A Test of Double Interspecific Introgression of Nucleoporin Genes in *Drosophila*

Kyoichi Sawamura,*,[1] Kazunori Maehara,[†] Yoko Keira,[‡] Hiroyuki O. Ishikawa,[‡] Takeshi Sasamura,[§] Tomoko Yamakawa,[§] and Kenji Matsuno[§]

*Faculty of Life and Environmental Sciences, and [†]Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, [‡]Department of Biology, Chiba University, Chiba, Chiba 263-8522, and [§]Department of Biological Sciences, Osaka University, Toyonaka, Osaka, Japan 560-0043

ABSTRACT In interspecific hybrids between *Drosophila melanogaster* and *Drosophila simulans*, the *D. simulans* nucleoporin-encoding *Nup96*^{sim} and *Nup160*^{sim} can cause recessive lethality if the hybrid does not also inherit the *D. simulans* X chromosome. In addition, *Nup160*^{sim} leads to recessive female sterility in the *D. melanogaster* genetic background. Here, we conducted carefully controlled crosses to better understand the relationship between *Nup96*^{sim} and *Nup160*^{sim}. *Nup96*^{sim} did not lead to female sterility in the *D. melanogaster* genetic background, and double introgression of *Nup96*^{sim} and *Nup160*^{sim} did not generally lead to lethality when one was heterozygous and the other homozygous (hemizygous). It appears that introgression of additional autosomal *D. simulans* genes is necessary to cause lethality and that the effect of the introgression is dominant to *D. melanogaster* alleles. Interestingly, the genetic background affected dominance of *Nup96*^{sim}, and double introgression carrying homozygous *Nup96*^{sim} and hemizygous *Nup160*^{sim} resulted in lethality. Thus, *Nup96*^{sim} and *Nup160*^{sim} seem to be two components of the same incompatibility.

KEYWORDS
Drosophila
hybrid inviability
hybrid sterility
nucleoporin
reproductive isolation
speciation

A handful of hybrid incompatibility genes that are responsible for reproductive isolation between species have been identified (Johnson 2010; Presgraves 2010; Maheshwari and Barbash 2011; Ferree and Prasad 2012; Sawamura 2012). Surprisingly, two of these genes in the genus *Drosophila* encode the nuclear pore proteins (nucleoporins = Nups), which were previously thought to be functionally conserved among diverse organisms. Approximately 30 different Nups assemble to form the nuclear pore complex (NPC) and are essential for nucleocytoplasmic transport, gene regulation, and kinetochore formation (Bapteste *et al.* 2005; Strambio-De-Castillia *et al.* 2010; Adams and Wente 2013). *Nup96* and *Nup160* have been identified as reproductive isolation genes by deficiency mapping in which male hybrids were rescued from the independent lethality by *Lethal hybrid rescue* (*Lhr*) mutation of *D. simulans*. *D. melanogaster*/*D. simulans* hybrids carrying the *D. simulans Nup96*^{sim} and *Nup160*^{sim} are lethal in hemizygotes (or homozygotes) if they do not inherit the *D. simulans* X chromosome (Figure 1, A and B), and *Nup160*^{sim} leads to recessive female sterility in the *D. melanogaster* genetic background (Presgraves *et al.* 2003; Tang and Presgraves 2009; Sawamura *et al.* 2010). Furthermore, positive natural selection and intermolecular coevolution have been demonstrated for several Nup genes including *Nup96* and *Nup160* in the genus *Drosophila* (Presgraves and Stephan 2007; Clark and Aquadro 2010; Mensch *et al.* 2013; Nolte *et al.* 2013).

Both Nup96 and Nup160 (yeast homologs are Nup145C and Nup120, respectively) are components of the conserved Nup107−160 complex that has a role in the initial assembly of the NPC and functions as a stable anchoring point for other Nups—referred to as central scaffold Nups (Walther *et al.* 2003; Rasala *et al.* 2006; Grossman *et al.* 2012). The Nup107−160 complex forms a Y-shaped structure composed of two short arms—one composed of Nup160 and the other of Nup85—and an extended stalk that is connected to the two arms by Nup96 (Lutzmann *et al.* 2002; Brohawn *et al.* 2008; Bilokapic and Schwartz 2012; Szymborska *et al.* 2013). Because Nup96 and Nup160 interact directly (Leducq *et al.* 2012), it is reasonable to speculate that the lethality caused by *Nup96*^{sim} and that caused by *Nup160*^{sim} in the *D. melanogaster*/*D. simulans* hybrids are two distinct

doi: 10.1534/g3.114.014027
Manuscript received July 23, 2014; accepted for publication August 24, 2014; published Early Online August 28, 2014.

[1]Corresponding author: University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan. E-mail: sawamura@biol.tsukuba.ac.jp.

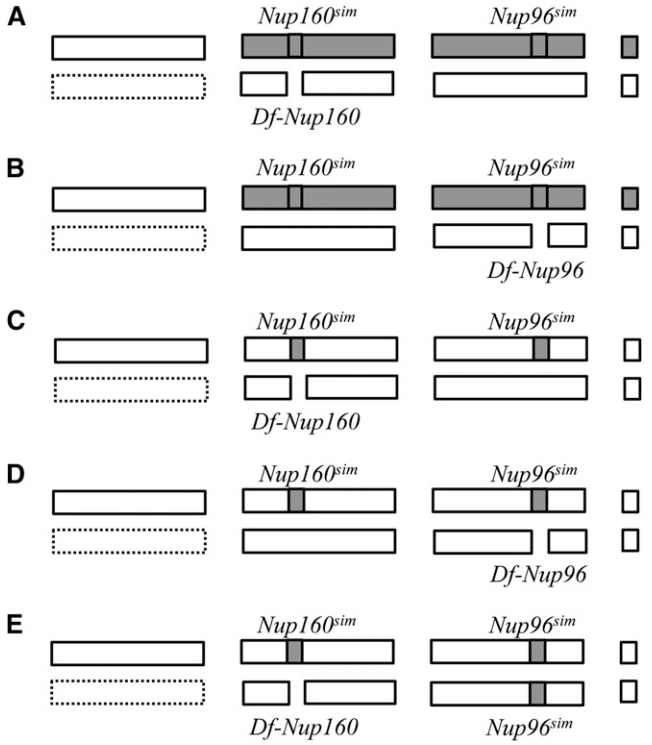

Figure 1 Genotypes examined previously and in this study. Pairs of bars represent chromosomes X, 2, 3, and 4 (left to right). Open bars (dashed if the presence is not obligate) indicate chromosomes/regions from *D. melanogaster*, and gray bars indicate chromosomes/regions from *D. simulans*. *D. simulans* alleles of *Nup160* and *Nup96* and the deficiencies on *D. melanogaster* chromosomes are also indicated. (A) Flies of this genotype all die according to Tang and Presgraves (2009) and Sawamura *et al.* (2010). (B) Flies of this genotype all die according to Presgraves *et al.* (2003). (C, D) These flies are viable according to the present analysis. (E) Flies of this genotype all die according to the present analysis. The genotypes in (A) and (B) are usually males carrying one X chromosome from *D. melanogaster*, but females carrying two *D. melanogaster* X chromosomes can also be obtained using the attached-X system (Presgraves *et al.*, 2003; Tang & Presgraves 2009). The genotypes in (C), (D), and (E) are females carrying two *D. melanogaster* X chromosomes or males carrying one *D. melanogaster* X chromosome.

aspects of the same incompatibility. In this context, it is notable that protein−protein interactions between Nup96 and Nup160 are species-specific, as revealed in yeast sibling species and their hybrids (Leducq *et al.* 2012).

We conducted interspecific crosses of *Drosophila* to address the following three questions. (1) Does $Nup96^{sim}$ lead to female sterility in the *D. melanogaster* genetic background as seen with $Nup160^{sim}$ introgression? (2) Does the $Nup96^{sim}$ and $Nup160^{sim}$ double introgression lead to lethality when one is heterozygous and the other homozygous (or hemizygous) in the *D. melanogaster* background (Figure 1, C and D)? (3) Does the $Nup96^{sim}$ and $Nup160^{sim}$ double introgression lead to lethality when both are homozygous (or hemizygous) in the *D. melanogaster* background (Figure 1E)? Based on these three tests, we ask whether the double introgression of $Nup96^{sim}$ and $Nup160^{sim}$ is necessary and sufficient condition for the incompatibility to the gene(s) on the *D. melanogastere* X chromosome. Dominance of the genes and the possible involvement of different genes to the hybrid lethality will also be discussed.

MATERIALS AND METHODS

A genomic fragment of ~20.9 kb, including three open reading frames (*CG10208*, *Nup98-96*, and *mbc*), was amplified from DSM1-010P23, a *D. simulans* bacterial artificial chromosome clone established by the National BioResource Project Drosophila (Murakami *et al.* 2008), by polymerase chain reaction using the primers LA-AscI-F (5′-AGGCGCGCCTTACTTGCGACGGAACACCTCGACCTTGAG-3′), LA-*Bam*HI-R (5′-CGCGGATCCACGCACCTGGACAATGCAAGAGGGTGATTTG-3′), RA-*Bam*HI-F (5′-CGCGGATCCGACCAGCATGAGCATTGCCAACAGCATGCT-3′), and RA-*Pac*I-R (5′-ACCTTAATTAATCAGCACACCGGGCATAAGGTATCCCTGCTC-3′). This fragment was subcloned into the vector *attB*-P[acman]-Cm^R by homologous recombination (Venken *et al.* 2006). The construct was injected into embryos from *D. melanogaster* strain *y sc v* $P\{y^{+t7.7} = nos\text{-}phiC31\backslash int.NSL\}X$; $P\{y^{+t7.7} = CaryP\}attP2$ to allow for ϕC31-targeted, site-specific recombination into the *attP* landing site (cytological position 68A4 on chromosome 3) (Groth *et al.* 2004; Bateman *et al.* 2006; Bischof *et al.* 2007). The resultant transgene is abbreviated as $P\{w^+\ Nup96^{sim}\}$ in the present report.

A $P\{w^+\ Nup96^{sim}\}$ *e* $Nup98\text{-}96^{339}$ chromosome was made by recombination between $P\{w^+\ Nup96^{sim}\}$ and *e* $Nup98\text{-}96^{339}$ chromosomes in the *w* genetic background (Figure 2). Here w^+ (68A4; red eye color) and *e* (93C7-D1; ebony/dark body color) were used as visible markers, and *Nup98-96* is at 95B1-5. To confirm that the recombinant chromosome carried the $Nup98\text{-}96^{339}$ mutation and that it was not lost by rare double recombination between *e* and $Nup98\text{-}96^{339}$, $P\{w^+\ Nup96^{sim}\}$ was removed from the established chromosome by further recombination with a wild-type chromosome using the w^+ and *e* markers. The resultant chromosome again exhibited recessive lethality that was not complemented by the *Nup98-96* deficiencies (*Df(3R)Exel9014* and *Df(3R)BSC489*), thus confirming that the chromosome examined carried $Nup98\text{-}96^{339}$. A balancer chromosome, *TM3*, was used to isolate the recombinant chromosome in a heterozygous state, and *CyO* and *SM1* were used as a chromosome 2 balancer. *Int(2L)D+S* is a chromosome 2 *D. simulans* introgression covering two cytological regions that include $Nup160^{sim}$ (Sawamura *et al.* 2000). Of note, the *Int(2L)D+S* introgression also carries other *Nup* loci (*Nup107* and *Nup154*), but we do not believe that this could affect our overall conclusion of this study. When

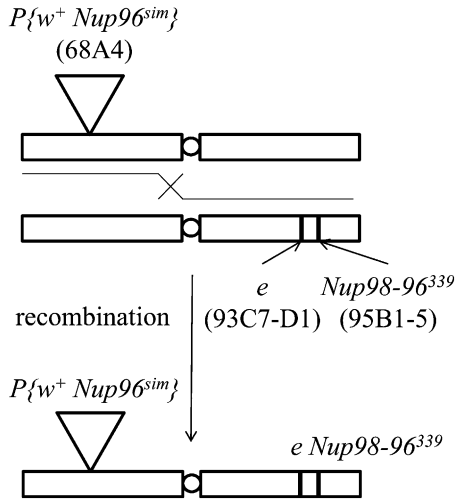

Figure 2 Construction of chromosome $P\{w^+\ Nup96^{sim}\}$ *e* $Nup98\text{-}96^{339}$ in the X-linked *w* mutant background. The w^+ *e* recombinant (potentially $P\{w^+\ Nup96^{sim}\}$ *e* $Nup98\text{-}96^{339}$) is produced by crossing $P\{w^+\ Nup96^{sim}\}$ and *e* $Nup98\text{-}96^{339}$.

Table 1 Hatchability of eggs from females crossed with wild-type *D. melanogaster* males

Maternal Genotype[a]	Number of Eggs		Hatchability, %
	Collected	Hatched	
Nup96^{sim} heterozygotes over *TM3*	200	191	95.5
Nup96^{sim} homozygotes	200	106	53.0
Nup96^{sim} hemizygotes over *Df(3R)Exel9014*	200	185	92.5
Nup96^{sim} hemizygotes over *Df(3R)BSC489*	200	177	88.5

[a] The full genotype of *Nup96^{sim}* is *P{w$^+$ Nup96^{sim}} e Nup98-96^{339}*.

necessary, only *Nup160^{sim}* was made hemizygous by a deficiency of the *Nup160* locus, *Df(2L)Nup160M190* (Maehara *et al.* 2012).

RESULTS

First, we established a *D. melanogaster* line carrying an extra segment of *D. simulans* chromosome 3 (including *CG10208*, *Nup98-96*, and *mbc*) inserted at cytological position 68A4 of the same chromosome. Note that *Nup98-96* is a dicistronic gene that produces the proteins Nup98 and Nup96 by autoproteolysis (Presgraves *et al.* 2003). Then, the endogenous *Nup98-96* at 95B1-5 of the line was replaced by the recessive lethal *Nup98-96^{339}* mutant allele (Figure 2), which has a stop codon at amino acid position 1726 (therefore, only *Nup96* was affected; Presgraves *et al.* 2003). Thus, we obtained a *D. melanogaster* chromosome 3 carrying *Nup96^{sim}* instead of the *D. melanogaster* wild-type allele of *Nup96*. The resultant chromosome (*P{w$^+$ Nup96^{sim}} e Nup98-96^{339}*) is referred to as the *Nup96^{sim}* introgression. Both male and female *Nup96^{sim}* introgression homozygotes (and hemizygotes) were viable and fertile, and the strain homozygous for *Nup96^{sim}* could be maintained indefinitely. Although females that were homozygous for *Nup96^{sim}* and hemizygous over *Df(3R)BSC489* exhibited lower fertility than heterozygous controls ($\chi^2 = 94.5$, $P < 0.001$ and $\chi^2 = 6.6576$, $P < 0.05$, respectively), fertility was not decreased in *Nup96^{sim}* hemizygotes over *Df(3R)Exel9014* ($\chi^2 = 1.5958$, $P > 0.2$) (Table 1). Therefore, *Nup96^{sim}* does not lead to female sterility in the *D. melanogaster* genetic background. We note the possibility that the chromosome harboring *Nup96^{sim}* might have a second-site recessive gene or genes responsible for lower female fertility.

Next, to examine possible synergistic and/or additive effects of *Nup160^{sim}* and *Nup96^{sim}* introgression, we produced *w*; *Int(2L)D+S, Nup160^{sim}/CyO*; *Nup96^{sim} e/+* males by conventional crosses. Then, these males were crossed to females heterozygous for a balancer and a mutation (or a deficiency) of *Nup160* or *Nup98-96*. If the introgressions were behaving similar to the F_1 hybrid, then *Nup160^{sim}/(Nup160^{sim}* or *Df-Nup160)*; *Nup96^{sim}/+* is expected to be lethal; however, that is not what is observed. Instead, the *Nup160^{sim}* homozygotes (or hemizygotes) were viable in the *Nup96^{sim}* heterozygous background (Figure 1C and Table 2). If the introgressions were behaving similar to the F_1 hybrid, then *Nup160^{sim}/+; Nup96^{sim}/(Nup96^{sim}* or *Df-Nup96)* is expected to be lethal; however, that is not what is observed. Instead, the *Nup96^{sim}* homozygotes (or hemizygotes) were viable in the *Nup160^{sim}* heterozygous background (Figure 1D and Table 3). Thus, the *Nup96^{sim}* and *Nup160^{sim}* double introgression did not lead to lethality when one was heterozygous and the other homozygous (or hemizygous).

Finally, we attempted to make a strain carrying both *Nup160^{sim}* and *Nup96^{sim}* introgressions maintained with chromosome 2 and 3 balancers but were not successful, presumably because *Int(2L)D+S* can cause dominant male semisterility in some genetic backgrounds (S. Parhad, personal communication). Therefore, we could not test the viability/fertility of *Nup96^{sim}* and *Nup160^{sim}* double introgression homozygotes. Instead, we made *w*; *Df(2L)Nup160M190/SM1*; *Nup96^{sim}/TM3* females and *w*; *Int(2L)D+S*, *Nup160^{sim}/SM1*; *Nup96^{sim}/+* males by conventional crosses and crossed them. *Int(2L)D+S, Nup160^{sim}/ Df(2L)Nup160M190*; *Nup96^{sim}/+* flies were viable as we previously noted (Table 2), although hemizygosity of *Nup160^{sim}* might have reduced their viability (Table 4). Unexpectedly, we found that *Int(2L)D+S, Nup160^{sim}/ Df(2L)Nup160M190*; *Nup96^{sim}/TM3* was semilethal (Table 4). This suggests that dominance of *Nup96^{sim}* may be affected by the genetic background. Furthermore, we found that *Int(2L)D+S, Nup160^{sim}/ Df(2L)Nup160M190*; *Nup96^{sim}/Nup96^{sim}* was also absolutely lethal (Table 4 and Figure 1E). Thus, the protein products of *Nup96^{sim}* and *Nup160^{sim}* seem to interact directly.

DISCUSSION

We found that *D. melanogaster* females homozygous (or hemizygous) for the *Nup96^{sim}* introgression were fertile (Table 1), in contrast to what has been observed for the *Nup160^{sim}* introgression, for which eggs produced by homozygotes (or hemizygotes) display karyogamy

Table 2 Viability of flies homozygous (or hemizygous) for *Nup160^{sim}* and heterozygous for *Nup96^{sim}*

Maternal genotype[a]	Number of Flies			
	Cy w	Cy w$^+$	Cy$^+$ w	Cy$^+$ w$^+$ (Viability[b])
w; Int(2L)D+S, Nup160^{sim}/CyO				
Genotype	*Nup160^{sim}/+; +/+*	*Nup160^{sim}/+; Nup96^{sim}/+*	*Nup160^{sim}/Nup160^{sim}; +/+*	*Nup160^{sim}/Nup160^{sim}; Nup96^{sim}/+*
Females	132	202	35[c]	25 (0.71)[c]
Males	146	206	39[c]	35 (0.90)[c]
w; Df(2L)Nup160M190/CyO				
Genotype	*(Nup160^{sim}* or *Df-Nup160)/+; +/+*	*(Nup160^{sim}* or *Df-Nup160)/+; Nup96^{sim}/+*	*Nup160^{sim}/Df-Nup160; +/+*	*Nup160^{sim}/Df-Nup160; Nup96^{sim}/+*
Females	180	201	77	20 (0.26)
Males	155	188	105	68 (0.65)
Segregation ratio expected	2	2	1	1

[a] Crossed with *w; Int(2L)D+S, Nup160^{sim}/CyO; Nup96^{sim}/+* males. The balancer *CyO* has *Cy* as a dominant marker.
[b] Calculated as (number of flies in the fourth class) divided by (number of flies in the third class).
[c] The viability of *Int(2L)D+S* homozygotes was low because of linked recessive lethals that presumably accumulated on the chromosome.

Table 3 Viability of flies heterozygous for *Nup160^{sim}* and hemizygous for *Nup96^{sim}*

Maternal genotype[b]	Number of Flies							
	Cy w Sb	Cy w Sb$^+$	Cy w$^+$ Sb	Cy w$^+$ Sb$^+$	Cy$^+$ w Sb	Cy$^+$ w Sb$^+$	Cy$^+$ w$^+$ Sb	Cy$^+$ w$^+$ Sb$^+$ (Viability[a])
w; Nup98-96^{339}/TM3								
Genotype	+/+; +/+	+/+; *l(3)Nup96*/+	+/+; *Nup96^{sim}*/+	+/+; *Nup96^{sim}/l(3)Nup96*	*Nup160^{sim}*/+; +/+	*Nup160^{sim}*/+; *l(3)Nup96*/+	*Nup160^{sim}*/+; *Nup96^{sim}*/+	*Nup160^{sim}*/+; *Nup96^{sim}/l(3)Nup96*
Females	38	123	50	84	72	102	84	72 (0.86)
Males	61	89	70	62	87	109	90	69 (1.11)
w; Df(3R)BSC489/TM6C								
Genotype	+/+; +/+	+/+; *Df-Nup96*/+	+/+; *Nup96^{sim}*/+	+/+; *Nup96^{sim}/Df-Nup96*	*Nup160^{sim}*/+; +/+	*Nup160^{sim}*/+; *Df-Nup96*/+	*Nup160^{sim}*/+; *Nup96^{sim}*/+	*Nup160^{sim}*/+; *Nup96^{sim}/Df-Nup96*
Females	123	170	63	142	98	151	62	92 (0.65)
Males	117	135	28	106	85	128	76	65 (0.61)
Segregation ratio expected	1	1	1	1	1	1	1	1

[a] Calculated as (number of flies in the eighth class) divided by (number of flies in the fourth class).
[b] They were crossed to *w; Int(2L)D+S, Nup160^{sim}/CyO; Nup96^{sim}/+* males. The balancers *TM3* and *TM6C* have *Sb* (and *Ser* in the former) as a dominant marker. *l(3)Nup96* stands for a recessive mutation of the *Nup96* gene, *Nup98-96^{339}*.

Table 4 Viability of flies hemizygous for *Nup160^{sim}* and homozygous for *Nup96^{sim}*

Maternal genotype[c]	Number of Flies[a]							
	Cy w Sb	Cy w$^+$ Sb$^+$	Cy w$^+$ Sb	Cy w^{++} Sb$^+$	Cy$^+$ w Sb	Cy$^+$ w$^+$ Sb$^+$	Cy$^+$ w$^+$ Sb (Viability[b])	Cy$^+$ w^{++} Sb$^+$ (Viability[b])
w; Df(2L)Nup160M190/ SM1; Nup96^{sim}/TM3								
Genotype	(*Nup160^{sim}* or *Df-Nup160*)/+; +/+	(*Nup160^{sim}* or *Df-Nup160*)/+; *Nup96^{sim}*/+	(*Nup160^{sim}* or *Df-Nup160*)/+; *Nup96^{sim}*/+ (*TM3*)	(*Nup160^{sim}* or *Df-Nup160*)/+; *Nup96^{sim}/Nup96^{sim}*	*Nup160^{sim}/ Df-Nup160*; +/+	*Nup160^{sim}/ Df-Nup160; Nup96^{sim}*/+	*Nup160^{sim}/ Df-Nup160; Nup96^{sim}*/+ (*TM3*)	*Nup160^{sim}/Df-Nup160; Nup96^{sim}Nup96^{sim}*
Females	436[d]	533	423	163	137	98[e]	1 (0.01)	0 (0)
Males	442[d]	547	452	190	145	177	8 (0.05)	0 (0)
Segregation ratio expected	2	2	2	2	1	1	1	1

[a] w^{++} means flies carrying two w$^+$ markers; distinguished by their darker eye color. A few flies ambiguous for the Cy phenotype were excluded.
[b] Calculated as (number of flies in the seventh or eighth class) divided by (number of flies in the sixth class).
[c] Crossed with *w; Int(2L)D+S, Nup160^{sim}/SM1; Nup96^{sim}/+* males. The balancers *SM1* and *TM3* have *Cy* and *Sb* (and *Ser*) as dominant markers, respectively.
[d] One was ebony presumably caused by a rare male recombination or a spontaneous mutation.
[e] One was a gynandromorph.

failure and female pronuclei never fuse to wild-type male pronuclei (Sawamura *et al.* 2004). Although Nup96 and Nup160 are functionally and structurally in close proximity in the Y-shaped Nup107−160 complex, the effects of interspecific substitution of these two components differed. The structural position of Nup96 and Nup160 might reflect the functional difference; Nup160 is on the surface of the pore ring (Bilokapic and Schwartz 2012; Szymborska *et al.* 2013) and might have more interactions with other proteins important for NPC function.

We found that flies with genotypes indicated in Figure 1, C and D were viable (Table 2 and Table 3), in contrast to the lethality observed for those with genotypes indicated in Figure 1, A and B (Presgraves *et al.* 2003; Tang and Presgraves 2009; Sawamura *et al.* 2010). The primary difference between these flies is the genetic background, with the remaining autosomal genes being from *D. melanogaster* in our flies and from *D. melanogaster* and *D. simulans* (heterozygous) in the previous studies. Apparently the presence of additional autosomal *D. simulans* genes is necessary to cause lethality, and these genes are dominant to the *D. melanogaster* alleles. Thus, more genes (maybe encoding other Nups) are involved in this hybrid incompatibility. *Nup107* and *Nup154* are excluded from the candidates because *Int (2L)D+S* also carries these genes from *D. simulans* but did not exhibit the dominant effect. One candidate for the interactant is *Nup75*, presumably the *Drosophila* homolog of *Nup85*. Further investigation of this system is necessary to better understand the genetic mechanisms of reproductive isolation.

Interestingly, dominance of $Nup96^{sim}$ was changed by the presence of a balancer *TM3* (Table 4). Reproductive isolation might be easily affected by the genetic background, as has been suggested in the other hybrid incompatibility (*Lhr vs. Hmr*) in the same species cross (Matute *et al.* 2014; Shirata *et al.* 2014). Finally, double introgression carrying homozygous $Nup96^{sim}$ and hemizygous $Nup160^{sim}$ resulted in lethality in the hybrids (Table 4 and Figure 1E). This is the first evidence suggesting that $Nup96^{sim}$ and $Nup160^{sim}$ are two components of the same incompatibility.

ACKNOWLEDGMENTS

We are grateful to the Bloomington Drosophila Stock Center at Indiana University and the Drosophila Genetic Resource Center at the Kyoto Institute of Technology for providing fly strains and the BAC clone. This work was supported by a Grant-in-Aid for Scientific Research (24570001) from the Japan Society for the Promotion of Science to K.S.

LITERATURE CITED

Adams, R. L., and S. R. Wente, 2013 Uncovering nuclear pore complexity with innovation. Cell 152: 1218–1221.

Bapteste, E., R. L. Charlebois, D. MacLeod, and C. Brochier, 2005 The two tempos of nuclear pore complex evolution: highly adapting proteins in an ancient frozen structure. Genome Biol. 6: R85.

Bateman, J. R., A. M. Lee, and C. T. Wu, 2006 Site-specific transformation of *Drosophila* via ϕC31 integrase-mediated cassette exchange. Genetics 173: 769–777.

Bilokapic, S., and T. U. Schwartz, 2012 Molecular basis for Nup37 and ELY5/ELYS recruitment to the nuclear pore complex. Proc. Natl. Acad. Sci. USA 109: 15241–15246.

Bischof, J., R. K. Maeda, M. Hediger, F. Karch, and K. Basler, 2007 An optimized transgenesis system for *Drosophila* using germ-line specific φC31 integrases. Proc. Natl. Acad. Sci. USA 104: 3312–3317.

Brohawn, S. G., N. C. Leksa, E. D. Spear, K. R. Rajashankar, and T. U. Schwartz, 2008 Structural evidence for common ancestry of the nuclear pore complex and vesicle coats. Science 322: 1369–1373.

Clark, N. L., and C. F. Aquadro, 2010 A novel method to detect proteins evolving at correlated rates: identifying new functional relationships between coevolving proteins. Mol. Biol. Evol. 27: 1152–1161.

Ferree, P. M., and S. Prasad, 2012 How can satellite DNA divergence cause reproductive isolation? Let us count the chromosomal ways. Genet. Res. Int. 2012: 430136.

Grossman, E., O. Medalia, and M. Zwerger, 2012 Functional architecture of the nuclear pore complex. Annu. Rev. Biophys. 41: 557–584.

Groth, A. C., M. Fish, R. Nusse, and M. P. Calos, 2004 Construction of transgenic *Drosophila* by using the site-specific integrase from phage φC31. Genetics 166: 1775–1782.

Johnson, N. A., 2010 Hybrid incompatibility genes: remnants of a genomic battlefield? Trends Genet. 26: 317–325.

Leducq, J. B., G. Charron, G. Diss, I. Gagnon-Arsenault, A. K. Dubé *et al.*, 2012 Evidence for the robustness of protein complexes to inter-species hybridization. PLoS Genet. 8: e1003161.

Lutzmann, M., R. Kunze, A. Buerer, U. Aebi, and E. Hurt, 2002 Modular self-assembly of a Y-shaped multiprotein complex from seven nucleoporins. EMBO J. 21: 387–397.

Maehara, K., T. Murata, N. Aoyama, K. Matsuno, and K. Sawamura, 2012 Genetic dissection of *Nucleoporin 160* (*Nup160*), a gene involved in multiple phenotypes of reproductive isolation in *Drosophila*. Genes Genet. Syst. 87: 99–106.

Maheshwari, S., and D. A. Barbash, 2011 The genetics of hybrid incompatibilities. Annu. Rev. Genet. 45: 331–355.

Matute, D. R., J. Gavin-Smyth, and G. Liu, 2014 Variable post-zygotic isolation in *Drosophila melanogaster/D. simulans* hybrids. J. Evol. Biol. 27: 1691−1705.

Mensch, J., F. Serra, N. J. Lavagnino, H. Dopazo, and E. Hasson, 2013 Positive selection in nucleoporins challenges constraints on early expressed genes in *Drosophila* development. Genome Biol. Evol. 5: 2231–2241.

Murakami, K., A. Toyoda, M. Hattori, Y. Kuroki, A. Fujiyama *et al.*, 2008 BAC library construction and BAC end sequencing of five *Drosophila* species: the comparative map with the *D. melanogaster* genome. Genes Genet. Syst. 83: 245–256.

Nolte, V., R. V. Pandey, R. Kofler, and C. Schlötterer, 2013 Genome-wide patterns of natural variation reveal strong selective sweeps and ongoing genomic conflict in *Drosophila mauritiana*. Genome Res. 23: 99–110.

Presgraves, D. C., 2010 The molecular evolutionary basis of species formation. Nat. Rev. Genet. 11: 175–180.

Presgraves, D. C., and W. Stephan, 2007 Pervasive adaptive evolution among interactors of the *Drosophila* hybrid inviability gene, *Nup96*. Mol. Biol. Evol. 24: 306–314.

Presgraves, D. C., L. Balagopalan, S. M. Abmayr, and H. A. Orr, 2003 Adaptive evolution drives divergence of a hybrid inviability gene between two species of *Drosophila*. Nature 423: 715–719.

Rasala, B. A., A. V. Orjalo, Z. Shen, S. Briggs, and D. J. Forbes, 2006 ELYS is a dual nucleoporin/kinetochore protein required for nuclear pore assembly and proper cell division. Proc. Natl. Acad. Sci. USA 103: 17801–17806.

Sawamura, K., 2012 Chromatin evolution and molecular drive in speciation. Int. J. Evol. Biol. 2012: 301894.

Sawamura, K., A. W. Davis, and C. I. Wu, 2000 Genetic analysis of speciation by means of introgression into *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 97: 2652–2655.

Sawamura, K., T. L. Karr, and M. T. Yamamoto, 2004 Genetics of hybrid inviability and sterility in *Drosophila*: dissection of introgression of *D. simulans* genes in *D. melanogaster* genome. Genetica 120: 253–260.

Sawamura, K., K. Maehara, S. Mashino, T. Kagesawa, M. Kajiwara *et al.*, 2010 Introgression of *Drosophila simulans* nuclear pore protein 160 in *Drosophila melanogaster* alone does not cause inviability but does cause female sterility. Genetics 186: 669–676.

Shirata, M., Q. Araye, K. Maehara, S. Enya, T. Takano-Shimizu *et al.*, 2014 Allelic asymmetry of the *Lethal hybrid rescue* (*Lhr*) gene expression in the hybrid between *Drosophila melanogaster* and *D. simulans*: confirmation by using genetic variations of *D. melanogaster*. Genetica 142: 43–48.

Strambio-De-Castillia, C., M. Niepel, and M. Rout, 2010 The nuclear pore complex: bridging nuclear transport and gene regulation. Nat. Rev. Mol. Cell Res. 11: 490–501.

Szymborska, A., A. de Marco, N. Daigle, V. C. Cordes, J. A. G. Briggs *et al.*, 2013 Nuclear pore scaffold structure analyzed by super-resolution microscopy and particle averaging. Science 341: 655–658.

Tang, S., and D. C. Presgraves, 2009 Evolution of the *Drosophila* nuclear pore complex results in multiple hybrid incompatibilities. Science 5915: 779–782.

Venken, K. J., Y. He, R. A. Hoskins, and H. J. Bellen, 2006 P[acman]: a BAC transgenic platform for targeted insertion of large DNA fragments in *D. melanogaster*. Science 314: 1747–1751.

Walther, T. C., A. Alves, H. Pickersgill, I. Loïodice, M. Hetzer *et al.*, 2003 The conserved Nup107–160 complex is critical for nuclear pore complex assembly. Cell 113: 195–206.

Communicating editor: B. J. Andrews