

Abundant genetic variability in *Drosophila simulans* for hybrid female lethality in interspecific crosses to *Drosophila melanogaster*

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Summary

Intrinsic postzygotic reproductive isolation is thought to result from the substitution of multiple harmless or beneficial genetic differences between species that are incidentally deleterious when combined in species hybrids, causing hybrid sterility or inviability. Genetic variability for hybrid sterility or inviability phenotypes is, however, rarely assessed in natural populations. Here, we assess variation for *Drosophila simulans*-encoded maternal factor(s) that cause lethality in *D. simulans*–*Drosophila melanogaster* F_1 hybrid females. First, we survey genetic variability in the strength of *D. simulans*-mediated maternal effect hybrid lethality among 37 geographic and laboratory isolates. We find abundant variability in the strength of maternal effect hybrid lethality, ranging from complete lethality to none. Second, we assess maternal effect hybrid lethality for a subset of wild isolates made heterozygous with two so-called hybrid rescue strains. The results suggest that the *D. simulans* maternal effect hybrid lethality involves a diversity of alleles and/or multiple loci.

1. Introduction

Speciation involves, among other reproductive barriers, the evolution of intrinsic postzygotic isolation (Coyne & Orr, 2004). The modern study of postzygotic reproductive barriers began with Sturtevant's (1920, 1921) analysis of interspecific genetic incompatibilities between *Drosophila melanogaster* and *Drosophila simulans* (Provine, 1991; Barbash, 2010). One direction of the cross, *D. melanogaster* females \times *D. simulans* males, produces sterile adult hybrid females and inviable hybrid males that die at the larval–pupal transition (Sturtevant, 1920; Hadorn, 1961). The genetic basis of this hybrid male lethality is now well worked out. In an F_1 hybrid male genetic background, the wild-type *D. melanogaster* allele of the X-linked gene, *Hybrid male rescue* (*Hmr*), is incompatible with the wild-type *D. simulans* allele of the second chromosome gene, *Lethal hybrid rescue* (*Lhr*), causing lethality (Barbash *et al.*, 2003; Brideau *et al.*, 2006).

The other direction of the cross, *D. simulans* females \times *D. melanogaster* males, produces sterile

adult hybrid males, while inviable hybrid females typically die as embryos (Sturtevant, 1920, 1921; Hadorn, 1961). The genetic basis of hybrid lethality in this direction of the cross is, however, somewhat less well characterized. In F_1 hybrid female embryos, a *D. melanogaster* X-linked factor, *Zygotic hybrid rescue* (*Zhr*), is incompatible with an uncharacterized maternal factor from *D. simulans*, *maternal hybrid rescue* (*mhr*), causing lethality (Sawamura *et al.*, 1993b). *Zhr* appears to correspond to a *D. melanogaster*-specific block of heterochromatin rich in 359-bp satellite repeats at the pericentromeric base of the X chromosome (Sawamura & Yamamoto, 1997; Ferree & Barbash, 2009). In F_1 hybrid female embryos, the *D. melanogaster* X fails to condense properly when heterochromatin is first established, resulting in mitotic defects, lagging chromatin and missegregation (Ferree & Barbash, 2009).

Progress in determining the genetic basis of F_1 hybrid lethality between these species has relied almost exclusively on the characterization of so-called hybrid rescue mutations – compatible alleles at otherwise incompatible loci. The hybrid rescue mutations, *Hmr*¹ and *Lhr*¹, are rare within *D. melanogaster* and *D. simulans* populations (Watanabe, 1979; Hutter & Ashburner, 1987), respectively, and both alleles have

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large insertions in their 5'-regions and behave as partial loss of function mutations (Barbash *et al.*, 2003; Brideau *et al.*, 2006). Similarly, the hybrid rescue mutation *Zhr¹* corresponds to a rare deletion of a substantial part of the 359-bp satellite block (Sawamura *et al.*, 1993b; Ferree & Barbash, 2009). The wild-type alleles at all three loci cause hybrid lethality (Sawamura & Yamamoto, 1997; Barbash *et al.*, 2000; Orr & Irving, 2000; Ferree & Barbash, 2009). Unlike these hybrid rescue mutations, the *D. simulans* alleles conferring *F₁* hybrid female rescue are not rare, with four separate surveys finding that $\frac{1}{4}$, $\frac{2}{9}$, $\frac{2}{11}$, and, surprisingly, $\frac{10}{12}$ *D. simulans* strains tested yield some level of hybrid female rescue (Bocquet & Tsacas, 1969; Lachaise *et al.*, 1986; Sawamura *et al.*, 1993a; Orr, 1996). Hybrid female lethality, once considered the wild-type state (Sturtevant, 1921), appears to be rarer in more recent samples, raising the speculation that rescue has increased in frequency in *D. simulans* populations over the last century (Orr, 1996; Carracedo *et al.*, 2000).

Genetic and molecular characterization of the *D. simulans mhr* factor has lagged behind the other hybrid rescue mutations. One challenge is that sexual isolation in this direction of the cross is prohibitively strong – *D. simulans* females are reluctant to mate with *D. melanogaster* males (Sturtevant, 1920, 1929; Watanabe & Kawanishi, 1979) – making genetic mapping of the rescue phenotype difficult. Another problem, however, is that characterization of the *D. simulans* side of hybrid female rescue has yielded conflicting results. Sawamura *et al.* (1993a) inferred that hybrid female rescue depends on a single recessive maternal factor, *mhr*; Orr (1996) inferred that rescue depends on a dominant maternal factor(s), unnamed; and Carracedo *et al.* (2000) inferred that rescue depends on a dominant zygotically acting factor, *Simulans hybrid females rescue (Shfr)*. All three rescue phenotypes map to the second chromosome.

Here, we survey genetic variability for hybrid female lethality among 37 *D. simulans* strains, sampled from a variety of geographic localities, in crosses with *D. melanogaster* males. We also assay levels of maternal effect hybrid female lethality resulting from crosses between *D. simulans* females heterozygous for wild-type chromosomes over previously described rescuing strains and *D. melanogaster* males. Our findings support the notion that *F₁* hybrid female rescue is not rare. Furthermore, our findings suggest that hybrid rescue is not new, being common even in presumed ancestral populations of *D. simulans* from the Indian Ocean and Eastern Africa (Dean & Ballard, 2004; Baudry *et al.*, 2006; Schöfl & Schlötterer, 2006). Finally, our results suggest that the *D. simulans* side of hybrid female rescue differs from other described rescue mutations in potentially having a multigenic basis. Our results help explain inconsistencies

in previous reports and have implications for how to identify the factors involved.

2. Materials and methods

We used isofemale lines derived from flies collected in natural populations from Zimbabwe (kindly provided by Todd Schlenke, Emory University), Seychelles, Mayotte and Eilat (kindly provided by Catherine Montchamp-Moreau, CNRS Gif-sur-Yvette). Laboratory stocks are available at the UC San Diego *Drosophila* species Stock Center except for *net b py sd pm* and *y w f; mhr* (kindly provided by H. Allen Orr, University of Rochester).

To assess the variability in hybrid female viability, we first crossed females from each *D. simulans* isofemale line to *D. melanogaster* Ore-R males. For most crosses, 2–13 independent replicates per line successfully produced more than ten hybrid progeny depending on the strain (Table 1). For interspecific crosses involving the *D. simulans* isofemale lines ZH65, ZH7 and E7, only one cross each yielded progeny despite several dozens of crosses attempted, due to the strong prezygotic isolation in this direction of the species cross. In general, the low number of replicates for some crosses reflects the challenge of strong sexual isolation. For isofemale line S0, one replicate cross that produced fewer than ten hybrids was included.

We performed further crosses to determine if hybrid female rescue was maternal or zygotic and to determine the dominance of rescue. We used reciprocal crosses between two known rescue strains (*y w f; mhr* and separately, C167.4: see Table 1) and nine strains with weak to no rescue (Table 2). The resulting *F₁* *D. simulans* females, heterozygous for the rescue factor(s), were then crossed to *D. melanogaster* Ore-R males to assess their hybrid female viability phenotype. All of these interspecific crosses were replicated independently between 2 and 5 times.

All interspecific crosses were performed under constant light on cornmeal-agarose medium at 18 °C, as hybrid female viability is usually higher at low temperature for rescuing strains. Independent replicate crosses were set up with 5–10 virgin *D. simulans* females and 15–20 1-day old *D. melanogaster* Ore-R males, and parent flies were transferred to new vials every 3–4 days until all females had died. Statistical analyses were performed in R (R Development Core team).

3. Results and discussion

We crossed females from 25 Eastern African and Indian Ocean isofemale lines (Zimbabwe, Madagascar, Mayotte, Kenya and Seychelles) and 12 other lines (Israel, North America, New Caledonia,

Table 1. Hybrid female lethality varies among crosses between *D. simulans* isofemale line females and *D. melanogaster* Ore-R males

Strain	Origin	N ^a	Hybrid females	Hybrid males	Total	Relative female viability
<i>Eastern Africa and Indian Ocean</i>						
ZH12	Harare, Zimbabwe	3	0	23	23	0.000
ZH15	Harare, Zimbabwe	2	0	28	28	0.000
S0	Seychelles	2	0	15	15	0.000
S1	Seychelles	3	0	152	152	0.000
ZH17	Harare, Zimbabwe	4	4	177	181	0.023
MD199S ^b	Joffreville, Madagascar	4	2	86	88	0.023
ZH24	Harare, Zimbabwe	5	13	490	503	0.027
ZK244	Lake Kariba, Zimbabwe	3	5	126	131	0.040
ZH6	Harare, Zimbabwe	2	1	25	26	0.040
ZH3	Harare, Zimbabwe	3	7	152	159	0.046
Rf85	Mayotte	2	3	51	54	0.059
ZH9	Harare, Zimbabwe	4	6	99	105	0.061
ZH58	Harare, Zimbabwe	4	20	174	194	0.115
ZH28	Harare, Zimbabwe	2	4	33	37	0.121
ZK105	Lake Kariba, Zimbabwe	3	14	93	107	0.151
ZH5	Harare, Zimbabwe	4	33	172	205	0.192
ZH19	Harare, Zimbabwe	5	158	623	781	0.254
MD106TS ^b	Ansirabe, Madagascar	13	757	2291	3048	0.330
ZH26	Harare, Zimbabwe	4	22	55	77	0.400
ZH36-2	Harare, Zimbabwe	2	9	15	24	0.600
Rf83	Mayotte	4	210	269	479	0.781
C167.4 ^b	Nanyuki, Kenya	6	426	500	926	0.852
ZH65	Harare, Zimbabwe	1	5	5	10	1.000
ZH49	Harare, Zimbabwe	2	59	54	113	1.093
ZH7	Harare, Zimbabwe	1	6	5	11	1.200
<i>Other strains</i>						
NC48S ^b	Noumea, New Caledonia	5	0	230	230	0.000
sim 6 ^b	Winters, California	5	5	192	197	0.026
net b py sd pm	Unknown	2	5	173	178	0.029
Clr	Unknown	5	4	101	105	0.040
Cy ^{NC}	North Carolina	2	3	26	29	0.115
sw Clr	Unknown	4	7	53	60	0.132
sim 4 ^b	Winters, California	4	24	100	124	0.240
f; net pr; st e; ey	Unknown	3	85	271	356	0.314
E7	Eilat, Israel	1	3	7	10	0.429
BS6	Sao Tome	2	72	92	164	0.783
ywf; mhr	Unknown	11	886	906	1792	0.978
w501 ^b	North America	9	649	627	1276	1.035

^a Number of replicate crosses between each *D. simulans* isofemale line and Ore-R.

^b Strains with genome sequence data (Begun *et al.*, 2007).

Sao Tome and four lab strains) of *D. simulans* to males from the *D. melanogaster* laboratory stock Ore-R (Table 1). The average viability of hybrid females relative to hybrid males from these crosses was 0.31, ranging from complete hybrid female lethality to complete rescue. Relative hybrid female viability from the Eastern African strains (mean = 0.314) does not differ significantly from the other strains (mean = 0.344; Kruskal–Wallis rank test: $\chi^2_1 = 0.254$, $P = 0.615$). For the entire sample of 37 *D. simulans* strains, 15 (40%) show substantial hybrid female rescue (arbitrarily defined as relative viability > 0.20). The frequency of such hybrid female rescue in Eastern Africa

($\frac{9}{25}$) is similar to that outside of Eastern Africa ($\frac{6}{12}$; Fisher's exact $P = 0.488$). These crosses show that hybrid female rescue is common worldwide. Eastern African populations, especially those in Madagascar (Dean & Ballard, 2004) – frequency of rescue in Madagascar/Mayotte/Kenya is $\frac{3}{5}$ – are believed to represent the ancestral range of *D. simulans* with new world populations having a relatively recent origin (Baudry *et al.*, 2006; Schöfl & Schlötterer, 2006). The similarly high frequency of hybrid female rescue in ancestral Eastern African and other populations of *D. simulans* thus argues against its sudden recent increase in frequency.

Table 2. Hybrid female rescue from crosses between heterozygous rescue/wild *D. simulans* females and *D. melanogaster* males

Wild strain	<i>mhr/+^a</i>				<i>+_i/mhr^d</i>				<i>C167.4/+^e</i>				<i>+_i/C167.4^e</i>			
	<i>+_i+^b</i>	N ^c	Hybrid females	Hybrid males	Relative female viability	Hybrid females	Hybrid males	Relative female viability	N ^c	Hybrid females	Hybrid males	Relative female viability	N ^c	Hybrid females	Hybrid males	Relative female viability
1 <i>f; net pr; st e; ey</i>	0.314	3	248	235	1.055	158	168	0.940	2	99	93	1.065	3	283	286	0.990
2 <i>net b py sd pm</i>	0.029	4	99	189	0.524	21	73	0.288	3	82	241	0.340	2	98	241	0.407
3 <i>sw Clr</i>	0.132	4	244	234	1.043	274	309	0.887	3	79	104	0.760	3	195	170	1.147
4 <i>Cy^{NC}</i>	0.115	3	133	152	0.875	67	88	0.761	3	10	28	0.357	—	—	—	—
5 <i>NC48S</i>	0.000	3	168	412	0.408	224	742	0.302	3	152	1143	0.133	3	94	666	0.141
6 <i>S1</i>	0.000	2	14	252	0.056	83	815	0.102	3	4	738	0.005	4	13	510	0.025
7 <i>ZH3</i>	0.046	5	363	479	0.758	218	349	0.625	5	339	793	0.427	3	58	259	0.224
8 <i>ZH17</i>	0.023	3	144	134	1.075	663	625	1.061	4	65	120	0.542	4	150	575	0.261
9 <i>ZH24</i>	0.027	3	74	128	0.578	254	625	0.406	4	232	563	0.412	4	195	437	0.446
Mean	0.076				0.708			0.597				0.449				0.455

^a Genetic background of heterozygous *D. simulans* females between a rescue strain (*y w f; mhr* or *C167.4*) and the *i*th non-rescue strain (1–9), resulting from a female/male cross.
^b Relative viability of females from homozygous non-rescue *D. simulans* females crossed to *D. melanogaster* Ore-R males (data from Table 1).
^c Number of replicate crosses between heterozygous *D. simulans* and Ore-R.

To investigate the inheritance of hybrid female rescue, we made reciprocal crosses between the rescuing strains *y w f; mhr* and separately, *C167.4*, with five non-African strains (four lab strains and one from New Caledonia) and four African strains (three Zimbabwean strains and one from the Seychelles) showing moderate to no rescue in the initial crosses (Table 2). We then crossed the resulting heterozygous *D. simulans* female progeny to *D. melanogaster* Ore-R males. When we compare hybrid female rescue from heterozygous *D. simulans* females generated from reciprocal crosses (i.e. *mhr/+_i* vs. *+_i/mhr* and *C167.4/+* vs. *+_i/C167.4* in Table 2), the strength of rescue was highly correlated (Spearman’s rank correlation for *mhr* crosses: $\rho=0.82$, $P=0.007$; for *C167.4* crosses: $\rho=0.89$, $P=0.003$; see Table 2), demonstrating that hybrid female rescue does not depend on the cytoplasm.

If hybrid female rescue is zygotic and dominant, then heterozygous *D. simulans* females will transmit the rescue allele to half of their hybrid progeny, resulting in ~ 50 % of the hybrid rescue effect produced by the homozygous rescuing strain. If, however, hybrid female rescue is maternal and dominant (recessive), then heterozygous *D. simulans* females should rescue 100 % (0 %) of the hybrid rescue produced by the homozygous rescuing strain. As Table 2 shows the pattern of rescue is complex. In some crosses, it seems that *y w f; mhr* rescue is maternal and recessive (Table 2, line 6, *S1* strain: the relative hybrid female viability is close to 0), as originally reported by Sawamura *et al.* (1993a). In other crosses, however, it seems that *y w f; mhr* rescue is maternal and dominant (Table 2, line 8, *ZH17* strain: the relative hybrid female viability is close to 1). Still other crosses produce intermediate levels of rescue (Table 2, line 5, *NC48S*), making it difficult to formally distinguish zygotic vs. partially dominant maternal hybrid rescue. However, as the *y w f; mhr* strain clearly has a maternal hybrid rescue effect (Table 2, lines 6 and 8), it seems most parsimonious to infer that its dominance depends on genetic background. Similar results hold for the hybrid female rescue of *C167.4* (Table 2).

In the original crosses, homozygous *y w f; mhr* females produced a slightly, albeit not quite significantly, stronger hybrid rescue than *C167.4* females (Table 1; Fisher’s exact $P=0.097$). The rescue produced by both strains when heterozygous was correspondingly different: the average rescue of *y w f; mhr* when heterozygous was higher than that of *C167.4* when heterozygous. These findings suggest either that *y w f; mhr* and *C167.4* have rescue alleles at the same locus with different strengths and/or dominance or, alternatively, that rescue is affected by multiple segregating factors that differ between the strains.

The data in Table 2 strongly suggest that hybrid female rescue involves more than a single locus. In

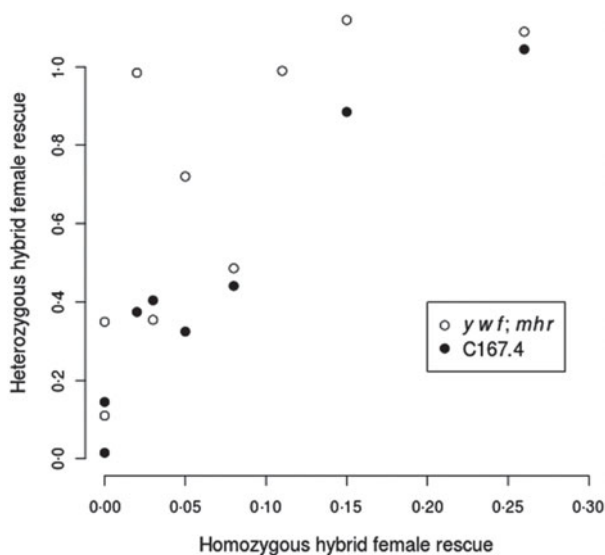


Fig. 1. Homozygous vs. heterozygous hybrid female rescue. Correlation between hybrid female rescue of non-rescue *D. simulans* strains and hybrid female rescue (averaged between reciprocal crosses) from heterozygous non-rescue/rescue strain (*y wf; mhr* (white circles) or C167.4 (black circles): data from Table 2). All relative viabilities of hybrid females vs. males result from crosses with *D. melanogaster* Ore-R males.

particular, not only do *y wf; mhr* and C167.4 have different average rescue effects, but the strength of rescue in heterozygous state depends on the wild strains to which they were crossed. For example, strains showing no rescue when homozygous tended to produce the weakest rescue when heterozygous with *y wf; mhr* or C167.4 (e.g. Table 2, line 6), whereas strains showing moderate rescue when homozygous tended to produce the strongest rescue when heterozygous with *y wf; mhr* or C167.4 (e.g. Table 2, line 1). Indeed, there is a strong correlation between the level of rescue from homozygous *D. simulans* strains and the level of rescue when the same strains are heterozygous with *y wf; mhr* or C167.4 (Spearman's rank correlation for *mhr* crosses: $\rho = 0.86$, $P = 0.002$; for C167.4 crosses: $\rho = 0.92$, $P = 0.001$; see Fig. 1). This observation implies either that virtually every strain bears a different allele at a single hybrid rescue locus or, more plausibly, that the basis for hybrid female rescue is multigenic.

4. Conclusions

Our findings help explain apparent discrepancies among previous reports. In particular, our results are consistent with both Sawamura *et al.* (1993a) and Orr (1996), showing that *D. simulans* maternal effect hybrid rescue can appear either recessive (Sawamura *et al.* 1993a) or dominant (Orr, 1996) depending on genetic background. We also note that the previous

finding that the *D. simulans* rescue mutation(s), *Shfr*, yields only partial (~50%) rescue when heterozygous is consistent not only with zygotic rescue, as inferred by Carracedo *et al.* (2000), but also with incompletely dominant maternal rescue, as observed here (e.g., Table 2, line 5).

The previous findings that *mhr*, Orr's (1996) unnamed rescue mutation(s), and *Shfr* all map to the second chromosome may, at face value, seem difficult to reconcile with the notion that maternal hybrid rescue is multigenic. After all, why should all of the multiple factors that contribute to hybrid rescue map to a single chromosome? While the previous studies showed that chromosome 2 had the largest effect on hybrid rescue, they did not demonstrate that all of the hybrid rescue phenotype could be explained by chromosome 2. Indeed, both Sawamura *et al.* (1993a) and Orr (1996) note that while chromosome 2 has the largest effect on rescue, their genetic analyses cannot exclude a role for other factors. We suggest that, indeed, multiple genetic factors contribute to hybrid female rescue, a fact that distinguishes *D. simulans* maternal hybrid female rescue from the other three rescue mutations, *Hmr*, *Lhr* and *Zhr* and a fact that will complicate future genetic mapping efforts.

Whatever the number of genes involved in hybrid female lethality, the abundant quantitative genetic variability described here may be useful in determining the molecular basis of hybrid lethality and, moreover, informative about the evolutionary history of the factors involved. First, as the incompatible locus *Zhr* on the *D. melanogaster* X chromosome comprises a large pericentromeric block of satellite DNA, the maternally transmitted factors from *D. simulans* that rescue hybrid female lethality are likely involved in the regulation of heterochromatin – e.g. chromatin-binding or -modifying proteins or small RNAs (Ferree & Barbash, 2007, 2009). We are testing the possibility that among strain variation in hybrid rescue correlates quantitatively with the amount of particular maternal products in the egg, thus providing a means for identifying candidate causative maternal factors.

Second, the variation in hybrid rescue found segregating among disparate geographic populations of *D. simulans* suggests that directional selection has been neither strong nor consistent species-wide. The rapid evolutionary turnover of heterochromatic satellite DNA sequences like the 359 bp repeats of *Zhr* – driven either by genetic conflict over transmission through the female germline (Henikoff *et al.*, 2001) or by nearly neutral processes (Charlesworth *et al.*, 1994) – ought to elicit correspondingly rapid compensatory evolution at interacting loci, like those affecting maternal hybrid rescue. The abundant functional genetic variation for hybrid rescue in *D. simulans* is therefore surprising. One possibility is

that genetic conflict-mediated arms races between selfish satellite DNAs in *D. simulans* and their interactors has, incidentally, maintained allelic variation that affects regulation of *D. melanogaster* *Zhr*-like repeats. Another possibility, however, is that the variability could reflect residual ancestral variation at loci historically involved in regulating a *D. melanogaster* *Zhr*-like satellite DNA that was subsequently lost (or simply diverged) in the lineage leading to *D. simulans*. Consistent with the latter model, some 359-bp satellite DNA exists in *D. simulans* but it is ~50-fold less abundant than in *D. melanogaster* and considerably diverged in sequence (Strachan *et al.*, 1985; Lohe & Roberts, 1988). Determining the molecular basis and evolutionary histories of the factors responsible for variable hybrid female rescue segregating in *D. simulans* may have general implications for the common observation of polymorphic interspecific incompatibilities, like those detected in plants, nematodes, insects, copepods and vertebrates (reviewed in Rieseberg & Blackman, 2010; Cutter, 2012).

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