a0005

# Sigma Rhabdoviruses

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## Glossary

g0005 **Permissive allele** A host allele, which allows or participates in viral propagation.

g0010 **Restrictive allele** A host allele, which opposes viral propagation.

g0015 **Stabilized lineage for a virus** A female or male lineage with infected primordial germinal cells, which transmits the virus to its progeny.

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## Introduction

p0005 Sigma virus (SIGV) is a rhabdovirus that naturally infects fruit flies (*Drosophila* spp.). SIGV infection causes increased sensitivity to carbon dioxide gas, which is anesthetic for flies. Whereas uninfected flies recover rapidly from the effects of $CO_2$ exposure upon return to a normal atmosphere, flies infected with sigma virus remain irreversibly paralyzed. This effect has been observed among both wild flies and laboratory strains of as many as 13 *Drosophila* species and in three of these (*D. melanogaster*, *D. affinis*, and *D. athabasca*) it has been shown to be the consequence of infection.

p0010 The discovery of SIGV originates from 1937, when a study by P. L'Héritier and G. Tessier described a $CO_2$-sensitive strain of *D. melanogaster*. While this gas is used as an anesthetic in fly genetics, these flies were irreversibly paralyzed when exposed to a $CO_2$-rich atmosphere. This paralysis was specific to $CO_2$, dependent on the gas concentration and temperature during the $CO_2$ exposure. For example, irreversible paralysis appears at 10 °C with $CO_2$ concentrations higher than 50% while $CO_2$ concentrations must reach 75% to induce irreversible paralysis at 16 °C.

p0015 Transmission of this character appeared hereditary but could not be linked to any chromosome. Considered as cytoplasmic, the agent was named Sigma. Its infectiosity was then demonstrated by inoculating sensitive-fly extracts to resistant flies. Inactivation by X-ray irradiation experiments estimated the sensitive volume diameter to 42 µm and filtration through a 180-µm-pore-size membrane eliminated 99% of infectiosity. In 1965, Sigma was finally described as a particle of $70 \times 140$ µm similar to vesicular stomatitis virus (VSV) or rabies virions. Thereafter, studies on SIGV focused on its hereditary transmission among flies. More recently, publications on SIGV are centered on population genetics inquiries into the SIGV–*Drosophila* couple and descriptions of the defence mechanisms developed by *D. melanogaster*.

## Classification

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p0020 SIGV is currently classified as an unassigned member of the family *Rhabdoviridae*. The virion morphology, genome organization, and sequence relationships of several structural proteins are clearly consistent with its assignment as a rhabdovirus. However, it is not closely related to other rhabdoviruses to be classified in any existing genus. It is closer to vesiculoviruses than to other rhabdoviruses according to phylogenetic studies (**Figure 1**) and biological properties (i.e., the $CO_2$ symptom induced by both vesiculoviruses and SIGV).

## Virion and Genome Structure

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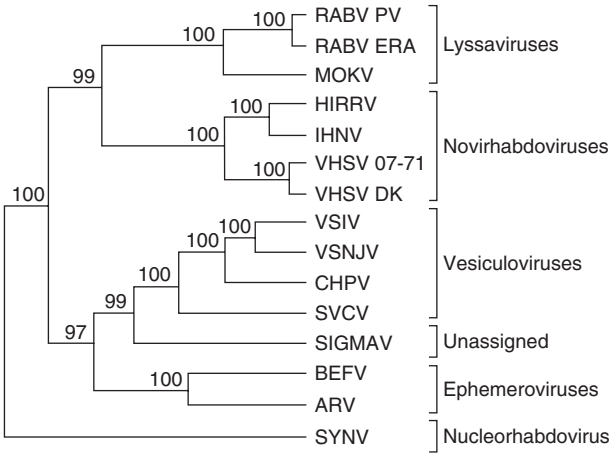
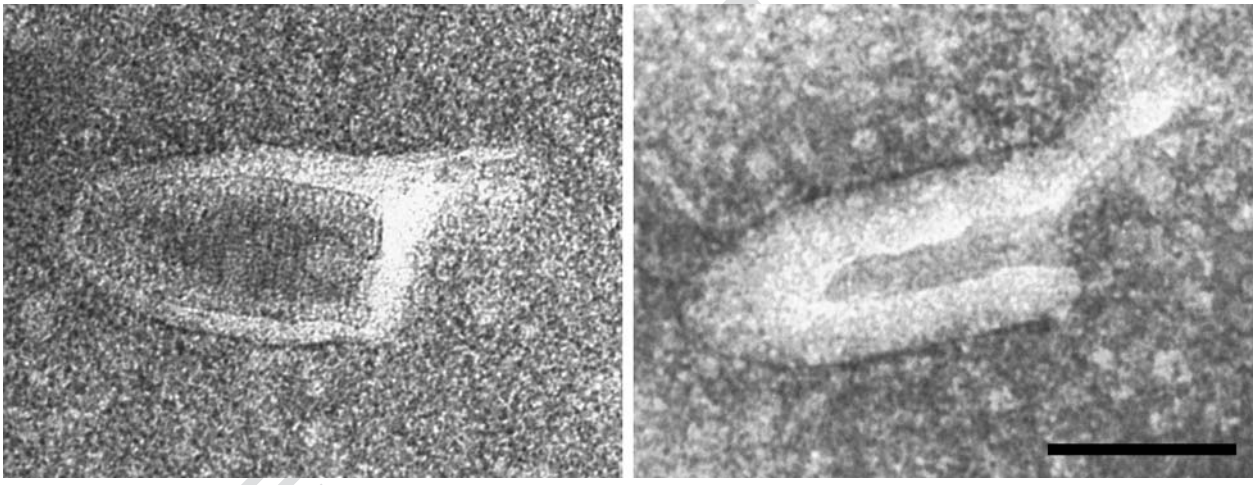
p0025 The virion is a spiked and enveloped bullet-shaped particle of approximately $75 \times 140$–$200$ nm containing a helical nucleocapsid (**Figure 2**). The genome is a negative-sense single-stranded RNA. It contains five genes arranged in the same order as in other rhabdoviruses (3′-*N*-*P*-*M*-*G*-*L*-5′) and encodes proteins with significant levels of sequence identity to the corresponding proteins of rhabdoviruses. For example, the P protein of SIGV is acidic and the distribution of charges is similar to what can be observed for other P proteins of *Rhabdoviridae*. Charge and size of the M protein of SIGV are also conserved among M proteins of vesiculoviruses and its main domains (basic domain, proline-rich domain, hydrophobic domain) are found in other M rhabdovirus proteins. The SIGV genome also contains an additional gene (*X*) between *P* and *M* genes (**Figure 3**). The *X* gene encodes a putative protein of 298 amino acids that is of unknown function, but which contains three conserved domains found in reverse transcriptases. Another unusual feature of the sigma genome is the overlapping mRNAs of *M* and *G* genes over 33 nucleotides. The CAACANC (+ sense) sequence is found at the beginning of the *P*, *X*, *M*, *G*, and *L* genes. The potential transcription stop $CAUG(A)_7$ (+ sense) ends the *N*, *P*, *X*, *M*, and *G* genes. Phylogenetic analyses based on the most conserved rhabdovirus proteins, N and G, indicate that SIGV clusters with vesiculoviruses (**Figure 1**).

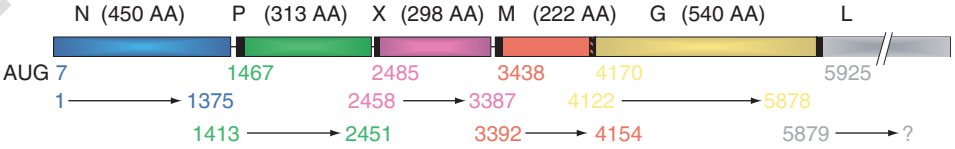



f0005 **Figure 1** Phylogenetic tree of 15 rhabdovirus glycoproteins. The values next to the branches indicate the boostrapping confidence limits. Also sigma virus is unassigned to any group of rhabdoviruses: it's most conserved proteins (including protein G) are closer to vesiculoviruses. Reproduced from Björklund HV, Higman KH, and Kurath G (1996) The glycoprotein genes and gene junctions of the fish rhabdoviruses spring viremia of carp virus and hirame rhabdovirus: Analysis of relationships with other rhabdoviruses. *Virus Research* 42: 65–80, with permission from Elsevier.





f0010 **Figure 2** Sigma virus virions as observed by negative-contrast electron microscopy. The membrane fragment that is seen on the right side of the viral particles shown is frequently but not always observed. Scale = 100 nm.




f0015 **Figure 3** Genome organization of the sigma virus. The single-strand RNA genome of sigma virus encodes six proteins (N, P, X, M, G, and L) for which the size is indicated. The X protein is also named PP3 for protein product 3. The genome has been sequenced with the exception of the L gene that is only partially sequenced on its 3′ end. The length of the L gene is evaluated to approximately 6000 nucleotides and would encode a 2000-amino-acid long protein. The genome would thus be approximately 12 000 nucleotides long. The different mRNAs transcribed are indicated under the genome scheme as well as the position of the AUG sites. The arrows indicate the transcription direction for each gene and are in between the position numbers of the transcription starting and termination points. It is important to note that the M and G transcripts overlap from position 4122 to position 4154.

s0020 ## The $CO_2$ Symptom

p0030 The molecular basis of $CO_2$ sensitivity is unknown. However, the $CO_2$-induced paralysis of wings and legs correlates with viral invasion of thoracic ganglia that are involved in the nervous control of locomotion. Following abdominal inoculation of flies, several cycles of replication are necessary for SIGV to reach the thoracic ganglia and induce $CO_2$ sensitivity. Following injection of one infectious dose at 25 °C, an incubation period of 20 days is required for expression of the symptom, and as the virus dose is increased the mean incubation period decreases. Moreover, a dose of virus that sensitizes flies to $CO_2$ in 10 days when injected into the abdomen, leads to $CO_2$-induced paralysis in only 3 days if injected into the thoracic ganglia.

p0035 The paralysis symptom is specific to $CO_2$ as other gases (e.g., $N_2$, $H_2$, CO, propane, and volatile acids) do not induce similar effects. This characteristic distinguishes SIGV from other viruses infecting *Drosophila* such as the entomobirnavirus drosophila X virus which induces sensitivity to both $CO_2$ and $N_2$ in what appears to be a general anoxia. There is evidence, however, that several vesiculoviruses, although not naturally infecting drosophila, induce $CO_2$-specific sensitivity in flies similar to that induced by SIGV. Each vesiculovirus tested to date, including vesicular stomatitis Indiana virus, cocal virus, pike fry rhabdovirus, and spring viremia of carp virus, is capable of replication in *D. melanogaster* and induces sensitivity to $CO_2$ but not to $N_2$ or propane. In contrast, the lyssavirus rabies virus (CVS strain) and two novirhabdoviruses of fish (infectious haematopoeitic nectosis virus and vesicular hemorrhagic septacaemia virus) do not replicate and do not induce $CO_2$ symptoms in *D. melanogaster*. The specificity of $CO_2$ to induce paralysis in flies infected with SIGV or vesiculoviruses suggests that the underlying molecular mechanism responsible for the symptom is likely to be similar. Nevertheless, $CO_2$ symptoms vary for different vesiculovirus. For example, cocal virus induces $CO_2$-dependent paralysis when the virus titer in the nervous system reaches the maximum. Later in infection, though the virus titer in the entire fly remains constant, the titer decreases in the thoracic ganglia and $CO_2$ sensitivity disappears. It has also been observed that, following infection of flies with vesicular stomatitis Indiana virus, a proportion of the exposed population displays a delayed $CO_2$ symptom while the remainder of the $CO_2$-sensitive flies are immediately paralyzed. The delayed sensitivity correlates with a more rapid invasion of the cephalic ganglia than the thoracic ganglia and results in death 2–3 days after exposure, presumably due to paralysis of the mouthparts. Direct injection of SIGV into the cephalic ganglia also leads to this delayed sensitivity and lethality.

## Host Range

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p0040 Sigma virus has been reported to trigger $CO_2$ sensitivity in flies of 15 of the 16 *Drosophila* species tested to date, the exception being *D. repleta*. However, some *D. melanogaster* genotypes are also resistant to SIGV replication and $CO_2$ sensitivity, and the observed resistance of *D. repleta* may not be valid for all genotypes.

p0045 One of the criteria used to demonstrate virus replication is the recovery of a virus yield following infection that is in excess of the original infecting dose. This criterion is very stringent. Indeed, the virus yield from infected flies decreases soon after infection due to the entry of the nucleocapsids into the cytoplasm of infected cells, and there is a period of latency before replication generates new progeny virus. Therefore, a less-stringent criterion for SIGV replication is the observation of a higher virus yield than can be recovered during the latent period. Using these criteria, of 13 *Diptera* species (*Phormia terranovae*, *Ceratitis capitata*, *Musca domestica*, *Calliphora erythrocephala*, *Sarcophaga argyrostoma*, *Glossina mortisans*, *Aedes albopictus*, *Aedes aegypti*, *Aedes detritus*, *Anopheles stephensi*, *Toxorhynchites amboinensis*, *Culex pipiens*, *Culex quinquefasciatus*) that have been tested, only the fleshfly (*Phormia terranovae*) and the mosquito (*Aedes albopictus*) appeared incapable of supporting SIGV replication. As for *D. repleta*, there may be genotypic variation in the susceptibility of these insects and it is possible that susceptibility can be increased by SIGV strain adaptation. This is supported by the observation that sigma virus can be adapted to replication in *A. albopictus* cell cultures by passage in the mosquito *Toxorynchites amboinensis*. The selected variants multiply in and induce $CO_2$ sensitivity of *A. albopictus*. Adaptation of sigma virus to replication in restrictive genotypes of *D. melanogaster* has also been observed. SIGV has not been observed to replicate in tests conducted in nine insect species representing five orders (*Blattaria*, *Orthoptera*, *Lepidoptera*, *Hymenoptera*, *Hemiptera*) other than *Diptera* or in cultured vertebrate cells.

## Virus Transmission

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p0050 Sigma virus is not transmitted between insects by casual contact, and no vector allowing horizontal transmission has been identified. In nature, SIGV transmission appears to be exclusively vertical and, although 100% prevalence of infection can be achieved in laboratory strains, only a proportion of natural populations of flies are infected.

p0055 There is no evidence of integration of the SIGV genome into host chromosomes and the virus appears to remain exclusively in the cytoplasm. SIGV establishes a stable infection in oogonia, and between 10 and 40 viral genomes can be detected per oogonium according to the

virus strain. Thereafter, there is an accumulation of genomes as oocyte volume increases. This balance reflects an autoregulation of viral infection in which cellular proteins may play a role and allows the virus transmission during cell division. The nature of the process also implies low cytopathogenicity of SIGV in oocytes. Furthermore, if viral genomes segregate stochastically following cell division, relatively rare events of completely asymmetrical distribution would lead to the presence of uninfected individuals in the progeny of an infected female.

p0060 In a lineage stabilized for sigma virus infection, some females will not transmit the virus to 100% of their progeny and infected females are less fertile. This should lead to a rapid elimination of the virus if the males were also not able to transmit the infection. The efficiency of viral transmission by males can be as high as 90% in the progeny of a cross between uninfected females and males infected by a recently isolated strain of wild virus. In *D. melanogaster*, the male progeny of such a cross does not transmit the virus to the next generation. However, the female progeny can transmit sigma virus to the next generation and some of their daughters are the source of stabilized lineages. These transmission rules are the roots of the invasive character of the virus and allow the virus to maintain itself in nature.

p0065 In *D. affinis* and *D. athabasca*, sigma virus transmission follows similar rules with the exception that the male progeny of an uninfected female crossed with an infected male can transmit the virus to the next generation.

## s0035 Virus Replication Cycle

p0070 The replication cycle of sigma virus has been studied *in vivo* following injection of a viral extract into the abdomen of flies. There is a rapid reduction in recoverable infectivity following inoculation such that after 1 h only 1% of the infecting dose can be recovered, and new viral production commences between 24 and 48 h post-infection. Studies of the viral replication cycle have been conducted using both wild-type viruses and temperature-sensitive (*ts*) mutants. Heat–shock experiments have identified three groups of *ts* mutants corresponding to three phases in the replication cycle. The first group of mutants identified a transiently thermolabile complex comprising the viral genome and viral proteins. The half-life of thermosensitivity is 4 h with a maximum span of 9 h post-infection. The molecular basis of the thermoresistance has not been determined but similar studies in vesiculoviruses suggest that this phase could include the steps that precede replication Au2 of the viral genome such as the release of the RNP complex from the endosome and primary transcription and protein synthesis.

p0075 A second group of *ts* mutants is affected in a replication phase that has a half-life of 9 h, commencing 7 h post-infection and terminating at 14 h post-infection. This phase corresponds to genome replication since hereditary transmission of these mutants is interrupted at the restrictive temperature. Late functions are also altered as have been observed in molecular studies of vesiculoviruses, which have shown that the N, P, and L proteins that are essential for genome replication and secondary transcription.

p0080 The third group of *ts* mutants is affected in the late phase of the viral cycle. At the restrictive temperature, these mutants can be hereditarily transmitted but no infectious particles are produced and $CO_2$-induced paralysis of the host is not observed. The proteins modified in these mutants could be the G, M, or X proteins. Since the N, P, and L proteins are involved in viral replication and the X protein is absent in vesiculoviruses, the $CO_2$ sensitivity must be due to G, M, or both G and M proteins.

p0085 At 20 °C, the viral replication cycle can vary in length between 25 and 90 h post-infection in different individuals with an average of 60 h. The cycle length varies according to temperature and is much faster at 25 °C, as is the metabolism of flies. The host genotype also influences the cycle length. In more permissive genotypes, the replication cycle is faster and in the most permissive genotypes the longest cycle is only 48 h in duration.

## s0040 Host Immunity

p0090 The reduced fertility that has been observed in female flies infected experimentally with sigma virus should be a strong selective pressure in nature. Surprisingly, poor fertility is not evident in natural populations. A loss of fertility is observed in crosses between infected females from a natural population and uninfected males from laboratory strains. These results suggest that the viral infection cycle is controlled by the host genome. Indeed, seven such genes are known: *ref(1)H*, *ref(2)M*, *ref(2)P*, *ref(3)G*, *ref(3)O*, *ref(3)D*, and *ref(3)V*. Each is polymorphic and with alleles segregating into two categories. Permissive alleles allow the viral infection cycle to proceed while restrictive alleles restrict virus cycling. The only exception is the *ref(3)V* gene for which the restrictive allele blocks hereditary transmission of sigma virus from stably infected males.

p0095 The most intensively studied of the *ref* genes is *ref(2)P*. Restrictive *ref(2)P* alleles modify the rules for hereditary transmission of sigma virus. The frequency of uninfected flies in the progeny of infected parents increases with the number of restrictive alleles in the genome of the female. Stabilized mothers that are homozygous for a restrictive allele do not display $CO_2$ sensitivity even though they remain capable of virus transmission. Their progeny are transmission-defective but may be $CO_2$-sensitive, depending on their genotype. Moreover, when stabilized

males are crossed with uninfected females, the proportion of progeny that is infected is twofold lower when the mother is heterozygous for *ref(2)P* (permissive/restrictive) and zero when the mother is homozygous restrictive for *ref(2)P*. These restrictive alleles are often encountered in natural populations and do not appear to be counter-selected in uninfected drosophila. When the populations are infected with currently observed virus strains, which are sensitive to this defense system, *ref(2)P* restrictive alleles are favored until their frequency reaches 0.3. At this frequency, the sensitive strain of virus is eliminated. Thereafter, the lack of counter-selection of restrictive alleles maintains their frequency around 0.3, which protects the population from any new invasion by a sensitive virus.

p0100 Three functional domains have been identified in both the Ref(2)P protein and its mammalian homolog, p62/Sequestosome-1. Two protein–protein interaction motifs are found: an amino-terminal PB1 (Phox and Bem 1) domain and a more central ZZ zinc finger. At the other end of both proteins there is a carboxy-terminal UBA (ubiquitin-binding area) domain. The Ref(2)P PB1 domain mediates the interaction with the drosophila atypical protein kinase C (DaPKC) and p62 binds mammalian aPKCs. The physiological function of Ref(2)P remains unknown even though it is essential for male fertility in some specific genotypes.

p0105 A comparison of susceptibility to the sigma virus between flies that are homozygous for permissive alleles of *ref(2)P* and flies *ref(2)P*$^{-/-}$ has shown that permissive Ref(2)P protein is required for the virus to multiply at highest efficiency. For SIGV strains that are the most sensitive to *ref(2)P* alleles, a 16-fold higher dose is required for infection of a *ref(2)P*$^{-/-}$ genotype and a 10 000-fold higher dose is required for infection of a homozygous restrictive genotype than to infect homozygous permissive flies.

p0110 A comparison of the 15 sequenced alleles of *D. melanogaster ref(2)P*, among which three are restrictive, and the reference sequence of *D. simulans ref(2)P* indicate that the ancestral gene was permissive. Three mutations affecting the PB1 domain of the protein are necessary and sufficient to convert a permissive *ref(2)P* allele into a restrictive allele. Both permissive and restrictive alleles with sequence variations in the PB1 domain have been shown to form a monophyletic group that shows less internal variability than the group of the ancestral permissive alleles. This suggests that the three variations in the PB1 domain that affect susceptibility to sigma virus infection have emerged relatively recently. The high frequency of these variants (up to 50% of the observed alleles) indicates their existence provides a selective advantage in drosophila populations.

p0115 The interaction between sigma virus and the *ref(2)P* gene is highly specific. None of the other viruses that replicate in *D. melanogaster*, such as drosophila X virus and vesiculoviruses, is sensitive to restrictive *ref(2)P* alleles. A unique amino acid change in the PB1 domain is sufficient to suppress the restrictive character of an allele. In a viral population, genotypes with a capacity to replicate efficiently in a restrictive environment exist, even if the virus has never been exposed to restrictive alleles. For this reason, the elimination of sensitive viruses in a natural fly population by restrictive *ref(2)P* alleles is often associated with a new infection of the population by adapted viruses. Therefore, one could doubt that restrictive alleles provide a real defense system against sigma virus but *ref(2)P* is not the only *ref* gene.

p0120 Each of the *ref* genes impacts independently on the viral replication cycle. For example, if a particular viral strain requires a tenfold higher dose to successfully infect flies that are restrictive at the *ref(2)P* locus, and a tenfold higher dose to infect flies restrictive at the *ref(2)M* locus, a 100-fold higher dose is required to overcome the in flies that are restrictive for both *ref* genes. Adapted viruses can be isolated from a clone sensitive to *ref(2)P* restrictive alleles and studied for their sensitivity to *ref(2)M* restrictive alleles. As observed in **Figure 4**, the viral mutants most adapted to *ref(2)P* restrictive alleles become more sensitive to restrictive alleles of *ref(2)M*. Increasing the frequency of *ref(2)M* restrictive alleles can also contribute to the elimination of viral mutants, only allowing viruses adapted to both *ref(2)P* and *ref(2)M* restrictive alleles to invade a fly population. It appears that the various *ref* genes cooperate to defend the fly population against the virus. For example, if the frequency of *ref(2)P* restrictive alleles was around 0.3, the frequency of *ref(2)M* restrictive alleles could rise while viruses sensitive to *ref(2)M* would disappear.

p0125 There are still sigma virus strains in nature which are sensitive to *ref(2)P* restrictive alleles. In addition, a virus adapted to *ref(2)P* with an increased sensitivity to *ref(2)M* (i.e., equivalent to the red circles of **Figure 4**) is yet to be found in nature. Moreover, analysis of *ref(2)P* allele genealogy suggests that, if all the viruses present in a region were adapted to *ref(2)P* restrictive alleles, a new mutation in the PB1 domain of the protein would be selected to generate a new restrictive allele. Therefore, Ref(2)P remains as an active defensive mechanism against natural sigma virus infections. The role of the other Ref proteins in host immunity remains unknown.

## Concluding Remarks

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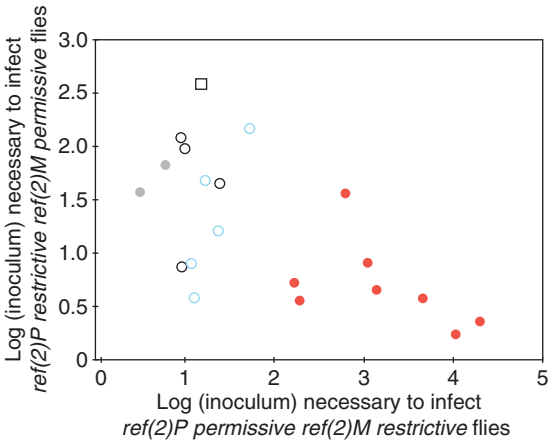
p0130 In earlier studies, the detailed rules of vertical transmission of sigma viruses in *D. melanogaster* populations illustrated how vector insects such as mosquitoes or sandflies can become the reservoirs of the virus. Currently, the two major interests in sigma virus research are the description of the host–virus interactions in natural populations




f0020 **Figure 4** Virus clones distribution according to the inoculum size necessary to infect different fly genotypes. The reference *Drosophila melanogaster* strain was homozygous permissive for both *ref(2)P* and *ref(2)M*. The size of inoculum necessary to infect these flies was arbitrarily defined as 1.00. The *y*-axis values are the logarithm of the smallest inoculum capable of infecting flies homozygous for both a permissive *ref(2)M* allele and homozygous for a restrictive *ref(2)P* allele. The *x*-axis values are the logarithm of the smallest inoculum capable of infecting flies homozygous for both a permissive *ref(2)P* allele and a restrictive *ref(2)M* allele. The square represents the parental virus while circles represent viral mutants adapted to *ref(2)P* restrictive alleles. Open circles symbolize mutants that are not significantly different from the parental virus when assayed in *ref(2)M* restrictive flies. Blue circles depict the results observed for thermosensitive mutants, while gray and red circles show the observation made for mutants that are slightly adapted to both *ref* genes and mutants that are more sensitive to *ref(2)M* restrictive alleles than the parental clone, respectively. Among the mutants that are at least 20 times more adapted to *ref(2)P* than the parental clone, three are thermosensitive and thus would not survive in nature. The majority (seven out of eight) of the other 'adapted mutants' are more sensitive to *ref(2)M* restrictive alleles.

together with the development of an improved understanding of defensive mechanisms mediated by the *ref* genes. This immunity has certain similarities to innate immunity since its components are hereditary, but they differ because of the high specificity of protection and the memory effect that is generated in host populations. Strictly speaking, Ref(2)P may be part of the innate immune response through its interaction with DaPKC and a more extensive study could reveal that other *ref* genes also form part of a complex innate host immune system in flies.

*See also:* Vesiculoviruses (Rhabdoviridae) (00368); Vesicular stomatitis virus (Rhabdoviridae) (00529); Fish rhabdoviruses (Rhabdoviridae) (00493).

## Further Reading

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