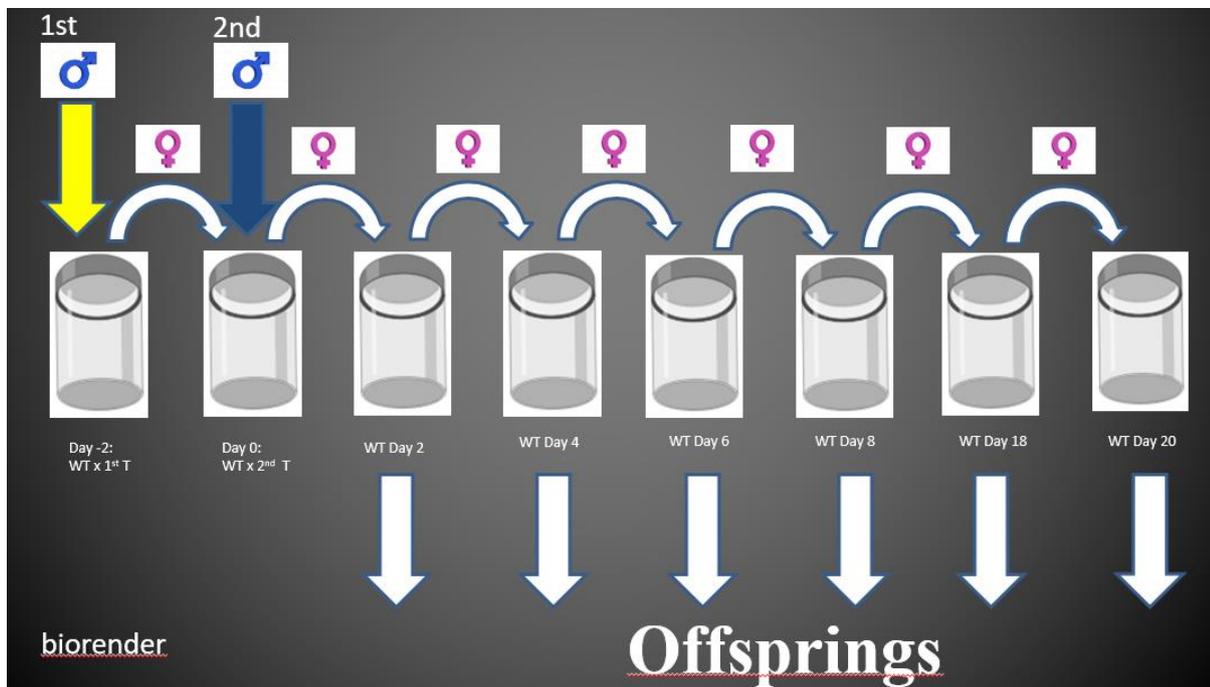


# Competitive sperm-marked beetles for monitoring approaches in genetic biocontrol and studies in reproductive biology

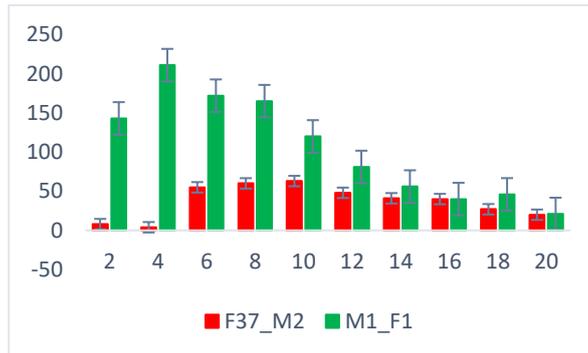
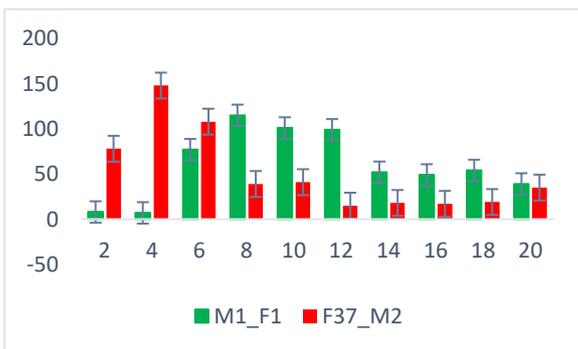
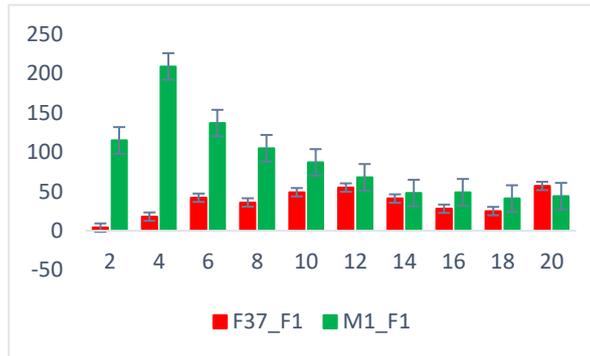
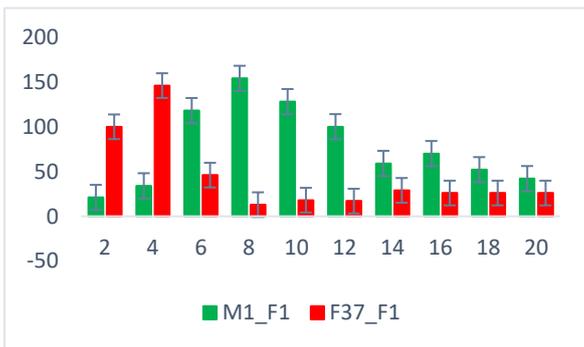
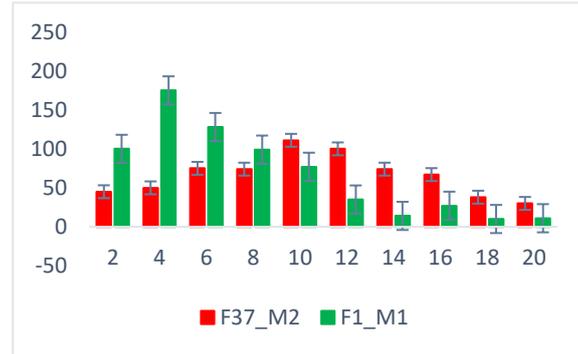
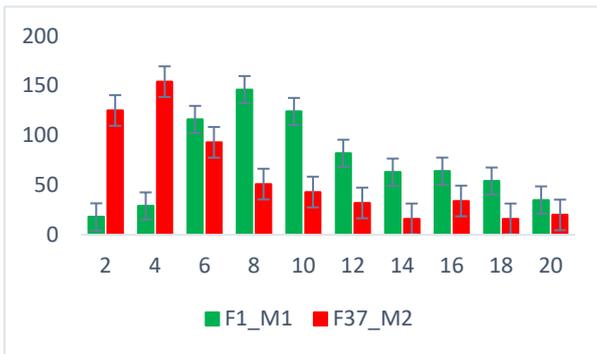
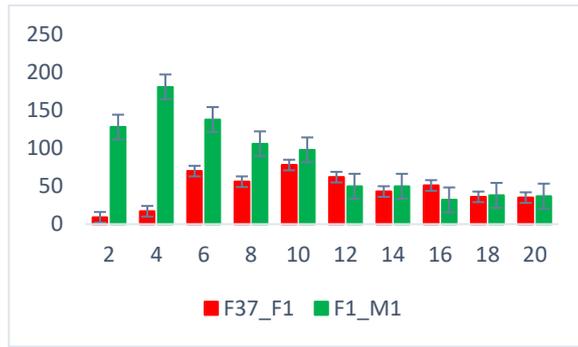
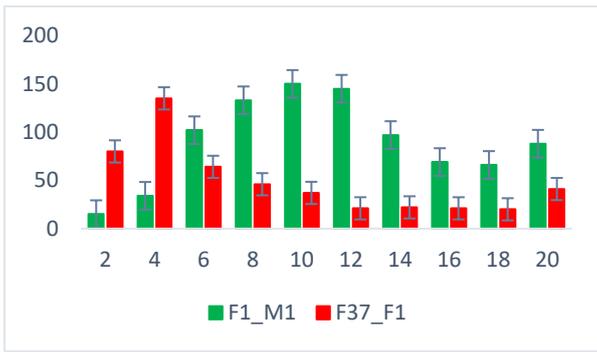
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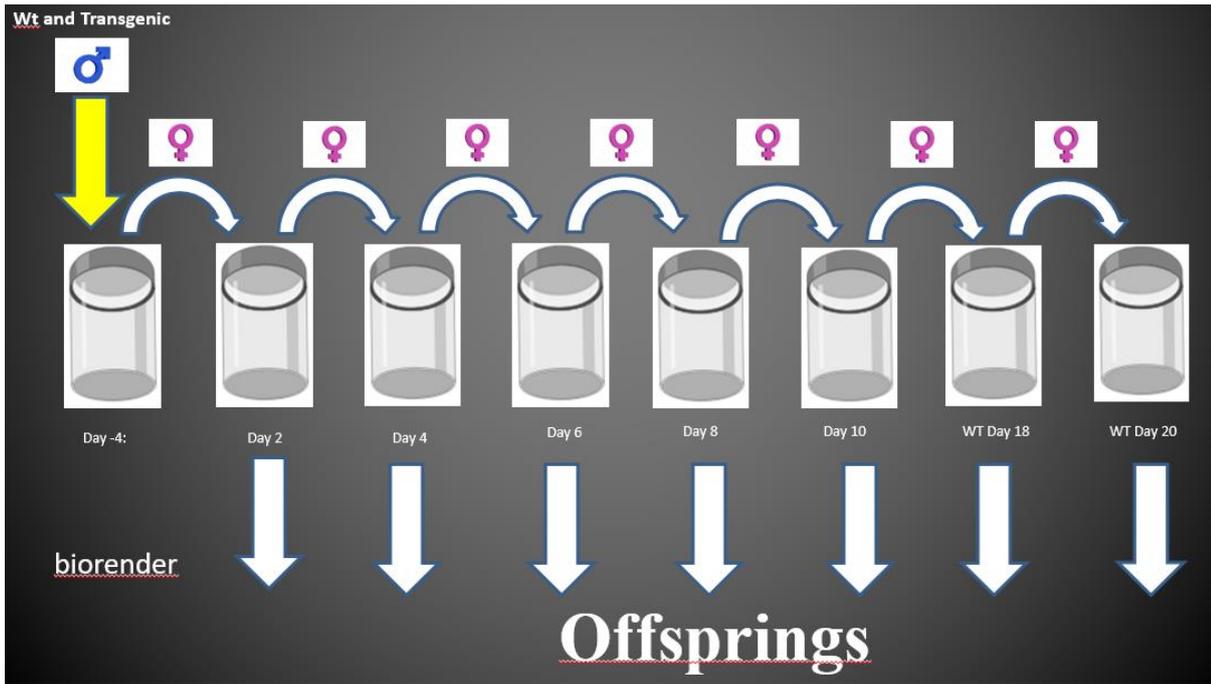
## Supplementary Materials:



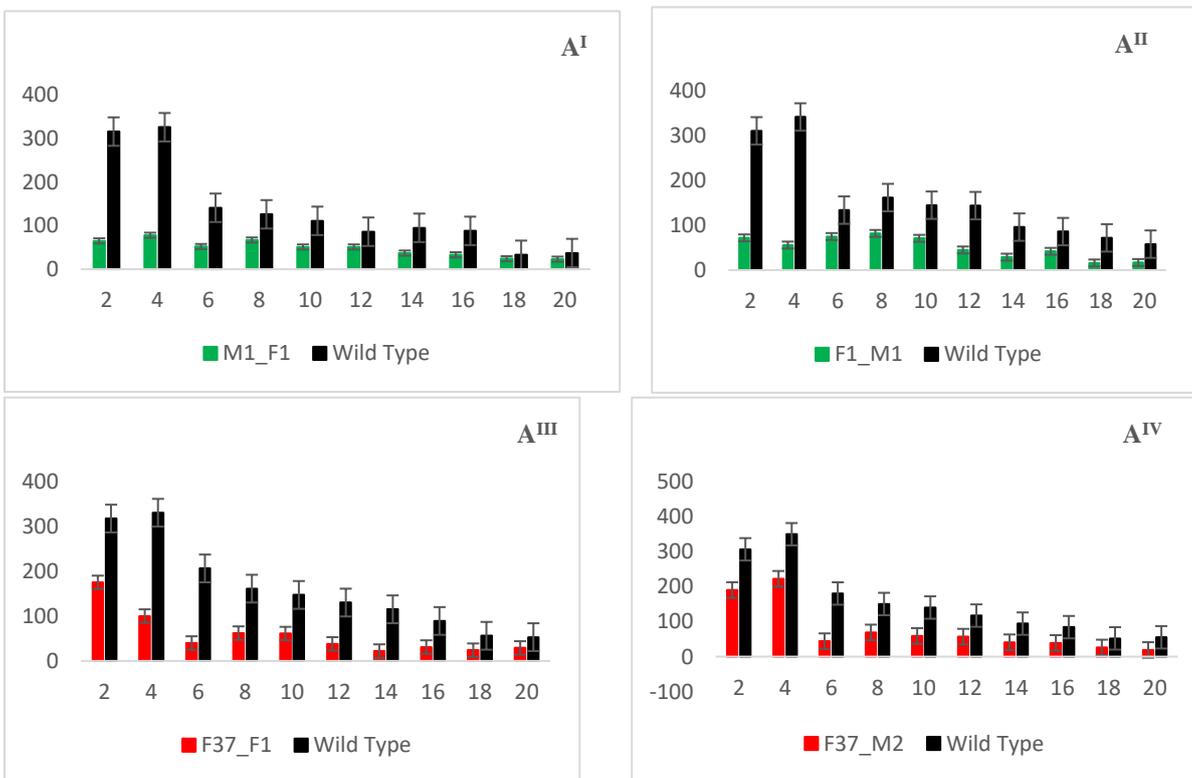
**Supplementary Figure S1:** Experimental protocol for Sperm utilization and competition of sequentially twice mated females.

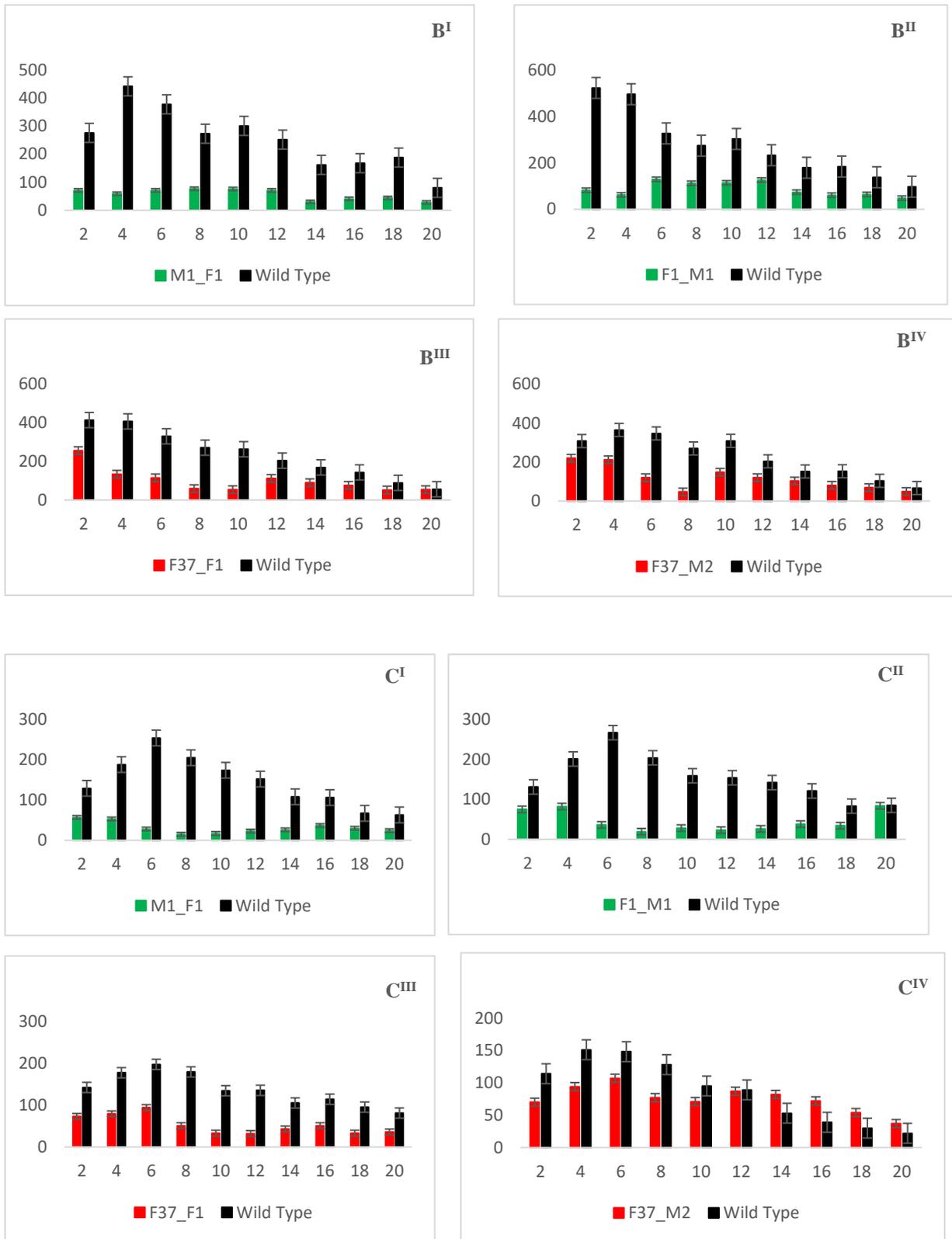


**Supplementary Figure S2:** Sperm utilization and competition of sequentially twice mated females. Matings were performed as indicated in Supplementary Figure S1 with the order of the respective lines as indicated below of each chart.

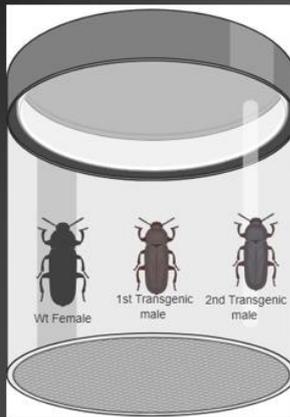


**Supplementary Figure S3:** Experimental protocol for simultaneous competition in a relaxed (5 transgenic males x 5 non-transgenic males x 10 non-transgenic females), a normal (5 transgenic males x 5 non-transgenic males x 5 non-transgenic females), and a high competition (10 transgenic males x 10 non-transgenic males x 5 non-transgenic females).

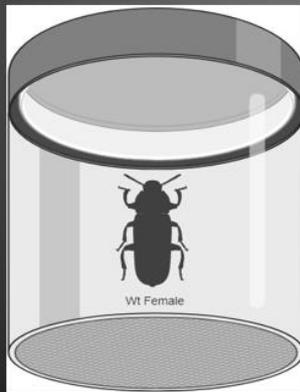




**Supplementary Figure S4.** Simultaneous competition of transgenic males compared to non-transgenic males. (a): 5 transgenic males x 5 non-transgenic males x 5 non-transgenic females (normal competition). (b) 5 transgenic males x 5 non-transgenic males x 10 non-transgenic females (relaxed competition). (c) 10 transgenic males x 10 non-transgenic males x 5 non-transgenic females (high competition). Matings were performed as indicated in Supplementary Figure S3 with the respective transgenic males competing against non-transgenic males as indicated below of each chart.



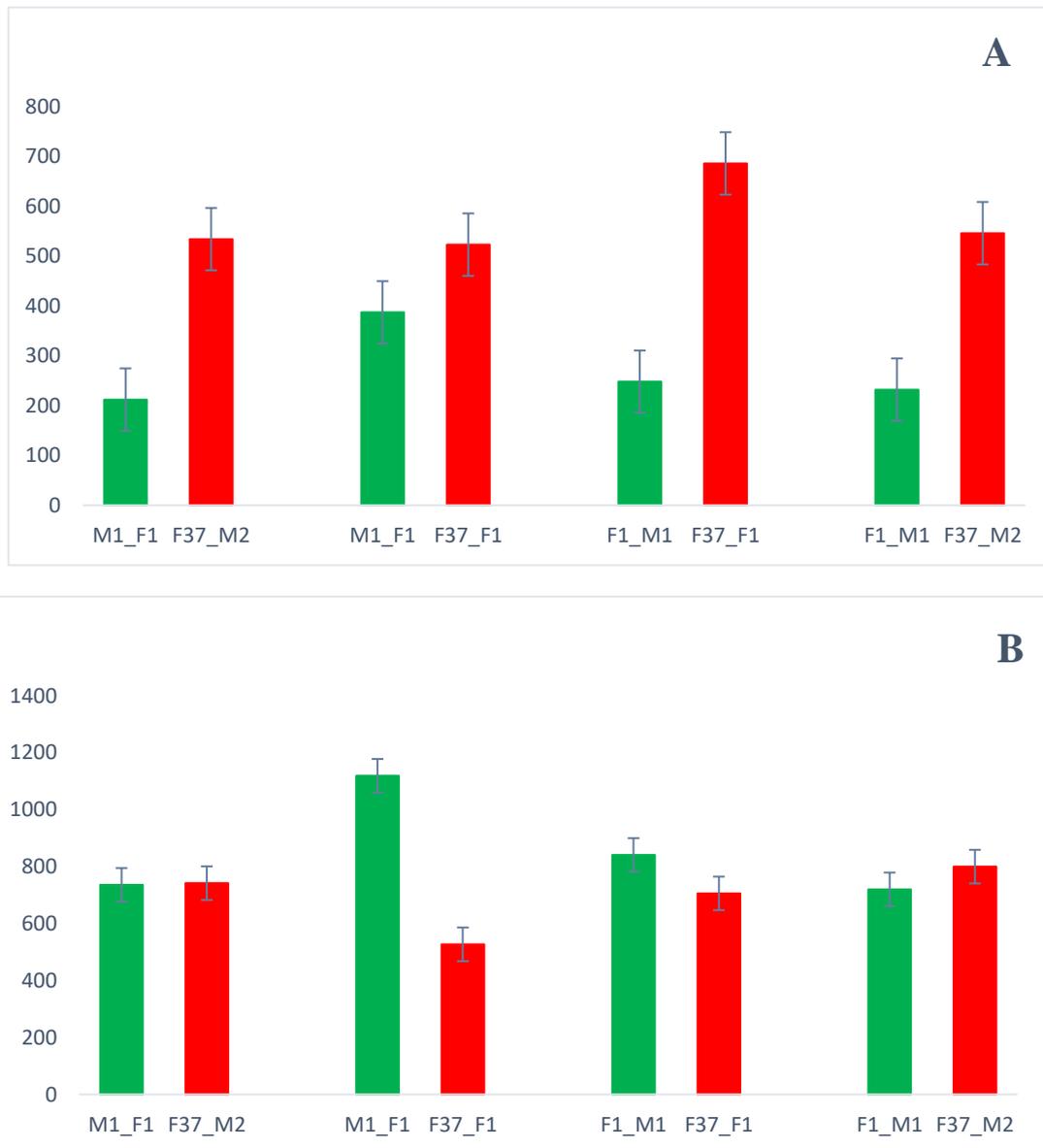
After 4 days the males were removed, and the female were transferred to a new flour



The female was moved to new flour and left for 21 days and the offsprings recorded.

biorender

**Supplementary Figure S5:** Experimental protocol for simultaneous competition between different transgenic males. Ten virgin non-transgenic females crossed with five males of two different transgenic lines each.



**Supplementary Figure S6:** Simultaneous competition of different transgenic males. (a) Progeny of the first 4 days after mating. (b) Progeny over 21 days after removal of the males. Matings were performed as indicated in Supplementary Figure S5 with the respective transgenic males competing against each other as indicated below of each column pair.

**Supplementary Table S1:** Transformation rate

Construct	No. of injected embryos	Hatched larvae	Pupae (G <sub>0</sub> )	Crosses with progeny	G <sub>0</sub> with transgenic progeny	Transformation rate
β2t-egfp	1000	139	77	55	3	5.5 %
β2t-dsred	1000	210	107	82	4	4.9 %

**Supplementary Table S2: Integration sites**

**F1\_M1 line = pBac {3xp3\_EGFP\_SV40-attP\_B2t (1Kb)\_DsRed\_SV40a}**

TGCAAAAAAATACATTGGCTAACAAAAGCAAACGATTTTACTATAATTTTACTCTTTGTTTCACATAAAAATATAAATTCCTCTAACTTTTACTATAAAAAGCATTTAC  
 TTAACCTTTATTTCATACGGCTCATTATTTCTATGAACAGTTAATTCCTGCTTTGAAATGCTATGATTATATCCGGTAGGCATTTTAATATTACGTACCTTAATCACTTT  
 AATGATTTATTTGTCTTATTTTTTGTGTTAACAATAATTAATAAATGCATTTTGTTTTTTGTATAATTAGGATTAGAACAATTATATTTTATTTTAAACAATTTAAA  
 CAAGT **TTAA-5'PiggyBac3'** -  
**TTAA**TTAATATTAATAAAAAATGTTGTTTTTTCCAGCGATTCAATTAAGGGATTAATTATAAAAAACAGTATGCTATGTAAGTCTGATTTAGGAGATAAACTCTGGATCCGGT  
 GCTCGTAATGATAACAAAACGGATATTAATTTCTGATAAGCAGCAATAAACCTAAT

**Integrated in first intron of TC007038.**

**M1\_F1 line = pBac {3xp3\_EGFP\_SV40-attP\_B2t (1Kb)\_DsRed\_SV40}**

TGGTAAATAAGTCCAACATTTTTGAAATAATTTTATTAGAAAAAAGCACAAAAGTTAATCTTGCACATCAATCAGCATCCTTAATCTTGTAAATAAATGTTTC  
 ACCAAAGAAAATAAAGTTGTTTCATCCGCAAGCATGATACCACAAAAACAAGACCTCACTTTCCCAGAATTTACAAAATATCCGCGGCATGTCATCCACAATCGGGCAA  
 TTTAATTTCCATTTTATCTTTTCCAGGAAAGTAAATCCACTTCTTGCACGCAAGAAAAATGTCATTGTGACAATTTATCTCACTATCATCTCTGGTATGTTTAAAT  
 TAAAAATTTCTCGTCGTTTTTCACTTTTCAACGACGATGATGAGTTTATTATGGAGGCCCTGGCAGGTGTTTTCAAAATTCGTACGCTGGGCTAG **TTAA-**  
**5'PiggyBac3'** -  
**TTAA**TAAAGCTAAATCAGGACCAAAACATGGCAAATATAGTCAATGTAACACGGGCTGCTGTCTGTCCATAATCAATATGCGTAAGATGGGTGTTCCGGTTTGGACAC  
 CTCCTTTCCGGTTTTTAATTTCCATTGCGGCAACGACCTTAGCGCGCCAGTTTCCGGACACAATAACGTTGGCAGACCTGCCACCATTTTACCAACTTTCT

**Integrated in first intron of TC012012.**

**F37\_F1 line = pBac {3xp3\_DsRed\_SV40-attP\_B2t (1Kb)\_EGFP\_SV40}**

TGAATCTTCAATAAATAAATCGTTAATTTGGCCTATCTTGATTAAGTGCATCTTGAAGTTTCAGCTACATACTTTGATTACTAGTTTGAAT**TTAA-**  
**5'PiggyBac3'** -TTAA

**Integrated in coding sequence of TC032496 (pangolin).**

**Supplementary Table S3: List of primers**

ID	NAME	Sequence 5' – 3'
MID#4	Tc-b2t-NcoI 1KB-F	ATGGCCATGGAGGTATCTCACAACCACCGCTTCC
MID#7	Tc- b2t-ClaI-R	GAGGATCGATTTTACTTTAACGTTAAACAAATTTATTAAGAAATACCG
MID#103	ClaI-EGFP-SV40_F2	GAGGATCGATATGGTGAGCAAGGGCGAG
MID#104	HindIII-EGFP-SV40_R	TTTAAGCTTGATGAGTTTGGACAAACCACAAC
No.5	iPCR5'F1	GACGCATGATTATCTTTTACGTGAC 1st round PCR
No.6	iPCR5'R1	TGACACTTACCGCATTGACA
No.7	iPCR5'F2	GCGATGACGAGCTTGTGGTGG 2nd round PCR
No.8	iPCR5'R2	TCCAAGCGGCGACTGAGATG
No.9	iPCR5'Seq	CGCGCTATTTAGAAAGAGAGAG
MFS227	iPCR3'F1	AGTCAGTCAGAAACAACCTTTGGCACATATC
MFS228	iPCR3'R1	CCTCGATATACAGACCGATAAAAACATGC
	3' Junction CH_PRSeq	TACGCATGATTATCTTTAACG
	Pjet 1.2 Forward	CGACTCACTATAGGGAGAGCGGC
	Pjet 1.2 Reverse	AAGAACATCGATTTTCCATGGCAG

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