

Supplementary Table 1: P-element polymorphism in hot evolved populations. The coverage (cov) and the fraction of reads supporting the minor allele (%) are provided for all three replicates (rep) during the invasion (generations are at the top). SNPs that occur in more than one replicate are shown in bold. Only SNPs where the minor allele has a minimum count of 20 (summed over all generations) are shown. pos. position of polymorphism within the P-element, refc. reference character [of the *D. simulans* P-element; see Kofler et al. (2015a)], ma. major allele, mi. minor allele

rep	pos	refc	ma	mi	0		10		20		30		40		50		60	
					cov	%	cov	%	cov	%	cov	%	cov	%	cov	%	cov	%
1	434	A	A	G	92	0.0	133	0.8	1024	2.1	727	0.7	1046	0.8	519	0.2	893	0.8
1	529	A	A	G	78	1.3	127	1.6	944	1.8	576	2.3	941	2.4	423	1.4	693	4.2
1	647	G	G	T	69	0.0	115	0.0	966	1.3	527	2.5	867	2.7	429	1.6	646	2.3
1	799	T	T	A	93	0.0	155	0.6	1030	0.0	661	0.2	975	0.7	553	0.9	869	1.0
1	800	C	C	T	91	0.0	150	0.7	1031	0.0	660	0.2	978	0.6	554	0.9	876	1.1
1	802	G	G	A	94	0.0	153	0.7	1037	0.0	649	0.2	970	0.5	561	0.9	879	1.1
1	845	T	T	A	80	0.0	121	0.0	1034	0.4	611	0.7	995	1.2	485	1.4	818	1.5
1	846	A	A	C	79	0.0	122	0.0	1027	0.4	616	0.6	996	1.2	482	1.7	798	1.5
1	1462	A	A	T	69	0.0	134	0.0	1015	0.3	721	0.6	1007	0.8	529	0.4	847	0.6
1	2330	C	C	T	70	0.0	137	0.0	1020	0.8	722	1.4	1007	2.0	512	1.6	879	0.8
1	2557	G	G	A	73	0.0	166	1.2	1102	4.4	886	2.6	1096	2.8	649	4.0	1060	3.9
3	425	T	T	C	120	1.7	192	0.0	1074	1.2	569	1.9	1053	2.2	460	2.2	455	2.0
3	529	A	A	G	116	0.0	164	2.4	1076	3.7	501	4.8	1016	3.4	459	4.1	434	1.4
3	1667	A	A	T	111	0.0	182	0.0	1047	1.2	519	2.7	993	1.6	449	2.2	491	3.7
3	1668	C	C	T	112	0.0	186	0.0	1046	1.2	522	2.7	997	1.5	452	2.2	498	3.8
3	1670	G	G	T	115	0.0	184	0.0	1050	1.2	535	2.6	996	1.5	455	2.2	501	3.8
3	1671	A	A	T	115	0.0	182	0.0	1046	1.2	536	2.6	998	1.5	453	2.2	492	3.9
3	1672	A	A	T	119	0.0	184	0.0	1040	1.3	542	2.6	1000	1.5	462	2.2	491	3.7
3	2242	A	A	G	102	0.0	148	0.0	1050	0.5	596	1.0	1015	0.6	450	0.7	447	1.3
3	2244	A	A	T	103	0.0	147	0.0	1074	0.5	591	1.0	1022	0.7	455	0.7	449	1.3
3	2258	T	T	A	102	0.0	152	2.6	1078	3.7	632	5.9	1087	7.3	460	5.4	445	7.2
3	2259	C	C	T	104	0.0	150	2.7	1094	3.6	634	6.2	1096	7.3	459	5.4	444	6.5
3	2304	C	C	A	116	0.0	171	2.3	1082	5.1	626	8.8	1060	8.8	500	10.0	507	12.4
3	2476	C	C	A	113	0.0	160	1.9	1088	5.1	642	7.3	1106	8.4	526	8.4	508	9.1
5	705	T	T	A	146	0.0	213	0.0	1075	0.7	385	1.0	886	0.8	597	1.0	466	0.2
5	708	T	T	A	149	0.0	213	0.0	1077	0.6	401	1.0	899	0.9	611	1.1	474	0.2
5	2283	G	G	A	143	0.0	283	0.0	1078	0.6	418	1.0	1005	0.8	678	0.9	585	0.2
5	2284	T	T	A	141	0.0	283	0.0	1072	0.7	421	1.0	1007	0.8	676	1.0	590	0.2
5	2692	T	T	C	146	0.0	255	0.0	1108	0.2	483	0.8	1015	1.0	700	0.4	576	0.3

Supplementary results 1; Variation with the P-element

As base substitutions within a TE can alter the activity and the invasion dynamics of a TE (Beall et al., 2002; Robillard et al., 2016) we investigated the segregating sites within the P-element under both conditions. The P-element in our experimental populations has the same sequence as the *D. simulans* P-element (Kofler et al., 2015a) (supplementary tables 1, 2). Only few base substitutions emerged during the experiment (supplementary tables 1,2). Hence the sequence of the P-element did not evolve during the invasion.

Supplementary Table 2: P-element polymorphism in the cold evolved populations. The coverage (cov) and the fraction of reads supporting the minor allele (%) are provided for all three replicates (rep) during the invasion (generations are at the top). SNPs that occur in more than one replicate are shown in bold. The high concordance between replicate 1 and 3 is due to alignment problems next to breakpoints of internally deleted P-elements being present in both replicates (SNPs at positions 361, 1960, 1964, 1965, 1966, 1967, 2599, and 2601 were visually inspected in IGV). Only SNPs where the minor allele has a minimum count of 5 (sum over all generations) are shown. pos. position of polymorphism within the P-element, refc. reference character [of the *D. simulans* P-element; see Kofler et al. (2015a)], ma. major allele, mi. minor allele

rep	pos	refc	ma	mi	0		10		20		30		40	
					cov	%	cov	%	cov	%	cov	%	cov	%
1	361	C	C	A	79	0.0	48	2.1	85	2.4	92	3.3	113	1.8
1	529	A	A	G	78	1.3	50	8.0	82	7.3	86	7.0	132	8.3
1	1960	T	T	C	73	0.0	51	0.0	89	2.2	99	3.0	142	4.9
1	1964	A	A	G	75	0.0	51	0.0	99	7.1	105	5.7	161	12.4
1	1965	G	G	C	74	0.0	50	0.0	98	7.1	105	6.7	165	11.5
1	1966	A	A	T	75	0.0	48	0.0	97	7.2	105	6.7	164	11.0
1	1967	A	A	G	76	0.0	45	0.0	99	7.1	104	6.7	168	11.9
1	2557	G	G	A	73	0.0	55	3.6	118	4.2	127	3.9	215	2.8
1	2599	T	T	C	63	0.0	51	2.0	109	1.8	116	2.6	222	0.9
1	2601	T	T	C	63	0.0	51	2.0	112	1.8	115	2.6	218	0.9
3	361	C	C	A	106	0.0	90	0.0	93	3.2	195	2.1	442	7.7
3	434	A	A	G	130	0.0	74	1.4	103	2.9	169	1.2	450	1.6
3	1960	T	T	C	117	0.0	94	2.1	72	2.8	185	4.9	530	11.7
3	1964	A	A	G	121	0.0	96	3.1	72	6.9	192	7.8	639	21.1
3	1965	G	G	C	119	0.0	93	3.2	70	7.1	187	7.5	634	21.0
3	1966	A	A	T	121	0.0	94	3.2	71	7.0	195	7.7	641	20.7
3	1967	A	A	G	121	0.0	93	3.2	73	6.8	198	8.1	645	21.2
3	2599	T	T	C	95	0.0	96	0.0	95	3.2	215	2.3	749	4.4
3	2601	T	T	C	92	0.0	99	0.0	97	3.1	219	1.8	774	4.3
5	425	T	T	C	149	3.4	51	0.0	49	0.0	89	0.0	116	0.0