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Viral infections reduce *Drosophila* lifespan through accelerated aging

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Summary

Viral infections induce symptoms reminiscent of aging, but their impact on the host's biological age remains unclear. Using a computational approach, we estimated the biological age of *Drosophila melanogaster* flies infected with various enteric viruses and demonstrated that enteric viral infections accelerate biological aging. Comparative analysis revealed that more pathogenic viruses induced a faster increase in acceleration of aging.

Introduction

Some viral infections induce aging-like phenotypes, accelerating cellular and immune senescence in their hosts[1,2]. To define the aging of an organism it is necessary to determine its biological age, which reflects the functional status of the organism and may deviate from its chronological age. Biological age can be estimated through various hallmarks of aging[3,4]. Currently, state-of-the-art computational methods combined with transcriptional data enable a precise estimation. Using these computational approaches, we aim to test whether viral infections impact their hosts' aging.

We focused on the *Drosophila melanogaster* fly, an ideal model organism for studying aging. Flies have a short lifespan, minimal ethical research concerns[5], abundant research resources available[7,8,9], and share a large number of functional homologies with humans, including disease and aging-related processes[5,10,11]. Specifically, we studied diverse RNA enteric viruses that primarily infect the intestine and mirror human enteric infections[12]. These infections induce symptomatology that includes intestinal stem cell overproliferation and barrier dysfunction[13,14], which in uninfected flies are established hallmarks of aging[11,15,16,17,18]. Hence, we hypothesize that enteric viral infections will accelerate aging in *Drosophila*.

Results

We began by investigating the effect of persistent viral infections on fly lifespan (Figure 1A). Uninfected flies had a lifespan of 55.5 ± 12.7 days (d, median ± SD), while infected flies exhibited significantly reduced lifespans, with the magnitude of reduction varying by virus. The strongest effects were observed with Drosophila C virus (DCV, 24.0 ± 17.1 d) and Drosophila A virus (DAV, 25.0 ± 7.2 d), followed by Bloomfield virus (33.0 ± 11.9 d), while Nora virus showed the mildest impact (46.5 ± 17.9 d). Notably, viral infections not only reduced host lifespan but also affected lifespan variability, as indicated by the differing standard deviations.

Next, we investigated whether the reduced lifespan was accompanied by acceleration of aging. To estimate the biological age of our samples, we employed RAPToR[19] using transcriptional time-series data[20]. Since no reference was available for adult flies, we created our own using whole-genome transcriptional data from uninfected animals. Our approach accurately estimated biological age in the uninfected flies used to create the reference, with a strong correlation between chronological and estimated biological age (Pearson's $r = 0.997$, 30 d.f., $P < 0.001$) (Figure 1B, SFig 1).

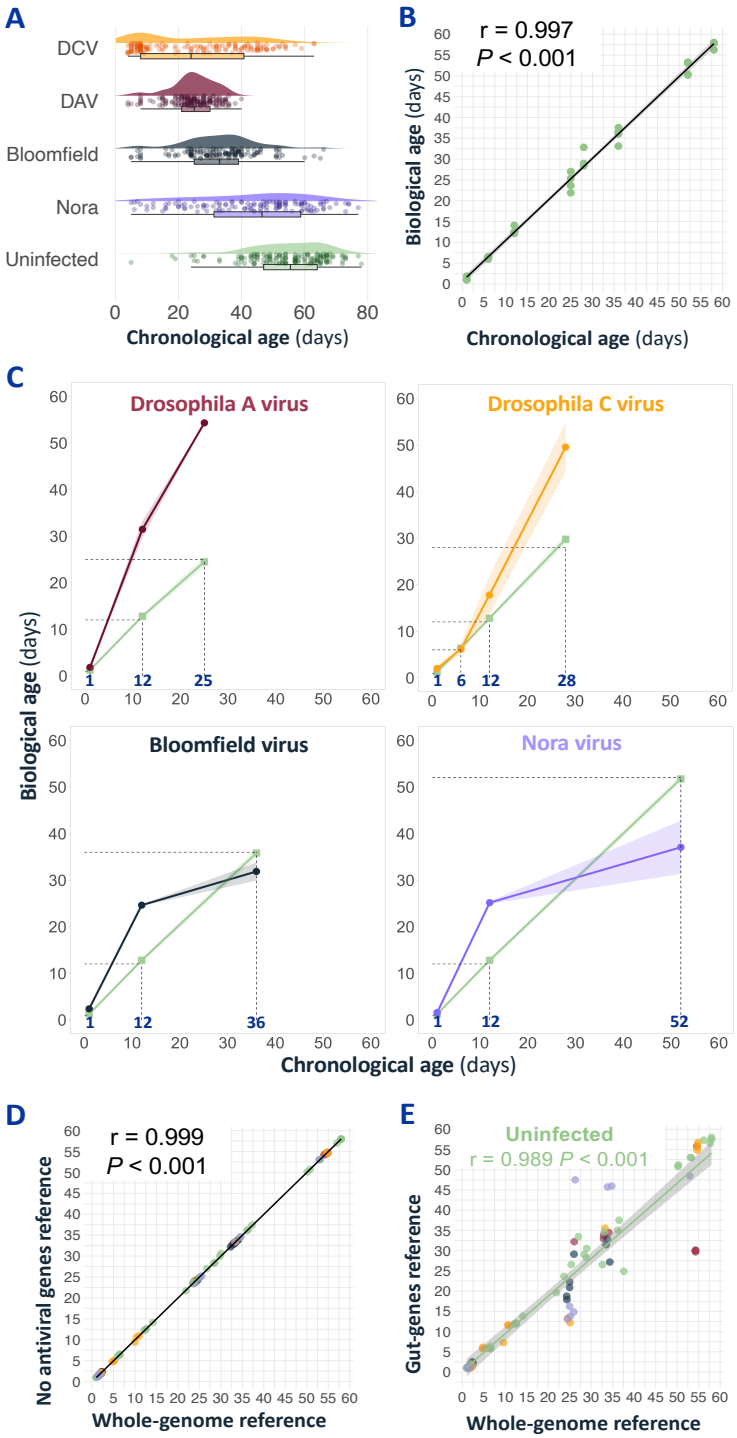

Figure 1. **(A)** Persistent viral infections reduce fly lifespan. Half-violin plot shows the distribution of flies lifespan, with each dot representing the day of death of individual flies and box plots summarizing the data per treatmeant. Data from [13]. **(B)** Chronological age correlates with the estimated biological age of the uninfected flies used to create an aging reference. **(C)** Viral infections disrupts fly aging. Panels show the biological age (Y-axis) at different time points (X-axis). Points represent the mean biological age, and the shaded area represents the standard error. **(D)** The biological age estimation is similar even when estimated without accounting for antiviral genes. **(E)** Using only gut genes to create the reference is sufficient to estimate the age of uninfected flies but not for infected ones.

For all panels, color legend: *uninfected*, *Nora virus*, *Bloomfield virus*, *DAV*, *DCV*.

With a validated reference, we assessed the biological age of virus-infected animals at three time points: an early stage at 1 day post-eclosion (dpe), an intermediate stage at 12 dpe, and later stages corresponding to 50% mortality of the host population (25 dpe for DAV, 28 dpe for DCV, 36 dpe for Bloomfield, and 52 dpe for Nora) (Figure 1C, Supplementary Figure 2). At 1 dpe, the biological age of uninfected animals was 1.2 ± 0.4 d (mean ± SD). Flies infected with Nora virus did not show a significant increase in aging (1.6 ± 0.2 d), but other viruses exhibited accelerated aging even at this early stage: 2.36 ± 0.2 d for Bloomfield virus, 1.8 ± 0.3 d for DAV, and 2.0 ± 0.2 d for DCV. By 12 dpe, the mean biological age of uninfected flies remained similar to their chronological age (12.8 ± 0.9 d), whereas all viral infections significantly increased the biological age: 31.5 ± 3.7 d for DAV, 17.8 ± 8.1 d for DCV, 24.6 ± 0.3 d for Bloomfield, and 25.2 ± 0.6 d for Nora. At later time points, infection by two viruses significantly increased the biological age: DAV-infected flies had a biological age of 54.3 ± 0.1 d by 25 dpe, and DCV-infected flies had a biological age of 49.6 ± 10.4 d at 28 dpe. For the other viruses, there was no accelerated aging at later time points; Bloomfield virus-infected flies showed a reduced but non-significant difference in biological age (31.9 ± 3.9 d at 36 dpe), and Nora virus-infected flies exhibited a significant slowing of aging (biological age of 37.1 ± 11.5 d at 53 dpe). These results demonstrate that enteric viral infections accelerate aging, with the magnitude of aging acceleration depending on the specific virus and the time post-infection.

We next wondered if the observed accelerated aging might be explained by the expression of specific genes triggered by viral infections, such as immune genes[21,22]. To address whether accelerated aging was driven by antiviral genes in the infected animals, we estimated biological age using a second reference generated with the same dataset but excluding known antiviral genes (SFile 1). The biological age estimates remained similar for both uninfected and infected animals, regardless of whether antiviral genes were included in the reference ($r = 0.999$, 82 d.f., $P < 0.001$, Figure 1D).

Given that intestinal dysfunction is a hallmark of aging in *Drosophila* and leads to reduced lifespan[16,23], we examined whether intestinal-related genes alone could suffice to determine biological age (SFile 2). Using a reduced subset of genes (≈3k intestinal genes vs ≈15k total genes), we found that a third reference made solely of intestinal genes accurately estimated biological age in uninfected animals ($r = 0.989$, 30 d.f., $P < 0.001$, Figure 1E). However, this estimation was perturbed in infected flies, suggesting that viral infections induce systemic aging beyond localized intestinal pathology.

We also tested various factors that could modulate the age disruption caused by viral infections. First, we examined host sex differences, as our initial analysis used female flies and *Drosophila* exhibits sex-specific transcriptional and immune responses[24,25]. Using a transcriptional dataset that included DCV-infected males and females[26], we found comparable biological ages in uninfected animals of both sexes and a similar significant acceleration of aging upon DCV infection (Figure 2A, left panel). Second, we studied the effect of *Wolbachia*, a common bacterial symbiont of insects known to reduce viral pathogenicity and transmission[27,28]. Using a transcriptional dataset of flies injected with DCV and either harboring or not *Wolbachia*[29], we observed that harboring *Wolbachia* mitigated the aging induced by DCV. At 3 dpe, *Wolbachia*-free flies injected with DCV had a biological age of 56.7 ± 0.6 d (median ± SD). In contrast, flies harboring *Wolbachia* had a significantly lower biological age of 34.6 ± 10.6 d (Figure 2A, central panel). The third factor studied was viral clearance. Although viral infections are typically persistent in fly populations, some individuals can clear DCV infection[20, 22]. Both the DCV-infected flies and virus-cleared flies showed significantly higher biological age than uninfected

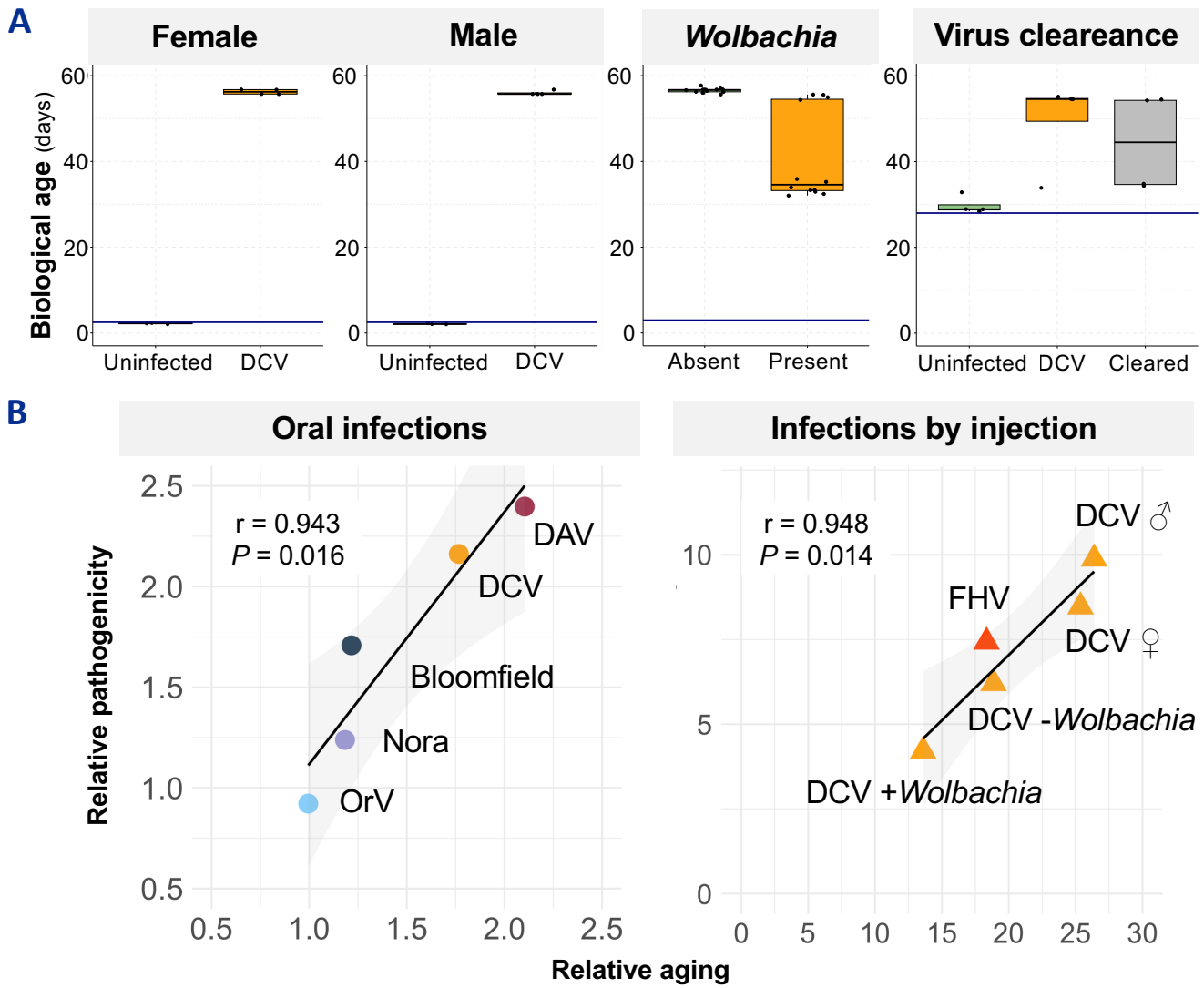

Figure 2. **(A)** Biological age of flies injected with DCV for males and females (left panel), of DCV-injected flies harboring or not harboring *Wolbachia* bacteria (central panel), and of DCV-persistently infected flies that cleared the infection (right panel). The horizontal line represents the chronological age of the samples, and the boxplots represent the estimated biological age. **(B)** Correlation between relative aging and pathogenicity for infection modes where the virus adquisition occurred by feeding (oral route, left panel) or by injection (right panel).

Color legend: *OrV*, *Nora virus*, *Bloomfield virus*, *DAV*, *DCV*, *FHV*.

flies; at 28 dpe the never-infected flies had a median biological age of 28.9 d meanwhile the DCV infected was 54.6 ± 10.4 d and for those that had cleared the DCV infection was 44.5 ± 11.4 d (Figure 2A, right panel), suggesting that once aging is triggered it cannot be totally reverted.

Finally, we wondered whether the pathogenicity (in here understood as the impact on lifespan) correlates with the aging that a virus induces. As all four persistent viruses have detrimental impacts on *Drosophila* lifespan (Figure 1A), we included the Orsay virus (OrV), an enteric virus of the nematode *Caenorhabditis elegans* with minimal host pathogenicity[30] and available time-series transcriptional data[31]. We observed that OrV infection has a minimal impact on the nematode's aging (Supplementary Figure 3) with a general slowing of aging in infected nematodes, except for

peaks of accelerated aging at 8 and 12 hours -that coincided with the viral load peak-[31]. This led us to investigate whether there is a correlation between the viral load of persistent viruses and the aging of flies. With only three time points per virus, we did not detect any statistically significant correlations. However, we noted a strong negative correlation between viral load and aging for Bloomfield and Nora viruses, a moderate positive correlation for DAV, and no correlation for DCV (Supplementary Figure 4). When considering the impact of viral infections on the host lifespan, we observed a significant positive correlation between infection pathogenicity and accelerated aging (r = 0.943, 3 d.f., P = 0.0162, Figure 2B, left panel). This allow us to propose that enteric viruses reduce host lifespan by accelerating their aging. We then questioned whether this correlation would hold for injected insects, as inoculating a virus by injection bypasses the gut barrier and increases its pathogenicity. We used data from injected DCV[26,29] and Flock House Virus (FHV)[32]. Indeed, we found that aging was higher in injected animals and positively correlated with pathogenicity (r = 0.948, 3 d.f., P = 0.0142, Figure 2B, right panel).

Discussion

Viruses have long been known to modulate their hosts in remarkable ways[33,34,35], but their role in modulating the aging process has only recently begun to be explored. Our work demonstrates that viral infections modify biological age, thus establishing viruses as age-distortersentities that interfere with their host's aging[36]. Most aging-associated gene families are hundreds of millions of years old[37], and viral proteins interact with host proteins in the aging pathway[38]. Considering this, we hypothesize that pathogenic viral infections significantly modulate the aging process across diverse organisms, with the magnitude and direction of this modulation (slowing or acceleration) depending on the virus's pathogenicity and the stage of infection. Further research is required to understand the prevalence of virus-induced aging and its underlying mechanisms.

Our findings on virus-induced aging provide a foundation for investigating aging mechanisms, identifying anti-aging targets, reducing infection pathogenicity, and leveraging viruses as tools to modulate aging.

Methods

Transcriptomic data

Persitent infected flies' data was generated by the team and raw sequencing reads can be found in the Sequence Read Archive under the BioProject PRJNA1235228. For injected flies raw sequencing reads were retrieved from the same archive: FHV from BioProject PRJNA644593, DCV and *Wolbachia* from BioProject PRJEB21984, DCV in male and female from BioProject PRJNA1084779. The *C. elegans* mapped reads were kindly provided by Santiago Elena's lab. Raw sequencing reads were mapped to the *D. melanogaster* genome release dmel_r6.56 with STAR (v2.7.11b)[39]. Feature counting was done with HTSeq (v0.11.2)[40] using the default settings.

Dataset biological information

All samples are made of several whole-body organisms. Unless otherwise indicated, all flies are *Wolbachia*-free and non-virgin. Persistent infected flies' data have four pools of 5 flies per virus and time point[20]. DCV male and female dataset has four pools of 4-7 dpe 10 flies per condition[26]. DCV and *Wolbachia* dataset has 12 pools of 6-9 dpe 10 flies per condition[29]. FHV dataset has four measures per condition of an undetermined number of 6-9 dpe male flies[32]. OrV dataset has 3 pools of 1200 animals per condition[31].

Age estimation

We estimated biological age from transcriptomic data using the RAPToR package (v1.2.0)[19] in R

(v4.3.2) in the RStudio development environment (v2024.04.2+764). First, a reference was created from uninfected flies collected on days 1, 6, 12, 25, 28, 36, 52, and 58 post-eclosion[20]. Gene expression counts were fitted to a generalized additive model via RAPToR's (v1.2.0) *ge_im* function using a cubic regression spline with eight knots. We then performed a principal component analysis (PCA) with the *prcomp* function in the 'stats' package, determining that 29 principal components should be retained (Supplementary Figure 1) by summing those with a cumulative variance below 0.99 and then adding one. The final reference was constructed using RAPToR's *make_ref* function with 1000 interpolation points. Subsequently, we estimated the biological ages of additional transcriptomic datasets by applying RAPToR's *ae* function to this reference. In specific cases described in the main text, new references were generated using the same approach but were restricted to subsets of genes of interest (Supplementary Files 1, 2, and 3) derived from the original uninfected dataset.

Calculating relative aging and pathogenicity

Relative aging was calculated by first summarizing the time series of estimated biological age and survival curves (Supplementary Files 4 and 5) into a single value using the Area Under the Disease Progress Stair (AUDPS) calculation[41]. These values were then used to calculate the relative aging or relative pathogenicity by dividing the AUDPS of the infected samples (either for aging or survival) by the AUDPS of their uninfected controls. The AUDPS calculation was performed using the 'agricolae' package (version 1.3-7) in R (version 4.3.2).

Statistical analysis

All statistical analyses were conducted using R (v4.3.2), with the significance level set at $P < 0.05$. General linear models were fitted using the *glm* function from the 'stats' package, specifying a Gamma distribution with a log link. In Figure 1A and 2A, the infection condition was used as a factor, whereas in Figure 1C, both infection condition and time point were included as factors. Pearson correlations were calculated using the *cor.test* function from the 'stats' package. Post hoc pairwise comparisons were performed using the *emmeans* function from the 'emmeans' package (v1.4.7) and Tukey adjustment.

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References

1. Mekker, A., Tchang, V.S., Haeberli, L., Oxenius, A., Trkola, A., and Karrer, U. (2012). Immune senescence: relative contributions of age and cytomegalovirus infection. PLoS Pathog 8, e1002850. https://doi.org/10.1371/journal.ppat.1002850.

2. Lopez Angel, C.J., Pham, E.A., Du, H., Vallania, F., Fram, B.J., Perez, K., Nguyen, T., Rosenberg-Hasson, Y., Ahmed, A., Dekker, C.L., et al. (2021). Signatures of immune dysfunction in HIV and HCV infection share features with chronic inflammation in aging and persist after viral reduction or elimination. Proc Natl Acad Sci USA. 118, e2022928118. https://doi.org/10.1073/pnas.2022928118.

3. López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., and Kroemer, G. (2023). Hallmarks of aging: An expanding universe. Cell 186, 243–278. https://doi.org/10.1016/j.cell.2022.11.001.

4. Aging Biomarker Consortium, Bao, H., Cao, J., Chen, M., Chen, M., Chen, W., Chen, X., Chen, Y., Chen, Y., Chen, Y., et al. (2023). Biomarkers of aging. Sci China Life Sci 66, 893–1066. https://doi.org/10.1007/s11427-023-2305-0.

5. Kiani, A.K., Pheby, D., Henehan, G., Brown, R., Sieving, P., Sykora, P., Marks, R., Falsini, B., Capodicasa, N., Miertus, S., Lorusso, L., Dondossola, D., Tartaglia, G.M., Ergoren, M.C., Dundar, M., Michelini, S., Malacarne, D., Bonetti, G., Dautaj, A., Donato, K., Medori, M.C., Beccari, T., Samaja, M., Connelly, S.T., Martin, D., Morresi, A., Bacu, A., Herbst, K.L., Kapustin, M., Stuppia, L., Lumer, L., Farronato, G., Bertelli, M. (2022). Ethical considerations regarding animal experimentation. J Prev Med Hyg 63, E255. https://doi.org/10.15167/2421-4248/JPMH2022.63.2S3.2768

6. Hales, K.G., Korey, C.A., Larracuente, A.M., Roberts, D.M., (2015). Genetics on the Fly: A Primer on the *Drosophila* Model System. Genetics 201, 815–842. https://doi.org/10.1534/genetics.115.183392

7. Lu, T.-C., Brbić, M., Park, Y.-J., Jackson, T., Chen, J., Kolluru, S.S., Qi, Y., Katheder, N.S., Cai, X.T., Lee, S., Chen, Y.-C., Auld, N., Liang, C.-Y., Ding, S.H., Welsch, D., D'Souza, S., Pisco, A.O., Jones, R.C., Leskovec, J., Lai, E.C., Bellen, H.J., Luo, L., Jasper, H., Quake, S.R., Li, H., (2023). Aging Fly Cell Atlas identifies exhaustive aging features at cellular resolution. Science 380, eadg0934. https://doi.org/10.1126/science.adg0934

8. Öztürk-Çolak, A., Marygold, S.J., Antonazzo, G., Attrill, H., Goutte-Gattat, D., Jenkins, V.K., Matthews, B.B., Millburn, G., Dos Santos, G., Tabone, C.J., FlyBase Consortium, Perrimon, N., Gelbart, S.R., Broll, K., Crosby, M., Dos Santos, G., Falls, K., Gramates, L.S., Jenkins, V.K., Longden, I., Matthews, B.B., Seme, J., Tabone, C.J., Zhou, P., Zytkovicz, M., Brown, N., Antonazzo, G., Attrill, H., Goutte-Gattat, D., Larkin, A., Marygold, S., McLachlan, A., Millburn, G., Pilgrim, C., Öztürk-Çolak, A., Kaufman, T., Calvi, B., Campbell, S., Goodman, J., Strelets, V., Thurmond, J., Cripps, R., Lovato, T (2024). FlyBase: updates to the *Drosophila* genes and genomes database. Genetics. 227, iyad211. https://doi.org/10.1093/genetics/iyad211

9. Ugur, B., Chen, K., and Bellen, H.J. (2016). *Drosophila* tools and assays for the study of human diseases. Dis Model Mech 9, 235–244. https://doi.org/10.1242/dmm.023762.

10. Tsurumi, A., and Li, W.X. (2020). Aging mechanisms—A perspective mostly from *Drosophila*. Adv Genet 1, e10026. https://doi.org/10.1002/ggn2.10026

11. Jasper, H. (2020). Intestinal Stem Cell Aging: Origins and Interventions. Annu Rev Physiol 82, 203–226. https://doi.org/10.1146/annurev-physiol-021119-034359

12. Apidianakis, Y., and Rahme, L.G. (2011). *Drosophila melanogaster* as a model for human intestinal infection and pathology. Dis Model Mech 4, 21–30. https://doi.org/10.1242/dmm.003970.

13. Nigg, J.C., Castelló-Sanjuán, M., Blanc, H., Frangeul, L., Mongelli, V., Godron, X., Bardin, A.J., and Saleh, M.-C. (2024). Viral infection disrupts intestinal homeostasis via Sting-dependent NF-κB signaling in *Drosophila*. Current Biology 34, 2785-2800.e7. https://doi.org/10.1016/j.cub.2024.05.009.

14. Franchet, A., Haller, S., Yamba, M., Barbier, V., Thomaz-Vieira, A., Leclerc, V., Becker, S., Kwang-Zin, L., Orlov, I., Spehner, D., Daeffler, L., Ferrandon, D. (2025). Nora virus proliferates in dividing intestinal stem cells and sensitizes flies to intestinal infection and oxidative stress. bioRxiv. https://doi.org/10.1101/2025.01.30.635658.

15. Biteau, B., Hochmuth, C.E., and Jasper, H. (2008). JNK activity in somatic stem cells causes loss of tissue homeostasis in the aging *Drosophila* gut. Cell Stem Cell 3, 442–455. https://doi.org/10.1016/j.stem.2008.07.024.

16. Rera, M., Clark, R.I., and Walker, D.W. (2012). Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in *Drosophila*. Proc Natl Acad Sci USA 109, 21528–21533. https://doi.org/10.1073/pnas.1215849110.

17. Regan, J.C., Khericha, M., Dobson, A.J., Bolukbasi, E., Rattanavirotkul, N., and Partridge, L. (2016). Sex difference in pathology of the ageing gut mediates the greater response of female lifespan to dietary restriction. eLife 5, e10956. https://doi.org/10.7554/eLife.10956.

18. Salazar, A.M., Aparicio, R., Clark, R.I., Rera, M., Walker, D.W. (2023). Intestinal barrier dysfunction: an evolutionarily conserved hallmark of aging. Dis Models Mech 16, dmm049969. https://doi.org/10.1242/dmm.049969.

19. Bulteau, R., and Francesconi, M. (2022). Real age prediction from the transcriptome with RAPToR. Nat Methods 19, 969–975. https://doi.org/10.1038/s41592-022-01540-0.

20. Castelló-Sanjuán, M., González, R., Romoli, O., Blanc, H., Nigg, J., Saleh, M.-C. (2025). Persistent viral infections impact key

biological traits in *Drosophila melanogaster*. bioRxiv. https://doi.org/10.1101/2025.03.12.642769doi.

21. Xu, J., and Cherry, S. (2014). Viruses and antiviral immunity in *Drosophila*. Dev Comp Immunol 42, 67–84. https://doi.org/10.1016/j.dci.2013.05.002.

22. Mondotte, J.A., and Saleh, M.-C. (2018). Antiviral Immune Response and the route of infection in *Drosophila melanogaster*. Adv Virus Res 100, 247–278. https://doi.org/10.1016/bs.aivir.2017.10.006.

23. Clark, R.I., Salazar, A., Yamada, R., Fitz-Gibbon, S., Morselli, M., Alcaraz, J., Rana, A., Rera, M., Pellegrini, M., Ja, W.W., et al. (2015). Distinct shifts in microbiota composition during drosophila aging impair intestinal function and drive mortality. Cell Rep 12, 1656–1667. https://doi.org/10.1016/j.celrep.2015.08.004.

24. Hill-Burns, E.M., and Clark, A.G. (2009). X-linked variation in immune response in *Drosophila melanogaster*. Genetics 183, 1477–1491. https://doi.org/10.1534/genetics.108.093971.

25. Vincent, C.M., and Dionne, M.S. (2021). Disparate regulation of IMD signaling drives sex differences in infection pathology in *Drosophila melanogaster*. Proc Natl Acad Sci USA 118, e2026554118. https://doi.org/10.1073/pnas.2026554118.

26. Salminen, T.S., Vesala, L., Basikhina, Y., Kutzer, M., Tuomela, T., Lucas, R., Monteith, K., Prakash, A., Tietz, T., and Vale, P.F. (2024). A naturally occurring mitochondrial genome variant confers broad protection from infection in *Drosophila*. PLoS Gent 20, e1011476. https://doi.org/10.1371/journal.pgen.1011476

27. Teixeira, L., Ferreira, Á., and Ashburner, M. (2008). The Bacterial Symbiont *Wolbachia* Induces Resistance to RNA Viral Infections in *Drosophila melanogaster*. PLoS Biol 6, e1000002. https://doi.org/10.1371/journal.pbio.1000002.

28. Moreira, L.A., Iturbe-Ormaetxe, I., Jeffery, J.A., Lu, G., Pyke, A.T., Hedges, L.M., Rocha, B.C., Hall-Mendelin, S., Day, A., Riegler, M., et al. (2009). A *Wolbachia* symbiont in *Aedes aegypti* limits infection with Dengue, Chikungunya, and *Plasmodium*. Cell 139, 1268–1278. https://doi.org/10.1016/j.cell.2009.11.042.

29. Martinez, J., Bruner-Montero, G., Arunkumar, R., Smith, S.C.L., Day, J.P., Longdon, B., and Jiggins, F.M. (2019). Virus evolution in *Wolbachia*-infected *Drosophila*. Proc R Soc B 286, 20192117. https://doi.org/10.1098/rspb.2019.2117.

30. Ashe, A., Bélicard, T., Le Pen, J., Sarkies, P., Frézal, L., Lehrbach, N.J., Félix, M.-A., and Miska, E.A. (2013). A deletion polymorphism in the *Caenorhabditis elegans* RIG-I homolog disables viral RNA dicing and antiviral immunity. eLife 2, e00994. https://doi.org/10.7554/eLife.00994.

31. Castiglioni, V.G., Olmo-Uceda, M.J., Villena-Giménez, A., Muñoz-Sánchez, J.C., Legarda, E.G., and Elena, S.F. (2024). Story of an infection: Viral dynamics and host responses in the *Caenorhabditis elegans* –Orsay virus pathosystem. Sci Adv 10, eadn5945. https://doi.org/10.1126/sciadv.adn5945.

32. Sheffield, L., Sciambra, N., Evans, A., Hagedorn, E., Goltz, C., Delfeld, M., Kuhns, H., Fierst, J.L., and Chtarbanova, S. (2021). Age-dependent impairment of disease tolerance is associated with a robust transcriptional response following RNA virus infection in *Drosophila*. G3 11, jkab116. https://doi.org/10.1093/g3journal/jkab116.

33. Christiaansen, A., Varga, S.M., and Spencer, J.V. (2015). Viral manipulation of the host immune response. Curr Opin Immunol 36, 54–60. https://doi.org/10.1016/j.coi.2015.06.012.

34. Blanc, S., and Michalakis, Y. (2016). Manipulation of hosts and vectors by plant viruses and impact of the environment. Curr Opin Insect Sci 16, 36–43. https://doi.org/10.1016/j.cois.2016.05.007.

35. González, R., Butković, A., and Elena, S.F. (2020). From foes to friends: Viral infections expand the limits of host phenotypic plasticity. Adv Virus Res 106, 85–121. https://doi.org/10.1016/bs.aivir.2020.01.003.

36. Teulière, J., Bernard, C., and Bapteste, E. (2021). Interspecific interactions that affect ageing: Age-distorters manipulate host ageing to their own evolutionary benefits. Ageing Res Rev 70, 101375. https://doi.org/10.1016/j.arr.2021.101375.

37. Bonnefous, H., Teulière, J., Lapointe, F.-J., Lopez, P., and Bapteste, E. (2024). Most genetic roots of fungal and animal aging are hundreds of millions of years old according to phylostratigraphy analyses of aging networks. GeroScience 46, 5037–5059. https://doi.org/10.1007/s11357-024-01234-9.

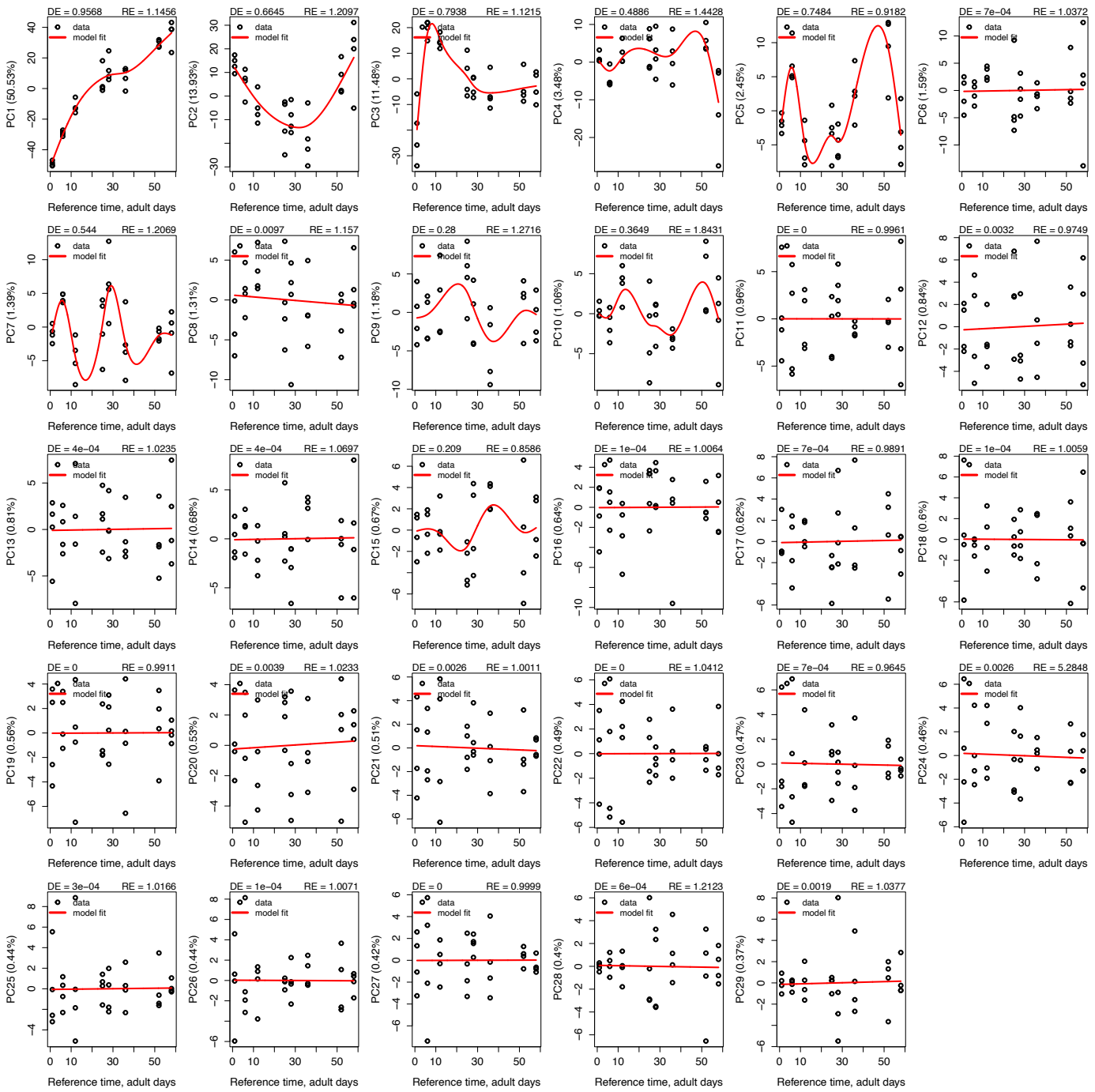
38. Teulière, J., Bernard, C., Bonnefous, H., Martens, J., Lopez, P., and Bapteste, E. (2023). Interactomics: Dozens of viruses, co-evolving with humans, including the influenza a virus, may actively distort human aging. Mol Biol Evol 40, msad012. https://doi.org/10.1093/molbev/msad012.

39. Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T.R. (2013). STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 15–21. https://doi.org/10.1093/bioinformatics/bts635.

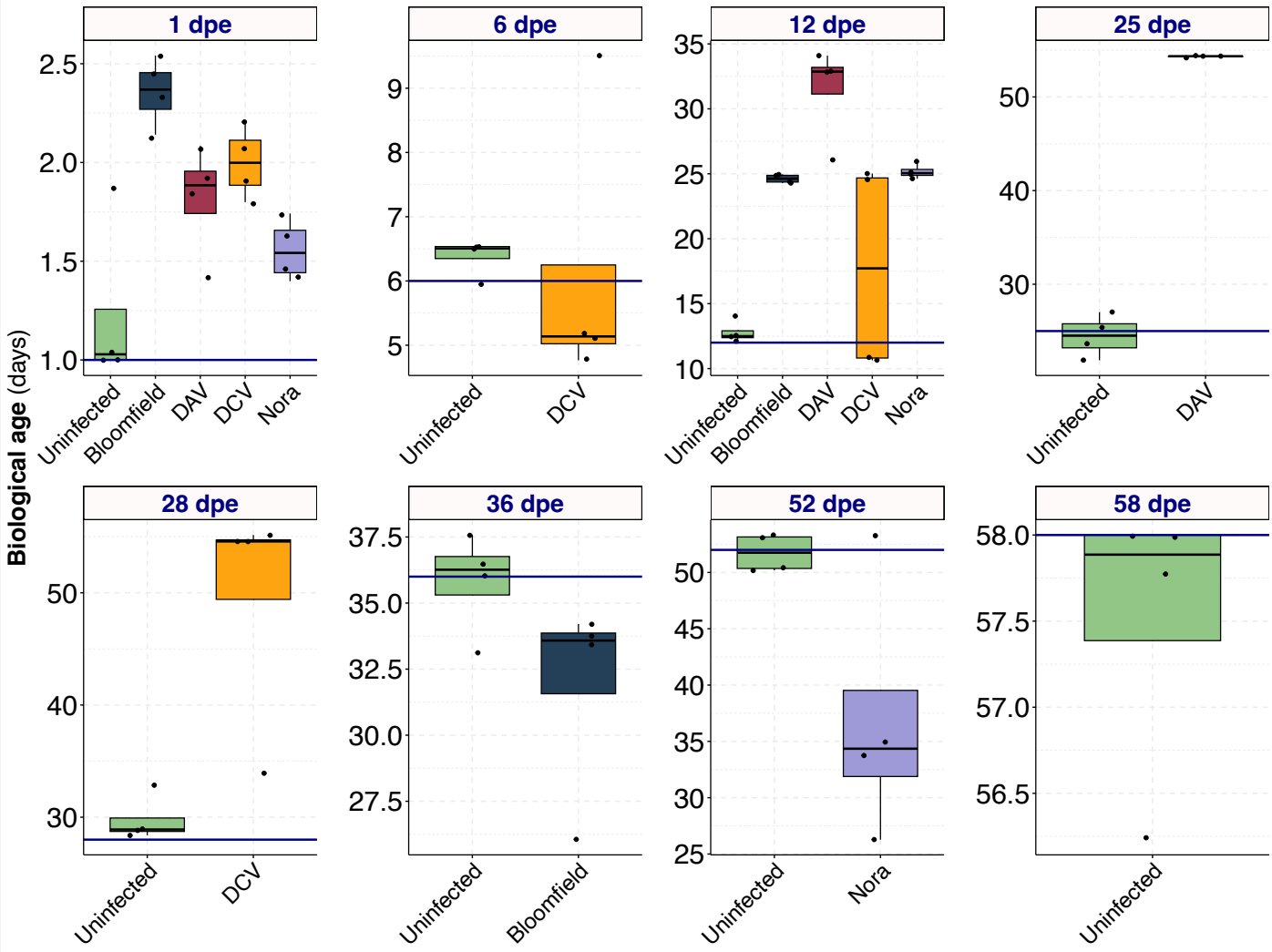
40. Putri, G.H., Anders, S., Pyl, P.T., Pimanda, J.E., and Zanini, F. (2022). Analysing high-throughput sequencing data in Python with HTSeq 2.0. Bioinformatics 38, 2943–2945. https://doi.org/10.1093/bioinformatics/btac166.

41. Simko, I., and Piepho, H.-P. (2012). The Area Under the Disease Progress Stairs: Calculation, advantage, and application. Phytopathology 102, 381–389. https://doi.org/10.1094/PHYTO-07-11-0216.

Supplementary Figures

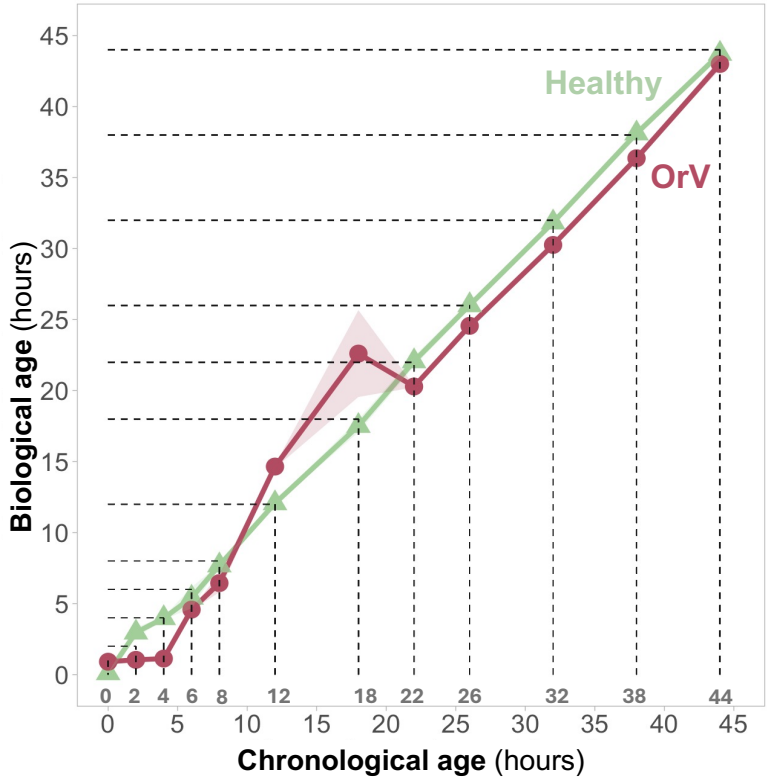


Supplementary Figure 1. Graphs with the 29 Principal Component Analysis (PCA), which decompose the expression matrix into components, used to create the adult fly reference.

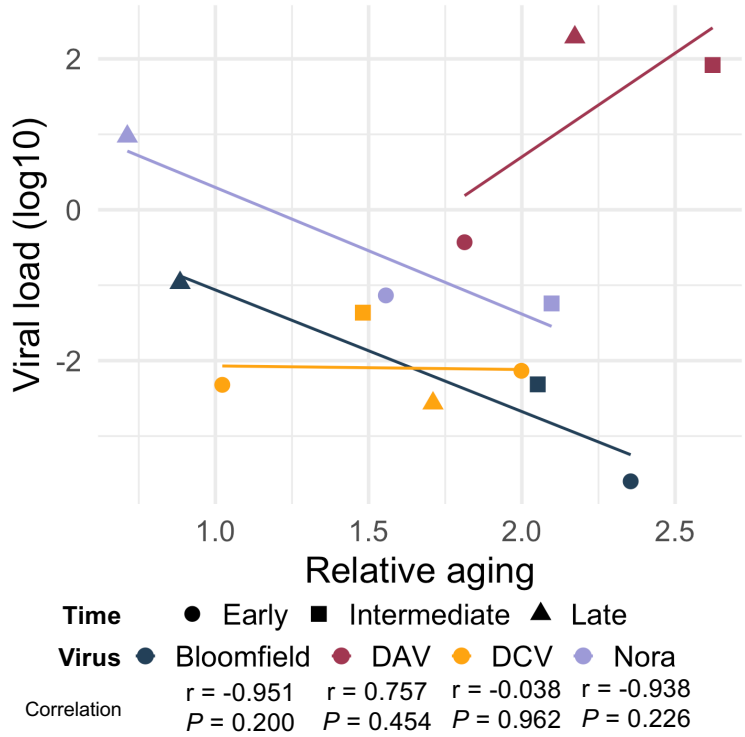


Supplementary Figure 2. Box plot representation of the biological age of persistently infected and uninfected flies from Figure 1C. Each panel represents a specific chronological time point, with the horizontal line indicating the chronological age of the samples.

For all panels, color legend: uninfected, Nora, Bloomfield, DAV, DCV.



Supplementary Figure 3. *C. elegans* biological age (Y-axis) at different chronological time points (X-axis) for uninfected animals (green triangles) and those infected with Orsay virus (red circles). Points represent the mean biological age, and the shaded area represents the standard error.



Supplementary Figure 4. Correlation between relative aging and viral load for the four persistent viral infections in *Drosophila*. Early time points are 1 and 6 days post-eclosion (dpe), the intermediate time point is 12 dpe, and the late time point is when 50% of the host population has died (25 dpe for DAV, 28 dpe for DCV, 36 dpe for Bloomfield, and 52 dpe for Nora).

Supplementary Files

Supplementary File 1. List of antiviral genes excluded from the time-series dataset to create a reference that do not take into account antiviral responses.

Supplementary File 2. List of gut genes subsetted from the time-series dataset to create a gut-only reference

Supplementary File 3. List of genes differentially expressed excluded from the reference to create a reference for virgin flies.

Supplementary File 4. Data used to generate all the figures.

Supplementary File 5. Files with the data for the AUDPS calculus for the pathogenicity of each virus. Survival data was extracted using computer vision assist (automeris.io) from survival plots already published: fly persistent infections come from 20, FHV from 32, DCV-injected male/female from 26, DCV-injected with or without *Wolbachia* from 29, and OrV from 30.