

**Cross-species transmission of *Drosophila melanogaster* Nora virus in other
Drosophila species and effect on geotaxis**

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ABSTRACT

This study was performed to determine the cross-species transmission of *Drosophila melanogaster* Nora virus in other *Drosophila* species and its effect on geotaxis. There are millions of known viruses, and new ones are discovered every year. A major source of new viruses is epizootic and enzootic animal viruses, seen when viruses typically occurring in animals adapt and mutate to infect humans. COVID-19 is an example of one of these host-switching viruses, as it originated in bats (Ji, 2020). Nora virus is a picorna-like virus whose only known pathogenic effect is a geotaxis defect. The cross-species transmission of this virus in fruit flies can be used to help scientists better understand host-switching in other viruses. It was predicted that the virus would infect the other species of *Drosophila* and that it would have an effect on their geotaxis. To test this hypothesis, Nora virus-positive males were allowed to defecate on fly food. Once they were removed, negative males and negative virgin females of each species were added to the vials. The geotaxis of their offspring was measured before they were collected for RNA analysis. RT-PCR was performed to determine infection, and all species tested positive in some fashion, showing Nora virus to be a host-switching virus. Geotaxis results showed a defect in the experimental groups in comparison to the control indicating that the virus does have a pathogenic effect on the other species. Learning more about cross-species transmission has increased importance in today's world as the number of zoonotic viruses increases.

INTRODUCTION

Nora virus is a picorna-like virus that infects the fruit fly, *Drosophila melanogaster* (Habayeb et al, 2006). Currently, the only known pathogenic effect of the virus is a geotaxis

defect or locomotor deficiency (Rogers et al, 2020). Viruses are the most abundant infectious agent on the planet, and they are a constant threat, causing significant mortality rates (Wang, 2010). New viruses emerge and are discovered every year. A major source of these new human viruses is epizootic and enzootic animal viruses (Parrish, 2008). This is when viruses originally seen in animals adapt and mutate to inhabit humans as their hosts (Parrish, 2008). As natural ecosystems are destroyed, more and more human to animal contact occurs, quadrupling the number of zoonotic diseases that infect people in the last decade (Rozenbaum, 2020). Examples of this can be seen in viruses such as SARS coronaviruses, Ebola virus, and human immunodeficiency virus type 1 (HIV-1) (Parrish, 2008). Recently, COVID-19, which has caused a global pandemic, was found to be a recombinant virus between the bat coronavirus and a virus of unknown origin (Ji, 2020).

The effects of cross-species host switching viruses can be major and life-threatening. For example, the Ebola virus, which originally infected bats, has had many outbreaks over the last five decades including the 2014-2016 outbreak in West Africa which resulted in 26,800 cases (CDC). COVID-19, or SARS-CoV-2, is another example of a host-switching virus. The virus is known to be able to infect cats, dogs, ferrets, minks, bats, and humans (CDC). These extreme species jumps show high adaptability of the virus and make it hard to contain.

Possible cross-species transmission of Nora virus in different *Drosophila* species can be used as a model to help scientists understand host-switching in other viruses. *Drosophila* make a good model for understanding human viruses because their genome is very closely related to that of mammals, and their genome has been widely documented (Habayeb et al, 2009). Also, 75% of known human genetic disease genes have homologs in flies (Wang, 2010). Therefore, understanding how the Nora virus can cross species in *Drosophila* can help us further understand

how COVID-19 mutated to infect humans, as these are both RNA viruses. Nora virus in *Drosophila* also makes a good model for viral research because it easily and readily infects fly stocks, as well as having little effect on the flies' overall health and longevity (Habayeb et al, 2009). Like many picornaviruses, the Nora virus is spread via the fecal-oral route (Habayeb et al, 2006).

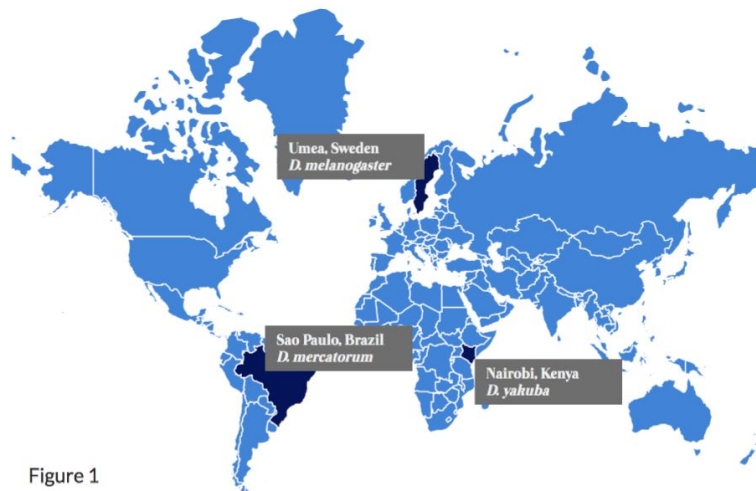


Figure 1

The current study was performed to determine Nora virus's ability to infect other species of *Drosophila*: *Drosophila yakuba* and *Drosophila mercatorum*. Fly species were chosen based upon their native continent of origin (Figure 1) to determine whether geographical distance from the species of origin would affect infectivity. Flies from Africa, South America, and Europe were used.

To test this, flies positive for the Nora virus were used to infect food in different vials. The flies were removed, and flies from different species were added. These flies were allowed to lay eggs before being removed as well. Once the eggs eclosed, the effect of the virus on their geotaxis was tested by recording the percentage of flies to return to the marked line near the top

of the vial after being knocked down. Finally, they were collected for RNA analysis to determine Nora virus infection.

In conclusion, understanding how the Nora virus can host-switch in *Drosophila* species will help us to further understand how cross-species transmission occurs in humans.

METHODS

Formula 4-24® Instant Blue *Drosophila* Medium from Carolina Biological Supply Company was prepared in twenty vials. Five Nora virus positive male *D. melanogaster* were added to vials 6-18 (experimental). After 96 hours, the flies were removed. Next, five negative males and five negative virgin females of each species: *D. yakuba*, *D. mercatorum*, and *D. melanogaster* were added. Negative virgin females and negative males of *D. melanogaster* were added to 1-5 (control). All these flies were removed once eggs were laid. The newly eclosed flies were tested for an effect on geotaxis. They were knocked to the bottom of the vial while a timer was set for 30 seconds. At the end of 30 seconds, the number of flies that reached a drawn line near the top was recorded. Finally, all flies were collected from each vial for RNA analysis via RT-PCR using Nora virus *ORF1* gene-specific primers for detection of infection.

RESULTS

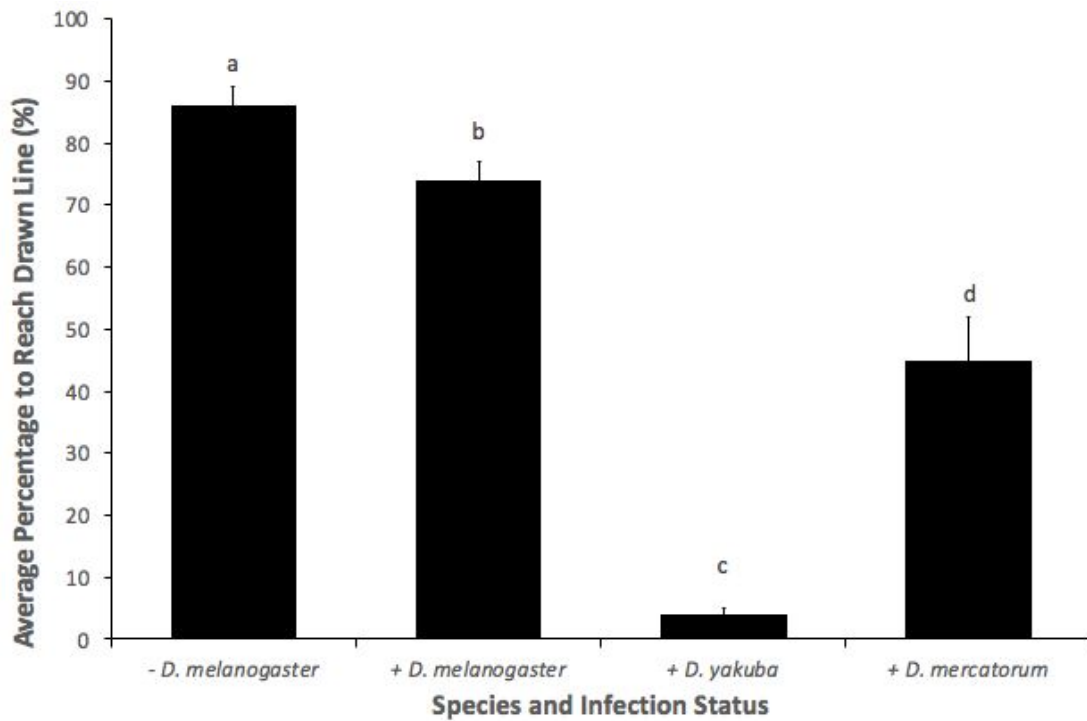


Figure 2 The average percentage of flies to reach the drawn line in 30 seconds by species and infection status. $n = 9, 6, 3, 6$. Error bars represent standard error. Letters indicate significance; $\alpha = 0.05$.

Figure 2 shows the average percentage of flies to reach the drawn line in 30 seconds by species and infection status. The mean percentage (86%) of the negative control group was higher than the mean percentage (74%) of the positive control group who were positive for Nora virus. Both controls had higher percentages than the experimental species: *D. yakuba* (3%) and *D. mercatorum* (45%).

Figure 3 The effect of Nora virus on geotaxis on Drosophila species.

	<i>D. melanogaster</i>		<i>D. yakuba</i>	<i>D. mercatorum</i>
Presence of Nora Virus	-	+	+	+
Percent to reach line	0.86	0.74	0.04	0.45
Standard deviation	0.10	0.079	0.033	0.12
Standard Error	0.035	0.032	0.013	0.067
Range	0.38	0.21	0.08	0.23
Maximum	1	0.85	0.08	0.56
Minimum	0.62	0.64	0	0.33
Trials	12	6	6	3

Figure 3 shows the mean, standard deviation, standard error, range, maximum, and minimum of the distance traveled by the experimental and control groups for each species. The standard deviation of the mercatorum (0.17) was slightly higher than those of the experimental groups (0.033, 0.079) and the control group (0.1). The standard error for all groups was extremely low ranging from 0.013-0.067. 82 negative *D. melanogaster*, 70 positive *D. yakuba*, 42 positive *D. mercatorum*, and 116 positive *D. melanogaster* flies were collected.

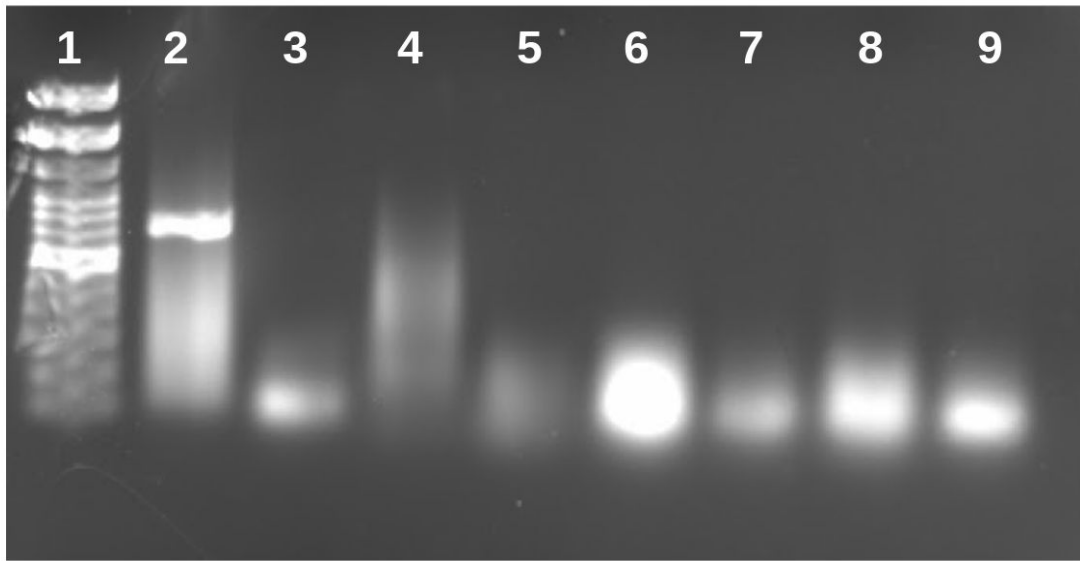


Figure 4 shows Nora virus *ORF1* RT-PCR of adult flies before testing. Lane 1: 100 bp ladder; lane 2: NV+ flies (+ control); lane 3: - control (water); lane 4-10: NV- *D. melanogaster* stocks.

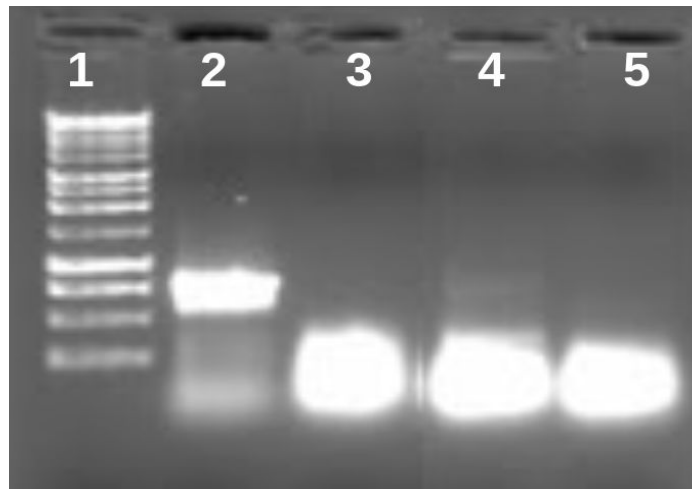


Figure 5 shows Nora virus *ORF1* RT-PCR of adult flies before testing. Lane 1: 100 bp ladder; lane 2: NV+ flies (+ control); lane 3: - control (water); lane 4: NV- *D. yakuba* stock; lane 5: NV- Nora virus *ORF1* stock.

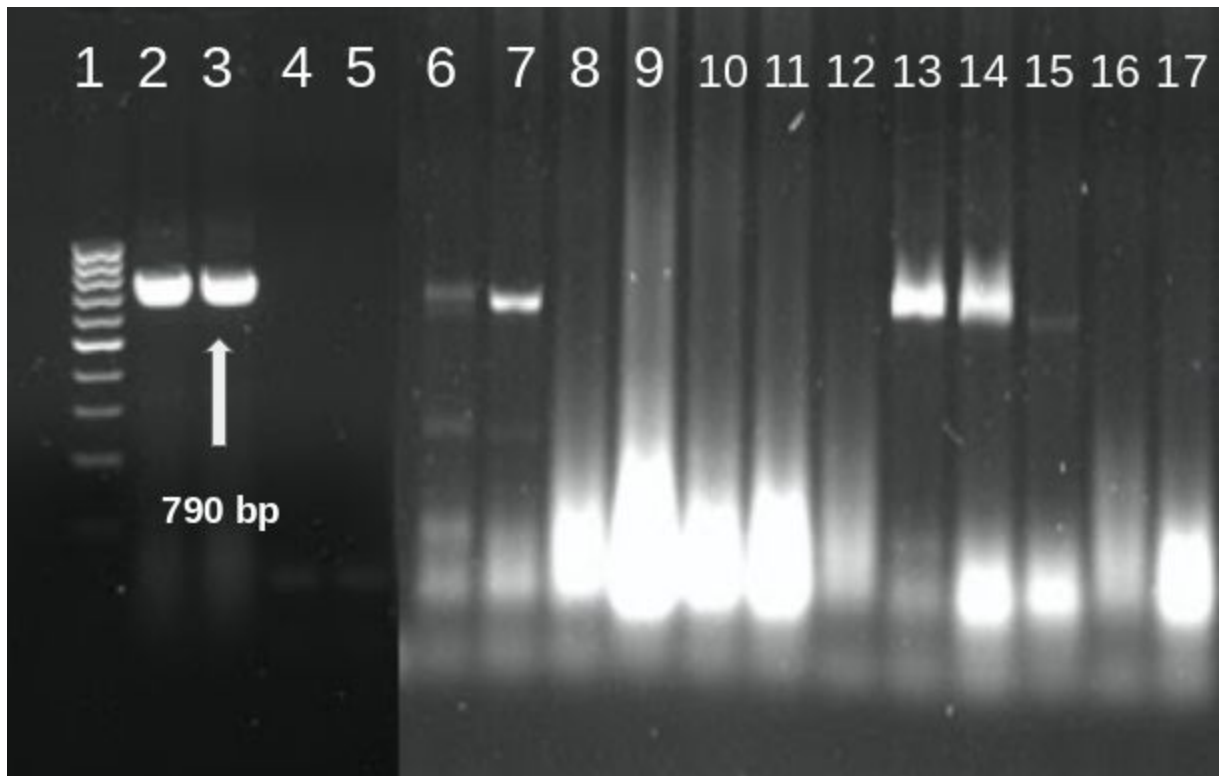


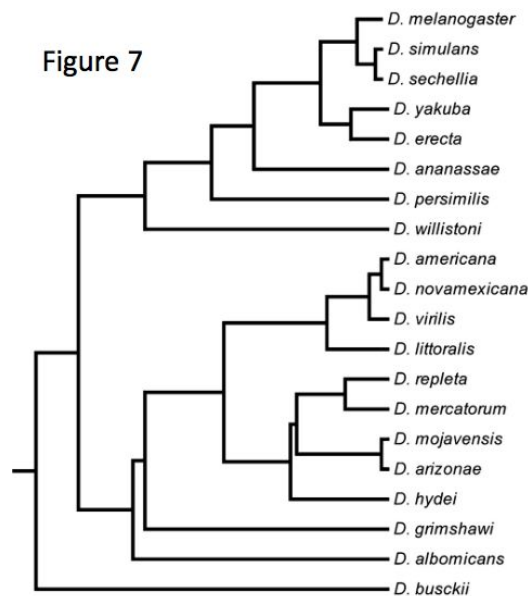
Figure 6 shows confirmation of infection status via Nora virus *ORF1* RT-PCR. Lane 1: 100 bp ladder; lane 2-3: NV+ flies (+ control); lane 4-5: - control (water); lanes 6-7: *D. melanogaster* NV+; lane 8: *D. melanogaster* NV-; lanes 9-11: *D. melanogaster* NV-; lane 12: *D. yakuba* NV-; lanes 13-14: *D. yakuba* NV+; lane 15: *D. mercatorum* NV+; lanes 16-17: *D. mercatorum* NV-. Positive infection is indicated by the ~790 bp PCR product in the lanes.

ANOVA tests were run between all groups, and all ANOVA tests resulted in P-values of < 0.05 . The null hypothesis that Nora virus would have no effect on the geotaxis was rejected because the P-value was lower than the critical value (0.05). This shows that there was a significant difference between the control and experimental groups. The negative flies reached the drawn line at a significantly higher percentage than the positive experimental species.

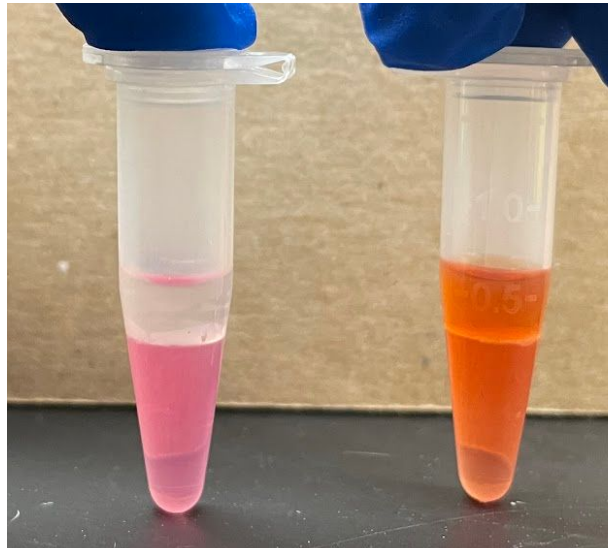
DISCUSSION

This experiment was conducted to determine the cross-species transmission of the *D. melanogaster* Nora virus to other species of *Drosophila*. The experiment was started with confirmed negative flies of three species (Figures 3 and 4). Once collected, the flies were tested for a geotaxis defect, the only known pathogenic effect of the virus, and all species displayed this effect. The null hypothesis that the virus would not display cross-species infectivity was not supported by the data, as they were able to be infected (Figure 5). This is the first time that the virus has been shown to infect other species of *Drosophila*. The null hypothesis that the virus would have no effect on the flies' geotaxis was also not supported by the data (Figure 1).

Another interesting finding was the level of infection present in each species. The species *D. yakuba* and *D. melanogaster* showed infection in two of the three vials, whereas the species *D. mercatorum* showed infection in only one of the vials. That one vial only showed a very faint PCR product as well. This could be because the virus was not as well adapted to the *D. mercatorum* species as it is more distantly related to *D. melanogaster*, both genetically (Figure 7) and geographically, than *D. yakuba* is.



It was also unique to see the difference in the color of ground fly parts during RT-PCR preparation. The type of *D. melanogaster* used has white eyes, and the mixture is a light pink color. On the other hand, the species *D. mercatorum* and *D. yakuba* have red eyes, and the mixture was a reddish color. The pigments from their eyes were not broken down as the rest of the fly was.



A limitation of this experiment is the number of trials, as more trials would provide a more accurate representation of the population. A greater number of species would better show the reach of the virus and could be done in future experiments.

Research of this type is important because as zoonotic viruses become more prevalent, it is important that we learn to understand them. Thirteen different zoonotic viruses cause 2.2 million deaths per year (Rozenbaum, 2020). COVID-19 is a great example of one of these viruses. Scientists around the world are working to learn more about the ways that viruses can mutate and adapt to infect new hosts in order to prevent their spread and identify them earlier. Understanding more about how viruses can host-switch will help us to better respond in the future and make the world a safer, healthier place.

REFERENCES

- Habayeb, Mazen S., et al. "The Drosophila Nora virus is an Enteric Virus, Transmitted via Feces." *Journal of Invertebrate Pathology*, vol. 101, no. 1, Apr. 2009, pp. 29-33.
- Habayeb, Mazen S., et al. "Nora virus, a Persistent Virus in Drosophila, defines a new Picorna-like virus Family." *Journal of General Virology*, vol. 87, no. 10, Nov. 2006, pp. 1-7.
- Ji, W, Wang, W, Zhao, X, Zai, J, Li, X. Cross-species transmission of the newly identified coronavirus 2019-nCoV. *J Med Virol*. 2020; 92: 433– 440.
- Parrish, Colin R et al. "Cross-species virus transmission and the emergence of new epidemic diseases." *Microbiology and molecular biology reviews : MMBR* vol. 72,3 (2008): 457-70. doi:10.1128/MMBR.00004-08
- Rogers, Abigail, et al. "Impaired Geotaxis as a Novel Phenotype of Nora Virus Infection of *Drosophila melanogaster*." *Scientifica*, vol. 2020, 30 July 2020, pp. 1-6.
- Rozenbaum, Mia. "The increase in zoonotic diseases: the WHO, the why and the when?" *Understanding Animal Research*, 6 July 2020. Accessed 14 Feb. 2021.
- Wang, Jing-Huan, et al. "Drosophila as a model for antiviral immunity." *World Journal of Biological Chemistry*, vol. 1, no. 5, 26 May 2010, pp. 151-59.
- Wang, Xiao-Hong, et al. "RNA interference directs innate immunity against viruses in adult *Drosophila*." *American Association for the Advancement of Science*, vol. 312, 21 Apr. 2006, pp. 452-54.