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Evolution of the *Drosophila* nuclear pore complex results in multiple hybrid incompatibilities

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Abstract

Speciation often involves the evolution of incompatible gene interactions that cause sterility or lethality in hybrids between populations. These so-called hybrid incompatibilities occur between two or more functionally divergent loci. We show that the *Nup160* gene of the fruitfly *Drosophila simulans* is incompatible with a factor(s) on the *D. melanogaster* X chromosome, causing hybrid lethality. *Nup160* encodes a nuclear pore complex protein and shows evidence of adaptive evolution. Furthermore, the protein encoded by *Nup160* interacts directly with that of another hybrid lethality gene, *Nup96*, indicating that at least two lethal hybrid incompatibility genes have evolved as by-products of divergent coevolution among interacting components of the *Drosophila* nuclear pore complex.

As species diverge from one another, they accumulate genetic substitutions that function within their own genomic background but, when brought together in hybrids, can disrupt gametogenesis (causing hybrid sterility) or development (causing hybrid lethality) (1,2). The evolution and genetics of these hybrid incompatibilities have been shown to follow specific rules. For instance, hybrid incompatibilities tend to accumulate gradually as species diverge (3); behave as partial recessives in hybrids (4); follow Haldane's rule [*i.e.*, the preferential sterility or inviability of hybrids of the heterogametic (XY or ZW) sex (5)]; and accumulate disproportionately on the X chromosome (*i.e.*, the large X-effect) (6,7). The molecular biology of hybrid incompatibilities has revealed that five of six hybrid incompatibility genes identified so far show signatures of recurrent adaptive evolution (8–13), suggesting that hybrid sterility and inviability generally evolve as incidental by-products of positive natural selection.

To test if this emerging molecular rule of speciation holds for additional hybrid incompatibility loci we performed a genetic screen for lethal hybrid incompatibilities between *Drosophila melanogaster* and *D. simulans*, two species that diverged ~3 Mya. As all hybrids between *D. melanogaster* and *D. simulans* are sterile, we performed a chromosomal deletion (deficiency) screen to identify recessive, lethal hybrid incompatibility factors in the *D. simulans* autosomal genome in the F₁ generation [Fig. 1A; (14)]. By screening ~70% of the *D. simulans* autosomal genome with ~200 deficiencies, we identified 20 small regions that cause hybrid lethality when combined with a hemizygous *D. melanogaster* X chromosome (14). In one of these 20 regions, we mapped a new hybrid lethality gene using chromosomal deficiencies from *D. melanogaster* to a region that includes 21 genes (Fig. 1B; Table 1, Lines 1–5) and then performed single-locus complementation tests (Table S1). Only one lesion, *PBac{RB}RfC38^{e00704}*, uncovers hybrid lethality (Table 1, line 6). To confirm that hybrid lethality is uncovered by the *PBac{RB}RfC38^{e00704}* mutation (a *piggyBac* transposon insertion) and not an unidentified linked mutation, we generated revertant chromosomes that were genetically identical to the original *PBac{RB}RfC38^{e00704}* chromosome except that the *piggyBac* insertion is precisely excised (Fig. S1). We recovered eight independent revertant chromosomes that

lacked the *piggyBac* insertion ($PBac\{RB\}RfC38^{e00704}-R1, R2, \dots R8$) and all were viable in hybrid males (Table S2), confirming that the *piggyBac* insertion unmasks hybrid lethality.

To determine which gene(s) is disrupted by $PBac\{RB\}RfC38^{e00704}$, we analyzed the genomic flanking sequence of the insertion (15) and found that the overlapping 3'-UTRs of two genes, *CG4738* and *RfC38*, are disrupted (Fig. 1B). Of these two genes, *CG4738* is the stronger candidate on the basis of three observations: DNA sequences are more diverged between species at *CG4738* than *RfC38* (58 fixed replacement differences *versus* one, respectively); disruption of *RfC38^{mel}* is not generally associated with hybrid lethality as two other transposable element insertions that disrupt *RfC38* but not *CG4738*, $P\{lacW\}RfC38^{k13807}$ and $PBac\{WH\}RfC38^{07177}$, do not unmask hybrid lethality (Table S1, Lines 2 and 9); and *CG4738* encodes the predicted protein sequence of the *Drosophila* homolog of the nucleoporin *Nup160*, a protein component of the nuclear pore complex. The latter finding is especially intriguing as we previously identified another nucleoporin, *Nup96*, as a hybrid lethality gene between these species (11).

While compelling, all three lines of evidence are circumstantial. We reasoned that if the *D. simulans* allele, *Nup160^{sim}*, causes recessive hybrid lethality, then introduction of the *D. melanogaster* allele, *Nup160^{mel}*, should rescue otherwise inviable hybrid males. To test this hypothesis, we cloned the *Nup160^{mel}* cDNA into a vector that carries a *w⁺* eye-color marker (conferring red eyes) and a GAL4-inducible UAS promoter (hereafter, we refer to this construct as $P\{UAS-Nup160^{mel}\}$). We transformed $P\{UAS-Nup160^{mel}\}$ into *D. simulans w; Lhr* flies and recovered four independent stocks with autosomal insertions of the $P\{UAS-Nup160^{mel}\}$ construct. We then crossed *D. melanogaster* $PBac\{RB\}RfC38^{e00704}/CyO$; $P\{Tub-Gal4\}/TM3, Ser$ females carrying a ubiquitously expressed GAL4-driver of $P\{UAS-Nup160^{mel}\}$ expression to *D. simulans w; Lhr* $P\{UAS-Nup160^{mel}\}$ males. These crosses produce four male zygotic genotypes in equal proportions (1:1:1:1), all of which inherit the $P\{UAS-Nup160^{mel}\}$ construct. As expected, the two kinds of hybrid males inheriting *CyO* are viable (Table 2, Line 1), and hybrid males inheriting the $PBac\{RB\}RfC38^{e00704}$ insertion but no induced *Nup160^{mel}* expression (i.e., no $P\{Tub-Gal4\}$) are lethal (Table 2, Line 1). However, in contrast to their $PBac\{RB\}RfC38^{e00704}/sim$; *TM3/sim* brothers, hybrid males inheriting the $PBac\{RB\}RfC38^{e00704}$ insertion and induced *Nup160^{mel}* expression, $PBac\{RB\}RfC38^{e00704}/sim$; $P\{Tub-Gal4\}/sim$, are viable (Table 2, Line 1). Moreover, the ratio of the three viable classes of hybrid males is not significantly different from 1:1:1 ($\chi^2 = 3.69$, $P = 0.158$). These data show that ubiquitous expression of *D. melanogaster* *Nup160* rescues the hybrid lethality caused by the insertion of $PBac\{RB\}RfC38^{e00704}$ and that *D. simulans* *Nup160* is a lethal hybrid incompatibility gene.

To confirm that *Nup160^{sim}* is incompatible with a factor(s) on the *D. melanogaster* X chromosome, we switched the species origin of the X in hybrids. We constructed a *D. melanogaster* *C(1)A, y/Y; PBac\{RB\}RfC38^{e00704}/CyO* stock, which possess two *X^{mel}* chromosomes fused to a single centromere that are transmitted together, and crossed females from this stock to *D. simulans w; Lhr* males. These crosses produced progeny with the same autosomal genotypes (and cytoplasm) as those in Fig. 1A, but hybrid males inherit their X from *D. simulans* whereas hybrid females inherit both X chromosomes from *D. melanogaster* (Fig. S2). [Attached-*X^{mel}* hybrid females also inherit a *Y^{sim}* chromosome, but the Y does not determine sex in *Drosophila* and is not essential for viability in these species (16).] These crosses show that, whereas *X^{mel}/Y^{sim}*; $PBac\{RB\}RfC38^{e00704}/sim$ hybrid males are lethal (Table 1, Line 6), *X^{sim}/Y^{mel}*; $PBac\{RB\}RfC38^{e00704}/sim$ hybrid males are viable (Table 1, Line 7; Fisher's Exact $P < 10^{-40}$). We also found that, whereas *X^{mel}/X^{sim}*; $PBac\{RB\}RfC38^{e00704}/sim$ hybrid females are viable (Table 1, Line 6), attached-*X^{mel}*, *C(1)A, y/Y^{sim}*; $PBac\{RB\}RfC38^{e00704}/sim$, hybrid females are lethal (Table 1, Line 7; Fisher's Exact $P < 10^{-29}$). These results show, respectively, that *Nup160^{sim}* causes hybrid lethality via an incompatible epistatic

interaction with a factor(s) on the *D. melanogaster* X chromosome and that *Nup160^{sim}*-dependent hybrid lethality occurs in both sexes.

To study the evolution of *Nup160*, we surveyed DNA sequence polymorphism and divergence within and between *D. melanogaster* and *D. simulans* from 12 isofemale lines of each species collected from Zimbabwe (see SOM). We tested the neutral mutation-drift model of protein evolution with the McDonald-Kreitman (MK) test (17). The MK test rejects the neutral hypothesis for *Nup160* and instead reveals a highly significant excess of fixed replacement differences between species (Table 3, Line 1), consistent with recurrent adaptive protein evolution. To distinguish if adaptive evolution at *Nup160* occurred during the history of one or both species, we polarized substitutions along lineages using sequence data from the outgroup species, *D. yakuba*, and contrasted polymorphic and fixed mutations in the *D. melanogaster* and *D. simulans* lineages separately. These lineage-specific MK tests showed that *Nup160* has experienced adaptive evolution in both lineages (Table 3, line 2–3).

Despite its history of recurrent positive selection, we found no evidence for a recent selective sweep at *Nup160* in either the *D. melanogaster* or *D. simulans* lineages. Selective sweeps should reduce local nucleotide diversity (18,19) and shift the distribution of allele frequencies towards an excess of both rare variants (20) and high-frequency derived variants (21). We found that mean silent nucleotide diversity, π , at *Nup160* is similar to other autosomal loci sampled from African populations of these species (22,23): $\pi = 0.0160$ in *D. melanogaster* and $\pi = 0.0335$ in *D. simulans*. Moreover, coalescent simulations show that neither Tajima's *D* (20) nor Fay and Wu's *H* (21), two summaries of the allele frequency spectrum, deviate significantly from standard neutral equilibrium expectations in either species (*D. melanogaster*: $D = -0.511$ and $H = -14.273$; in *D. simulans*, $D = 0.134$ and $H = 7.455$; $P \geq 0.124$ in all tests). These findings suggest that recurrent positive selection drove sequence evolution at *Nup160* but that none of the selected substitutions occurred recently (*i.e.*, within the last $\sim 0.1N_e$ generations, where N_e is the effective population size).

Our current and previous (11) findings show that two rapidly evolving autosomal genes from *D. simulans*, *Nup160^{sim}* and *Nup96^{sim}*, are incompatible with a factor(s) on the *D. melanogaster* X chromosome. NUP160 and NUP96 are members of the NUP107 subcomplex, a subset of interacting nucleoporins that together form a stable, architectural component of the nuclear pore complex (NPC), the macromolecular structures that mediate all molecular traffic between the nucleus and cytoplasm (24). The structure, the function, and the interactions among particular nucleoporins seem largely conserved among eukaryotes (25). In addition to NPC functions, members of the NUP107 subcomplex also contribute to kinetochore function (26), cell cycle progression (27), and dosage compensation in *Drosophila* males (28). In *D. melanogaster*, three other *Nup107* subcomplex genes (including *Nup96*) have histories of adaptive evolution, similar to that observed here for *Nup160*, whereas in *D. simulans* six others have histories of adaptive evolution (29). Interestingly, the components of the dosage compensation complex (30,31) and its X chromosome binding sites have evolved by positive selection between *D. melanogaster* and *D. simulans* (32). However, it is unlikely that the rapid evolution of *Nup160*, and other members of the *Nup107* subcomplex, reflects coevolution with the dosage compensation pathway or that hybrid inviability involves a disruption of dosage compensation in hybrids. For one, the rapid evolution of dosage compensation is largely limited to the *D. melanogaster* lineage (30–32) and thus cannot explain the especially rapid evolution of *Nup160* and the other *Nup107* subcomplex genes in *D. simulans*. For another, *Nup160^{sim}* kills *X^{mel}/Y^{sim}* hybrid males and *X^{mel}.X^{mel}/Y^{sim}* hybrid females, excluding a loss-of-function disruption of dosage compensation—a male-specific problem—as the cause of hybrid lethality. Instead, the recurrent bouts of adaptation of *Nup107* subcomplex genes are most likely driven by selection arising from evolutionary conflict involving pathogens, retrotransposons, or meiotic drive elements (29,33).

As part of the NUP107 subcomplex, NUP160 and NUP96 interact directly (24), raising the possibility that *Nup160^{sim}* and *Nup96^{sim}* are incompatible with the *same* X-linked factor(s) from *D. melanogaster*. The *D. melanogaster* allele, *Nup153^{mel}*, is a strong candidate X-linked partner for both *Nup96^{sim}* and *Nup160^{sim}*: *Nup153* has a history of adaptive evolution (29), its protein interacts physically with the NUP107 subcomplex (34), and it is the only known interacting nucleoporin encoded on the X chromosome. However, it is not clear if *Nup160* and *Nup96* are involved in two distinct two-locus hybrid incompatibilities with the *D. melanogaster* X or if they are components of one complex hybrid incompatibility (*i.e.*, one involving more than two loci). Theory predicts that complex hybrid incompatibilities should evolve more readily than simple hybrid incompatibilities (35), and there is the suggestion that complex hybrid incompatibilities may be typical (*e.g.*, refs. (9,36)). Regardless of whether nucleoporin-based hybrid lethality has a simple or complex basis, our study suggests that the adaptive coevolution of a large multi-protein complex may have given rise to multiple hybrid incompatibility genes. These findings suggest that divergent coevolution among the interacting partners of macromolecular complexes, particularly those prone to evolutionary conflicts, may drive the evolution of molecular incompatibilities that contribute to speciation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1A

Figure 1B

Figure 1.

(A) Deficiency mapping recessive X-autosome hybrid lethals in F_1 hybrids between *D. melanogaster* (*mel*, gray) and *D. simulans* (*sim*, white). The sex chromosomes (left, Y chromosome with hook) and one representative pair of autosomes (right) are shown, where *Df* = autosomal deficiency chromosome, and *CyO* = balancer chromosome. *D. melanogaster Df/CyO* females were crossed to *D. simulans Lhr* males; *Lhr* is a mutation that rescues ordinarily lethal hybrid males. Of the four zygotic genotypes produced, only *Df*-bearing hybrid males are forced to develop using only *D. simulans* material for the autosomal region exposed by the deficiency and only *D. melanogaster* material for the X chromosome. Thus, if only *Df*-bearing hybrid males die, we infer that a *D. simulans* autosomal region (red) is incompatible with *D. melanogaster* X chromosome, causing lethality (for more details see ref. (14)). Note that the hybrid lethal interactions identified in this screen would normally afflict advanced-generation (e.g., F_2), but not F_1 , hybrids as both partners in these X-autosome incompatibilities are recessive. (B) Fine-scale mapping results in region 32D on chromosome arm 2L. Three deficiencies fail to complement hybrid lethality in males (black bars): *Df(2L)FCK-20*, *Df(2L)Exel6027*, and *Df(2L)BSC36*; two deficiencies complement hybrid lethality in males (gray bars): *Df(2L)Exel6028* and *Df(2L)Exel6029*. Together, these deficiencies implicate one of 21 genes in hybrid lethality that maps to a 89.6-kb region in the cytological interval 32D02;32D05.

Table 1
Interspecific complementation mapping of a hybrid lethal factor on *D. simulans* chromosome arm 2L

<i>D. melanogaster</i> maternal genotype	Cytological breakpoints	Hybrid females		Hybrid males	
		– / <i>sim</i>	<i>CyO/sim</i>	– / <i>sim</i>	<i>CyO/sim</i>
1 <i>Df</i> (2L) <i>FCK-20/CyO</i>	32D1;32F1-3	471	504	0	88
2 <i>Df</i> (2L) <i>Exel6027/CyO</i>	32D2;32D5	338	296	1	261
3 <i>Df</i> (2L) <i>BSC36/CyO</i>	32D1;32F3	134	137	0	76
4 <i>Df</i> (2L) <i>Exel6028/CyO</i>	32D5;32E4	204	183	151	127
5 <i>Df</i> (2L) <i>Exel6029/CyO</i>	32E4;32F2	265	274	254	192
6 <i>PBac</i> (<i>RB</i>) <i>R</i> (<i>C</i> 38 ^{el0704} / <i>CyO</i>)	32D4	359	306	0	219
7 <i>C</i> (1) <i>A</i> , <i>y</i> ; <i>PBac</i> (<i>RB</i>) <i>R</i> (<i>C</i> 38 ^{el0704} / <i>CyO</i>)	32D4	0	93	114	120

Table 2

Ubiquitous expression of a *D. melanogaster* *Nup160* transgene rescues hybrid lethality.

		Hybrid autosomal genotypes			
Line	2 nd chromosome genotype: 3 rd chromosome genotype:	<i>PBac(RB)rfC38⁰⁰⁷⁰⁴/sim; TM3, Ser/sim</i>	<i>CyO/sim; TM3, Ser/sim</i>	<i>PBac(RB)rfC38⁰⁰⁷⁰⁴/sim; P{Tub-Gal4}/sim</i>	<i>Cyo/sim; P{Tub-Gal4}/sim</i>
1	Hybrid males, <i>X^{mel}/X^{sim}</i>	0	119	112	141
2	Hybrid females, <i>X^{mel}/X^{sim}</i>	191	204	224	219

Progeny counts are pooled results from four different *D. simulans* *Lhr P[UAS-Nup160^{mel}]* stocks as similar results hold across replicates. All progeny from these crosses inherit *Lhr* and *P[UAS-Nup160^{mel}]*, which, for simplicity, are not shown.

Table 3
Recurrent adaptive evolution at the hybrid lethality gene, *Nup160*, in both *D. melanogaster* and *D. simulans*.

	Polymorphic			Divergent			Fisher's Exact	
	R	S	R/S	R	S	R/S	P-value	
1 <i>D. melanogaster</i> - <i>D. simulans</i> pooled	27	154	0.175	58	64	0.906	9.3×10^{-10}	
2 <i>D. melanogaster</i> lineage	10	48	0.208	19	33	0.576	0.0299	
3 <i>D. simulans</i> lineage	11	85	0.129	26	21	1.238	7.1×10^{-8}	