakness, etc.
❖ Be judicious in protecting and treating guppies for disease. Concentrate more on breeding disease-resistant fish. Some disease outbreaks (e.g., flukes and Costia) can be used to cull individuals lacking genes for normal immunity.
❖ Avoid breeding for rigid strain uniformity. Instead, celebrate the fantastic color polymorphism unique to the species *Poecilia reticulata*.

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33 With longevity selection (i.e., saving progeny from older fish), there is plenty of time to pair non-virgin females with chosen males and get progeny from planned matings. (*My next article will focus on guppy reproduction.*)
4. Discussion (October 2018) about longevity on the Guppy Gene Collectors forum
   [https://www.facebook.com/groups/GuppyGeneCollectors/](https://www.facebook.com/groups/GuppyGeneCollectors/)
58. Allendorf [51], p. 419.
65. Allendorf [51], p. 360.
66. Bias, Alan S. 2011. Breeding strategies & genetic consequences in guppies. (The 11 page PDF can be freely downloaded from: https://independent.academia.edu/AlanBias)
68. Allendorf [51], p. 368 citing paper by C.C. Muheled (2009).
70. Allendorf [51], p. 374.
75. Monson CA and KC Sadler. 2010. Inbreeding depression and outbreeding depression are evident in wild-type zebrafish lines. *ZEBRAFISH* 7: 189-197.
81. Personal communication (2020) with C. Pélabon
97. Discussion about male fin shredding (March 2020) on the Guppy Gene Collectors forum https://www.facebook.com/groups/GuppyGeneCollectors/

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