CHARACTERIZATION AND MAPPING OF THE GENE CONFERRING RESISTANCE TO RIFT VALLEY FEVER VIRUS HEPATIC DISEASE IN WF.LEW RATS

A Dissertation

by

RALPH JENNINGS CALLICOTT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2008

Major Subject: Genetics

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Approved by:

Chair of Committee,	James Womack
Committee Members,	James Derr
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	Loren Skow
Intercollegiate Faculty Chair of Genetics,	James Wild

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ABSTRACT

Characterization and Mapping of the Gene Conferring Resistance to Rift Valley Fever Virus Hepatic Disease in WF.LEW Rats. (December 2008)

Ralph Jennings Callicott, D.V.M., Louisiana State University

Chair of Advisory Committee: Dr. James Womack

Rift Valley Fever Virus is a plebovirus that causes epidemics and epizootics in sub-Saharan African countries but has expanded to Egypt and the Arabian Peninsula. The laboratory rat (*Rattus norvegicus*) is susceptible to RVFV and has been shown to manifest the characteristic responses of humans and livestock. The rat has frequently been used as a model to study RVFV pathogenesis. Several strains have been infected and some found to be resistant to hepatic disease while others were not. This resistance was found to be associated with a dominant gene inherited in Mendelian fashion. The congenic rat strain WF.LEW and several substrains of the parental strains were used to try and locate the resistance gene. Microsatellites and single nucleotide polymorphisms were used to characterize the genomes of various rat substrains in an attempt to map the gene. Breeding and viral challenge experiments were used to further characterize the strains and assign a location to the resistance gene.

The LEW/SsNHsd rats showed approximately 37% genomic difference as compared with LEW/MolTac rats, and 8% difference as compared with LEW/Crl rats. WF/NHsd rats demonstrated a difference of approximately 8% as compared with

WF/CrCrl rats. Genotyping of the congenic WF.LEW revealed Lewis markers on RNO3 and RNO9. Subsequent backcross experiments and viral challenge experiments assigned the resistance gene to the distal end of RNO3.

DEDICATION

This Dissertation is dedicated to my son Ralph Jennings Callicott III.

ACKNOWLEDGEMENTS

I would like to thank my mentor Dr. Womack and my committee members Drs. Kier, Derr, and Skow, for guiding me through this process and having the patience to see it to the end with me. Thanks to all the members of the Womack lab for their support and friendship. Thanks go to Catherine Busch and Scott Ballard for their help with the genotyping experiments.

I would also like to acknowledge the hard work by my collaborators at UTMB, Drs. Peters and Morrill and Melissa Worthy. Thanks for all your help with the viral challenge experiments.

Finally, thanks to the friends I made at Texas A&M, Robert Rose, Quynh Tran, Chris Seabury, all the group at CMP, and the many others I didn't name, for their support and encouragement along the way.

NOMENCLATURE

LEW	Lewis
RNO3	Rat chromosome 3
RNO9	Rat chromosome 9
RVFV	Rift Valley Fever Virus
SSLP	Simple Sequence Length Polymorphism
SNP	Single Nucleotide Polymorphism
TFIIH	Transcription Factor II H
WF	Wistar-Furth

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CHAPTER I

INTRODUCTION: RIFT VALLEY FEVER VIRUS AND RATS

Rift Valley Fever virus (RVFV) is a member of the family *Bunyaviridae*. This family is divided into the five genera of *Bunyavirus, Phlebovirus, Hantavirus, Nairovirus, and Tospovirus* (Frese et al. 1996). RVFV is a plebovirus that causes epidemics and epizootics in sub-Saharan African countries but has expanded to Egypt (Ritter et al. 2000) and the Arabian Peninsula (Morrill and Peters 2003). Its transmission occurs mainly by *Aedes* and *Culex* mosquitoes (Le May et al. 2004), although transmission may also occur by other mosquito species or possibly by blood sucking arthropods. RVFV is responsible for devastating disease in livestock with ruminants being the most susceptible. Humans may be infected and suffer from a mild influenza-like illness. However, in a small proportion of cases the disease may progress in severity and result in hepatitis in conjunction with hemorrhagic fever, retinitis, or meningoencephalitis (Laughlin et al. 1979). The first human deaths reported to be directly caused by RVFV occurred in 1975 (Anderson et al. 1987). In ruminants the disease is usually associated with a fulminant hepatitis in young naïve animals and abortions in older animals. Sheep are particularly sensitive and experience mortality from 25% in adults up to 90% in lambs (Ritter et al. 2000). Recently, concern has been raised about the use of RVFV as a bioterrorism agent due to its ability to infect

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This dissertation follows the style of Mammalian Genome.

humans as well a livestock. Subsequently it has been classified as a select agent by both the Centers for Disease Control and the United States Department of Agriculture.

Rift Valley Fever virus like all members of the *Bunyaviridae* family carries a three part single-stranded RNA genome. The three segments are designated large (L), medium (M), and small (S). Both the L and M segments are of negative polarity with the L coding for the L RNA –dependant polymerase and the M segment coding for the glycoprotein precursor that is cleaved to produce the envelope glycoproteins G1 and G2 and two nonstructural proteins, 14K and 78K. The S segment codes for the nucleoprotein N and the nonstructural protein NSs in an ambisense fashion (Vialat et al. 2000). The NSs protein forms filamentous structures in the nuclei of infected cells that were found to inhibit host transcription (Le May et al. 2004; Vialat et al. 2000). The basal transcription factor, TFIIH, was shown to be targeted by the virus. Le May et al. (2004) hypothesized that the NSs protein bound to the p44 subunit of TFIIH and was transported to the nucleus. The binding of p44 coupled with the binding of the XPB subunit by NSs limits the quantity of TFIIH that can be assembled. This results in a reduced concentration of TFIIH in the nucleus and a reduction in transcription (Le May et al. 2004). In addition Le May et al. (2008) found that the NSs protein forms a SAP30 complex that serves to inhibit expression of IFN-β through transcriptional repression. This repression occurred at 3-6 hours post infection before the filamentous structure formation as opposed to the previously reported TFIIH inhibition which started at eight hours post infection (Le May et al. 2004; Le May et al. 2008).

The laboratory rat (*Rattus norvegicus*) is susceptible to RVFV and has been shown to manifest the characteristic responses of humans and livestock (Peters and Slone 1982). The rat has frequently been used as a model to study RVFV pathogenesis. Several strains have been infected and some found to be resistant to hepatic disease while others were not. This resistance was found to be associated with a dominant gene inherited in Mendelian fashion (Anderson and Peters 1988). More specifically, Lewis rats (LEW/Mai) were shown to be resistant to hepatic disease and Wistar-Furth rats (WF/Mai) were more sensitive (Anderson et al. 1987; Peters and Slone 1982). Viral titers for WF/Mai were found to be higher than LEW/Mai in every sample from the earliest timepoints (Anderson et al. 1987). The resistance was documented to be host-genotype dependent as well as age and dose dependent (Anderson et al. 1987; Anderson et al. 1991). Based on these findings a congenic rat strain was developed by backcrossing the resistance gene from the LEW/Mai strain onto the WF/Mai background. Rats of each generation were challenged with live virus and the survivors were mated with WF/Mai rats for the subsequent generation (Anderson et al. 1991). However, the resistance gene for RVFV hepatic disease was not located or identified. The congenic strain WF.LEW was terminated but embryos were cryopreserved and tissues collected before the colony was phased out. Interestingly, Ritter and colleagues (2000) reported experiments that demonstrated the WF strain as resistant to RVFV and LEW rats as succumbing to fatal hepatic disease. However, a different viral strain and different substrains of rats, LEW/Mol and WF/Mol, were used. Subsequently, the LEW/Mol substrain was reported to contain approximately 37% non-Lewis genome

when compared to the LEW/Ztm substrain. The presence of the relatively large amount of non-Lewis genome was attributed to a past crossbreeding event (Olofsson et al. 2004). Inbred strains of the laboratory rat are commonly used models in biomedical and behavioral research. Rats are second only to mice as the most frequently used laboratory mammal (Kohn and Clifford 2002). Inbred strains are produced and generally maintained with a brother–sister (full-sib) mating scheme. The genetic homogeneity achieved through this process eliminates the variability associated with genetic factors and reduces the number of animals needed per experiment. However, this homogeneity may be affected by several mechanisms that lead to divergence of an inbred strain into differing substrains. Genetic contamination caused by breeding errors, incomplete inbreeding with residual allogenicity, mutation, and genetic drift all are known to contribute to substrain divergence (Sharp et al. 2002; Simpson et al. 1997) . Therefore, colonies of inbred strains from various suppliers likely contain differing amounts of genetic variation.

CHAPTER II

GENOMIC COMPARISON OF SUBSTRAINS*

Rationale

The LEW/Mai, WF/Mai, and WF/Mol commercial rat colonies were all discontinued and as a result those substrains are extinct. In addition the Lewis strain has been shown to have phenotypic and genetic variation among the various substrains (Canzian 1997; Olofsson et al. 2004; Ritter et al. 2000). Therefore the first step undertaken was to compare the genetic makeup of the commercially available Lewis and Wistar-Furth substrains. Microsatellite markers were chosen for a genome scan and genotyped for three commercially available Lewis substrains and two commercially available Wistar-Furth substrains. This was done for later comparison with the WF.LEW congenic and to decide which substrains to use in future breeding experiments with the congenic.

Materials and Methods

DNA sources. LEW/SsNHsd and WF/NHsd spleens were purchased from Harlan Bioproducts for Science (Indianapolis, IN). LEW/Crl and WF/CrCrl rats were purchased from Charles River Laboratories (Boston, MA). LEW/MolTac rats were purchased from Taconic (Germantown, NY). Rats were housed in a facility accredited

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by the Association for the Assessment and Accreditation of Laboratory Animal Care, International, and were maintained on animal use protocols approved by the University Laboratory Animal Care and Use Committee at Texas A & M University. Live rats were euthanized humanely, and spleens were removed for DNA isolation. Genomic DNA was extracted from spleen by phenol extraction with ethanol precipitation (Moore 1996).We genotyped two rats for each of the LEW/SsNHsd and WF/NHsd substrains and one rat for each of the remaining substrains.

Selection of simple sequence-length polymorphisms (SSLPs). SSLPs were selected by use of the Genome Scanner tool provided by the Rat Genome Database (http://rgd.mcw.edu). Markers were chosen at approximately 15- to 20-cM intervals across the rat genome. A minimum of four markers were selected for each chromosome.

Genotyping protocol. A previously described allele-sizing method using M13-tailed primers was used to genotype samples of genomic DNA (Boutin-Ganache et al. 2001). Briefly, forward primers were synthesized with the M13 sequence at the 5' ends. Three M13 sequence primers were 5' labeled with the fluorescent dyes 6-FAM, HEX, and NED (Applied Biosystems, Foster City, CA). Each SSLP was amplified by use of standard polymerase chain reaction (PCR) techniques (Kramer and Coen 1995). Reactions contained 1 µl 10× PCR buffer with 15 mM $MgCl_2$ (Applied Biosystems), 0.2 mM each dNTP, 0.5 U AmpliTaq Gold (Applied Biosystems), 250 nM each of the forward and reverse primers, 50 ng genomic DNA, and enough double-distilled H_2O to yield a 10-µl reaction. The M13-labeled forward SSLP primers and the fluorescent-labeled M13 primers were mixed together in a 1:15 ratio for the forward primer

component of each reaction. Thermocycler reaction conditions were set at 94 °C for 10 min followed by 35 cycles of 94 °C for 30 s, 57 °C annealing for 30 s, and extension at 72 °C for 30 s, with final extension for 5 min at 72 °C. Postreaction products were analyzed automatically (3130*xl* Genetic Analyzer, Applied Biosystems), and genotypes were scored with GeneMapper version 3.7 (Applied Biosystems). Results were exported to a spreadsheet (Excel, Microsoft, Redmond, WA) for analysis and substrain comparisons.

Results

Genotyping. We performed genome scans consisting of 159 SSLP markers on DNA samples from the five substrains (three Lewis, two Wistar-Furth). The amplification products for three markers of different sizes were loaded together into a single well for injection into the genetic analyzer. All three Lewis substrains were scored simultaneously for each SSLP to eliminate variation due to the different genetic analyzer run times (Fig. 1). All markers were genotyped at least three times to assess repeatability. The Wistar-Furth samples were genotyped in the same manner. All rats genotyped were found to be homozygous for the markers tested.

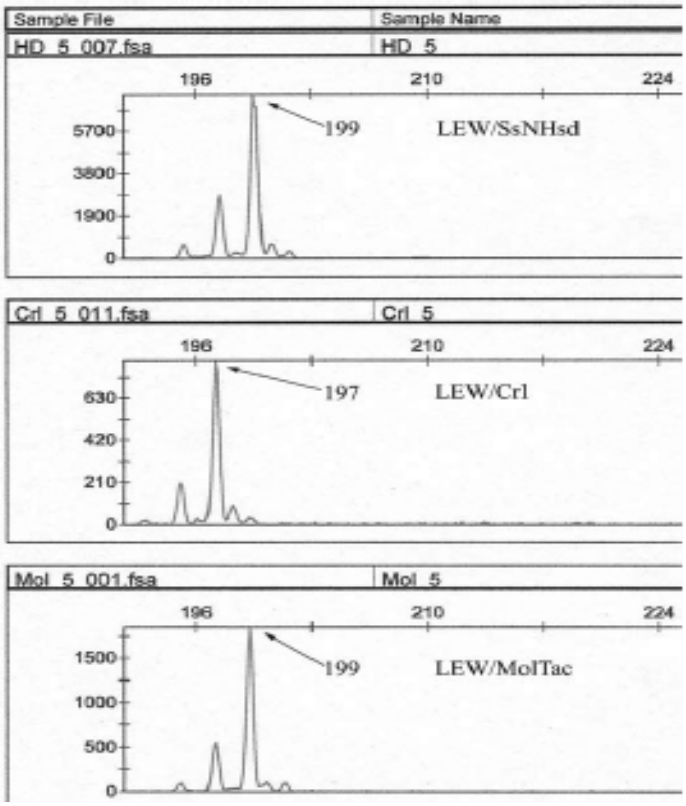

Fig. 1 Sample electropherogram. Genotypes scores (in basepairs) for the 3 Substrains of Lewis rats.

Lewis substrains. LEW/SsNHsd showed approximately 37% genomic difference when compared with LEW/MolTac. When compared with LEW/Crl, the LEW/SsNHsd substrain showed only an 8% difference. LEW/MolTac compared with LEW/Crl demonstrated an approximate 45% difference (Fig. 2).

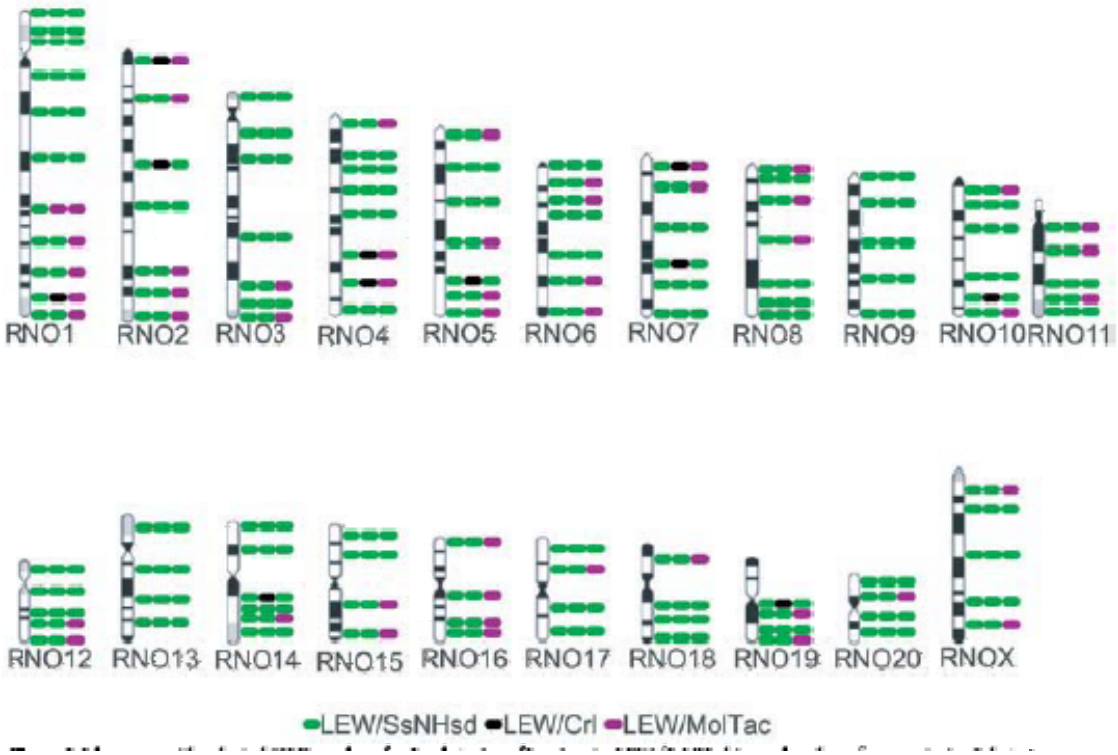

Fig. 2 Ideogram with selected SSLP markers for 3 substrains of Lewis rats. LEW/SsNHsd is used as the reference strain. Alleles specific to a particular substrain are denoted by the corresponding color.

Wistar-Furth substrains. WF/NHsd compared with WF/CrCrl demonstrated a difference of 8%, similar to the LEW/SsNHsd to LEW/Crl comparison (Fig. 3).

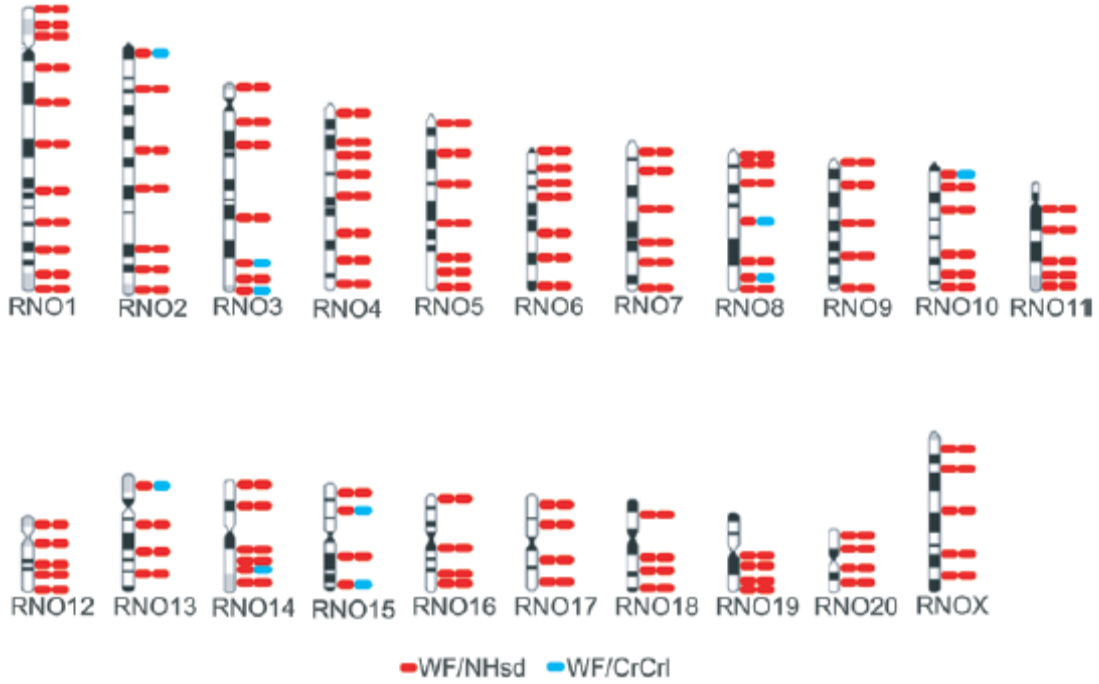

Fig. 3 Ideogram with selected SSLP markers for 2 substrains of Wistar-Furth Rats. WF/NHsd is used as the reference strain. Alleles specific to a particular substrain are denoted by the corresponding color.

Discussion

Smits and colleagues (2004) examined 80 single-nucleotide polymorphisms in 11 Lewis substrains and four Wistar-Furth substrains and found approximately 20% and 19% genetic variation within each inbred strain, respectively. Inbred strains are known to have residual allogenicity if separated at the 20th generation (Bailey 1982). Once past F40, at which residual allogenicity becomes negligible, the strain is still subject to mutation (Bailey 1982). The founder effect could be an important factor, considering that inbred colonies usually are created by use of small numbers of animals. With unfixed alleles segregating in a strain, genetic drift and founder effect may lead to fixation or loss of certain alleles, thereby creating substrains when colonies are separated.

The Lewis and Wistar-Furth strains both were created from outbred Wistar stock. The various Lewis substrains we used in this study have been separated since the late 1950s. Most notably, the LEW/MolTac substrain has been transferred to several locations (Fig. 4); overall the Wistar-Furth substrains have been transferred to fewer locations. LEW/SsNHsd and WF/NHsd rats potentially were derived from similar source colonies as were the LEW/Mai and WF/Mai rats, respectively, and are thought to be the living substrains most closely related to the extinct LEW/Mai and WF/Mai substrains.

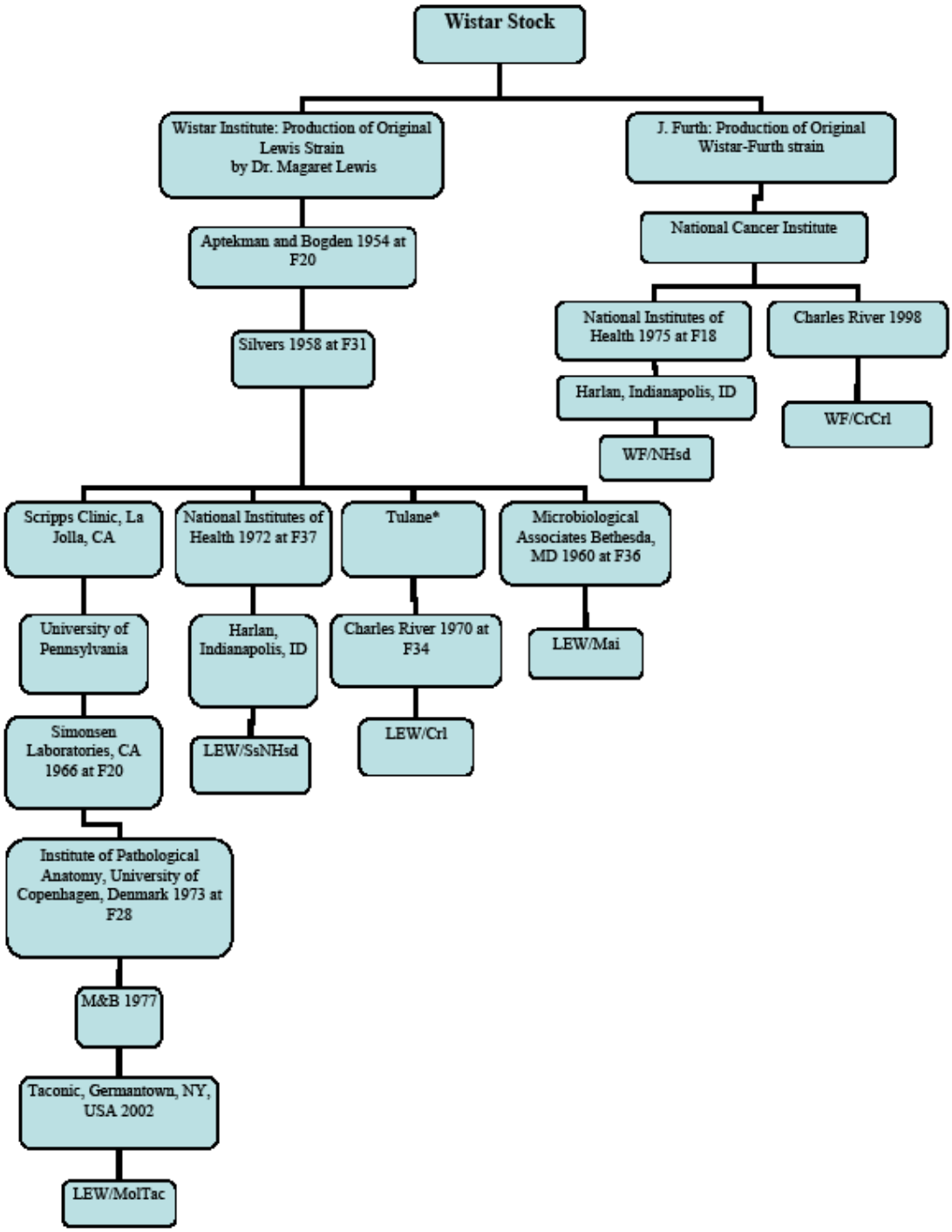

Fig. 4 Chart depicting the lineage of the Lewis and Wistar-Furth substrains. Information compiled from public databases, publications, and personal communication. *Information regarding exact origin of Tulane colony was unavailable. Therefore a possibility of an intermediate source exists.

LEW/Mol rats have long been known to have a different level of susceptibility to induction of autoimmune disease, compared with other Lewis substrains. Kallen and Lodgberg (1982) found that LEW/Mol rats failed to mount a host-versus-graft response to LEW/Mai rats. Those investigators concluded that LEW/Mol carried a mutation that was responsible for the difference in susceptibility and that the difference was not due to accidental crossbreeding of the strain (Kallen and Lodgberg 1982).However, no molecular genetic markers were evaluated. Kallen and Lodgberg (1982) mentioned the LER strain, which had its origin at Simonsen Laboratories (Gilroy, CA) as did LEW/Mol. It was later suggested that the LER strain was the result of contamination of the Lewis strain by crossbreeding with the Buffalo strain (Goldmuntz 1993).

Our data comparing the LEW/MolTac substrain with the LEW/SsNHsd substrain show similar results to the comparison of LEW/Mol with LEW/Ztm made by Olofsson and colleagues (2004). The presence of approximately 37% non-Lewis genome in the LEW/MolTac substrain leads to the conclusion that a crossbreeding event occurred somewhere in the history of this substrain. Comparison of our genotyping data with the public records of inbred strains failed to demonstrate a likely source for the contamination, perhaps because of contamination of other inbred strains reported in the public database (Olofsson et al. 2004). Other possibilities include outcrossing of the Lewis strain with an outbred stock or outcrossing to multiple inbred strains over time. Subsequent studies are needed to compare the LEW/MolTac substrain with a more robust group of control DNAs to determine the likely source of the contamination. We support the claim made by Olofsson and colleagues (2004) that LEW/MolTac should not

be considered a substrain of the Lewis strain. We submit that LEW/MolTac is a separate inbred strain and that the nomenclature should be updated accordingly to reflect this. Therefore, LEW/MolTac rats should not be used in studies for which standard Lewis genetics are needed as controls.

As evidenced by the data we present, genetic monitoring is an important management tool for any entity maintaining colonies of inbred rodents. Investigators should consider the background genetics of the particular strains used for their research projects and should use strains from a single source when feasible.

CHAPTER III

MAPPING OF RESISTANCE GENE AND VIRAL CHALLENGE

Rationale

With the ultimate goal of locating the gene conferring resistance to RVFV induced hepatic disease in rats, embryos were obtained and the WF.LEW congenic rat strain was rederived. These rats were then genotyped and the results compared with the substrains previously genotyped in order to locate the congenic segment and determine which substrains were most closely related to the original parental strains used to make the WF.LEW congenic. Once that was accomplished breeding experiments were performed in an effort to reduce the size of that segment and further clarify the resistant gene location. Finally, viral challenge experiments were undertaken to confirm and clarify the phenotypes of the various substrains and the congenic.

Materials and Methods

DNA and live rat sources. LEW/SsNHsd and WF/NHsd spleens were purchased from Harlan Bioproducts for Science (Indianapolis, IN). LEW/SsNHsd and WF/NHsd rats were purchased from Harlan (Indianapolis, IN). LEW/Crl and WF/CrCrl rats were purchased from Charles River Laboratories (Boston, MA). LEW/MolTac rats were purchased from Taconic (Germantown, NY). Embryos from the WF.LEW strain were frozen and maintained at National Institutes of Health (NIH). Live rats were rederived from WF.LEW embryos obtained from NIH by the Rat Resource and Research Center (RRRC), University of Missouri (Columbia, MO). Breeding pairs were then sent to

Texas A&M University to found a colony of WF.LEW rats. WF.LEW rats were mated with WF/NHsd rats to produce a generation of (WF.LEW X WF/NHsd)F1s. Female F1s were then backcrossed to WF/NHsd males to produce an N1 generation of (WF.LEW X WF/NHsd)F1 X WF/NHsd rats. Rats were housed in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International, and were maintained on animal use protocols approved by the University Laboratory Animal Care and Use Committee at Texas A&M University. Live rats were euthanized humanely, and spleens were removed for DNA isolation. Genomic DNA was extracted from spleen by phenol extraction with ethanol precipitation (Moore 1996). Tail biopsies were taken from neonatal rat pups from the N1 backcross litters and DNA extracted by a previously described technique (Truett et al. 2000).

Selection of simple sequence-length polymorphisms (SSLPs) and single nucleotide polymorphisms (SNPs). SSLPs were selected by use of the Genome Scanner tool provided by the Rat Genome Database (http://rgd.mcw.edu). Markers were chosen at approximately 15- to 20-cM intervals across the rat genome. A minimum of four markers were selected for each chromosome. Additional markers were chosen to further characterize regions where markers with LEW genotypes were located in the congenic strain. Appendix A lists the SSLP markers chosen for each chromosome and the allele sizes for each substrain. SNPs were selected with the GBrowse function of the Rat Genome Database (http://rgd.mcw.edu) and retrieved from the dbSNPs database (http://www.ncbi.nlm.nih.gov/SNP/). SNPs were chosen at regular intervals to fine map

regions of interest identified by SSLP markers. See Appendix B for the list of SNPs chosen for each region and the genotype for each substrain.

Genotyping protocols. The allele sizing protocol previously described in Chapter II was used to genotype the SSLP markers.

SNP's were genotyped using the SNaPshot Multiplex Kit (Applied Biosystems). Primers flanking the SNP retrieved from dbSNPs database were designed using Primer3 (http://primer3.sourceforge.net/). The 30 bases immediately 5' to the SNP were used for each specific SNaPshot primer. Initial PCR reactions contained 1 µl 10× PCR buffer with 15 mM $MgCl_2$ (Applied Biosystems), 0.2 mM each dNTP, 0.5 U AmpliTaq Gold (Applied Biosystems), 250 nM each of the forward and reverse primers, 50 ng genomic DNA, and enough double-distilled H_2O to yield a 10-µl reaction. These products were then purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, CA). SNaPshot PCR reactions consisted of 2 µl of SNaPshot Multiplex Ready Reaction Mix, 3 µl of purified PCR product, 250nM of SNaPshot primer, and enough double-distilled H_2O to yield a 10-µl reaction. Thermal cycling conditions were set per manufacturers instructions and post extension treatment with Shrimp Alkaline Phosphatase was performed per manufacturer's instructions. Postreaction products were analyzed automatically (3130*xl* Genetic Analyzer, Applied Biosystems), and genotypes were scored with GeneMapper version 4.0 (Applied Biosystems). Results were exported to a spreadsheet (Excel, Microsoft, Redmond, WA) for analysis and substrain comparisons similar to the SSLP data.

Viral challenges. Groups of adult rats 10+ weeks of age were anesthetized and inoculated subcutaneously with 0.1 ml of $5X10^5$ ZH501 strain of RVFV. Commercially available inbred substrains tested included LEW/SsNHsd, LEW/Crl, LEW/MolTac, WF/NHsd, WF/CrCrl. In addition (WF.LEW X WF/NHsd)F1s and three backcross litters of N1s were challenged using the same protocol as the commercial strains. Control rats from the substrains LEW/SsNHsd and WF/NHsd were inoculated with Hank's Balanced Salt Solution. All viral challenge work was done in the ABSL-4 facility at the University of Texas Medical Branch, Galveston, Texas.

Results

Genome scan. Since the original parental substrains, LEW/Mai and WF/Mai, are extinct, an initial genome scan of 137 SSLP markers was performed to compare the WF.LEW strain with the LEW/SsNHsd, LEW/Crl , LEW/MolTac , WF/CrCrl, and WF/NHsd substrains. This served to evaluate how closely the commercially available substrains and the original parental strains were related and to locate the congenic region within the WF.LEW genome. The Harlan substrains were found to share the most markers in common with the original strains used to construct the WF.LEW congenic. Lewis markers were located on RNO3 and RNO9 (Fig. 5). Four markers tested failed to match any of the other five substrains chosen. These included D9Rat30, D15Rat81, D15Rat60, and D18Rat55.

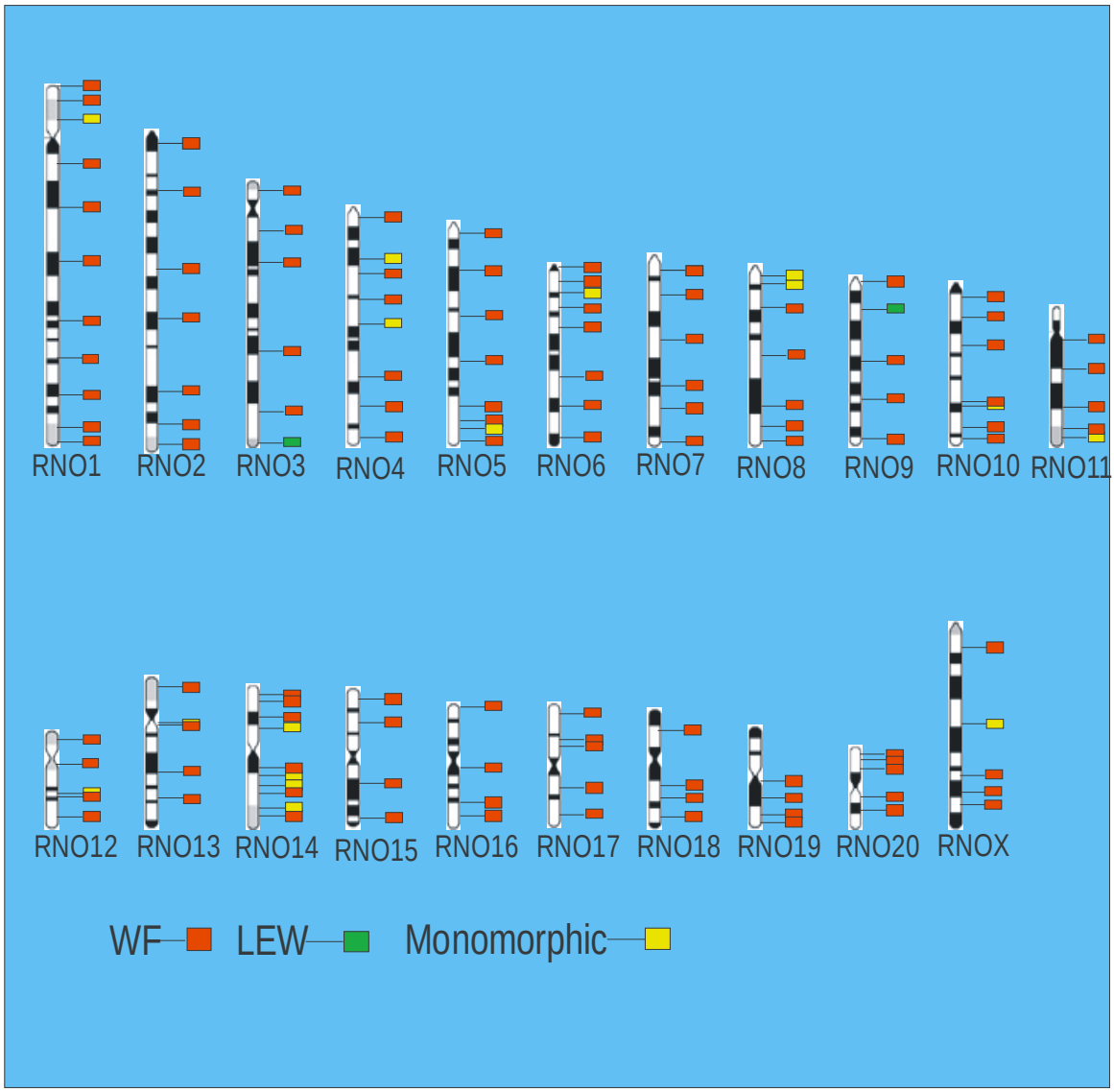

Fig . 5 Rat ideogram showing selected genomic scan marker locations.

Fine mapping of regions of interest. To further characterize the two regions of interest 22 additional SSLP markers were chosen, 15 on chromosome three and seven on chromosome nine. Genotyping experiments utilizing these 22 SSLPs to compare WF.LEW strain with the other substrains revealed two more Lewis markers on RNO3 and no additional Lewis markers on RNO9 (Figs. 6 and 7).

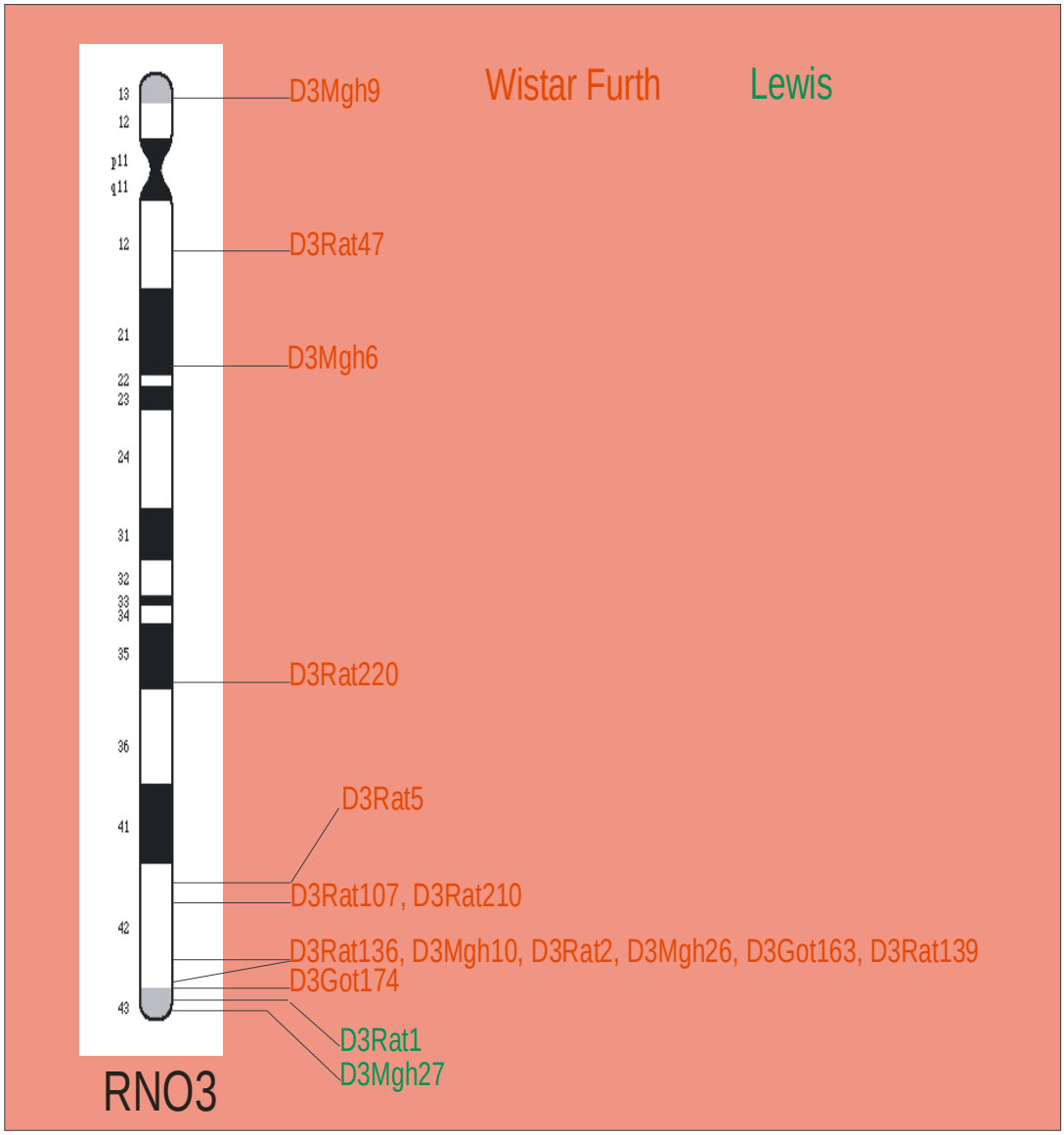

Fig. 6 RNO3 ideogram showing selected SSLP marker locations.

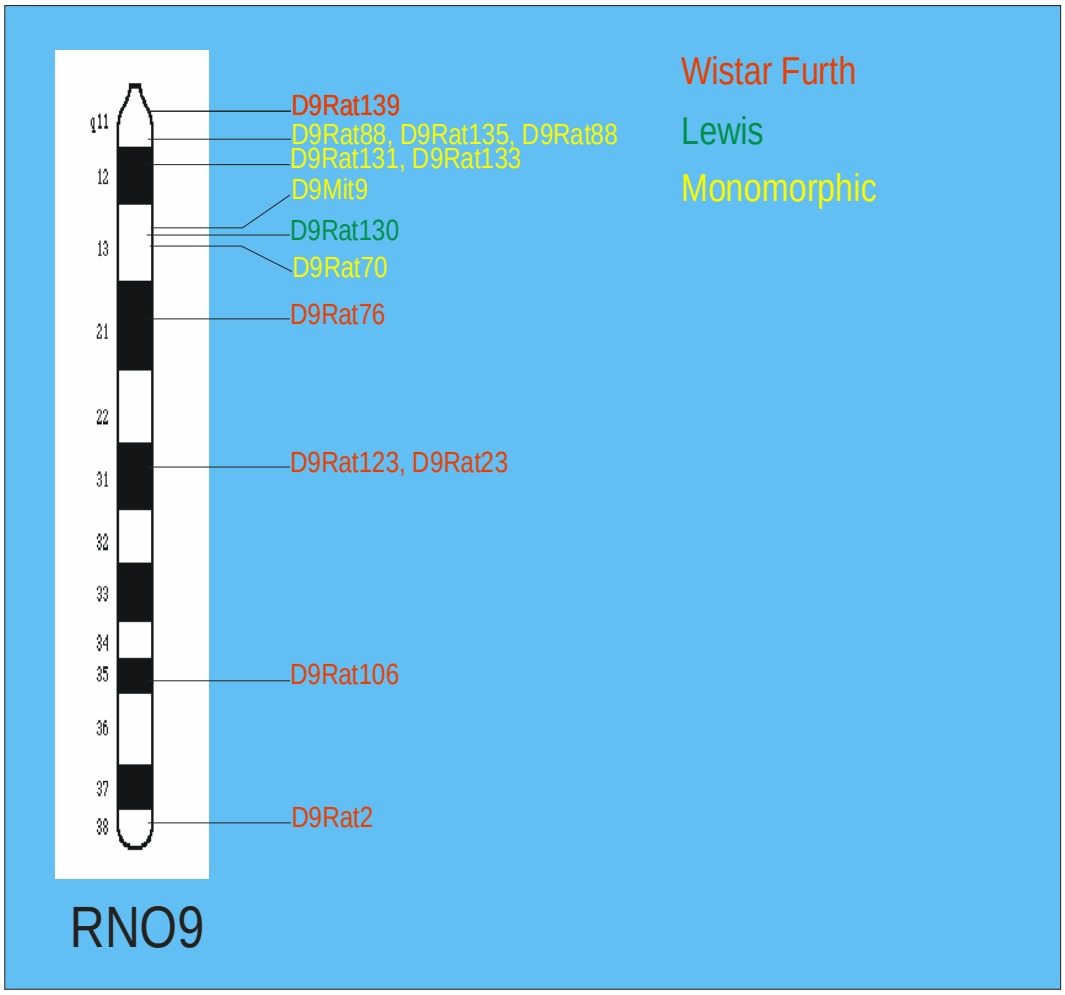

Fig. 7 RNO9 ideogram showing selected SSLP marker locations.

SNP's were then chosen in the regions of interest to increase coverage between the SSLP markers. A total of 32 SNP's, 24 on RNO3 and eight on RNO9, were genotyped across the five substrains and the congenic (Figs. 8 and 9).

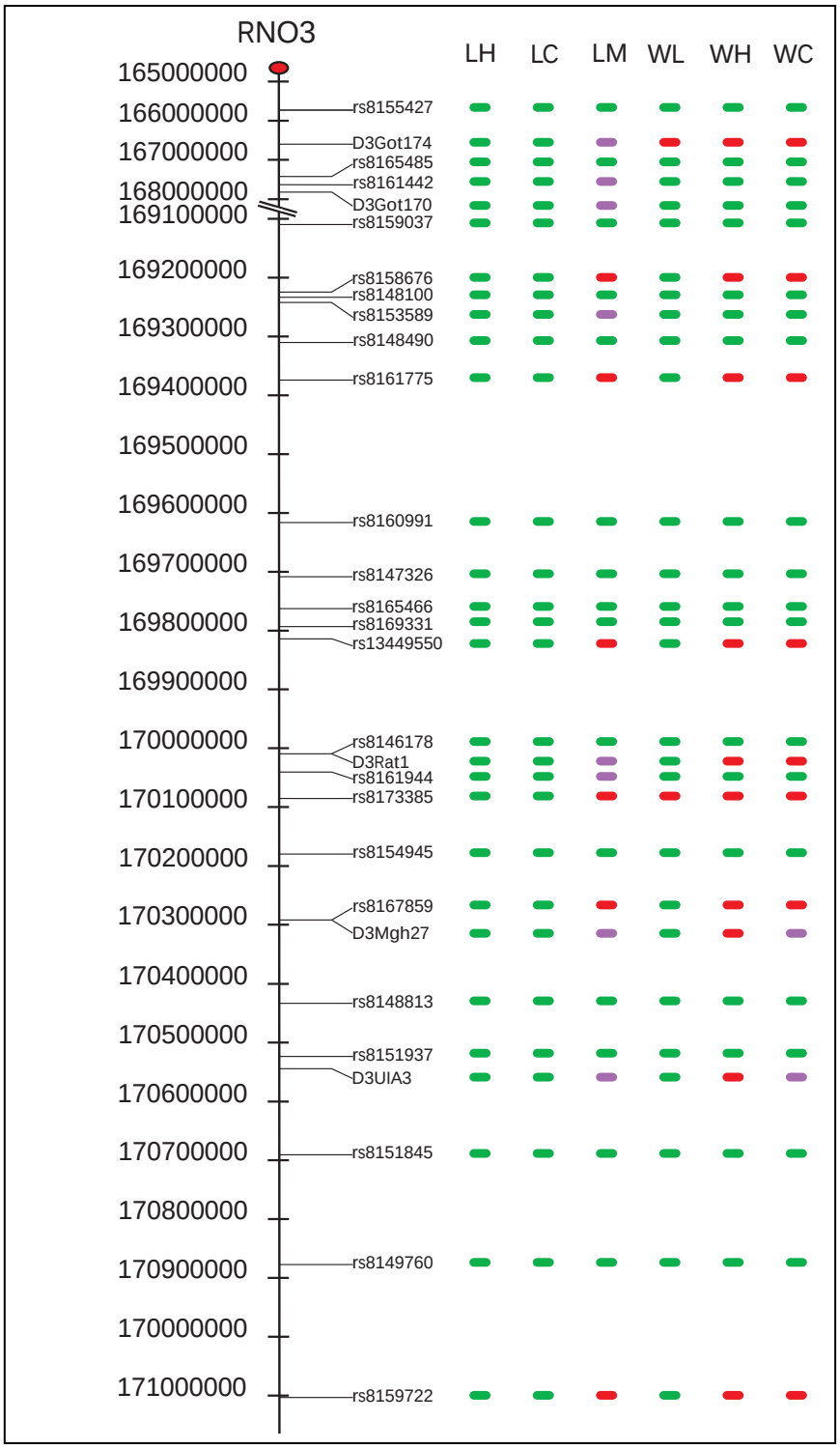

Fig. 8 RNO3 SNP marker locations. . LH=LEW/SsNHsd, LC=LEW/Crl, LM=LEW/MolTac, WL=WF.LEW, WH=WF/NHsd, WC=WF/CrCrl, Green=LEW, Red=WF, Orange=WF.LEW.

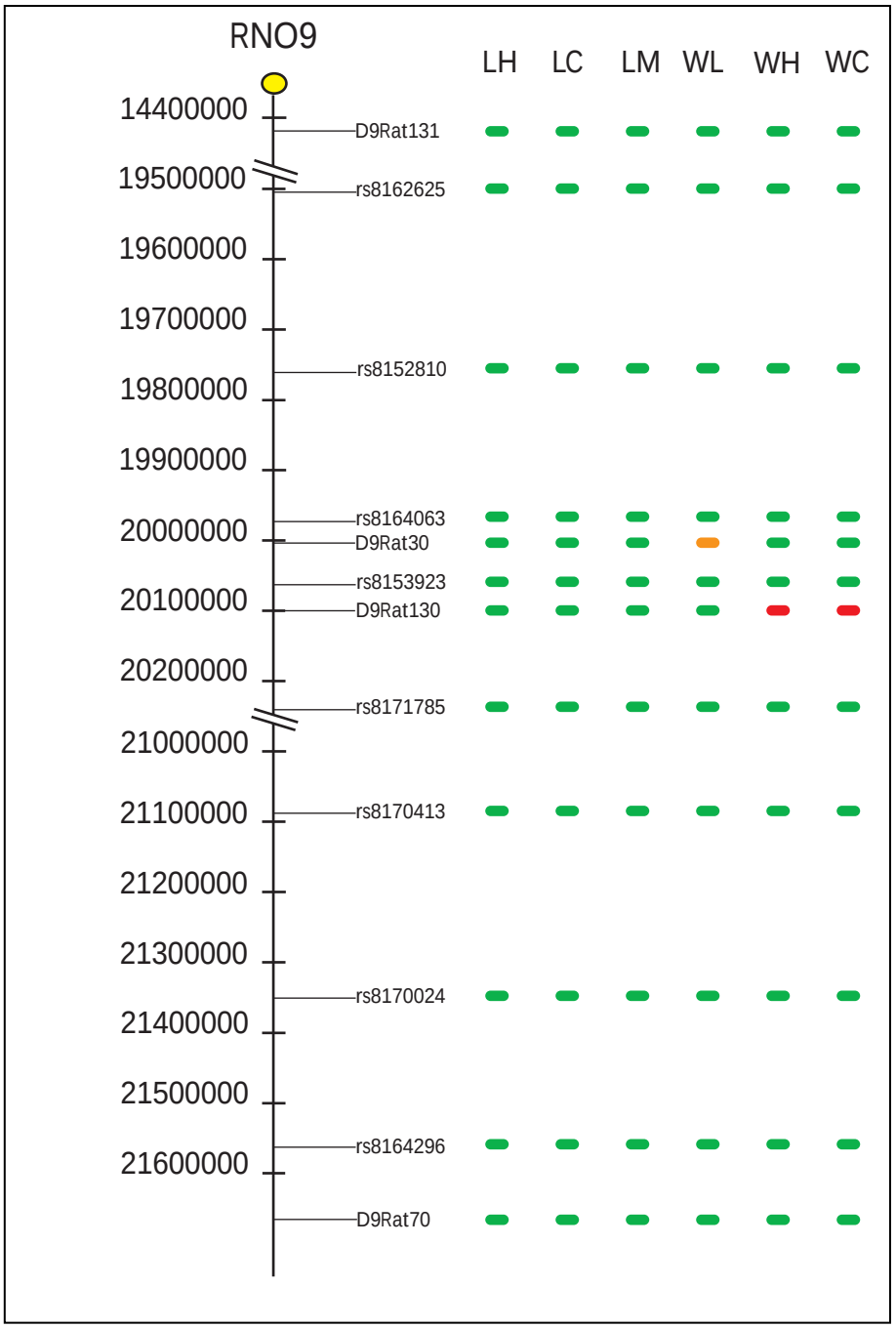

Fig. 9 RNO9 SNP marker locations. LH=LEW/SsNHsd, LC=LEW/Crl, LM=LEW/MolTac, WL=WF.LEW, WH=WF/NHsd, WC=WF/CrCrl, Green=LEW, Red=WF, Orange=WF.LEW.

Breeding and genotyping of N1 offspring. Since two regions of interest on two different chromosomes were located, breeding experiments were designed to separate the two regions and reduce the size of the larger one on RNO3 if possible. WF.LEW females were mated to WF/NHsd males to create an F1 hybrid generation. Females from the F1 generation were then mated to WF/NHsd males to produce a backcross N1 generation.

The single Lewis marker on RNO9 (D9Rat130) and 3 SNP markers (rs8158676, rs8164532, rs8159722) from RNO3 were chosen to allow the N1 offspring to be designated according to which chromosome the Lewis markers were located. The three SNP markers on RNO3 were used to evaluate if recombination had occurred in the larger segment on that chromosome. The N1 offspring were genotyped for the selected markers and assigned to one of four groups as shown in Table 1. No recombinant offspring were found in the first three litters.

Table 1 Genotypic classes of N1 litters

Litter	No Lewis Markers	Lewis Markers RNO 3	Lewis Markers RNO 9	Lewis Markers RNO 3 & 9
A	3	2	1	2
B	0	3	4	2
C	3	3	1	1
Total	6	8	6	5

Viral challenges. Groups of five rats from each of the commercially available substrains of Lewis and Wistar-Furth, the WF.LEW congenic and the F1 generation were challenged with live RVFV. Results were similar to those previously reported. (Anderson et al. 1987; Anderson et al. 1991; Ritter et al. 2000) (Table 2 ,Figs. 10 and 11)

Table 2 Viral challenge survival

Strain	Inoculated	Survived
LEW/Crl	5	4
LEW/SsNHsd	5	2
LEW/MolTac	5	0
WF/NHsd	5	0
WF/CrCrl	5	0
WF.LEW	5	4
(WF.LEWxWF/NHsd)F1	5	4

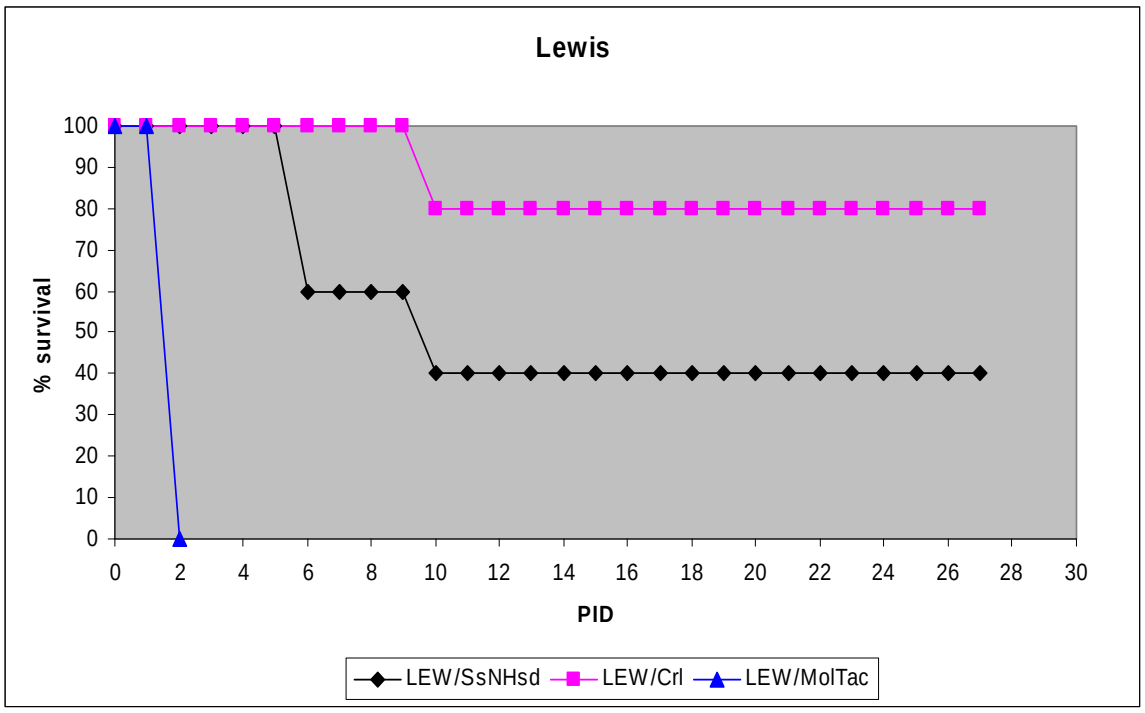

Fig. 10 Lewis substrain survival comparison.

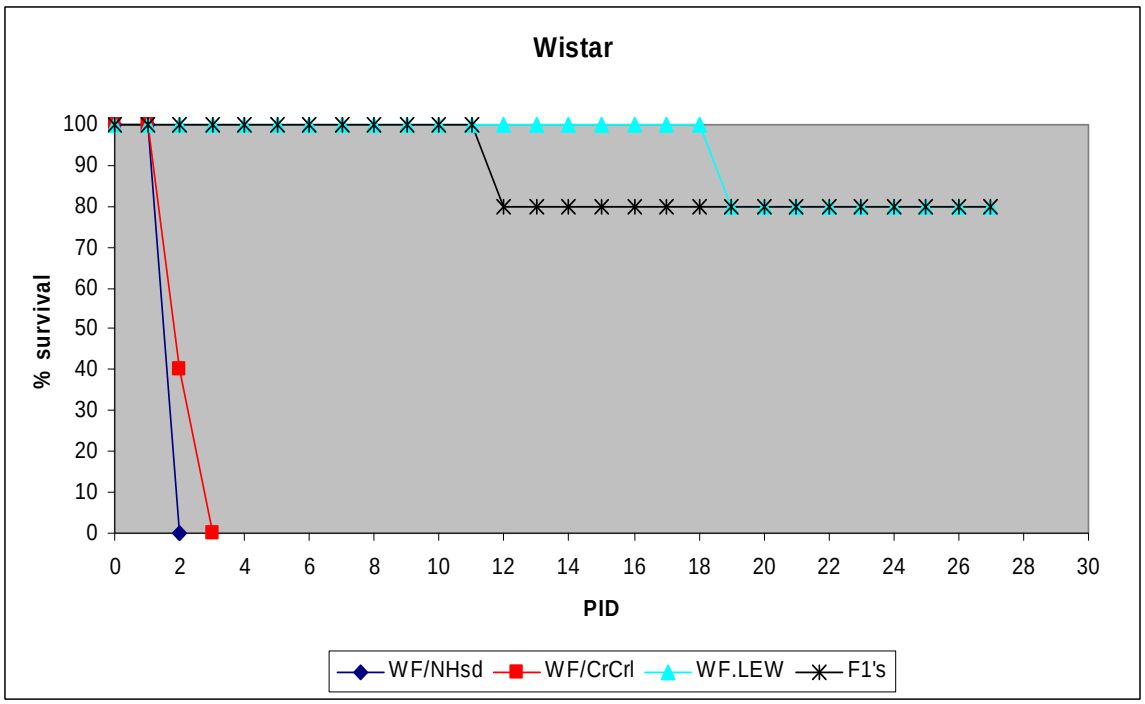

Fig. 11 Wistar-Furth substrain survival comparison.

The 4 groups of N1 offspring were challenged in an attempt to discern which chromosome, RNO3 or RNO9, carried the locus responsible for the resistance. The results indicated RNO3 correlated with the major resistance to hepatic disease. (Table 3, Fig. 12)

Table 3 Viral challenge survival of N1 groups

Group	Inoculated	Survived
No Lewis Markers	6	0
Lewis Markers RNO3	8	6
Lewis Markers RNO9	6	0
Lewis Markers RNO3 & 9	5	5

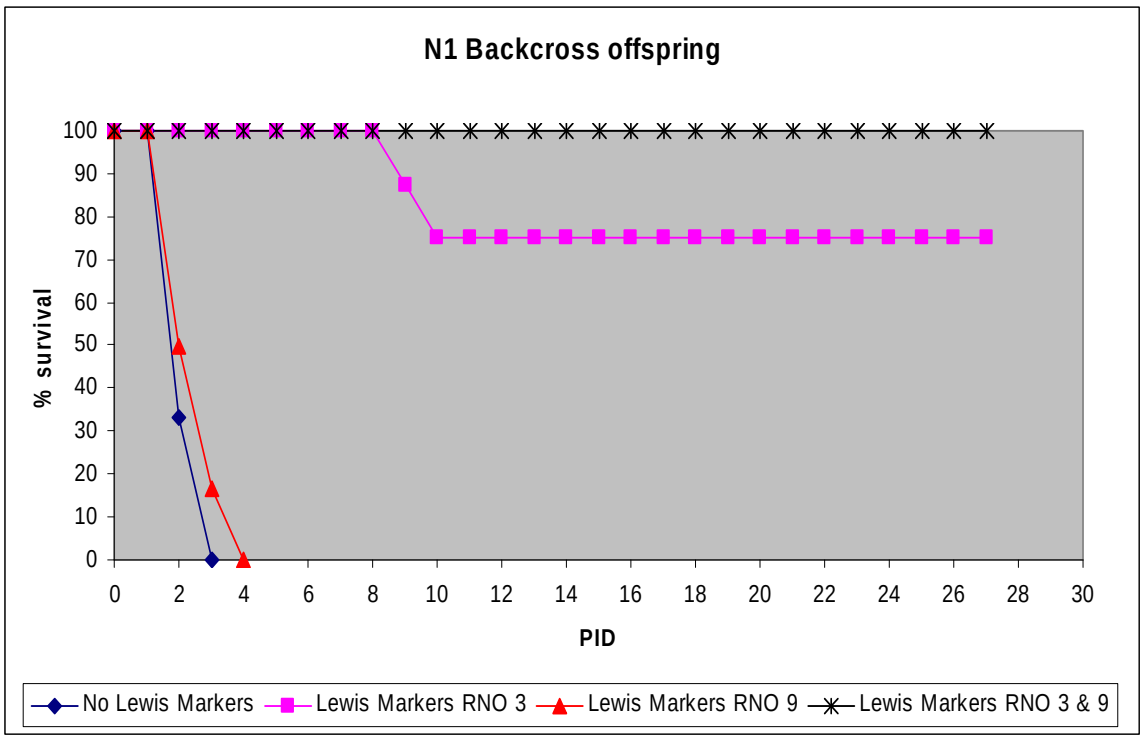

Fig. 12 N1 survival comparison.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Summary

Genotyping experiments were performed to survey the genetic variation among commercially available substrains of Lewis and Wistar-Furth rats. The results were compared to the genotyping results of the WF.LEW congenic. Lewis markers were identified on chromosomes RNO3 and RNO9 of the WF.LEW congenic. With the region found on RNO3 being the larger of the two regions. The LEW/MolTac substrain contained a striking amount of genomic difference from the other Lewis substrains as previously has been reported (Olofsson et al. 2004).

The results of the viral challenge experiments of the commercial Lewis and Wistar-Furth strains were similar to those previously reported (Anderson et al. 1987; Anderson et al. 1991; Ritter et al. 2000). The Lewis substrains were confirmed to be resistant with the exception of the Lew/MolTac which had been reported to be susceptible (Ritter et al. 2000). The Wistar-Furth substrains were found to be susceptible. The congenic WF.LEW and the F1's created from crossing the congenic with the WF/NHsd substrain were both resistant. Four groups of N1 backcross offspring created from mating the F1's with WF/NHsd rats were challenged and two groups were found to be resistant, those having Lewis markers on RNO3 and those having Lewis markers on both RNO3 and RNO9.

Conclusions

Rodent colonies separated after 20 generations of inbreeding but before 40 generations contain residual allogenicity that will lead to separate lines of differing substrains if inbreeding is continued. Inbred colonies more than 20 generations from a common ancestor may contain enough genetic variation due to mutation and genetic drift to quality as different substrains. The genotyping experiments demonstrated that commercially available substrains of Lewis and Wistar-Furth rats do contain genetic variation. In the case of the Lewis strain this variation can be quite considerable even to the point of possibly being a distinct inbred strain. Reinforcing the point that genetic monitoring of rodent colonies is very important. Investigators should be mindful if changing vendors that although purchasing the same strain the genetics may be somewhat different among the substrains of any particular strain. Comparison of the WF.LEW congenic to the commercial substrains demonstrated that the Lew/SsNHsd and the WF/NHsd substrains were the most closely related of the commercial substrains to the original substrains used to make the congenic. Comparison of the results from the various substrains to the WF.LEW congenic revealed two regions of interest for the gene conferring resistance to RVFV induced hepatitis. Results from the viral challenges of the N1 offspring suggest that the gene is located in the region identified on RNO3. The resistance of the F1's and the survival pattern of the N1 groups is in agreement with the previous finding that the resistance was inherited from a single dominant gene in a Mendelian fashion (Anderson et al. 1987). The Lewis marker identified on RNO9 is thought to be an artifact left over from the Mai substrains that were initially used to

create the congenic. Although a slight possibility exists that a gene on RNO9 may modify the survival later in the course of the disease, this possibility is greatly reduced due to the fact that the WF.LEW and F1's had a similar survival rate to the N1 group with Lewis markers only on RNO3. The susceptible LEW/MolTac substrain carries the same markers as the susceptible substrains in the region of interest on RNO3 and the same markers as the resistant groups on RNO9. Although most of the evidence points to the Lewis marker on RNO9 as an artifact, the N1 group with Lewis markers on RNO3 and RNO9 did have 100% survival at 28 days post infection. In addition the N1 group with Lewis markers only on RNO9 had a couple of animals that lived slightly longer than the other susceptible groups. Thus a greater number of animals would need to be tested to examine the hypothesis that a gene on RNO9 modifies the course of the disease.

Unfortunately, no strong candidate genes were identified on the region located at the distal end of RNO3. There are, however, several genes related to the process of transcription found in that area. These include *transcription elongation factor A (SII)2* (Tcea2), *SRY (sex determining region Y)-box 18* (Sox18), *myelin transcription factor 1* (Myt1) and *death inducer-obliterator 1* (Dido1). It has been shown that RVFV inhibits host cell transcription through interactions with TFIIH and a SAP30 complex (Le May et al. 2004; Le May et al. 2008). Therefore one may hypothesize one of the transcription related genes or some unknown transcription factor located on the distal portion of RNO3 is responsible for the resistance to RVFV. A reduction in size of the region on

RNO3 through further backcross experiments would be useful to help locate a putative candidate.

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APPENDIX A

SSLP MARKERS AND SIZES*

Marker	LEW/ SsNHsd	LEW/ MolTac	LEW/ Crl	WF. LEW	WF/ NHsd	WF/ CrCrl
D1Rat232	242	242	242	248	248	248
D1Rat5	181	181	181	203	203	203
D1Arb3	349	349	349	349	349	349
D1Rat257	134	134	134	126	126	126
D1Rat266	177	177	177	175	175	175
D1Rat183	256	256	256	246	246	246
D1Rat281	134	132	132	138	138	138
D1Rat70	133	131	133	171	171	171
D1Rat169	148	160	148	160	160	160
D1Rat166	157	165	159	163	163	163
D1Rat312	220	216	220	214	214	214
D2Rat3	137	143	139	141	141	145
D2Rat200	142	134	142	138	138	138
D2Rat123	200	200	198	202	202	202
D2Rat35	183	183	183	175	175	175
D2Mgh12	184	158	184	180	180	180
D2Rat64	202	206	202	202	202	202
D2Rat245	135	117	135	131	131	131
D2Rat70	193	187	193	185	185	185
D3Mgh9	166	166	166	168	168	168
D3Rat47	143	143	143	135	135	135
D3Mgh6	126	126	126	122	122	122
D3Rat220	234	234	234	248	248	248
D3Rat5	181	197	181	183	183	183
D3Rat107	224	218	224	220	224	220
D3Rat210	165	165	165	176	176	176
D3Rat136	235	237	235	233	233	233
D3Mgh10	138	138	138	126	126	126
D3Rat2	192	192	192	188	189	189
D3Mgh26	223	225	223	217	217	219
D3Rat77	218	234	218	226	226	226
D3Got163	183	183	183	191	191	191
D3Got157	267	263	267	265	265	265
D3Rat139	170	170	170	162	162	162
D3Got171	240	245	240	240	240	240
D3Got174	222	220	222	224	224	224

Marker	LEW/ SsNHsd	LEW/ MolTac	LEW/ Crl	WF. LEW	WF/ NHsd	WF/ CrCrl
D3Got170	396	400	396	396	396	396
D3Rat1	162	182	162	162	176	176
D3Mgh27	169	161	169	169	157	161
D3UIA3	285	293	285	285	289	293
D4Rat3	161	173	161	165	165	165
D4Rat13	140	140	140	140	140	140
D4Rat15	168	168	168	184	184	184
D4Rat27	140	140	140	146	146	146
D4Rat35	176	176	176	176	176	176
D4Rat116	259	251	261	253	253	253
D4Mgh7	179	179	179	161	161	161
D4Rat107	254	250	256	250	250	250
D4Rat90	216	220	216	220	220	220
D4Rat204	225	225	225	235	235	235
D5Rat120	168	154	168	154	154	154
D5Rat70	284	284	284	268	268	268
D5Rat12	162	162	162	168	168	168
D5Rat60	274	272	274	271	271	271
D5Rat36	192	192	194	164	164	164
D5Rat67	224	232	224	218	218	218
D5Rat205	243	243	243	243	243	243
D5Rat51	152	156	152	142	142	142
D6Rat46	155	155	155	147	147	147
D6Rat68	278	278	278	264	264	264
D6Rat105	246	244	246	246	246	246
D6Rat74	253	255	253	253	253	253
D6Rat144	185	181	185	181	181	181
D6Rat135	169	169	169	177	177	177
D6Rat124	260	260	260	270	270	270
D6Rat116	152	148	152	148	148	148
D6Rat1	238	252	238	246	246	246
D7Rat158	137	127	137	137	137	137
D7Rat113	132	116	136	116	116	116
D7Rat152	144	148	144	148	148	148
D7Rat69	251	251	251	247	247	247
D7Mgh6	154	154	152	162	162	162
D7Rat122	189	189	189	199	199	199
D7Rat80	239	239	239	225	225	225

Marker	LEW/ SsNHsd	LEW/ MolTac	LEW/ Crl	WF. LEW	WF/ NHsd	WF/ CrCrl
D8Rat58	190	198	190	190	190	190
D8Rat57	192	192	192	192	192	192
D8Rat162	268	258	268	264	264	264
D8Rat150	230	222	230	222	222	224
D8Rat126	188	188	188	184	184	184
D8Rat119	201	201	201	191	191	193
D8Rat171	250	250	250	270	270	270
D9Rat139	140	140	140	142	142	142
D9Rat88	220	220	220	220	220	220
D9Rat135	176	176	176	176	176	176
D9Rat131	122	122	122	122	122	122
D9Rat130	163	163	163	163	165	165
D9Rat30	176	176	176	180	176	176
D9Rat70	207	207	207	207	207	207
D9Rat76	248	248	248	240	240	240
D9Rat123	236	236	236	242	242	242
D9Rat23	162	162	162	150	150	150
D9Rat106	213	213	213	229	229	229
D9Rat2	146	146	146	156	156	156
D10Rat94	208	208	208	208	208	208
D10Rat218	166	152	166	164	162	164
D10Mit16	107	107	107	102	102	102
D10Rat166	163	163	163	161	161	161
D10Rat21	157	157	157	145	145	145
D10Rat11	208	208	210	188	188	188
D10Rat4	175	185	175	177	177	177
D11Rat40	256	250	256	260	260	260
D11Rat35	239	245	239	245	245	245
D11Rat64	253	253	253	257	257	257
D11Mit8	229	239	229	241	241	241
D11Rat1	189	189	189	189	189	189
D12Rat58	178	178	178	200	200	200
D12Rat47	235	235	235	237	237	237
D12Rat46	185	185	185	185	185	185
D12Rat96	220	222	220	222	222	222
D12Rat49	145	132	145	132	132	132

Marker	LEW/ SsNHsd	LEW/ MolTac	LEW/ Crl	WF. LEW	WF/ NHsd	WF/ CrCrl
D13Rat2	183	183	183	181	181	179
D13Rat25	175	175	175	175	175	175
D13Arb8	250	250	250	214	214	214
D13Rat85	153	153	153	157	157	157
D13Mit4	135	135	135	127	127	127
D14Rat70	197	197	197	193	193	193
D14Rat72	199	199	199	189	189	189
D14Rat50	270	270	270	248	248	248
D14Rat36	161	161	161	161	161	161
D14Rat62	240	240	240	240	240	240
D14Rat87	247	247	243	245	245	245
D14Rat40	124	124	124	124	124	124
D14Rat91	176	174	176	172	172	172
D14Arb10	278	278	278	320	320	320
D14Rat34	245	245	245	245	245	245
D14Rat22	185	185	185	199	197	199
D15Rat77	253	253	253	251	251	251
D15Rat81	162	162	162	184	182	178
D15Rat94	195	193	195	199	199	199
D15Rat60	217	221	217	227	223	221
D16Rat12	139	141	139	129	129	129
D16Rat29	164	160	164	166	166	166
D16Rat56	244	238	244	252	252	252
D16Rat15	153	163	153	163	163	163
D17Rat53	157	157	157	139	139	139
D17Rat117	226	224	226	214	214	214
D17Rat83	141	141	141	151	151	151
D17Rat40	191	191	191	193	193	193
D17Rat133	164	164	164	166	166	166
D18Mit1	310	300	310	274	274	274
D18Rat55	113	113	113	135	133	133
D18Rat43	246	246	246	226	226	226
D18Rat5	159	159	159	169	169	169
D18Rat44	222	222	222	222	222	222

Marker	LEW/ SsNHsd	LEW/ MolTac	LEW/ Crl	WF. LEW	WF/ NHsd	WF/ CrCrl
D19Rat74	144	144	148	148	148	148
D19Rat25	132	118	132	118	118	118
D19Rat66	164	164	164	166	166	166
D19Rat2	168	178	168	178	178	178
D20Rat21	172	172	172	184	184	184
D20Rat46	163	163	163	187	187	187
D20Rat33	187	211	187	211	211	211
D20Rat10	181	181	181	175	175	175
D20Rat19	203	203	203	199	199	199
DXRat74	152	160	152	160	160	160
DXRat90	232	232	232	232	232	232
DXRat95	223	223	223	217	217	217
DXRat97	166	166	166	168	168	168
DXRat104	163	169	163	173	173	173

* Allele sizes include the additional 19 base pairs of the M13 primer.

APPENDIX B

SNP MARKERS

RNO3 Markers	LEW/ SsNHsd	LEW/ MolTac	LEW/ Crl	WF. LEW	WF/ NHsd	WF/ CrCrl
rs8155427	C	C	C	C	C	C
rs8165485	A	A	A	A	A	A
rs8161442	T	C	T	T	T	T
rs8159037	G	G	G	G	G	G
rs8158676	A	G	A	A	G	G
rs8148100	C	C	C	C	C	C
rs8153589	G	A	G	G	G	G
rs8148490	G	G	G	G	G	G
rs8161775	A	T	A	A	T	T
rs8160991	G	G	G	G	G	G
rs8147326	T	T	T	T	T	T
rs8165466	G	G	G	G	G	G
rs8169331	T	T	T	T	T	T
rs13449550	A	G	A	A	G	G
rs8146178	G	G	G	G	G	G
rs8161944	T	C	T	T	T	T
rs8173385	C	T	C	T	T	T
rs8154945	G	G	G	G	G	G
rs8167859	T	C	T	T	C	C
rs8148813	G	G	G	G	G	G
rs8151937	C	C	C	C	C	C
rs8151845	T	T	T	T	T	T
rs8149760	C	C	C	C	C	C
rs8159722	G	T	G	G	T	T

RNO9 Markers	LEW/ SsNHsd	LEW/ MolTac	LEW/ Crl	WF. LEW	WF/ NHsd	WF/ CrCrl
rs8162625	C	C	C	C	C	C
rs8152810	G	G	G	G	G	G
rs8164063	C	C	C	C	C	C
rs8153923	G	G	G	G	G	G
rs8171785	C	C	C	C	C	C
rs8170413	A	A	A	A	A	A
rs8170024	C	C	C	C	C	C
rs8164296	G	G	G	G	G	G

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