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Outcrossing: The Rare Male Advantage

(by Diana Walstad, November 2023)

Outcrossing guppies is fun. I find it more interesting than breeding fish species where all progeny look like their parents. For when you outcross guppies, you never know what you will get (**Fig 1**).

The 'Rare Male Advantage' (RMA) increases reproductive success when outcrossing guppies. In general, female guppies control the mating game [1]. And their enhanced willingness to mate with males sporting new and unfamiliar color patterns has been shown repeatedly since 1977.

Female guppies can use 'old' sperm stored from prior matings to fertilize future broods for several months. Even though the last male she mates with gets siring priority, often he may not sire 100% of the female's next brood. For she still could produce broods sired by earlier males, especially if she is in 'mid-cycle' where she is carrying embryos (representing eggs that have already been fertilized). That said, RMA dramatically increases the odds that the female's offspring will be fertilized by the new outcross male.

In 1977, Farr [2] documented RMA in lab guppies. He housed 10 virgin females together with 10 males in 7 different tanks. Nine males had the same color pattern while one male (the 'rare' one), had a different color pattern.

Farr [2] used male color pattern to determine the paternity of the resulting offspring. Rare males sired 43% of 21 broods, even though they represented only 10% of available males. They were significantly better in acquiring females ($P < 0.001$). [1]

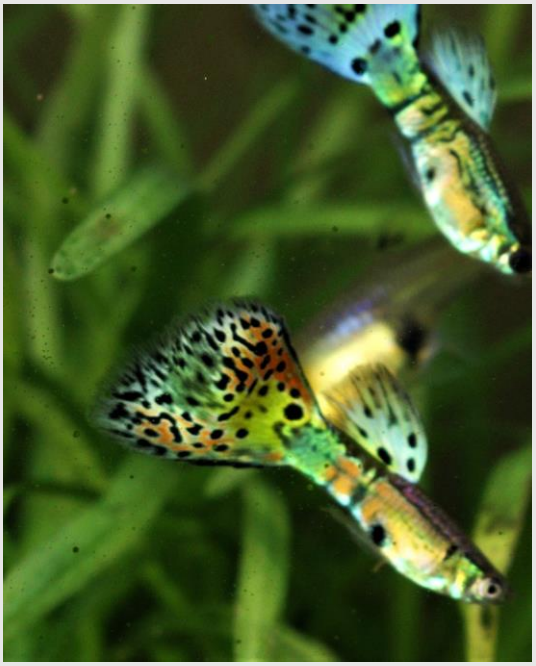
Hughes' 1999 study [3] used "experienced" females instead of virgins. This complicates reproductive studies, because non-virgins have stored sperm that will compete with fresh sperm for fertilizing eggs. But it is more realistic since most female guppies are non-virgins. Moreover, experienced females are more choosy in selecting mates than virgins [4].

Hughes [3] started with a virginity-elimination step whereby virgin females were mated for one week to

Fig 1. Swordtail X Blue Grass Outcross

Lower Swordtail Male bred by Alan S. Bias contained a boat-load of color genes masked here by a recessive allele for this male's solid blue body color.

Blue Grass female shown here with a male of the same strain



Male Progeny (F1s) showing expression of 'buried' color genes from their sire.

[1] *P*-values quantitate statistical probability. $P < 0.001$ in Farr's study [2] means that the probability that the observed skewed siring results (from male rarity) are merely random are less than 0.001 (i.e., 0.1%). [*P*-values equal to or greater than 0.05 ($P \geq 0.05$), are generally considered non-significant.] Thus, Farr's results are highly significant.

males of the M1 strain. Then, each pregnant female was transferred to a 10-gal tank where she could see 10 males. (Tanks had a clear glass divider separating the 10 males from the female.) Half the females (16 'Treatment' females) were exposed/familiarized to M7 strain males, whereas 15 'Control' females were exposed/familiarized to non-M7 males.

One day after giving birth, each female was housed with an M7 and a GP male for 24 hr. where she could mate with either (or both) males. Afterwards, the female's resulting first two broods were collected and their paternity determined via male color. (The M1, M7, and GP strains involved all had their own inheritable and characteristic male coloration.)

The **Table** compares male paternity as a function of the female's previous familiarization experience. None of the treatment females—familiarized with M7 males beforehand—produced pure broods sired by M7 males. In contrast, 5 control females—familiarized with other, *non*-M7 males beforehand—produced broods sired solely by M7 males. Other females (10 treatment and 10 control females) produced mixed broods, sired by both M7 and GP males. Thus, RMA was only revealed by the 11 females (5 + 6 = 11) that mated with a single male.

Table. Summary of Familiarization Effect [3]

Table shows the number of females that produced male offspring sired by M7 and GP males. 'Treatment" females (*n*=16) were those familiarized beforehand with M7 males; 'Control' females (*n*=15), were familiarized with other (i.e., non-M7) males.

SIRES:	Male Mating Success:	
	Treatment Females	Control Females
M7	0	5
GP	6	0
M7 + GP	10	10

Fig 2 from the same study [3] differs from the table in that it includes *all* male progeny from the 31 females. For females did produce progeny via stored sperm from the M1 males used in the virginity elimination step. Both 'Control' and 'Treatment' females produced slightly different numbers of M1 offspring (Column 1 versus Column 2), but the difference was not significant (*P* = 0.30). Thus, the prior M1 mating did not substantially alter the main RMA results. [2]

Columns 3 and 4 (Fig 2) show that control females (unfamiliar with M7 males) produced more M7-sired offspring than treatment females (familiarized to M7 males). Instead, treatment females produced more offspring sired by unfamiliar GP males (Column 6) than control females (Column 5).

The strong showing for M7 males in Column 3 suggests that the study's females were not

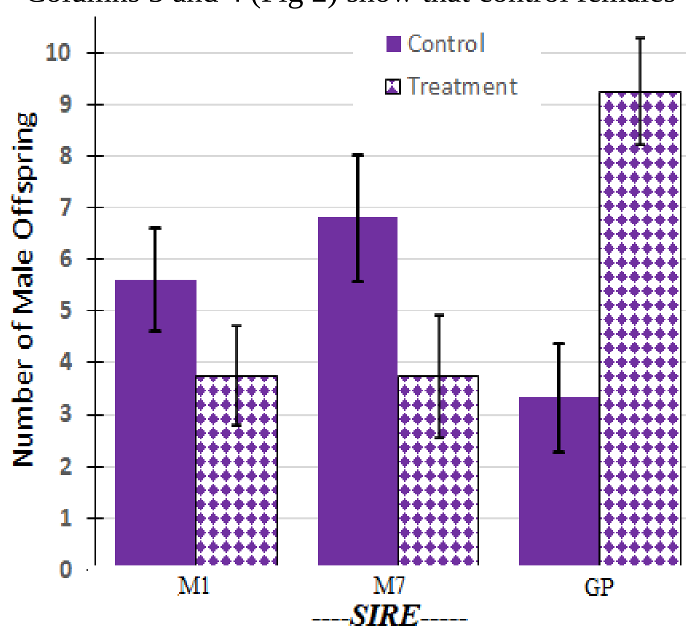


Fig 2. Paternity of Males in Study [3]

Columns show the average number (±S.E.) of male offspring sired by the M1, M7, and GP males. Females from 'Treatment' group were familiarized to M7 males before mating; Control females were familiarized to other, *non*-M7 males. M1-sired progeny resulted from the study's 'Virginity Elimination' step.

{I drew graph from data in Hughes' Table 1.}

[2] A full 29% of all progeny were sired by stored sperm from M1 males, while 71% were sired by fresh sperm from M7 and GP males. I view M1-sired progeny as 'background noise' to the main comparison (effect of M7 familiarization).

innately biased against M7 males. (Indeed, M7 males were apparently attractive to these females.) The females were only biased against M7 when they were familiar!

In summary, the overall difference between treatment and control groups was highly significant (*P*<0.001). Hughes' 1999 classic study [3] provides strong support for RMA.

However, to make sure that RMA was not just a laboratory artifact, investigators [5] later conducted a field study of wild guppies. They captured all guppies within 17 small natural pools from 3 different river sites in Trinidad, the native habitat of guppies. Captured males were sorted into two groups: 'drab' (>75% of tail was transparent) or 'colorful' (50% of tail was colored). Both males and females were individually tagged with a small paint injection for later identification.

Males were returned to the natural pools in differing ratios of drab versus colorful. In half the pools, the ratio of drab: colorful males was 1:3, making drab males rarer; in the other half of pools, the ratio was reversed to 3:1 making colorful males rarer. Females were returned to their home pools containing these artificially constructed male groups.

Sixteen days later—after many of the females would have mated—, the females were recaptured from the pools and isolated to give birth. Investigators genotyped the adults plus 693 fry in order to determine paternity.

Results (**Fig 3**) were dramatic. Rarer males mated with at least two times more females and sired at least two times more offspring than common males (i.e., the more numerous male phenotype in the pools). Whether a male was drab or colorful, large or small, etc. made no statistical difference in his reproductive success. The only factor that apparently mattered to the females was that he was less common (i.e., "rarer") (*P* = 0.003).

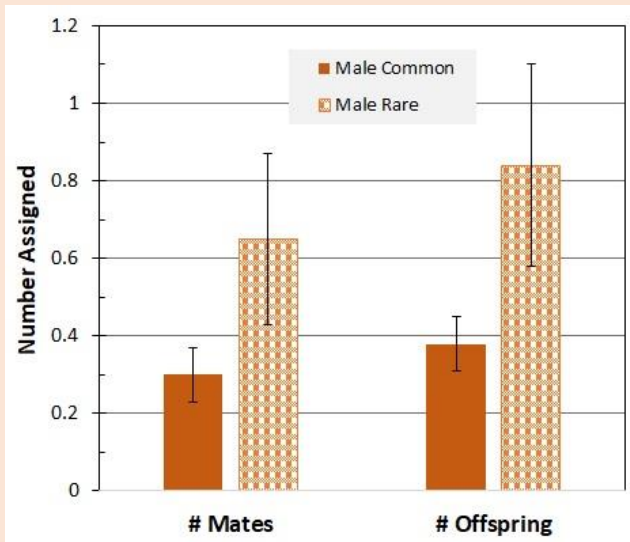


Fig 3. Rare Males have Greater Mating Success [5] Solid brown columns show # of female mates and # of offspring that the investigators assigned to 'common males', while patterned columns show assignments to 'rare males'. Vertical bar on each column shows variation (S.E.) within the group. Differences due to the rareness of males were statistically significant (i.e., *P*<0.005 for # of female mates and *P*=0.01 for # of offspring.)
{Adapted from Hughes (2013)}

Habituation could explain the RMA phenomenon [6]. Habituation is an adaptive mechanism widespread among animals. It allows organisms to filter out repetitive sensory 'noise' in their environment so that they can focus on novel stimuli that may be more biologically relevant. Females habituate to males with familiar color patterns. A 'rare' male is a new stimulus to which the female is not habituated. Male color also serves as a convenient cue by which females can easily identify a new genotype. By mating with rare and novel males, she lessens inbreeding and increases the genetic variability of her progeny.

The female guppy's preference for new color patterns (i.e., RMA) is also believed to contribute to the guppy's unique color polymorphism (the seemingly infinite variety of male color patterns).

RMA has widespread implications and is of keen interest to scientists currently studying evolution [7]. For it seems that not all evolution is driven by standard, textbook-described processes—"survival of the fittest," mutations, and genetic drift. Apparently, female mating preferences like RMA can change the course of evolution, affect speciation, and increase genetic heterozygosity.

For guppy breeders outcrossing strains, I believe RMA increases the siring success of the males involved. In my own experience, RMA may have helped to rescue the genes of one gorgeous but very fragile male. The male died within a week of purchase but still managed to impregnate one of my homebred females.

Other outcrosses have been surprisingly successful (**Fig 4**). These matings seem to work better than planned matings with familiar males. I had never thought of female acceptance as a factor in mating success. (For I had attributed a male's mating success solely to his vigor and competitive ability.) Nor had I heard of the Rare Male Advantage. However, the ease with which outcrosses have repeatedly worked for me has made me rethink this aspect of guppy breeding.

Fig 4. Swordtail X Blue Grass Outcross

Lyretail Males, newly purchased from a pet store, are shown courting a Blue Grass female. She produced a brood 21 days later. About half of her ~50 offspring were sired by these novel (to her) males. (The two males carry the Japan Blue gene for blue body color.)

Beautiful Male (F1) from the outcross inherited the enhanced blue body color from his sire and the elaborate finnage from his dam.

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