

Title: The *transformer-2* and *fruitless* characterisation with developmental expression profiles of sex-determining genes in *Bactrocera dorsalis* and *B. correcta*

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Supplementary Table S1. Identity percentages from the pairwise comparison of Transformer-2 (TRA-2) amino acid sequences

Family			Tephritidae										Drosophilidae
Genus			<i>Bactrocera</i>					<i>Zeugodacus</i>	<i>Anastrepha</i>			<i>Ceratitis</i>	<i>Drosophila</i>
Species			<i>Bd</i>	<i>Bc</i>	<i>Bj</i>	<i>Bt</i>	<i>Bo</i>	<i>Zcu</i>	<i>Ao</i>	<i>Af</i>	<i>As</i>	<i>Cc</i>	<i>Dm</i>
Tephritidae	<i>Bactrocera</i>	<i>Bd</i>											
		<i>Bc</i>	98										
		<i>Bj</i>	98	98									
		<i>Bt</i>	97	97	98								
		<i>Bo</i>	96	96	96	95							
		<i>Zcu</i>	96	95	96	94	94						
	<i>Anastrepha</i>	<i>Ao</i>	89	88	88	87	86	89					
		<i>Af</i>	89	89	88	87	87	89	98				
		<i>As</i>	89	88	88	87	86	89	97	98			
	<i>Ceratitis</i>	<i>Cc</i>	91	91	90	89	89	91	89	89	89		
		<i>Dm</i>	45	44	45	43	45	43	42	42	42	44	
Drosophilidae	<i>Drosophila</i>	<i>Dm</i>	45	44	45	43	45	43	42	42	42	44	
Number of amino acids			251	251	251	255	251	250	249	249	249	251	264

The pairwise comparisons were performed within the same genus, *Bactrocera* or *Anastrepha*, among different genera of the family Tephritidae, and with *D. melanogaster* from the family Drosophilidae. Fruit fly species are indicated as the following: *Bd*, *B. dorsalis*; *Bc*, *B. correcta*; *Bj*, *B. jarvisi*; *Bt*, *B. tryoni*; *Bo*, *B. oleae*; *Zcu*, *Z. cucurbitae*; *Ao*, *A. obliqua*; *Af*, *A. fraterculus aff.1*; *As*, *A. suspensa*; *Cc*, *C. capitata*; *Dm*, *D. melanogaster*. GenBank Acc. Nos. of TRA-2 amino acid sequences are presented in Supplementary Table S3.

Supplementary Table S2. The connection between transcript classes and zinc-finger exons of *Bdfu* and *Bcfu*

<i>fru</i> transcript classes	Zinc-finger exons							
	Male				Female			
	A	B	C	D	A	B	C	D
P1 (exon M)	✓	✓	✓	-	-	-	-	-
P2 (exon U1)	✓	✓	✓	✓	✓	✓	✓	✓
P3 (exons U2 and U4)	✓	✓	✓	✓	✓	✓	✓	✓
P4 (exon U3)	✓	-	✓	✓	✓	-	✓	✓

Supplementary Table S3. GenBank accession numbers of *transformer-2* (*tra-2*) and *fruitless* (*fru*) sequences used in this study

Species	GenBank accession numbers	References
<i>transformer-2</i>		
<i>Bactrocera dorsalis</i>	MT900512, MT900514	In this study
<i>Bactrocera correcta</i>	MT900513, MT900515	In this study
<i>Bactrocera dorsalis</i>	KP342061	1
<i>Bactrocera oleae</i>	AJ547623	-
<i>Bactrocera tryoni</i>	KJ443723	2
<i>Bactrocera jarvisi</i>	KJ443722	2
<i>Zeugodacus cucurbitae</i>	KR056217	3
<i>Anastrepha obliqua</i>	FN658607	4
<i>Anastrepha fraterculus aff.1</i>	FN658608	4
<i>Anastrepha suspensa</i>	JN597290	5
<i>Ceratitis capitata</i>	EU999754	6
<i>Drosophila melanogaster</i>	NM_057416	-
<i>fruitless</i>		
<i>Bactrocera dorsalis</i>	MT900516 - MT900519, MT900524 - MT900528	In this study
<i>Bactrocera correcta</i>	MT900520 - MT900523, MT900529 - MT900533	In this study
<i>Bactrocera dorsalis</i>	XM_011202587 - XM_011202592	-
<i>Bactrocera oleae</i>	XM_014238789 - XM_014238790	-
<i>Bactrocera latifrons</i>	XM_018927950 - XM_018927953	-
<i>Zeugodacus cucurbitae</i>	XM_011191466 - XM_011191472	-
<i>Ceratitis capitata</i>	EU999755	6
	XM_020861837 - XM_020861839	-
<i>Musca domestica</i>	KC750909 - KC750915	7
<i>Drosophila melanogaster</i>	NM_169820, NM_001275783, NM_206514, NM_169816, NM_169822	-
<i>Anopheles gambiae</i>	AY785361, AY785360, AY725820, AY725819	8
<i>Tribolium castaneum</i>	JQ409470, JQ409471, JQ409472, XM_008202773	-
<i>Nasonia vitripennis</i>	GQ338170, GQ338167, NM_001164125, NM_001164122, NM_001164128, NM_001164123, NM_001164130, NM_001164134, GQ338183.1	9

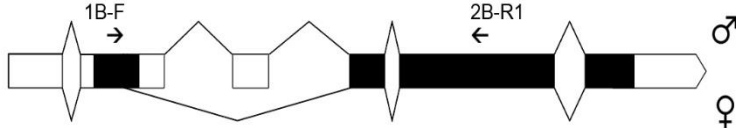
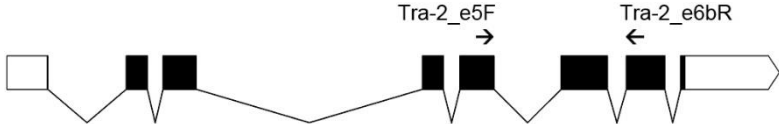
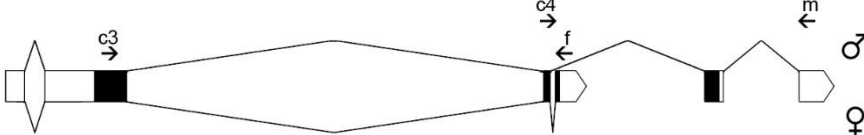
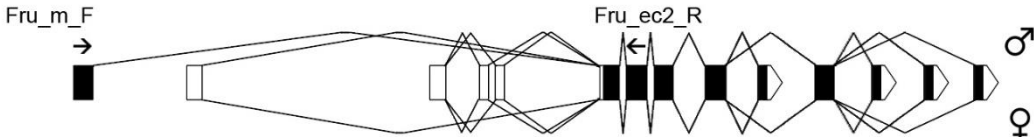
Supplementary Table S4. Primers used in this study for isolation of *transformer-2* (*tra-2*) and *fruitless* (*fru*) genes

Purpose	Primer name	Sequence (5' → 3')
Isolation of <i>tra-2</i> transcripts		
RT-PCR	Tra-2_e2F	CGTAGCCGCAGTATTTCCC
	Tre-2_e6aR	AGTGTGCGGTCTTTGCGTTG
5'RACE	UPM_long	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCA GAGT
	UPM_short	CTAATACGACTCACTATAGGGC
	Tra-2_e6bR	TTGCGACTGTGATAAGGTGAG
3'RACE	Tra-2_e2F	CGTAGCCGCAGTATTTCCC
	UPM_long	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCA GAGT
	UPM_short	CTAATACGACTCACTATAGGGC
Isolation of <i>tra-2</i> exon/intron sequences		
PCR	Tra-2_F	AGCATATCGAGCGAGTCCAT
	Tra-2_e2R	AACTGGCGACTTGGTGTAAC
	Tra-2_e2F	CGTAGCCGCAGTATTTCCC
	Tre-2_e6aR	AGTGTGCGGTCTTTGCGTTG
	Tra-2_e5F	GGACCCATTGAACGAATACAAG
	Tra-2_e7R	CAGAGTAAATGCCAAACATGG
Isolation of <i>fru</i> transcripts		
RT-PCR	Fru_ec1F1	TGGAACAATCACCCGACGAA
	Fru_ec4R2	GCATGACTGAGACCAATACTA
	Fru_ec4F2	TAGTATTGGTCTCAGTCATGC
	Fru_zAR-2	ACATAATGACTGAAGAAACGATC
	Fru_zBR	TATCATTCACCTGTGCGCATTGT
	Fru_zCR	GAGTGAAAGTGATGCCATCG
	Fru_zDR	TATCCTCGCCCACATTTATCG
Isolation of <i>fru</i> transcripts		
5'RACE	UPM_long	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGC AGAGT
	UPM_short	CTAATACGACTCACTATAGGGC

	Fru_ec3R2-AP	GATTACGCCAAGCTTGCTAACACTACGCTCACTATTTCGCATCG
	Fru_ec3R1_nested-AP	GATTACGCCAAGCTTGCTCAAAGCTGCTGCTGCTGCGATGTG
	Fru_m_R	GGCACCTGTGCTCGGCTCGATGTCTGCA
3'RACE	UPM_long	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT
	UPM_short	CTAATACGACTCACTATAGGGC
	Fru_ec4F3L	GTAGCGGTAGTGTTAGTGCGATTCCCAGTAG
Study of the connection between transcript classes and zinc-finger encoding exons		
RT-PCR	Fru_m_F (P1)	ATGTTGGCCATGTCACAAGG
	Fru_5U-1 (P2)	GTGCTGAACGCCCAAACTC
	Fru_5U-2 (P3)	TCAACTCGCATCGCATCATC
	Fru_5U-3 (P4)	TGGCGTATCGTAAAATGCTAAG
	Fru_ec2_R	GCTGTTTTGAGGAACATCGG
	Fru_zAF	GGACAACATTGATGGTGATGATGG
	Fru_zAR-2	ACATAATGACTGAAGAAACGATC
	Fru_zBF	TGTTATCTGCACCGCTGCAAAGT
	Fru_zBR	TATCATTCACTTGTCGCATTGT
	Fru_zCF	GCCCACCATCACATCTCTACAATC
	Fru_zCR	GAGTGAAAGTGATGCCATCG
	Fru_zDF	ATCGCATCGTTTGTGCTACGAACG
	Fru_zDR	TATCCTCGCCCACATTTATCG
Study of putative female-specific exon		
PCR and RT-PCR	Fru_m_F	ATGTTGGCCATGTCACAAGG
	Fru_mfj_F	GCCGCCGAGGTAAGGTTAC
	Fru_f_F	GTAAGGTTACGCCAAGCGGC
	Fru_f_R	AATGCTAGTAAAATGCGAGAGTGA
	Fru_ec1_F	TGGAACAATCACCCGACGAA
	Fru_ec2_R	GCTGTTTTGAGGAACATCGG
Study of the connection between transcript classes and developmental stages		
RT-PCR	Fru_m_F (P1)	ATGTTGGCCATGTCACAAGG
	Fru_5U-1 (P2)	GTGCTGAACGCCCAAACTC

	Fru_5U-2 (P3)	TCAACTCGCATCGCATCATC
	Fru_5U-3 (P4)	TGGCGTATCGTAAAATGCTAAG
	Fru_ec2_R	GCTGTTTTGAGGAACATCGG

Supplementary Table S5. Schematic representation of relative locations and amplified regions of primers used for expression analysis of sex-determining genes in different developmental stages

Gene	Schematic representation of primer locations and amplified regions
<i>slam</i>	
<i>MoY</i>	None
<i>tra</i>	
<i>tra-2</i>	
<i>dsx</i>	
<i>fru</i>	
<i>gapdh</i>	

The representation of gene structure is not to scale. Boxes and lines represent exons and introns, respectively. The white and black regions indicate untranslated and translated regions, respectively. Arrows with names indicate primers used in the analysis. The sequences of these primers are in Supplementary Table S6.

Supplementary Table S6. Primers used in this study for sexual identification and expression analysis in different developmental stages

Gene	Primer name	Sequence (5' → 3')	Species	Product size (bp)	
				Male	Female
Primers for sexual identification of single embryos, larvae, and pupae using genomic DNA as templates					
<i>MoY</i> ⁽¹⁾	MoY_F	AAATGATATAGAAGAGCATGGGAT C	<i>Bd, Bc</i>	190	-
	MoY_R	TTCATTAATTTTTTTGAATTCTGTGC			
Primers for expression analysis using cDNA as templates					
<i>slam</i>	Slam_F	TACACGCAAATCGAAGGTTAAGC	<i>Bd, Bc</i>	493	493
	Slam_R	CTCTAATTCACGTGCCAGCTC			
<i>MoY</i> ⁽¹⁾	MoY_F	AAATGATATAGAAGAGCATGGGAT C	<i>Bd, Bc</i>	190	-
	MoY_R	TTCATTAATTTTTTTGAATTCTGTGC			
<i>tra</i> ⁽²⁾	1B-F	GAAGTTGTTATTAAGCGTAGATT C	<i>Bd</i>	980	626
	2B-R1	CTTTCCCGTTCGCGTTTACTATTG	<i>Bc</i>	951	599
<i>tra-2</i>	Tra-2_e5F	GGACCCATTGAACGAATACAAG	<i>Bd, Bc</i>	296	296
	Tra-2_e6bR	TTGCGACTGTGATAAGGTGAG			
<i>dsx</i> ⁽³⁾	C3	GCTTAACCGCCGATCGTCAG	<i>Bd, Bc</i>	-	677
	Female-specific(f)	GTATTCGTTTACGACATGTTGGC			
	C4	GCTGGGGCAGATATTGAAGAG	<i>Bd, Bc</i>	484	-
	Male-specific(m)	CGGTACTAAGGGTTTAGTCATC			
<i>fru</i>	Fru_m_F	ATGTTGGCCATGTCACAAGG	<i>Bd, Bc</i>	506	-
	Fru_ec2_R	GCTGTTTTGAGGAACATCGG			
Primers for expression analysis using cDNA as templates					
<i>gapdh</i>	Gapdh_F	GTCTTCACCACAATCGATAAGGCT TCTGC	<i>Bd, Bc</i>	400	400
	Gapdh_R	GGAATGCCATACCAGTCAGTTTGC			

		CG			
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Bd: *B. dorsalis* and *Bc*: *B. correcta*

- (1) Primers were designed based on *BdMoY* sequence¹⁰.
- (2) Primers are from the previous study of *Bdtra* and *Bctra* sequences¹¹.
- (3) Primers are from the previous study of *Bddsx* and *Bcdsx* sequences¹².

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