

Supplement to Tirant stealthily invaded natural *Drosophila melanogaster* populations during the last century

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**Supplementary figures**

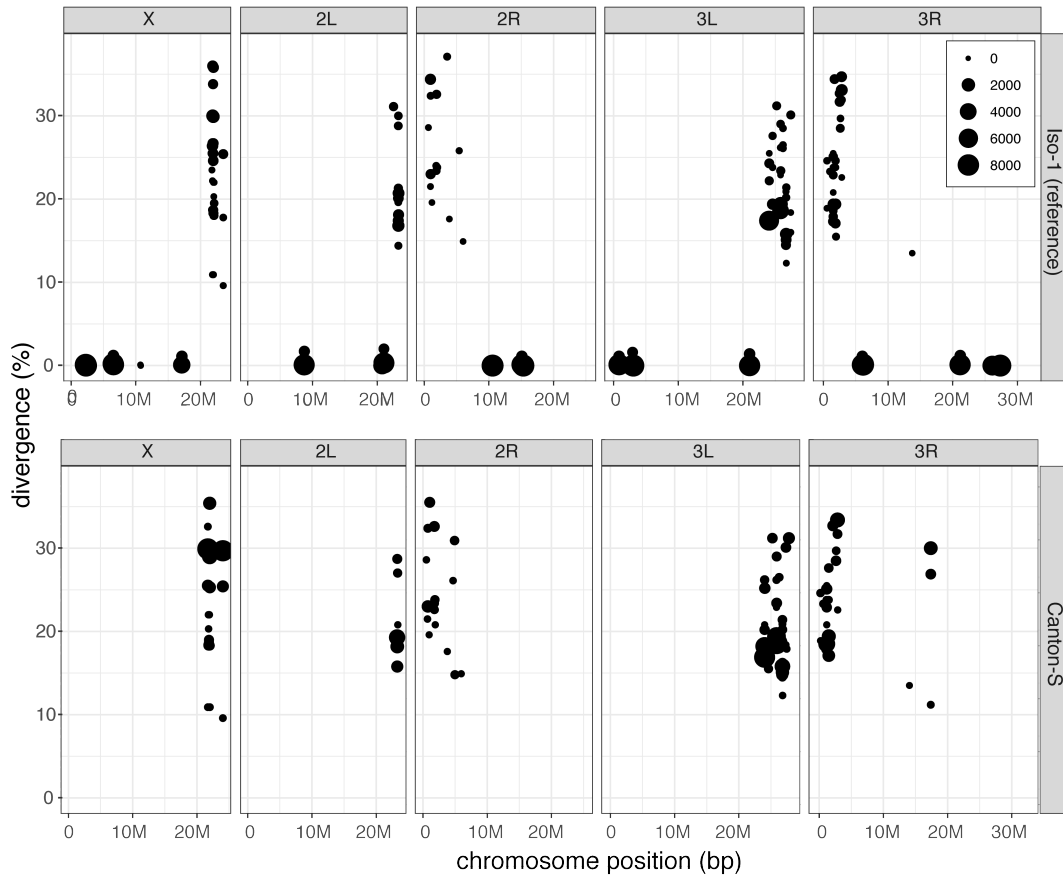


Figure 1: Canonical Tirant insertions are present in Iso-1 but not Canton-S. The Canton-S assembly was generated by Chakraborty et al. (2019) with PacBio reads (the Canton-S assembly shown in the main manuscript was generated by Wierzbicki et al. (2020) with ONT reads). For each Tirant insertion we show the position in the assembly, the length (size of dot), and the similarity to the consensus sequence (divergence).

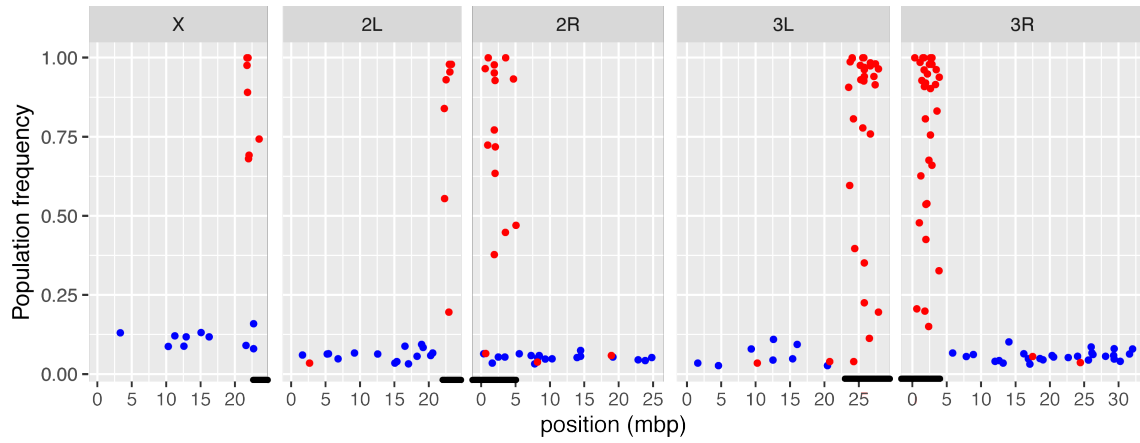


Figure 2: Position and population frequency of canonical (blue) and degraded (red) Tirant insertions in a population from France (Viltain) (Kapun et al., 2018). Canonical Tirant insertions are mostly euchromatic and segregating at a low population frequency whereas degraded insertions are mostly heterochromatic and segregating at high frequency. Black bars indicate (peri)centric heterochromatin (Riddle et al., 2011; Hoskins et al., 2015).

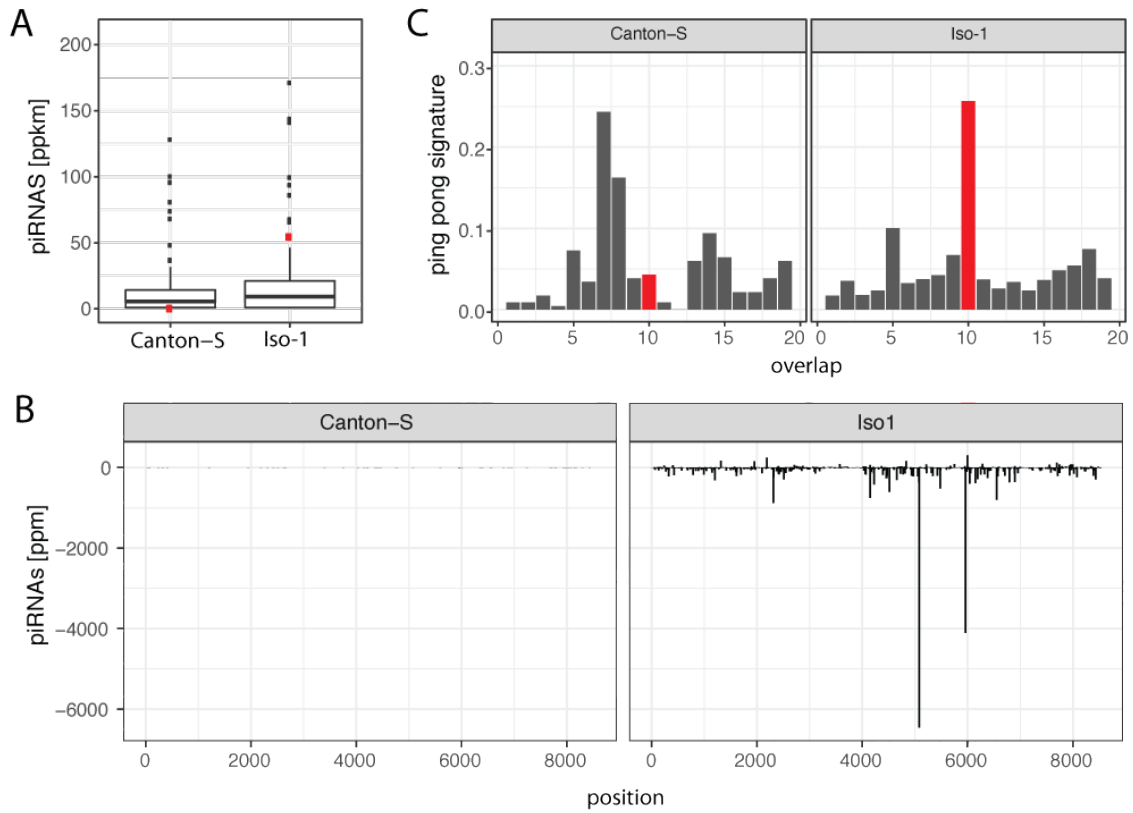


Figure 3: Tirant piRNAs in Iso-1 and Canton-S. A) Abundance of Tirant piRNAs (red) compared to piRNAs complementary to the other TEs of *D. melanogaster*. B) Abundance of sense (positive y-axis) and antisense (negative y-axis) piRNAs along the sequence of Tirant. C) Ping-pong signature for Iso-1 and Canton-S. A pronounced peak at position 10 (red) suggest secondary amplification of piRNAs by the ping-pong cycle.

### Tirant

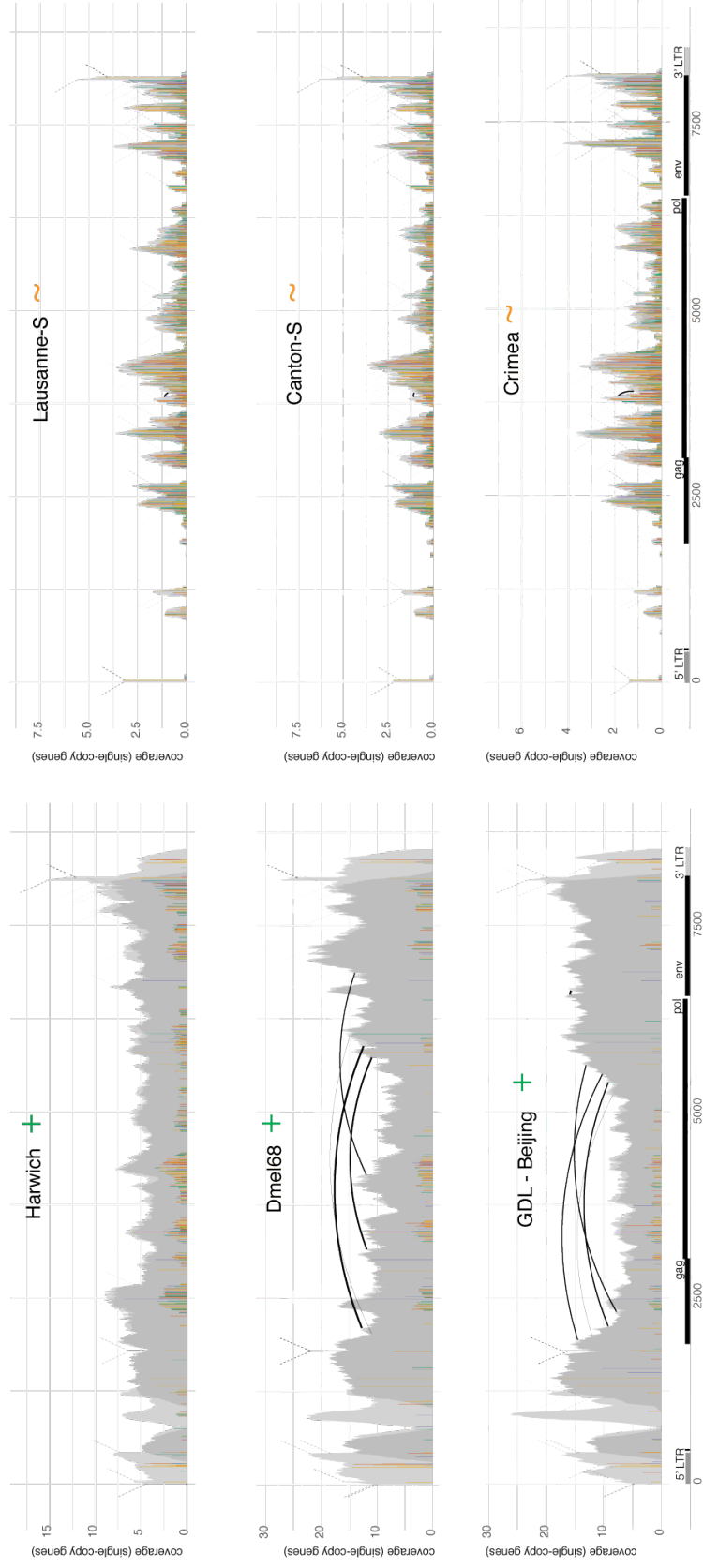


Figure 4: DeviaTE plots for three strains having non-degraded (i.e. canonical) Tirant sequences (+) and three strains solely having degraded Tirant sequences (-). Such plots were used for classifying the Tirant content of the different *D. melanogaster* strains (see supplementary table 1).

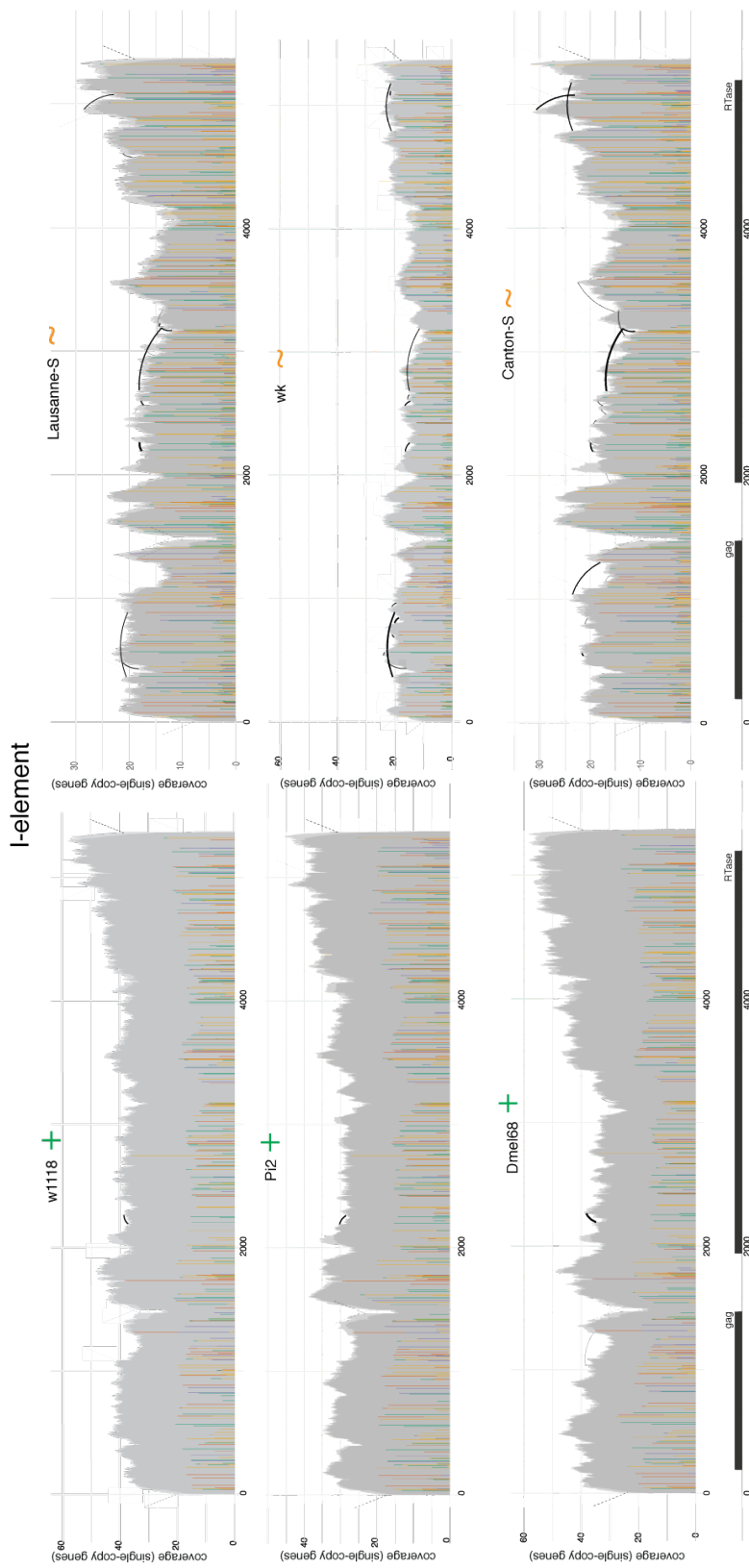


Figure 5: DeviaTE plots for three strains having non-degraded I-element sequences (+) and three strains solely having degraded I-element sequences (~). Such plots were used for classifying the I-element content of the different *D. melanogaster* strains (see supplementary table 1).

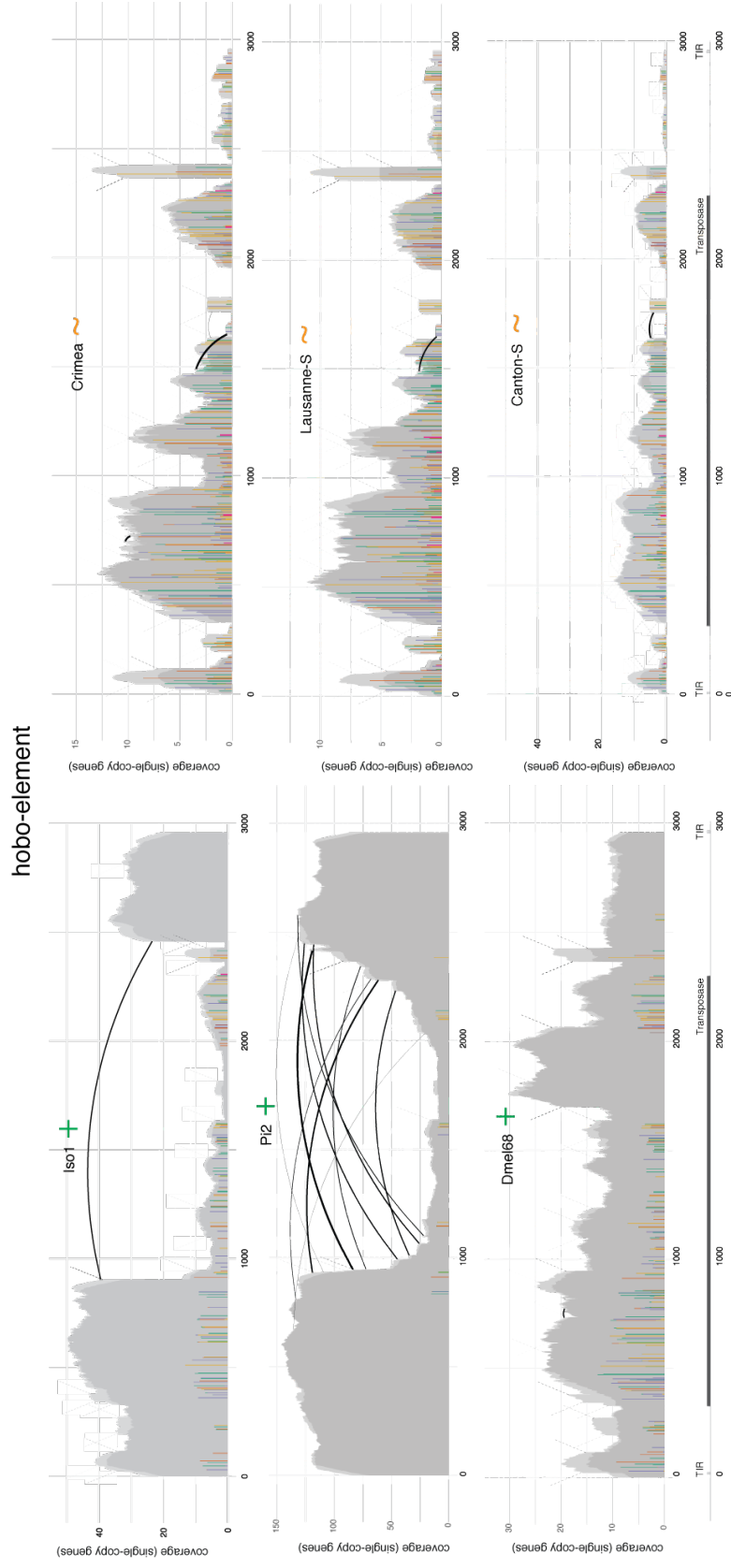


Figure 6: DeviaTE plots for three strains having non-degraded hobo sequences (+) and three strains solely having degraded hobo sequences (~). Such plots were used for classifying the hobo content of the different *D. melanogaster* strains (see supplementary table 1).

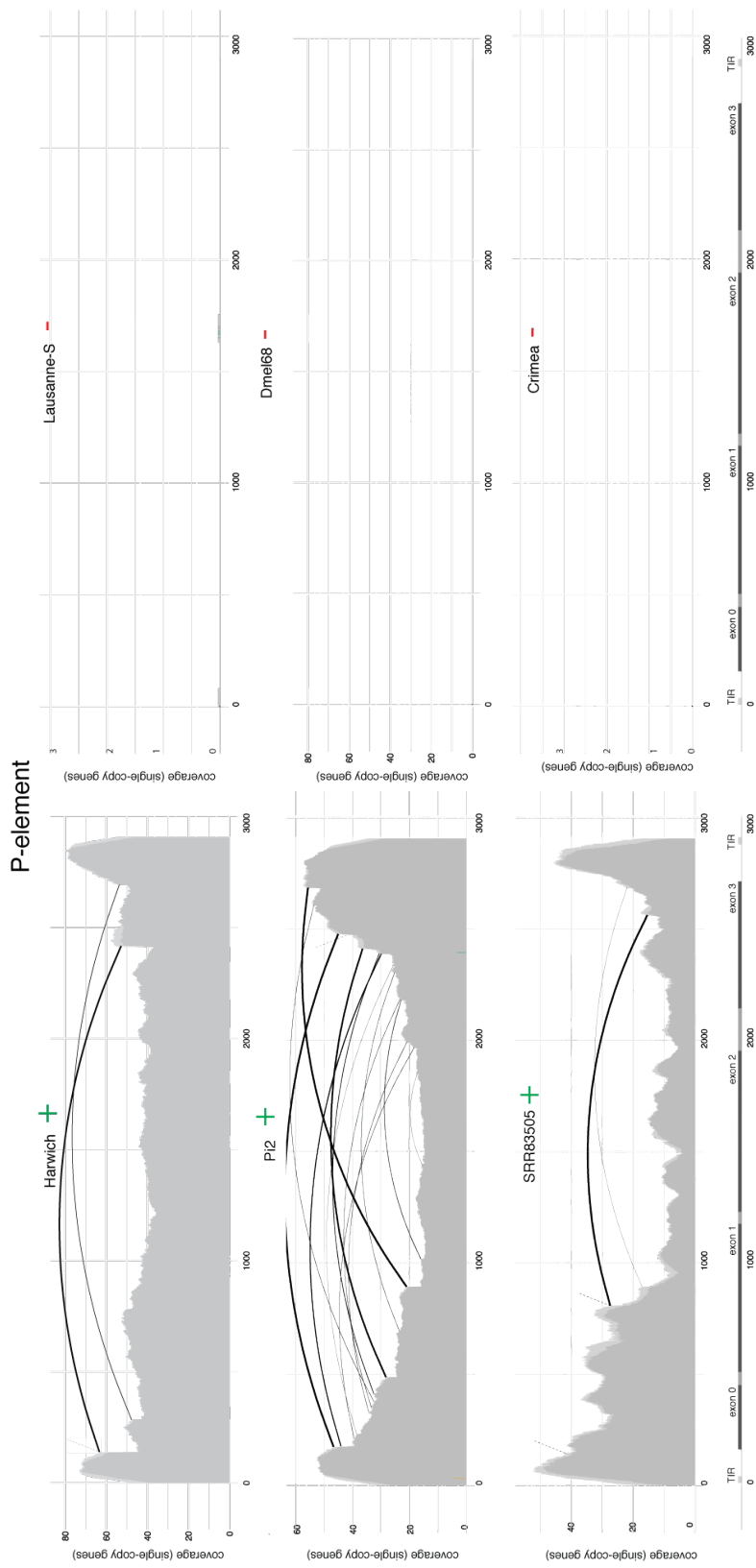


Figure 7: DeviaTE plots for three strains having P-element sequences (+) and three strains not having P-element sequences (-). Such plots were used for classifying the P-element content of the different *D. melanogaster* strains (see supplementary table 1).



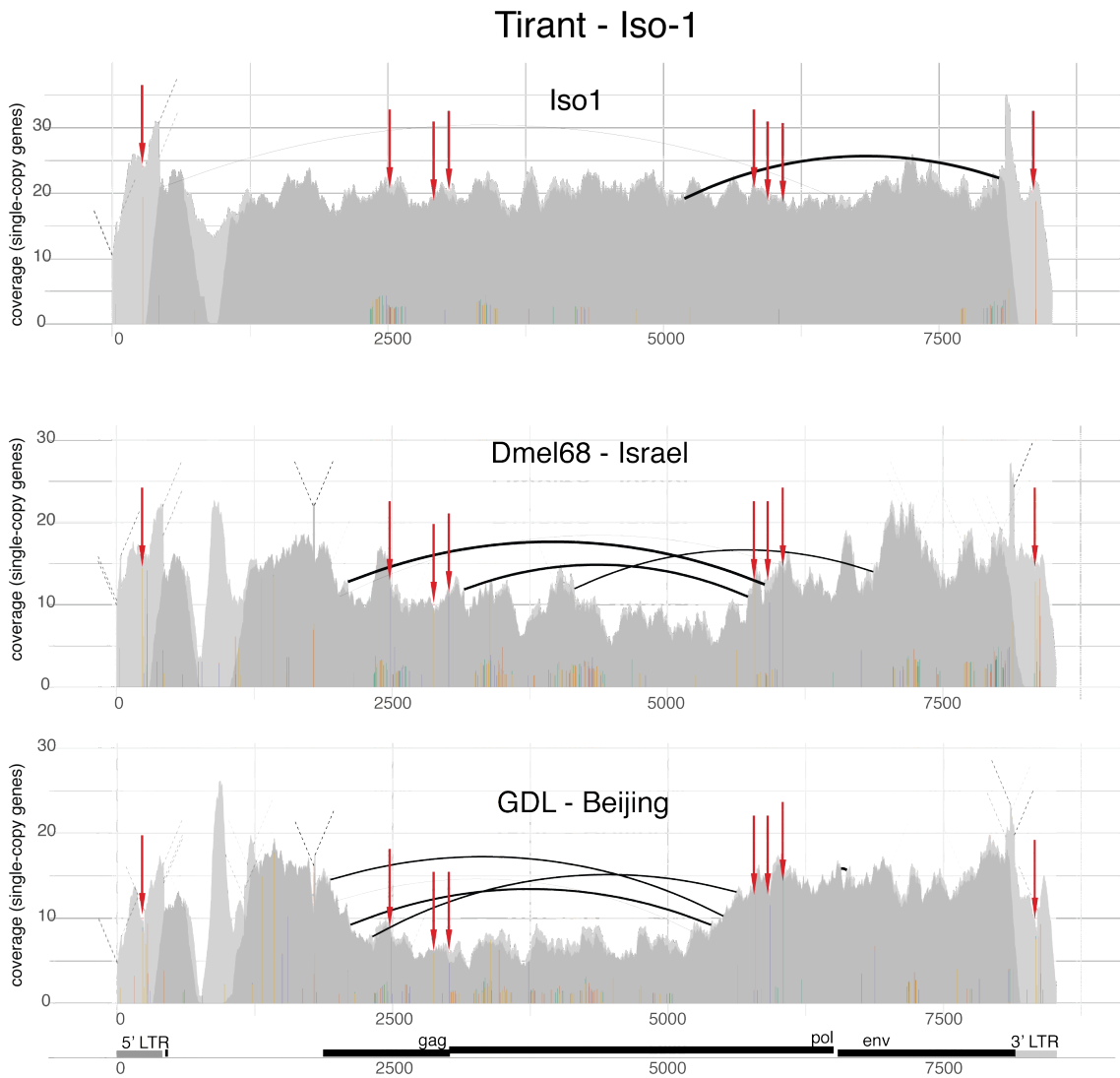


Figure 8: Abundance and diversity of Tirant in the reference strain Iso-1 and two strains collected from natural *D. melanogaster* populations (Dmel68 and a GDL line from Beijing). Eight SNPs found in natural populations but not in Iso-1 are marked by red arrows.

## Tirant - Tasmania

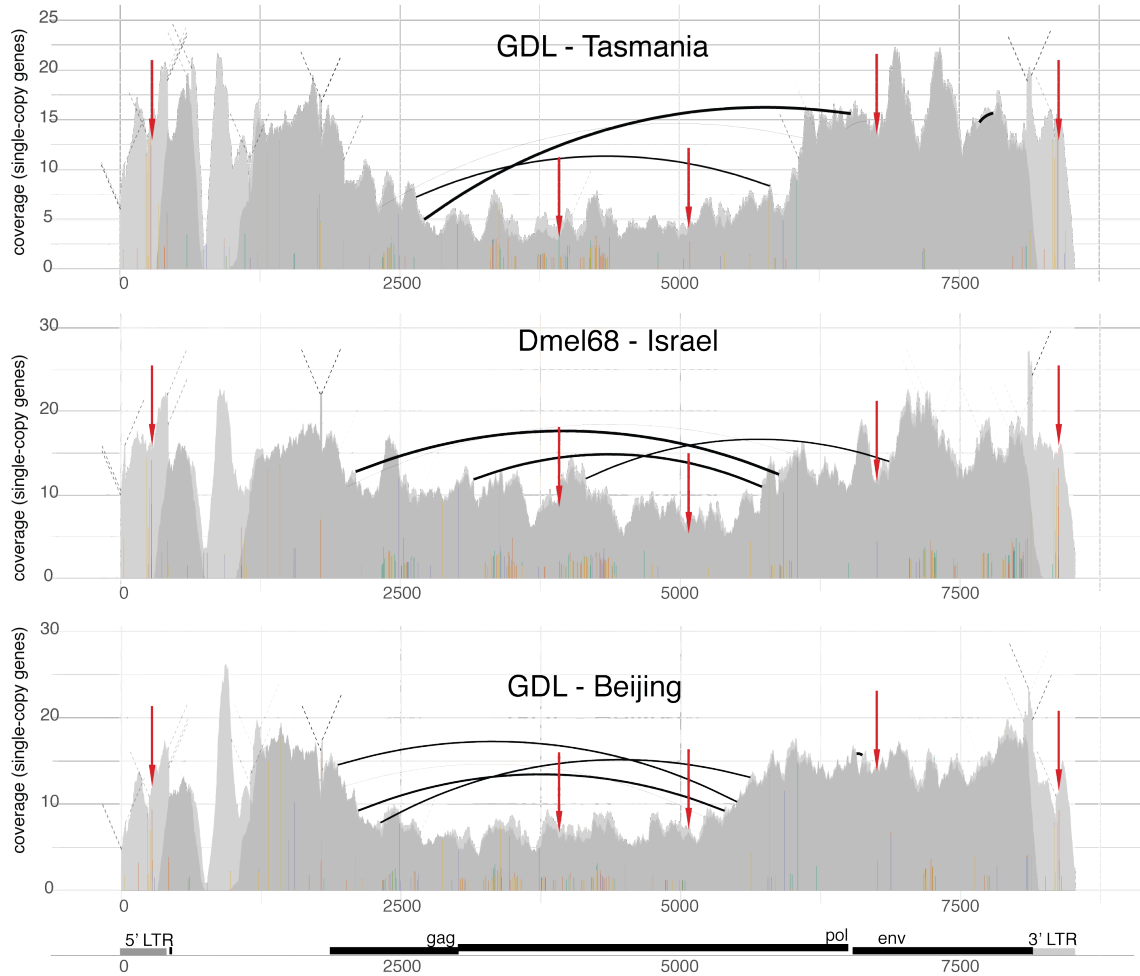


Figure 9: Abundance and diversity of Tirant sequences in a natural population from Tasmania and from other geographic locations. Five SNPs, marked by red arrows, have notably different allele frequencies between populations from Tasmania and the other geographic locations.

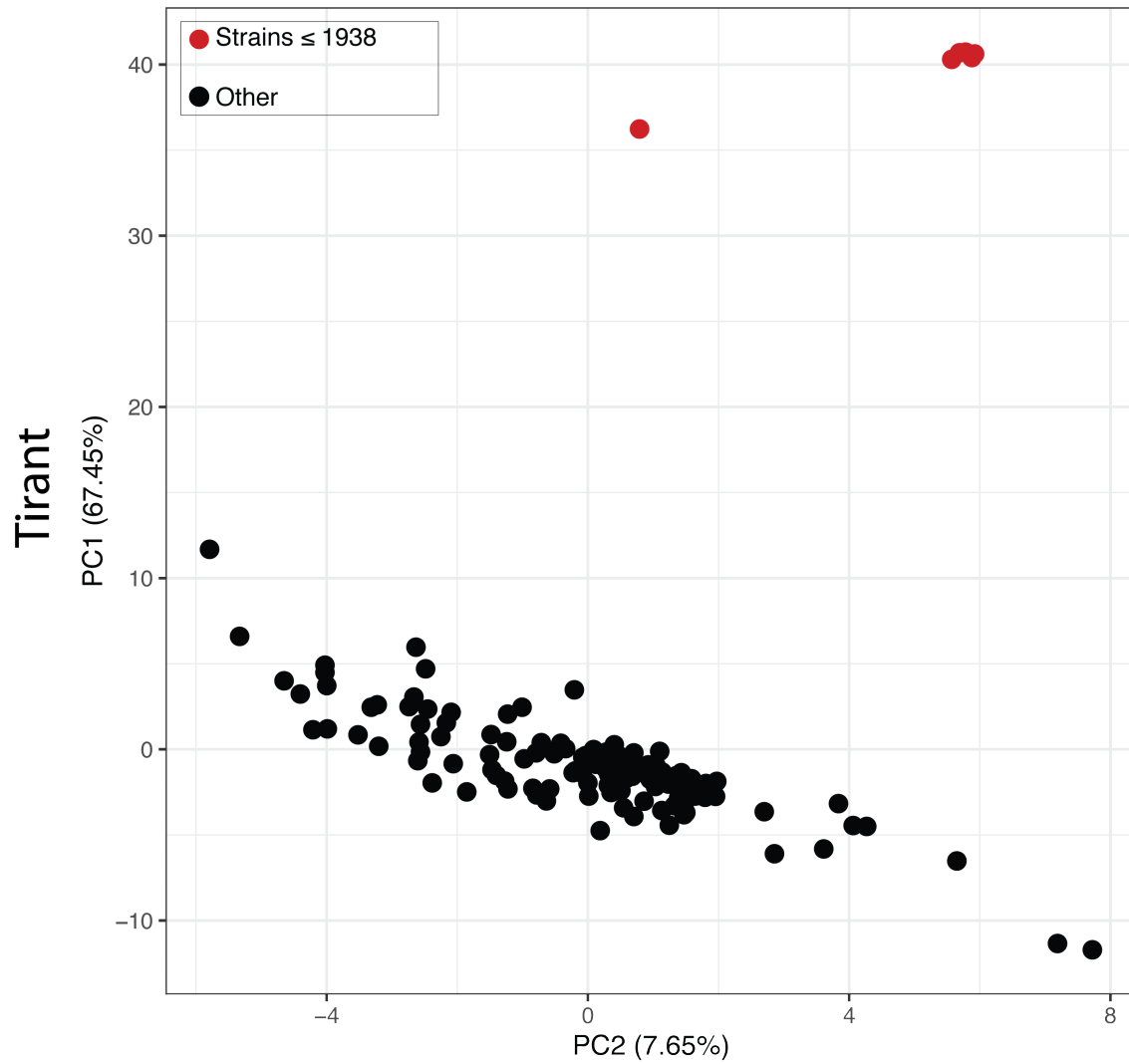


Figure 10: PCA based on the allele frequencies of SNPs in Tirant for different *D. melanogaster* strains and population samples. Strains sampled before or at 1938 form a separate cluster (due to the absence of canonical Tirant insrtions). In addition to the strains shown in the manuscript (fig. 3) we used DGRP, DrosEU and Dros-RTEC lines well as lines sampled by Bergland et al. (2014) and Lack et al. (2015) (see supplementary table 1 for details).

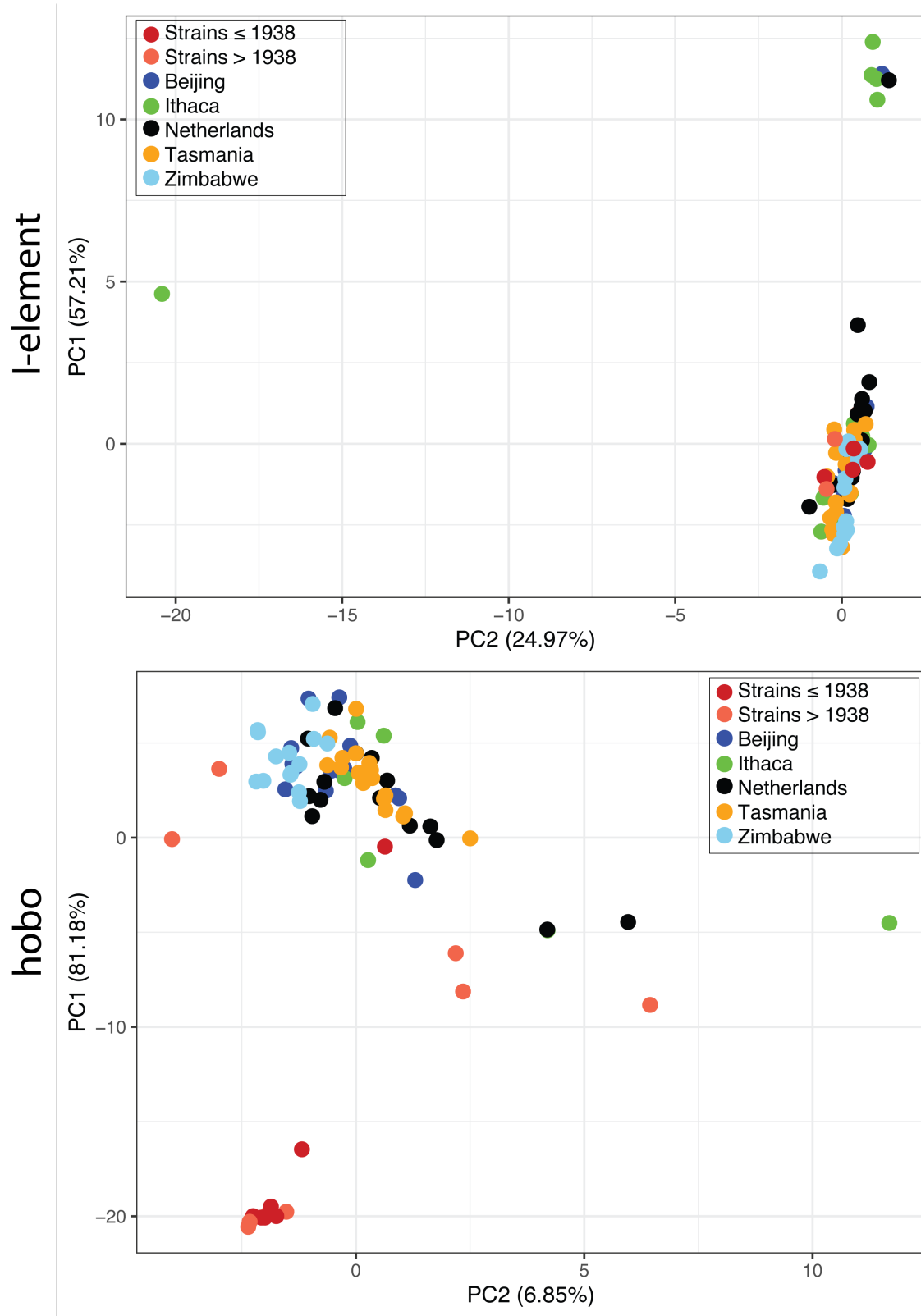


Figure 11: PCA based on the allele frequencies of SNPs in the I-element and hobo. Tasmanian populations cluster with strains from other geographic regions for both TEs<sub>12</sub>

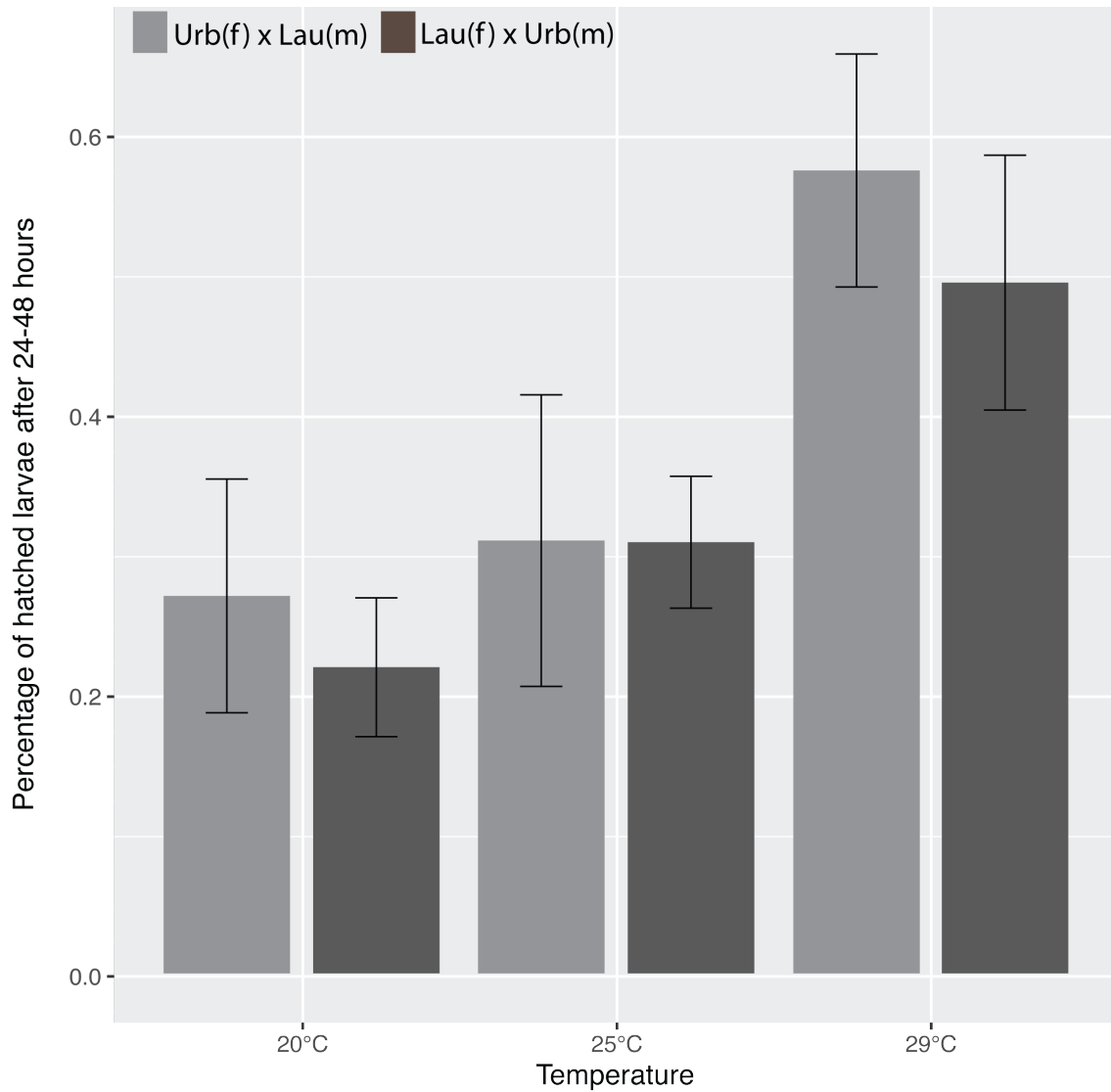


Figure 12: Fraction of hatched F2 eggs for reciprocal crosses between a strain having recent Tirant insertions (Urb: Urbana-S) and a strain not having recent Tirant insertions (Lau: Lausanne-S). Crosses were performed at three temperatures and three replicates were used for each cross. We did not detect significant differences in the abundance of hatched F2 eggs between the reciprocal crosses (Wilcoxon rank sum test;  $p_{20} = 0.4$ ,  $p_{25} = 0.7$ ,  $p_{29} = 0.4$ ). Differences in absolute number of hatched eggs between the temperatures are caused by an increased hatching rate at higher temperatures; m males, f females. .



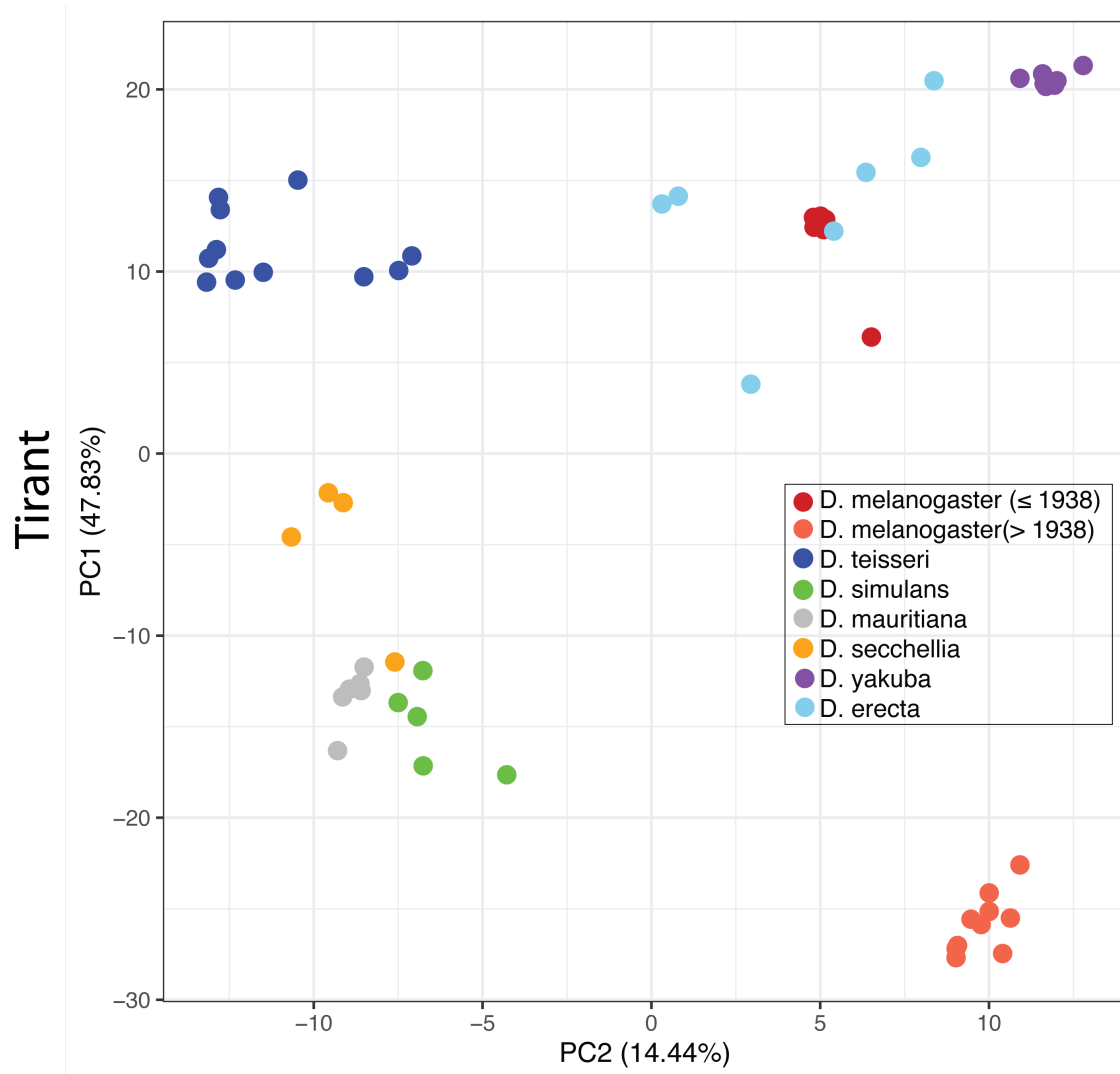


Figure 14: PCA based on the allele frequencies of SNPs in Tirant. Data are shown for several lines of different species from the *Drosophila melanogaster* species subgroup. Note that old lab strains of *D. melanogaster* cluster with *D. erecta* while more recently collected strain are closest to *D. simulans*.

## **Supplementary tables**



Table 1: Overview of the abundance of Tirant, I-element, hobo, and P-element sequences in different *D. melanogaster* strains. Strains are ordered by their estimated collection date. For each family and strain, we classified the TE content into three distinct categories: 'red' absence of any TE sequence, 'yellow' solely degraded TE sequences are present, 'green' non-degraded sequences, with a high similarity to the consensus sequence are present. Numbers in brackets represent the average coverage normalized to single-copy genes ( $\approx$  TE copy numbers per haploid genome). Strains sequenced in this work are marked by a star (\*). † latest possible collection date was inferred from death of C. Bridges (1938), who collected the strain (Lindsley and Grell, 1968). coll. date collection date, FlyBase <https://flybase.org/>, NDSSC <https://www.drosophilaspecies.com/>

strain	coll. date	Tirant	I-ele.	hobo	P-ele.	location	source
Oregon-R	1925	~ (0.6)	~ (19.9)	~ (5.8)	- (0)	Oregon, USA	Lindsley and Grell 1968
Canton-S	1935	~ (0.9)	~ (19.5)	~ (5.9)	- (0)	Ohio, USA	Anxolabéhère <i>et al.</i> 1988
Samarkand	1936	~ (0.7)	~ (17.6)	~ (4.3)	- (0)	Samarkand, Uzbekistan	Lindsley and Grell 1968
Crimea*	1936	~ (0.5)	~ (18.5)	~ (4.5)	- (0)	Crimea, Eastern Europe	Anxolabéhère <i>et al.</i> 1988
Lausanne-S*	1938	~ (0.5)	~ (18.8)	~ (3.7)	- (0)	Wisconsin, USA	Lindsley and Grell 1968
Swedish-C*	<1938(1923)	~ (0.6)	+ (37.5)	+ (27.7)	~ (0.3)	Stockholm, Swe- den	Lindsley and Grell 1968
Urbana-S*	<1938	+ (2.4)	~ (21.9)	~ (5.5)	- (0)	Illinois, USA	Bridges†, (Lindsley and Grell, 1968)
Berlin-K*	<1950	+ (6.6)	+ (32.1)	~ (3.6)	- (0)	Berlin, Germany	Ruebenbauer <i>et al.</i> 2008
Hikone-R*	1950-59	+ (6.2)	~ (17.9)	~ (5.8)	- (0)	Japan	Galindo <i>et al.</i> 1995
Florida-9*	<1952	+ (7.6)	+ (31.4)	+ (26.9)	+ (77.3)	Florida, USA	Lindsley and Grell 1968
Dmel68*	1954	+ (14.1)	+ (40.6)	+ (16.2)	- (0)	Israel	NDSSC
B1(BER1)	1954	+ (15.8)	+ (30.0)	~ (2.6)	- (0)	Bermuda	FlyBase
A3(BS1)	1954	+ (13.7)	+ (23.3)	~ (2.4)	- (0)	Barcelona, Spain	FlyBase
B2(CA1)	1954	+ (8.2)	+ (33.7)	~ (1.3)	- (0)	Capetown, South Africa	FlyBase
B3(QI2)	1954	+ (10.2)	+ (30.3)	+ (11.1)	- (0)	Israel	FlyBase
A2(BOG1)	1962	+ (18.2)	+ (40.7)	+ (19.5)	- (0)	Bogota, Colom- bia	FlyBase
A4(KSA2)	1963	+ (3.0)	+ (29.4)	~ (1.3)	- (0)	Koriba Dam, Zim- babwe	FlyBase
B4(RVC3)	1963	+ (15.8)	+ (44.1)	+ (64.7)	- (0)	California, USA	FlyBase
A5(VAG1)	1965	+ (7.7)	+ (31.4)	+ (10.9)	- (0)	Athens, Greece	FlyBase
A6(wild5B)	1966	+ (15.2)	+ (31.3)	+ (84.2)	- (0)	Georgia, USA	FlyBase
Harwich	1967	+ (5.5)	+ (55.2)	+ (12.9)	+ (60.1)	Massachusetts, USA	NDSSC
Pi2*	1975	+ (8.8)	+ (31.7)	+ (98.9)	+ (39.7)	N.A.	Engels 1979
w1118*	<1987	+ (5.2)	+ (40.5)	+ (35.5)	- (0)	N.A.	first used by Black <i>et al.</i> 1987
AB8 (Sam;ry506)	N.A.	~ (0.5)	~ (28.1)	~ (2.5)	- (0)	N.A.	N.A.
wk*	N.A.	+ (10.4)	~ (18.0)	~ (4.7)	- (0)	N.A.	N.A.
Amherst-3*	N.A.	+ (11.8)	+ (35.2)	~ (4.7)	- (0)	Massachusetts, USA	N.A.
Iso1	N.A.	+ (20.9)	+ (32.0)	+ (28.6)	- (0)	N.A.	N.A.

Table 2: Position in Tirant (pos.), reference allele (ref.) and frequency of the reference allele for SNPs with notable allele frequency differences between Iso-1 and natural populations (GDL, DrosEU and Dros-RTEC). For an overview of all SNPs in Iso-1 and some GDL lines see supplementary fig. 8.

pos	refbase	Iso-1 (SRR1663590)	GDL-Other (SRR1663540)	GDL-Other (SRR1663560)	GDL-Other (SRR1663600)	DrosEU (SRR5647729)	DrosEU (SRR5647776)	Dros-RTEC (SRR3590550)	Dros-RTEC (SRR3939104)
230	C	0.948	0.013	0	0	0.011	0	0	0
2485	A	0.792	0	0	0	0.046	0.036	0.038	0.057
2872	G	0.937	0	0	0	0.054	0.027	0.028	0.073
3014	A	0.891	0	0.167	0	0.032	0.023	0.033	0.05
5793	C	0.908	0	0	0	0	0	0	0
5925	A	0.931	0.134	0.152	0.227	0.131	0.14	0.042	0.103
6045	G	0.882	0	0	0	0	0.012	0.053	0.047
8337	C	0.931	0	0	0	0.004	0	0	0.003

Table 3: Position in Tirant (pos), reference allele (ref.) and frequency of the reference allele for SNPs with notable allele frequency differences between populations from Tasmania and other geographic locations (GDL). For an overview of all SNPs in Tasmanian and non-Tasmanian populations see supplementary fig. 9.

pos	ref.	GDL-Tasm. (SRR1663590)	GDL-Tasm. (SRR1663591)	GDL-Tasm. (SRR1663592)	GDL-Other (SRR1663540)	GDL-Other (SRR1663560)	GDL-Other (SRR1663600)
275	T	0.071	0.04	0.097	0.93	0.336	0.873
3921	G	0.075	0.111	0.084	0.68	0.824	0.791
5091	T	0.396	0.682	0.923	1	1	1
6757	A	0.932	1	0.993	0.734	0.366	0.439
8382	T	0.068	0.066	0.137	0.871	0.354	0.788

Table 4: Number of dysgenic and not-dysgenic ovaries in the F1 of reciprocal crosses between a strain having recent Tirant insertions (Urbana-S) and a strain not having recent Tirant insertions (Lausanne-S). Crosses were performed at two temperatures and three replicates were used for each cross. The direction of the cross had no significant influence on the fraction of dysgenic ovaries at both temperatures (Cochran–Mantel–Haenszel test;  $p_{25} = 0.736$ ,  $p_{29} = 0.742$ ).

female	male	temp.	rep.	not-dysgenic	dysgenic
Urbana-S	Lausanne-S	25°C	1	17	0
Urbana-S	Lausanne-S	25°C	2	13	0
Urbana-S	Lausanne-S	25°C	3	13	0
Urbana-S	Lausanne-S	29°C	1	12	1
Urbana-S	Lausanne-S	29°C	2	18	0
Urbana-S	Lausanne-S	29°C	3	18	0
Lausanne-S	Urbana-S	25°C	1	13	0
Lausanne-S	Urbana-S	25°C	2	15	0
Lausanne-S	Urbana-S	25°C	3	15	0
Lausanne-S	Urbana-S	29°C	1	18	0
Lausanne-S	Urbana-S	29°C	2	11	0
Lausanne-S	Urbana-S	29°C	3	15	0

Table 5: Publicly available short read data used in this work

Mackay et al. (2012)	SRR018294, SRR018305, SRR018517, SRR018521, SRR018574, SRR018580, SRR018582, SRR018593, SRR018601, SRR834538, SRR834536, SRR834540, SRR834542, SRR834550, SRR834548, SRR048925, SRR834549, SRR834524, SRR834525, SRR834528, SRR834529, SRR834531, SRR834532, SRR834533, SRR834534, SRR834535, SRR834504, SRR834530, SRR834510, SRR834505, SRR834506, SRR834507, SRR834513, SRR834515, SRR834516, SRR834544, SRR834539, SRR834554, SRR834520, SRR835025, SRR835023, SRR835028, SRR835026, SRR835029, SRR051592, SRR051594, SRR835024, SRR835027, SRR835031, SRR835032, SRR835033, SRR835037, SRR835035, SRR835036, SRR835038, SRR835040, SRR835041, SRR835042, SRR835045, SRR835046, SRR051896, SRR835081, SRR835101, SRR051905, SRR835082, SRR835083, SRR835085, SRR835099, SRR835088, SRR835089, SRR835091, SRR835092, SRR835093, SRR835094, SRR835095, SRR835051, SRR835052, SRR835057, SRR835053, SRR835064, SRR835065, SRR835066, SRR835070, SRR835067, SRR835068, SRR835071, SRR835074, SRR835075, SRR835076, SRR835078, SRR835080, SRR835084, SRR835069, SRR835073, SRR834546, SRR835103, SRR060062, SRR835100, SRR060098, SRR835090, SRR835058, SRR834537, SRR834543, SRR835030, SRR835049, SRR060821, SRR835072, SRR834541, SRR835096, SRR835054, SRR834521, SRR834517, SRR834545, SRR835050, SRR933564, SRR835223, SRR835228, SRR835236, SRR835242, SRR835252, SRR835256, SRR933571, SRR933572, SRR933575, SRR933577, SRR835333, SRR933578, SRR933580, SRR933582, SRR933585, SRR933586, SRR933587, SRR933588, SRR933591, SRR933592, SRR933593, SRR933594, SRR933595, SRR933596, SRR933597, SRR933598, SRR933600, SRR835247, SRR933563, SRR932121, SRR835221, SRR933566, SRR933569, SRR933570, SRR933573, SRR835331, SRR835326, SRR933581, SRR933589, SRR933593, SRR933594, SRR835341, SRR835345, SRR933599, SRR835349, SRR835329, SRR933583, SRR933592, SRR933601, SRR933579, SRR835343, SRR835399, SRR189040, SRR1686796, SRR1688222, SRR189389, SRR306623, SRR306611, SRR203502, SRR306616, SRR306618
Lack et al. (2015)	SRR1525685, SRR1525694, SRR1525695, SRR1525696, SRR1525697, SRR1525698, SRR1525699, SRR1525768, SRR1525769, SRR1525770, SRR1525771, SRR1525772, SRR1525773, SRR1525774, SRR2006283
Bergland et al. (2014)	SRR5647729, SRR5647730, SRR5647731, SRR5647732, SRR5647733, SRR5647734, SRR5647735, SRR5647736, SRR5647737, SRR5647738, SRR5647739, SRR5647740, SRR5647741, SRR5647742, SRR5647743, SRR5647744, SRR5647745, SRR5647746, SRR5647747, SRR5647748, SRR5647749, SRR5647750, SRR5647751, SRR5647752, SRR5647753, SRR5647754, SRR5647755, SRR5647756, SRR5647757, SRR5647758, SRR5647759, SRR5647760, SRR5647761, SRR5647762, SRR5647763, SRR5647764, SRR5647765, SRR5647766, SRR5647767, SRR5647768, SRR5647769, SRR5647770, SRR5647771, SRR5647772, SRR5647773, SRR5647774, SRR5647775, SRR5647776
Kapun et al. (2018)	SRR3590550, SRR3590551, SRR3590554, SRR3590555, SRR3590556, SRR3590557, SRR3590558, SRR3590559, SRR3590560, SRR3590561, SRR3590562, SRR3590563, SRR3939042, SRR3939043, SRR3939044, SRR3939045, SRR3939046, SRR3939047, SRR3939048, SRR3939049, SRR3939050, SRR3939051, SRR3939052, SRR3939054, SRR3939056, SRR3939057, SRR3939058, SRR3939059, SRR3939076, SRR3939077, SRR3939078, SRR3939080, SRR3939081, SRR3939082, SRR3939083, SRR3939084, SRR3939085, SRR3939086, SRR3939087, SRR3939088, SRR3939089, SRR3939091, SRR3939092, SRR3939093, SRR3939094, SRR3939095, SRR3939096, SRR3939097, SRR3939098, SRR3939099, SRR3939100, SRR3939101, SRR3939102, SRR3939103, SRR3939104, SRR8061818, SRR8061819
Machado et al. (2019)	SRR1663528, SRR1663529, SRR1663530, SRR1663531, SRR1663532, SRR1663533, SRR1663534, SRR1663535, SRR1663536, SRR1663537, SRR1663538, SRR1663539, SRR1663540, SRR1663541, SRR1663542, SRR1663543, SRR1663544, SRR1663545, SRR1663546, SRR1663547, SRR1663548, SRR1663549, SRR1663550, SRR1663551, SRR1663552, SRR1663553, SRR1663554, SRR1663555, SRR1663556, SRR1663557, SRR1663558, SRR1663559, SRR1663560, SRR1663561, SRR1663562, SRR1663563, SRR1663564, SRR1663565, SRR1663566, SRR1663567, SRR1663568, SRR1663569, SRR1663570, SRR1663571, SRR1663572, SRR1663573, SRR1663574, SRR1663575, SRR1663576, SRR1663577, SRR1663578, SRR1663579, SRR1663580, SRR1663581, SRR1663582, SRR1663583, SRR1663584, SRR1663585, SRR1663586, SRR1663587, SRR1663588, SRR1663589, SRR1663590, SRR1663591, SRR1663592, SRR1663593, SRR1663594, SRR1663595, SRR1663596, SRR1663597, SRR1663598, SRR1663599, SRR1663600, SRR1663601, SRR1663602, SRR1663603, SRR1663604, SRR1663605, SRR1663606, SRR1663607, SRR1663608, SRR1663609, SRR1663610, SRR1663611
Grenier et al. (2015)	SRR11460805, SRR11460802, SRR11460799
Wierzbicki et al. (2020)	SRR5851905, SRR5851906
Jakšić et al. (2017)	SRR1560275
Garrigan et al. (2014)	SRR1774232
Turissini et al. (2015)	SRR3113258
Hill et al. (2016)	SRR5860571, SRR5860572, SRR5860576, SRR5860577, SRR5860582
Schrider et al. (2018)	SRR5860615, SRR5860617, SRR5860619, SRR5860620, SRR5860621, SRR5860622
Lanno et al. (2019)	SRR6425993
Miller et al. (2018)	SRR6714726
Kang et al. (2019)	SRR7698174
Melvin et al. (2018)	SRR8834567, SRR8834568, SRR8834569
Meany et al. (2019)	SRR8840592
Cooper et al. (2019)	SRR9030358, SRR9030360
Garrigan et al. (2012)	SRR9699990, SRR9700000
Rogers et al. (2014)	SRR9700024, SRR9700028, SRR9700036, SRR9700038, SRR9700041, SRR9700049, SRR9700063
Stewart and Rogers (2019)	

## References

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