Pervasive Adaptive Evolution among Interactors of the Drosophila Hybrid Inviability Gene, Nup96

Daven C. Presgraves*† and Wolfgang Stephan*

*Section of Evolutionary Biology, Biocenter, University of Munich, Planegg-Martinsried, Germany; and †Department of Biology, University of Rochester

Nup96 is involved in a lethal hybrid incompatibility between 2 fruit fly species, Drosophila melanogaster and Drosophila simulans. Recurrent adaptive evolution drove the rapid functional divergence of Nup96 in both the D. melanogaster and the D. simulans lineages. Functional divergence of Nup96 between these 2 species is unexpected as Nup96 encodes part of the Nup107 subcomplex, an architectural component of nuclear pore complexes, the macromolecular channels in nuclear envelopes that mediate nucleocytoplasmic traffic in all eukaryotes. Here we study the evolutionary histories of 5 of Nup96’s protein interactors—3 stable Nup107 subcomplex proteins (Nup75, Nup107, and Nup133) and 2 mobile nucleoporins (Nup98 and Nup153)—and show that all 5 have experienced recurrent adaptive evolution. These results are consistent with selection-driven coevolution among molecular interactors within species causing the incidental evolution of incompatible interactions seen in hybrids between species. We suggest that genetic conflict–driven processes may have contributed to the rapid molecular evolution of Nup107 subcomplex genes.

Introduction

New species arise through the evolution of reproductive isolation (Dobzhansky 1937; Coyne and Orr 2004). Among diverging allopatric Drosophila species, prezygotic isolation (e.g., sexual isolation) and intrinsic postzygotic isolation (e.g., hybrid sterility or inviability) evolve gradually and do so at roughly equal rates (Coyne and Orr 1989, 1997). The evolution of intrinsic postzygotic isolation corresponds to the accumulation of alleles that have deleterious epistatic interactions in species hybrids—so-called hybrid incompatibilities (Dobzhansky 1937; Muller 1940, 1942; Orr 1995). Many classical genetic analyses have confirmed that alleles fixed in 1 species are often functionally incompatible with the genetic backgrounds of closely related species (Coyne and Orr 2004). However, the normal functions and evolutionary histories of hybrid incompatibility genes have been determined in only a few cases, in part because the particular loci involved have proven difficult to identify (Orr et al. 2004; Wu and Ting 2004; Orr 2005).

In Drosophila, just 4 hybrid incompatibility genes have been identified. Ods is a duplicated homeobox transcription factor that is misexpressed in the testes of Drosophila simulans–Drosophila mauritiana hybrids, causing male sterility (Ting et al. 1998; Barbash and Ashburner 2003; Barbash et al. 2003; Ting et al. 2004). Hmr is a single-copy gene encoding a Myb-related protein with putative DNA-binding properties, which causes lethality and female sterility in F1 Drosophila melanogaster–D. simulans hybrids (Barbash and Ashburner 2003; Barbash et al. 2003). The D. simulans allele of Nup96, a single-copy gene, encodes a nuclear pore protein that causes recessive lethality when combined with a hemizygous D. melanogaster X chromosome (Presgraves et al. 2003). And, JYAlpha, which encodes a male fertility−essential Na+/K+ ATPase, was recently found to cause sterility due to its change in genetic map position: JYAlpha is on the fourth chromosome in D. melanogaster but on the third chromosome in D. simulans; hybrids homozygous D. melanogaster at all major chromosomes except the fourth are therefore sterile as they lack JYAlpha (Masly et al. 2006).

It is unclear what role, if any, these particular genes played in reproductive isolation in nature, but they nevertheless serve as important models whose molecular properties and evolutionary histories are informative about the evolution and genetics of hybrid incompatibilities in general. For example, a striking, but still tentative, pattern has already emerged from 3 of these genes: each has a history of recurrent positive selection at its protein-coding sequence (Ting et al. 1998; Presgraves et al. 2003; Barbash et al. 2004). (The remaining gene, JYAlpha, causes hybrid sterility not by its functional divergence but by a change in map position [Lynch and Force 2000]). Adaptive divergence at Ods is confined to the D. mauritiana lineage (Ting et al. 1998), whereas Hmr and Nup96 evolved adaptively in both the D. melanogaster and the D. simulans lineages (Presgraves et al. 2003; Barbash et al. 2004).

Hybrid incompatibility genes are not unconditionally deleterious but instead depend on alleles at interacting loci. By identifying specific loci, we can make inferences about the interactions that lead to hybrid sterility and inviability. For example, the D. simulans allele of Nup96 causes lethality in hybrids that are hemizygous for the D. melanogaster X chromosome but not in hybrids hemizygous for the D. simulans X (Presgraves 2003; Presgraves et al. 2003). This is not surprising as the D. simulans allele of Nup96 is not expected to be incompatible with loci on the D. simulans X chromosome. On the contrary, the functional divergence of Nup96 in D. simulans must have coevolved with functional divergence at 1 (or more) of its interacting genes. Because Nup96 does not encode a transcription factor and because the relevant divergence in Nup96 seems to be concentrated in its protein-coding region (Presgraves et al. 2003), its hybrid inviability likely involves a protein–protein incompatibility rather than a protein–DNA incompatibility (Landry et al. 2005).

Fortunately, a good deal is known about Nup96’s function and potential protein interactors. Nup96 is 1 of ~30 different nucleoporins that together form a single nuclear pore complex (NPC) (Allen et al. 2000, 2002; Rout et al. 2000; Tran and Wente 2006). Thousands of NPCs perforate the nuclear envelopes of eukaryotic cells, mediating molecular...
traffic into and out of the nucleus. The NPC is largely conserved throughout eukaryotic evolution in its modular architecture, complement of nucleoporins, and interactions among particular subsets of nucleoporins (Allen et al. 2000, 2002; Rout et al. 2000; Vasu and Forbes 2002; Bapteste et al. 2005; Devos et al. 2006; Tran and Wente 2006). Nup96 is 1 of 8 known proteins that form the Nup107 subcomplex (known as the Nup84 subcomplex in yeast), a stable structural component of the NPC, along with Nup75, Nup107, Nup133, Nup160, Seh1, Nup37, and Nup43 (Siniossoglou et al. 1996; Rappsilber et al. 2000; Siniossoglou et al. 2000; Vasu et al. 2001; Allen et al. 2002; Lutzmann et al. 2002; Walther et al. 2003; Devos et al. 2004). From what is now known, Nup96 occupies a central position in the Nup107 subcomplex and interacts directly with Nup75 and Nup107 (Lutzmann et al. 2002). In addition to these stable Nup107 subcomplex proteins, 3 dynamic nucleoporins (Nup98, Nup153, and Sec13) are known to shuttle between the nuclear interior and the NPC, where they physically dock at the Nup107 subcomplex (Griffis et al. 2002). All 3 of these dynamic nucleoporins interact directly with Nup96 (Vasu et al. 2001; Hodel et al. 2002; Enninga et al. 2003); Nup153 and Nup98 are also known to bind with Nup107, Nup133, and Nup160 (Vasu et al. 2001).

Because Nup96 is a hub for multiple interactions in the Nup107 subcomplex—and because Nup96 has experienced repeated bouts of adaptive evolution in the *D. melanogaster* and the *D. simulans* lineages—we investigated the possibility of adaptive coevolution between Nup96 and some of its interacting proteins. We surveyed DNA sequence divergence and polymorphism in *D. melanogaster* and *D. simulans* at 5 additional nucleoporins: Nup75, Nup107, Nup133, Nup98, Nup153. Here we report that divergence at every 1 of these genes was driven by adaptive evolution.

**Materials and Methods**

**Genes and Fly Stocks**

We identified Nup75 (CG5733), Nup107 (CG6743), Nup133 (CG6958), Nup98 (CG10198), and Nup153 (CG4453) using Blast searches of the *D. melanogaster* and *D. simulans* whole-genome sequences using mouse, human, *Xenopus*, and yeast homologs as queries. All of these nucleoporins appear to be encoded by single-copy genes in the 2 *Drosophila* genomes. For the 2 large shuttling nucleoporins, we sequenced 2.4 kb from the 5′ end Nup153 (positions 27–2387 of 9246 bp) and 1.3 kb from 3′ end of Nup98 (positions 1906–3223 of 3223 bp) as these are known to encode the protein domains that mediate interactions with the Nup107 subcomplex (Enarson et al. 1998; Vasu et al. 2001; Hodel et al. 2002). For the 3 Nup107 subcomplex nucleoporins, we sequenced their entire protein-coding regions. For completeness, we also included the previously sequenced entire protein-coding region of Nup96 in our analyses below (Presgraves et al. 2003).

For each gene, we sampled 11–15 chromosomes from Zimbabwean populations of both *D. melanogaster* and *D. simulans*. The lines were kindly provided by Dr Charles Aquadro (Cornell University). We studied sequences from Zimbabwe to minimize the confounding effects of the recent demographic expansion associated with the out-of-Africa events of both species (Glinka et al. 2003; Baudry et al. 2004; Ometto et al. 2005; Thornton and Andolfatto 2006). To extract and isogenize autosomes in *D. melanogaster*, we used standard crosses involving balancer chromosomes. For those lines for which autosomes could not be made homozygous (e.g., due to the presence of balanced recessive lethals), we extracted genomic DNA from deficiency heterozygotes in which the deficiency deletes a small chromosomal region including the relevant nucleoporin (i.e., from flies hemizygous for a Zimbabwe chromosome at the locus of interest). Details about the particular stocks and crosses used for each nucleoporin are available upon request.

We obtained *Drosophila sechellia* and *Drosophila yakuba* sequences for each gene using Blast searches of the complete genome sequences available from the Broad Institute and the Washington University Genome Sequencing Center, respectively.

**Sequencing and Analysis**

We extracted genomic DNA from ~20 females from each line using Puregene DNA isolation kits (Gentra Systems, Minneapolis, MN). Polymerase chain reaction and internal sequencing primers were designed using the available *D. melanogaster* (Adams et al. 2000) and *D. simulans* (Washington University Genome Sequencing Center) genome sequences as guides. We purified PCR products with EXOSAP-IT (USB, Cleveland, OH) and sequenced both strands. Some sequencing was done at the University of Munich, using the DYEnamic ET terminator cycle sequencing kit (Amersham Biosciences, Buckinghamshire, UK), and some at the University of Rochester, using ABI BigDye terminator chemistry (PerkinElmer, Wellesley, MA). Sequences were assembled and edited using Sequencher v. 4.5 (Gene Codes, Ann Arbor, MI) and manually aligned with Se-Al v. 2.0 (Rambaut 1996). All sequences have been deposited in GenBank, under accession numbers EF057923–EF058045.

We used DnaSP v. 4.0 for most population genetic analyses (Rozas et al. 2003). To estimate probabilities for Tajima’s *D* (Tajima 1989) and Fay and Wu’s *H* (Fay and Wu 2000) statistics (with and without recombination), we simulated 5,000 neutral genealogies, conditioning on the observed *θ* (Watterson 1975), using J. Fay’s web application (http://www.genetics.wustl.edu/jf/Sub/htest.html). The population recombination rate, *ρ* = 4*Nr* (Hudson 1987), used in these simulations was estimated from the data.

**Results**

**Adaptive Evolution of the Nup107 Subcomplex**

We used MK tests (McDonald and Kreitman 1991) to determine if patterns of protein polymorphism and divergence at 6 nucleoporins, Nup75, Nup107, Nup133, Nup98, Nup153, and, for completeness, Nup96 (Presgraves et al. 2003), are consistent with histories of neutral mutation and drift. Under a neutral model of molecular evolution, in which only neutral mutations contribute to variation within and divergence between species, the ratio of replacement to synonymous polymorphisms will equal the ratio of replacement to synonymous fixed differences (Kimura 1983;
Table 1
MK Tests for Nucleoporins in Drosophila melanogaster and Drosophila simulans

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphisms</th>
<th>Fixed Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Nup75</td>
<td>24</td>
<td>95</td>
</tr>
<tr>
<td>Nup96</td>
<td>27</td>
<td>108</td>
</tr>
<tr>
<td>Nup107</td>
<td>29</td>
<td>105</td>
</tr>
<tr>
<td>Nup133</td>
<td>29</td>
<td>127</td>
</tr>
<tr>
<td>Nup98</td>
<td>14</td>
<td>44</td>
</tr>
<tr>
<td>Nup153</td>
<td>10</td>
<td>32</td>
</tr>
<tr>
<td>Pooled</td>
<td>133</td>
<td>511</td>
</tr>
</tbody>
</table>

McDonald and Kreitman (1991). Table 1 shows that this null neutral model can be rejected for all 6 genes. Each—the 4 stable Nup107 subcomplex proteins (Nup75, Nup96, Nup107, and Nup133) and the 2 dynamic shuttling nucleoporins (Nup98 and Nup153)—shows a significant excess of amino acid replacement fixations between species, consistent with recurrent bouts of adaptive protein evolution. Sliding window analyses of replacement/synonymous (R/S) polymorphism and divergence are provided as supplementary figures, Supplementary Material online.

Lineage-Specific Adaptive Evolution

To determine whether the adaptive evolution of any particular nucleoporin gene was restricted to the D. melanogaster lineage or to the D. simulans lineage, or occurred in both lineages, we performed lineage-specific MK tests. Table 2 shows the results of 3 of these tests for each gene. For the first test, D. simulans and D. yakuba sequences were used as near and distant outgroups, respectively, to polarize mutations in the D. melanogaster lineage. For the second test, D. melanogaster and D. yakuba were used as near and distant outgroups, respectively, to polarize mutations in the D. simulans lineage. These 2 tests include fixations that occurred over the 3–5 Myr of independent evolution in D. melanogaster and in D. simulans, respectively (Hey and Kliman 1993; Li 1997; Tamura et al. 2004). For the third test, D. sechellia and D. melanogaster sequences were used as near and distant outgroups, respectively, to polarize substitutions in the most recent ~400,000 years of evolution in the D. simulans lineage (Kliman et al. 2000). The latter test, therefore, asks about patterns of substitution in the more recent history of D. simulans. (Speciation between D. simulans and D. sechellia was sufficiently recent that some of the history of the alleles in our sample could predate the species split [Kliman et al. 2000], possibly confounding polymorphism and divergence. The D. sechellia–D. simulans MK tests should therefore be interpreted with caution.) It is important to note that lineage-specific MK tests suffer reduced statistical power, for 2 reasons. First, mapping mutations onto each lineage reduces the number of observations in each cell of the MK table. Second, mutations that cannot be accurately polarized, due to, for example, multiple substitutions or alignment gaps that make inference of ancestral and derived states ambiguous, are excluded from the analysis.

Table 2
Lineage-Specific MK Tests for Nucleoporins in Drosophila melanogaster and Drosophila simulans

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphisms</th>
<th>Fixed Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Nup75</td>
<td>17</td>
<td>59</td>
</tr>
<tr>
<td>Nup96</td>
<td>15</td>
<td>51</td>
</tr>
<tr>
<td>Nup107</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Nup113</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Nup98</td>
<td>15</td>
<td>41</td>
</tr>
<tr>
<td>Nup153</td>
<td>12</td>
<td>34</td>
</tr>
<tr>
<td>Pooled</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

* Fisher’s exact probability.
In the *D. melanogaster* lineage, 4 of 6 nucleoporins have experienced an excess of replacement fixations (i.e., R/S for fixations is greater than R/S for polymorphism). Two of 4, *Nup75* and *Nup96*, show individually significant excesses of replacement fixations consistent with adaptive evolution (table 2). A third gene, *Nup133*, shows a marginally nonsignificant excess of replacement fixations.

In the *D. simulans* lineage, 4 of 6 nucleoporins show individually significant evidence for adaptive evolution (*Nup96, Nup107, Nup133, and Nup153; table 2). Similar histories of adaptive evolution cannot be excluded for the remaining 2 genes because *Nup75* shows excess amino acid divergence that is marginally nonsignificant (table 2, line 15); and *Nup98* shows a significant excess of replacement fixations since the split from *D. sechellia* (table 2, line 15). Thus, at least 5 (nearly 6) of 6 nucleoporins show some evidence of recurrent adaptive protein evolution in *D. simulans*.

*Nup107* and *Nup153* have experienced recurrent bouts of positive selection strictly limited to the *D. simulans* lineage (table 2, lines 8 and 17), whereas none of the genes show *D. melanogaster*–limited bouts of positive selection. Only 1 gene, *Nup96*, shows strong evidence for parallel bouts of positive selection in both species (table 2, lines 4 and 5). The data for *Nup75* and *Nup133* are suggestive of similar parallel bouts of positive selection, but in 1 of the species (*D. simulans* and *D. melanogaster*, respectively), the lineage-specific tests are marginally nonsignificant. Overall, the *Nup107* subcomplex appears to have diverged more in *D. simulans* than in the *D. melanogaster* (table 2, lines 19–21).

**Little Evidence for Recent Selective Sweeps**

Recent selective sweeps caused by the fixation of new, favorable mutations can leave characteristic signatures in the level and distribution of linked neutral nucleotide variability (Maynard Smith and Haigh 1974; Kaplan et al. 1989; Nielsen 2005), for example, by depressing silent variability and causing an excess of rare variants. Table 3 shows summary statistics of the levels of silent (synonymous and noncoding) variability and of the frequency spectra of silent mutations for each of the 6 nucleoporins. Levels of silent polymorphism at each locus are not unusual for African populations of these species (Andolfatto 2001; Mousset and Derome 2004). Consistent with its previously inferred larger effective population size (Aquadro et al. 1988; Akashi 1996), *D. simulans* shows ~50% higher levels of silent diversity, on average, than *D. melanogaster* (table 3). None of the nucleoporin genes in either species exhibit the dramatically reduced variability expected after a recent, strong selective sweep.

The frequency spectra of silent polymorphisms, summarized by Tajima’s *D* statistic, are more skewed toward rare alleles in *D. simulans* than in *D. melanogaster* for 5 of 6 genes. A comparison of the distribution of all silent polymorphisms pooled across loci differs significantly between species (Kolmogorov–Smirnov test, *P* = 0.0002). This species difference is consistent with either a stronger signal of population expansion or stronger selective constraints on silent sites in *D. simulans* than in *D. melanogaster* (Akashi 1995). The sole exception to this pattern is *Nup107*: in *D. melanogaster*, silent variability at *Nup107* shows a negative Tajima’s *D* (table 3; without and with recombination, *P* = 0.092 and 0.013, respectively) and a negative Fay and Wu’s *H* (without and with recombination, *P* = 0.114 and 0.057, respectively). These patterns of variability are consistent with a fairly old selective sweep at *Nup107* in *D. melanogaster* (relative to the approximate detection limit of ~0.1Ne generations in *Drosophila*), but it is difficult to rule out alternative (e.g., demographic) explanations. Thus, neither the levels nor the frequency spectra of silent variability at *Nup107* subcomplex genes show particularly compelling evidence for recent, strong selective sweeps in either species.

**Discussion**

The structure and function of NPCs are largely conserved among eukaryotes. It is therefore surprising that all 4 *Nup107* subcomplex nucleoporins and both interacting shuttling nucleoporins studied have histories of recurrent adaptive evolution during the last 3–5 Myr of divergence between *D. melanogaster* and *D. simulans*. Several recent analyses of polymorphism and divergence data across loci in *Drosophila* have revealed an overall excess of amino acid divergence consistent widespread adaptive evolution (Fay et al. 2002; Smith and Eyre-Walker 2002; Sawyer et al. 2003). However, even in such multilocus surveys, only 5–10% of genes show *individually significant* evidence for adaptive evolution (Smith and Eyre-Walker 2002;
Presgraves and Stephan (2005). The fact that each of 6 genes in our survey shows individually significant evidence for adaptive evolution is therefore striking. This finding—along with the fact that these proteins are known interactors—suggests that the bouts of adaptation at these 6 genes are not independent but rather reflect histories of molecular coevolution. We note, however, that our evidence is circumstantial as only functional tests can confirm coevolution. These tests require that the particular functional domains of these nucleoporins become better characterized.

What might cause such a large fraction of the proteins of the otherwise conserved NPC to diverge so dramatically? The pattern of divergence observed here suggests that a simple scenario of ecological adaptation to the extrinsic environment—say, adaptation to a new host plant—is unlikely. *D. melanogaster* and *D. simulans* are thought to have evolved in allopatry, in southeastern sub-Saharan Africa and in Madagascar, respectively (Lachaise et al. 1988; Dean and Ballard 2004; Lachaise and Silvain 2004; Lachaise et al. 2004). For an ecological scenario to account for the divergence seen here, these 2 historically geographically isolated species must have independently experienced an ecological challenge—at about the same time in their evolutionary histories—and responded to that challenge with multiple beneficial substitutions at the same (or related) sets of genes. Although we cannot rule out such a scenario, an alternative class of explanation seems more plausible.

In particular, the parallel bouts of adaptive evolution at nucleoporins in both *D. melanogaster* and *D. simulans* suggest that these 2 species may have inherited an unresolved conflict involving NPCs from their common ancestor. Under this scenario, targeted nucleoporins in these 2 species would have evolved in response to a conflict using different sets of substitutions, with interacting nucleoporins coevolving. This adaptive divergence would then incidentally lead to a hybrid incompatibility. There are at least 3 types of genetic conflicts in which NPCs—the “gatekeepers of the nucleus” (Wente 2000)—might plausibly be involved.

### Genetic Conflict between Host and Pathogens

First, there is ample evidence that nucleoporins are involved in antagonistic interactions with viruses and virus-like particles of retrotransposons (Cronshaw and Matunis 2004; Smith and Helenius 2004; Fontoura et al. 2005; Irwin et al. 2005). Some viruses (e.g., adenovirus and herpes simplex virus) are too large to pass through the nuclear pore and instead dock on the cytoplasmic face of NPCs to inject their genomes into the nucleus (Cronshaw and Matunis 2004; Smith and Helenius 2004; Fontoura et al. 2005). Other viruses directly target particular nucleoporins for disruption. In human cells, the matrix protein of the vesicular stomatitis virus inhibits mRNA export by specifically disrupting Nup98 (Enninga et al. 2002). In response, host cells upregulate expression of Nup98 and Nup96 via an interferon-mediated immune response. Similarly, poliovirus (Gustin and Sarnow 2001) and rhinovirus (Gustin and Sarnow 2002) impair specific nuclear import pathways by proteolyzing Nup153. Furthermore, HIV-1 and Tf1 retrotransposons also interact with Nup153 (Varadarajan et al. 2005). Although relatively little is known about the molecular interactions between flies and viruses (but see Dostert et al. 2005; Wang et al. 2006), it is easy to imagine that fly pathogens have evolved similar strategies, leading to conflicts that drive the divergence of *Drosophila* nucleoporins.

### Genetic Conflict over Centromeric Drive

Meiotic drive of selfish centromeres during oogenesis represents a second genetic conflict potentially fueling the divergence of the Nup107 subcomplex. Of the 4 meiotic products of oogenesis, only 1 becomes the primary oocyte and the other 3 are discarded as polar bodies. This inherent asymmetry in oogenesis thus creates an opportunity for centromeres, the sites of kinetochore assembly and spindle attachment, to compete for access to the primary oocyte (Zwick et al. 1999; Henikoff et al. 2001). There is direct evidence for such centromeric drive in plants and animals (Pardo-Manuel de Villena and Sapienza 2001; Fishman and Willis 2005), as well as some indirect evidence suggested by the rapid evolution of both centromeric heterochromatin and centromere-binding proteins in plants and animals, including *Drosophila* (Malik and Henikoff 2001; Malik et al. 2002; Talbert et al. 2002). Centromeric drive is predicted to have deleterious side effects for both female and male fertility, including nondisjunction (Zwick et al. 1999) and other meiotic defects (Henikoff and Malik 2002; Malik 2005). Centromere-binding proteins may therefore evolve rapidly in order to suppress selfish centromeres. How might the Nup107 subcomplex be involved in centromeric drive? One of the surprises to emerge from recent work on the Nup107 subcomplex is its localization during cell division: during mitosis the nuclear envelope breaks down and NPCs disassemble; however, the Nup107 subcomplex remains intact, specifically localizes to the kinetochores of chromosomes, and promotes spindle assembly (Belgareh et al. 2001; Loidice et al. 2004). The members of the Nup107 subcomplex therefore represent another class of rapidly evolving, centromere-associated proteins potentially involved in suppressing centromeric drive.

### Genetic Conflict over Segregation Distortion

Segregation distortion represents a third genetic conflict that might involve the NPC. In *D. melanogaster*, a male-specific meiotic drive complex called *Segregation Distorter* (*SD*) resides on 1–5% of second chromosomes in natural populations (Hartl and Hiraizumi 1976; Kusano et al. 2003). During spermatogenesis in *SD*/*SD* males, *SD*+-bearing sperm are incapacitated so that virtually all progeny inherit *SD* instead of the 50% expected under Mendelian inheritance. The 3 major loci of the *SD* complex are 1) the *Segregation distorter* locus (*Sd*), the main driver that encodes a truncated duplicate of RanGAP (Merrill et al. 1999); 2) *Enhancer of SD* (*E(SD)*), an upward modifier of the strength of distortion; and 3) *Responder* (*Rsp*), the target of distortion. *Rsp* is an array of AT-rich 120-bp repeats for which repeat copy number is correlated with sensitivity to distortion—sensitive *Rsp* alleles comprise 700–2500 repeats, whereas insensitive alleles comprise a few dozen to ~200 (Wu et al. 1988). Wildtype RanGAP localizes to the cytoplasm where it functions as a major regulator of nuclear transport; in contrast, truncated Sd-RanGAP was
recently shown to mislocalize to the nuclear interior, where it causes distortion by disrupting nuclear transport during spermatogenesis (Kusano et al. 2001). Genes able to influence nuclear transport—including nucleoporins—could in principle suppress or compensate for the deleterious effects of distortion and might therefore evolve in response to this genetic conflict. We have argued elsewhere that RanGAP-mediated distortion may be an ancient genetic conflict, predating the D. melanogaster–D. simulans split, which has driven rapid divergence of several nuclear transport genes, including RanGAP (Presgraves DC, unpublished data).

Thus, the rapid molecular evolution of the Nup107 subcomplex and its interactors could plausibly reflect histories of 1) host–pathogen conflict, 2) conflict over centromeric drive, or 3) conflict over nuclear transport–related segregation distortion. Although admittedly speculative, such genetic conflict–driven scenarios seem likely to explain the simultaneous bouts of recurrent adaptive substitutions concentrated in the Nup107 subcomplex genes of D. melanogaster and D. simulans.

Coevolutionary Consequences for Hybrid Incompatibilities

Population genetic models of the evolution of hybrid incompatibilities usually assume that substitutions occur independently (Orr 1995). For instance, in the simple 2-locus, 2-allele Dobzhansky–Muller model, an ancestral aabb genotype first becomes Abb in 1 of 2 lineages; then, with equal probability, a B substitution can occur either in the same lineage yielding ABB (scenario 1) or in the sister lineage yielding aABB (scenario 2). The pattern of molecular evolution among the Nup107 subcomplex genes implies, however, that the histories of interacting genes are not independent—the Nup107 subcomplex genes appear to coevolve. Such nonindependence among coevolving interactors has 2 consequences for the evolution of hybrid incompatibilities. First, coevolution accelerates the rate of evolution of hybrid incompatibilities relative to a model with independent substitutions because substitutions at 1 locus increase the probability of substitutions at interacting loci—within a lineage an a→A substitution can drive a b→B substitution.

Second, coevolution decreases the probability of evolving incompatibilities between 2 derived alleles (derived–derived) versus incompatibilities between 1 derived and once ancestral allele (derived–ancestral). Under scenario 1 in the model above, the AABB × aabb species cross will produce AaBb hybrids exposed to a possible incompatibility between the derived B allele and the ancestral a allele (as “B” has never been tested by natural selection on an “a” genetic background). Similarly, under scenario 2, the AAbb × aABB species cross will produce AaBb hybrids exposed to incompatibilities between the derived A and derived B alleles. Coevolution among interacting loci will cause substitutions to be concentrated in 1 lineage (scenario 1), increasing the probability of derived–ancestral incompatibilities relative to a model with independent substitutions (Orr 1995). Thus, the nonindependence of substitutions in coevolving genes should increase the rate of evolution of derived–ancestral hybrid incompatibilities.

Coevolution between Nup96 and a Partner on the X Chromosome?

Genetic analysis shows that the D. simulans allele of Nup96 causes hybrid inviability via an epistatic interaction with an incompatible “partner” gene (or genes) on the X chromosome of D. melanogaster (Presgraves 2003; Presgraves et al. 2003). Of the 8 known members of the Nup107 subcomplex and the three shuttling nucleoporins that dock with it, only 1 is encoded by a gene on the X chromosome: the shuttling nucleoporin, Nup153. Interestingly, Nup153 shows evidence of adaptive evolution only in the D. simulans lineage, suggesting that the D. simulans allele of Nup96 is functional in the context of the newly evolved allele of Nup153 in D. simulans. If true, then this putative hybrid incompatibility could represent a derived–ancestral incompatibility—the derived allele of Nup96 (from D. simulans) is incompatible with the ancestral allele of Nup153 (from D. melanogaster). It is important to note, however, that Nup153 remains only a candidate partner gene until functional tests demonstrate its role in the Nup96 incompatibility.

Supplementary Material

Supplementary figures and Accession Numbers EF057923–EF058045 are available at Molecular Biology and Evolution online (http://www.mbe.oxfordjournals.org/).

Acknowledgments

We thank Andrea Betancourt, Victoria Cattani, J.P. Masly, Allen Orr, and Shanwu Tang for comments on the manuscript. This work was supported by an Alexander von Humboldt Research Fellowship and funding from the University of Rochester to D.C.P. and by a grant from the VW-Foundation (I/78815) to W.S.

Literature Cited


Downloaded from https://academic.oup.com/molecularbiologyevolution/article-abstract/24/1/306/1074651 by guest on 25 December 2018


Jianzhi Zhang, Associate Editor

Accepted October 17, 2006