

Supplemental Figure Legends

Supplemental Figure 1: Progeny of a dihybrid cross between a non-striped, yellow female GloFish and a wildtype male zebrafish. Progeny were photographed on a bright green surface to emphasize the contrast between the yellow and grey progeny. Note the rounded, fat ventral side of the females and the straighter, sleeker ventral side of the males. Data and chi-square analysis for this cross are found in Table 2 and Supplemental Table 2. Lateral views, anterior to the left and dorsal to the top. Key: Yellow, striped: 1, 2, 3, 5, 8, 9, 10, 11, 14; Grey, striped 4, 6, 7, 12, 13; Male 1, 2, 3, 4, 7, 9, 10, 14; Female 5, 6, 8, 11, 12, 13.

Supplemental Figure 2: Progeny of a dihybrid cross between two parents heterozygous for *Glo^{YFP}* and *gol*. Data and chi-square analysis for this cross are found in Table 4. Lateral views, anterior to the left and dorsal to the top. Key: Grey, striped: 11, 13, 25, 27, 29, 32; Grey, non-striped: 4, 6, 17, 22; Yellow, striped: 1, 5, 9, 10, 14, 15, 19, 21, 23, 26, 30, 31, 33, 34; Yellow, non-striped: 2, 3, 7, 8, 12, 16, 18, 20, 24, 28.

Supplemental Figure 3: Progeny of a trihybrid cross between a yellow GloFish and red GloFish, both heterozygous for *gol*. Data and chi-square analysis for this cross are found in Table 5. Lateral views, anterior to the left and dorsal to the top. Key: Red, striped: 9, 10, 15, 18, 25, 29, 32; Red, non-striped: 1, 7, 34; Yellow, striped: 6, 13, 19, 21, 24, 28; Yellow, non-striped: 2, 30; Grey, striped: 3, 8, 11, 14, 31, 33, 36; Grey, non-striped: 16, 20, 23, 26; Orange, striped: 5, 12, 17, 27, 35, 37; Orange, non-striped: 4, 22.

Supplemental Figure 4: Progeny of cross between fish heterozygous for the *Glo transgene* and *lof* mutation. Data and chi-square analysis for this cross is included in Table 6. Lateral views, anterior to the left and dorsal to the top. Key: Orange, short fins: 4, 15; Orange, long fins: 1, 8, 10, 13; Grey, short fins: 2, 6, 11, 16; Yellow, short fins: 9; Yellow, long fins: 3; Red, short fins: 5, 12; Red, long fins: 7, 14.

Supplemental Figure 5: Progeny of cross between two *cyc* heterozygotes. Embryos at approximately 3 dpf were purposely not positioned in a specific orientation to give students the experience of looking at fish through a microscope. Since zebrafish embryos typically hatch at 3 dpf, some are inside their chorion, while others are not. Data and chi-square analysis for this cross is included in Table 7. Key: two eyes: 2, 4, 6, 7, 8, 9, 11, 12, 13, 14, 20, 21, 22, 23, 24, 25, 26, 27, 28, 30, 31, 32, 33, 34, 37, 39, 40, 41, 42, 44, 45, 46, 47, 48, 50, 51, 52, 53, 55, 56, 57, 58, 59, 60, 62, 63, 64, 65, 67, 68, 69, 70, 71, 73, 76, 77, 80, 81, 82, 83, 84, 85, 87, 88, 89, 90, 91, 92, 93, 94, 96, 97, 98, 99, 100, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117; cyclopic eye: 1, 3, 5, 10, 15, 16, 17, 18, 19, 29, 35, 36, 38, 43, 49, 54, 61, 66, 72, 74, 75, 78, 79, 86, 95, 101.

Supplemental Figure 6: Progeny of cross between two *sqt* heterozygotes. Larva at approximately 5 dpf were aligned in an orientation that best exposed the eye(s), typically in a ventral or rotated ventral view with anterior to the left. Data and chi-square analysis for this cross is included in Table 9. Key: two eyes: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 23, 24, 25, 26, 27, 28; cyclopic eye: 12, 22.

Supplemental Figure 7: Cross between two WT fish to determine inheritance of gender. The parental (P_0) generation consisted of a female and male WT fish. The F_1 generation was produced through a single mating of the P_0 pair, with the progeny raised under standard conditions. Males and females were identified through their dimorphic morphological characteristics (<http://www.zfic.org/common%20techniques/Gender%20identification%20guide.pdf>), Images are lateral views, anterior to the left and dorsal to the top. Images of all progeny from this cross are included in Supplemental Figure 9.

Supplemental Figure 8: Hypothesis for gender inheritance pattern. The hypothesis for the inheritance pattern presented as a Punnett square.

Supplemental Figure 9: Progeny of cross between two wildtype fish. Data and chi-square analysis for this cross are included in Supplemental Table 1. Females were identified by their rounded belly and whiter color and males were identified by their flatter belly and yellowish tinge. Lateral views, anterior to the left and dorsal to the top. Key: Female: 1; Male: 2- 16.