S2 TABLE – Expanded Breedings and Results

The Cellular Expression and Genetics of an Established Polymorphism in *Poecilia reticulata*; "Purple Body, (*Pb*)" is an Autosomal Dominant Gene

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1. Breeding: **C** *(hetero. or homo. Pb Bias Ginga Sulphureus / P) x* **A** *(homo. Pb Roebuck Purple Delta / P)*

Sire & Dam

Results: Litter ***471*** F1 [DOB 4.10.14] - 8 males, all Pb. 10 females, all Pb.

Offspring

S2 Fig 1. Male posterior peduncle spots are masked by Zebrinus (*Ze,* Winge 1927). However, posterior peduncle iridophore base is modified light wild-type purple coloration. Pb males express a visible "reflective purple crescent" at caudal base, used to verify presence or not of Purple Body gene. Caudal is variegated purple bi-color. Female caudal's are purple. These results do not distinguish between Y-linked, X-linked or autosomal modes of inheritance.

2. Breeding: **C** *(hetero. or homo. Pb Bias Ginga Sulphureus / P) x* **CA** *(F1 hetero. or homo. Pb / 471)*

Sire & Dam (no photo available)

Results: Litter ***524*** TC1 [DOB 7.28.14] – 15 males, all Pb. Females not counted.

Offspring

S2 Fig 2. Male posterior peduncle spots visible in a portion of males and are modified pinkish-purple. Peduncle spots in a portion of males are masked by Zebrinus (*Ze*). However, posterior peduncle iridophore base in all males is modified light wild-type purple coloration. Caudal is purple bi-color. Female caudal's are purple. These results do not distinguish between Y-linked, X-linked or autosomal modes of inheritance.

3. Breeding: **CA** (*F1 hetero. or homo. Pb / 471*) *x* **CA** (*F1 hetero. or homo. Pb / 471*)

Sire & Dam (no photo available)

Results: Litter ***519*** F2 [DOB 9.14.14] – 7 males, all Pb. Females not counted.

Offspring

S2 Fig 3. Both posterior peduncle spots and anterior shoulder spots in males modified pinkish-purple. Peduncle iridophore base in all males is modified light wild-type purple coloration. Caudal is purple bi-color. These results do not distinguish between Y-linked, X-linked or autosomal modes of inheritance.

4. Breeding: **C** *(hetero. or homo. Pb Bias Ginga Sulphureus / P) x* **A** *(homo. Pb Roebuck Purple Delta / P)*

Sire & Dam

Results: Litter ***475*** F1 [DOB 6.12.14] - 10 males, all Pb. 15 females, all Pb.

Offspring

S2 Fig 4. Male posterior peduncle spots are masked by Zebrinus (*Ze*). However, posterior peduncle iridophore base is modified light wild-type purple coloration. Pb males express a visible "reflective purple crescent" at caudal base, used to verify presence or absence of Purple Body gene. Caudal is variegated purple bi-color. Female caudal's are purple. These results do not distinguish between Y-linked, X-linked or autosomal modes of inheritance.

5. Breeding: **D** (*homo. non-Pb Bias Panda Moscow / P*) *x* **A** *(homo. Pb Roebuck Purple Delta / P)*

Sire & Dam

Results: Litter ***496*** F1 [DOB 6.1.14] - 15 males, all Pb. 10 females, all Pb.

Offspring

S2 Fig 5. Male posterior peduncle spots are masked by Zebrinus (*Ze*). However, posterior peduncle iridophore base is modified light wild-type purple coloration. Shoulder pattern is Y-link dark Moscow (*Mw,* Kempkes 2007). Pb males express a visible "reflective purple crescent" at caudal base, used to verify presence or absence of Purple Body gene. Female caudals are purple. All Pb males and all Pb females indicate either an X-linked or autosomal dominant mode of inheritance.

6. Breeding: **D** *(homo. non-Pb Bias Panda Moscow / P) x* **DA** *(F1 hetero. Pb / 496)*


Sire & Dam

Results: Offspring: ***546*** TC1 Litter [DOB 11.6.14] – 19 males in litter; 12 Pb and 7 non-Pb (green). Females not counted.



Offspring

S2 Fig 6. Males recombined into expected phenotypes for body and finnage. 10 Moscow Snakeskin delta; 6 Pb and 4 non-Pb (green). Verified by Peduncle iridophore base modification to light wild-type purple coloration. 9 Panda Moscow roundtail; 6 Pb and 3 non-Pb (green). Verified by Peduncle and caudal modification to wild-type purple coloration. Results indicate an X-linked or autosomal dominant mode of inheritance.

7. Breeding: **D / DA** (*TC1 hetero. Pb Purple Panda / 546) x* **D / DA** *(TC1 hetero. Pb Purple Panda / 546)*

Sire & Dam

Results: Litter ***614*** F2 [DOB 5.14.15] – 10 males in litter: 5 Pb and 5 non-Pb (green). 17 females in litter: 11 Pb, 3 non-Pb (color neutral and presumed green), 3 lost with no color identification.

Offspring

S2 Fig 7. Males express much green coloration in the peduncle and spotting is fully masked by snakeskin pattern and later solid colored peduncle at maturity. Expression of green color in the peduncle can be expected in males of recent green delta ancestry. Pb and non-Pb males and Pb and especially the non-Pb females indicate an autosomal dominant mode of inheritance.

8. Breeding: **D / DA** (*TC1 hetero. Pb Purple Panda / 546) x* **B** (*homo non-Pb Shubel Green Delta / P*)


Sire & Dam (representative female)

Results: Litter ***618*** TC2 **[**DOB 5.24.15] – 17 males in litter: Pb detected in high percentage of males. Non-Pb male status inconclusive. 15 females in litter; 6 Pb, 8 non-Pb, 1 color & tail neutral with no color identification.



Offspring

S2 Fig 8. The combined genotype involved in this breeding resulted in a complex phenotype making accurate visual assessment of males for Pb or non-Pb status difficult. Female assessment was less complex. Pb females express purple & black bi-color caudal with "reflective purple crescent." Non-Pb females express green & black bi-color caudal. Color & tail neutral female expressed clear caudal with no pigmentation. Female count appears accurate.

Though male visual assessment of male coloration not conclusive, a high degree, at least 50%, express Pb characteristics. All males express green coloration in the peduncle over white leucophores to point adjoining anterior Y-link Moscow (*Mw*) violet-blue iridophore shoulder pattern. A portion of males express purple coloration in shoulder, peduncle and finnage, while others appear blue. Expression of Moscow shoulder pattern is highly mood dependent, thus motile.

Male posterior peduncle spots are near completely masked by Snakeskin Body pattern (*Ssb,* Phang and Fernando 1989) and/or Zebrinus (*Ze*) in co-expression with heterozygous Pink (*p,* Luckman 1990; *pi* Kempkes 2007). Though where visible expected non-Pb orange is modified to a Pb "pinkish-purple" coloration. Male caudal and/or peduncle is heavily masked by excessive green coloration. Making it difficult to distinguish and verify if males express a visible "reflective purple crescent" at caudal base, used to verify presence or not of Purple Body Gene. Male caudal is green bi-color delta. Pb and non-Pb males and Pb and non-Pb females indicate an autosomal dominant mode of inheritance.

9. Breeding: **E** *(hetero. Pb Bias Vienna LS / P) x* **F** *(homo. non-Pb Magoschitz Vienna DS / P)*


Sire and Dam

Results: Litter **680** F1 by female #1 [DOB 10.12.15] – 30 males; 9 non-Pb and 21 Pb. Females; Not counted for lack of color.

Offspring

S2 Fig 9. Pb male posterior peduncle spot is modified pinkish-purple and body iridophore base is modified light wild-type purple coloration. Non-Pb male posterior peduncle spot is orange and body iridophore base is green coloration. Pb and non-Pb males seem to indicate an autosomal mode of inheritance. This fairly small sample of 30 males by itself is somewhat problematic. The probability of these male results by chance alone, assuming an autosomal dominant gene, is Chi-square = 4.8, 1 df, $0.05>P>0.01$. By itself, we would reject the hypothesis of autosomal linkage. But then the alternative explanation with Y-linkage would give an estimated recombination frequency of 30% for the non-Pb males, which does not fit our other data shown here either. The discussion for Figures 1 and 2 of the main text of the paper make sex-linkage very unlikely.

10. Breeding: **EF** *(F1 hetero. Pb Vienna DS / 680) x* **I** *(homo. non-Pb Mousseau Green delta / P)*

Sire and Dam

Results: Litter **782** TC2 by female #1 [DOB 3.6.16] – 11 males; 5 Pb and 6 non-Pb. 8 females; 2 Pb and 1 non-Pb and 5 color/tail neutral.

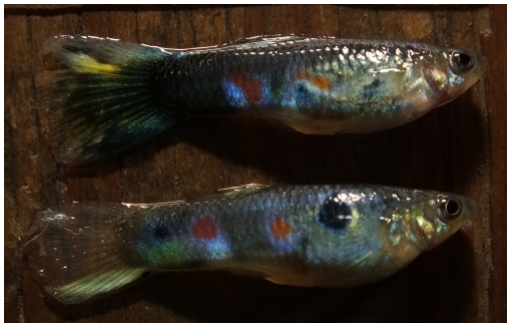
Offspring

S2 Fig 10. Delta and Swordtail genotype of sire segregated in both male and female offspring in this breeding. Males - 6 Non-Pb males; 2 delta and 4 swordtails. 5 Pb males; 4 delta and 1 swordtail. Females - 1 non-Pb delta, 2 Pb delta, 5 color/tail neutral swordtails. Pb and non-Pb males and Pb and non-Pb females indicate an autosomal dominant mode of inheritance.

11. Breeding: **EF** *(F1 hetero. Pb Vienna DS / 680) x* **I** *(homo. non-Pb Mousseau Green delta / P)*

Sire and Dam

Results: Litter **762** TC2 by female #2 [DOB 3.16.16] – 11 males: 5 Pb and 6 non-Pb. 9 females: 3 Pb, ~~and~~ 2 non-Pb and 4 color/tail neutral.



Offspring

S2 Fig 11. Delta and Swordtail genotype of sire segregated in both male and female offspring in this breeding. Males - 6 non-Pb males: 2 delta and 4 swordtails. 5 Pb males: 1 delta and 4 swordtail. Females - 2 non-Pb delta, 3 Pb delta, 4 color/tail neutral swordtails. Pb and non-Pb males and Pb and non-Pb indicate an autosomal dominant mode of inheritance.

12. Breeding: **E** *(hetero. Pb Bias Vienna LS / P) x* **F** *(homo. non-Pb Magoschitz Vienna DS / P)*

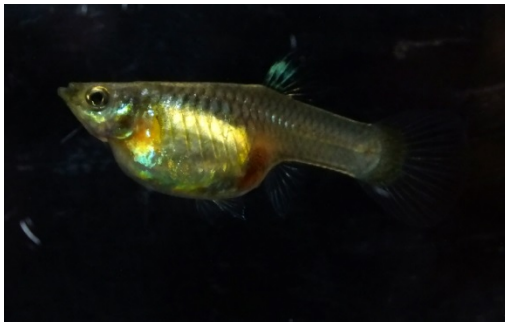

Sire and Dam

Results: Litter **699** F1 by female #1 [DOB 11.10.15] – 22 males: 12 non-Pb and 10 Pb. Females: Not counted for lack of color.


Offspring

S2 Fig 12. Pb male posterior peduncle spot is modified pinkish-purple and body iridophore base is modified light wild-type purple coloration. Non-Pb male posterior peduncle spot is orange and body iridophore base is green coloration. Pb and non-Pb males indicate an autosomal dominant mode of inheritance.

13. Breeding: **E** *(hetero. Pb Bias Vienna LS / P) x* **F** *(homo. non-Pb Magoschitz Vienna DS / P)*

Sire and Dam

Results: Litter **687** F1 by female #2 [DOB 10.19.15] – 19 males: 7 non-Pb and 12 Pb. Females: Not counted for lack of color.


Offspring

S2 Fig 13. Pb male posterior peduncle spot is modified pinkish-purple and body iridophore base is modified light wild-type purple coloration. Non-Pb male posterior peduncle spot is orange and body iridophore base is green coloration. Pb and non-Pb males indicate an autosomal dominant mode of inheritance.

14. Breeding: **E** *(hetero. Pb Bias Vienna LS / P) x* **F** *(homo. non-Pb Magoschitz Vienna DS / P)*

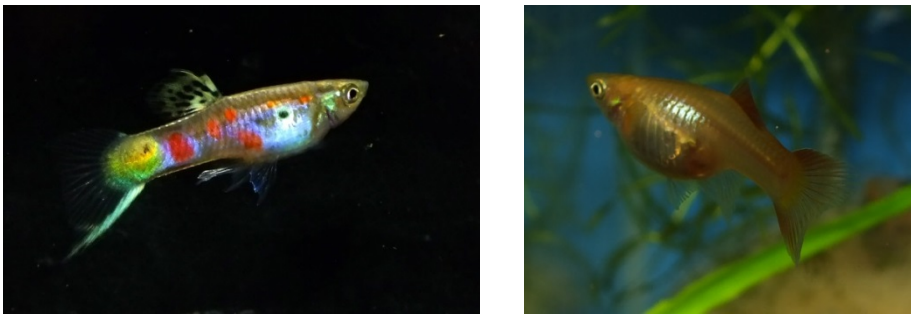
Sire and Dam

Results: Litter **694** F1 by female #3 [DOB 10.27.15] – 12 males: 7 non-Pb and 5 Pb. Females: Not counted for lack of color.

Offspring

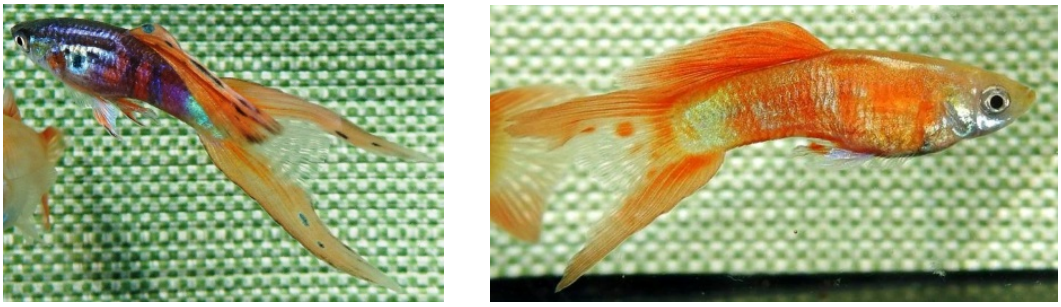
S2 Fig 14. Pb male posterior peduncle spot is modified pinkish-purple and body iridophore base is modified light wild-type purple coloration. Non-Pb male posterior peduncle spot is orange and body iridophore base is green coloration. Complete integrity of litter count not guaranteed. Dam dropped fry over a period of three days and cannibalism not ruled out. Pb and non-Pb males indicate an autosomal dominant mode of inheritance.

15. Breeding: **G** *(hetero. Pb Bias Red LS / P) x* **G** *(homo. non-Pb Bias Red DS / P)*



Sire and Dam

Results: Litter **703** F1 [DOB 12.5.15] – 14 males: 8 Pb and 6 non-Pb. Females: Not counted for non-verifiable color.



Offspring

S2 Fig 15. Sire is a grey body heterozygous Pb, Lowersword out of Doublesword breeding. Dam is blond (*b*, Goodrich 1944) non-Pb Red Doublesword female. Of notable interest non-Pb offspring males are all blond (*b*) and Pb offspring males are all grey. Pb and non-Pb males indicate an autosomal dominant mode of inheritance.

16. Breeding: **H** *(homo. Pb Mousseau Purple delta / P) x* **I** *(homo. non-Pb Mousseau Green delta / P)*

Sire and Dam

Results: Litter **753** F1 by female #1 [DOB 4.5.16] – 15 males: 15 Pb and 0 non-Pb. 15 females: 15 Pb and 0 non-Pb.

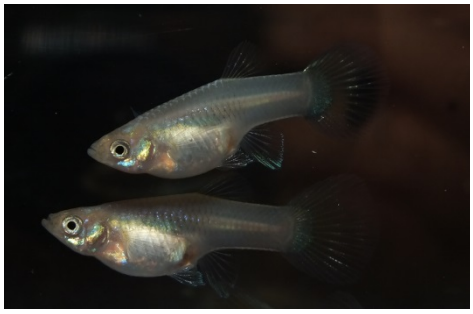
Offspring

S2 Fig 16. All males express Pb modified orange body spotting and purple caudal fins with varying degrees of Variegation. Females express variegated caudal fins comprised of black melanophores and yellow metal gold coloration. Pb females express a visible "reflective purple crescent" at caudal base, used to verify presence or not of Purple Body Gene. All Pb males and all Pb females indicate either both X and Y-linkage in the male or an autosomal dominant mode of inheritance.

17. Breeding: **H** *(homo. Pb Mousseau Purple delta / P) x* **I** *(homo. non-Pb Mousseau Green delta / P)*

Sire and Dam

Results: Litter **764** F1 by female #1 [DOB 4.5.16] – 25 males: 25 Pb and 0 non-Pb. 27 females: 27 Pb and 0 non-Pb.



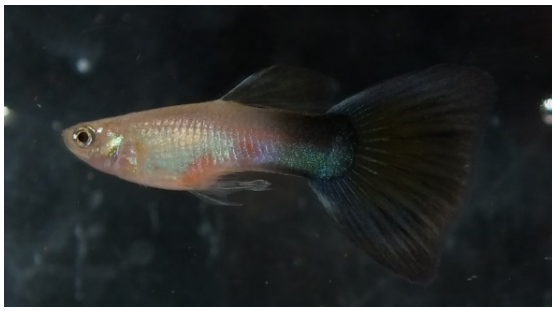
Offspring

S2 Fig 17. All males express Pb modified orange body spotting and purple caudal fins with varying degrees of Variegation. Females express variegated caudal fins comprised of black melanophores and yellow metal gold coloration. Pb females express a visible "reflective purple crescent" at caudal base, used to verify presence or not of Purple Body Gene. All Pb males and all Pb females indicate either both X and Y-linkage or an autosomal dominant mode of inheritance.

18. Breeding: **H** *(homo. Pb Mousseau Purple delta / P) x* **I** *(homo. non-Pb Mousseau Green delta / P)*

Sire and Dam

Results: Litter **768** F1 by female #2[DOB 4.12.16] – 13 males; 13 Pb and 0 non-Pb. 12 females; 12 Pb and 0 non-Pb.



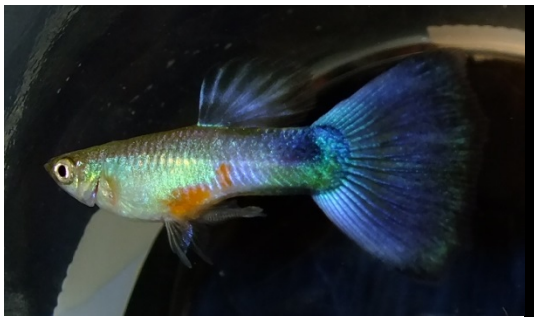
Offspring

S2 Fig 18. All males express Pb modified orange body spotting and purple caudal fins with varying degrees of Variegation. Females express variegated caudal fins comprised of black melanophores and yellow metal gold coloration. Pb females express a visible "reflective purple crescent" at caudal base, used to verify presence or not of Purple Body Gene. All Pb males and all Pb females indicate an autosomal dominant mode of inheritance.

19. Breeding: **J** *(homo. non-Pb Anderson Green delta / P) x* **K** *(homo. Pb Anderson Purple delta / P)*

Sire and Dam

Results: Litter **800** F1 [DOB 5.24.16] – 15 males:15 Pb and 0 non-Pb. 14 females: 14 Pb and 0 non-Pb.



Offspring

S2 Fig 19. All males express Pb modified orange body spotting and purple caudal fins with varying degrees of Variegation. Females express variegated caudal fins comprised of black melanophores and yellow metal gold coloration. Pb females express a visible "reflective purple crescent" at caudal base, used to verify presence or not of Purple Body Gene. All Pb males and all Pb females suggestive of X-linked or autosomal dominant mode of inheritance.

20. Breeding: **J** *(homo. non-Pb Anderson Green delta / P) x* **K** *(homo. Pb Anderson Purple delta / P)*

Sire and Dam

Results: Litter **805** F1 [DOB 6.10.16] – 1 males: 1 Pb and 0 non-Pb. 5 females: 5 Pb and 0 non-Pb.

Offspring

S2 Fig 20. All males express Pb modified orange body spotting and purple caudal fins with varying degrees of Variegation. Females express variegated caudal fins comprised of black melanophores and yellow metal gold coloration. Pb females express a visible "reflective purple crescent" at caudal base, used to verify presence or not of Purple Body Gene. All Pb males and all Pb females suggestive of X-linked or autosomal dominant mode of inheritance.

21. Breeding: **J** *(homo. non-Pb Anderson Green delta / P) x* **K** *(homo. Pb Anderson Purple delta / P)*

Sire and Dam

Results: Litter **806** F1 [DOB 6.12.16] – 3 males: 3 Pb and 0 non-Pb. 6 females: 6 Pb and 0 non-Pb.

Offspring

S2 Fig 21. All males express Pb modified orange body spotting and purple caudal fins with varying degrees of variegation. Females express variegated caudal fins comprised of black melanophores and yellow metal gold coloration. Pb females express a visible "reflective purple crescent" at caudal base, used to verify presence or absence of Purple Body Gene. All Pb males and all Pb females suggestive of X-linked or autosomal dominant mode of inheritance.

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