Electronic Supplementary Material

Detailed Materials & Methods

Analysis of Drosophila Activity

Single flies were either systemically infected or control pricked as described above, and immediately placed in a single DAM tube and allocated a random slot in one of 8 DAM monitor units (each unit is capable of housing a maximum of 32 tubes). We tested the effect of DCV on locomotor activity in *D. melanogaster* by comparing infected and uninfected individuals, rather than monitoring the same individual before and after infection, as this would require removing and reintroducing the flies into the DAM. Our design therefore compares locomotion in healthy vs infected flies without the potentially confounding source of stress and damage that would come from manipulating the same fly. At least one slot of each monitor unit was left empty and another contained an empty tube, as negative controls. While flies were monitored continuously for 4 complete days. Flies that died during this 4-day period were removed from the dataset. In total we analysed the activity of 872 flies, with n=18-27 flies for each combination of sex and genetic background (Table S1). Raw activity data was processed using the DAM System File Scan Software [6], and the resulting data was manipulated using Microsoft Excel. Activity counts for each individual fly were combined into 5-minute bins. We analysed fly activity data using three metrics: total locomotor activity, proportion of time spent asleep and the average activity when awake [7]. Total locomotor activity refers to the sum of all recorded movements during the 4-day measuring period and is an outcome of how often a fly sleeps and how much it moves during bouts of awake activity. In *Drosophila*, sleep is defined as five minutes of continuous inactivity, sharing several features with mammalian sleep, such as being followed by an increased arousal threshold, and being regulated independently from the circadian clock [8]. To assess the proportion of time spent asleep, we used the proportion of all 5-min bouts (n=1152) where no activity was logged. To quantify awake activity, we calculated the average level of locomotor activity across every 5-min period where at least one instance of movement was recorded. Average activity when awake can help characterise lethargy when individuals are active, an important behavioural symptom of infection [9].

Genetic Background	Male		Female	
	Control	Infected	Control	Infected
RAL-59	24 (7)	24 (4)	22 (5)	22 (5)
RAL-75	20 (3)	27 (2)	20 (5)	20 (6)
RAL-138	21 (5)	20 (2)	18 (4)	19 (5)
RAL-373	20 (1)	26 (2)	21 (4)	28 (10)
RAL-379	20 (4)	22 (3)	21 (3)	20 (2)
RAL-380	24 (5)	20 (4)	23 (4)	24 (7)
RAL-502	23 (3)	21 (3)	21 (4)	20 (2)
RAL-738	21 (4)	21 (7)	20 (4)	20 (1)
RAL-765	26 (8)	28 (7)	20 (5)	21 (5)
RAL-818	21 (5)	20 (2)	20 (6)	22 (9)

Table S1. The sample size of alive individuals from each treatment group, representing every combination of sex, genetic background and infection status, that was used to measure locomotor activity. Values in brackets represent the number of individuals that died during the experiment and were removed from the final dataset.

Results

Response Variable	Predictor	Df	X^2	p
Alive after 4 days	Genetic Background	9	16.45	0.058
	Sex	1	0.0013	0.97
	Infection	1	0.0018	0.97
	Genetic Background × Sex	9	9.15	0.42
	Genetic Background × Infection	9	6.64	0.67
	Sex × Infection	1	0.74	0.39
	Genetic Background × Sex × Infection	9	1.05	0.99

Table S2. Model outputs for the binomial logistic regression conducted on the number of flies alive after 4 days of locomotor activity measurements in the DAM.

DCV Susceptibility

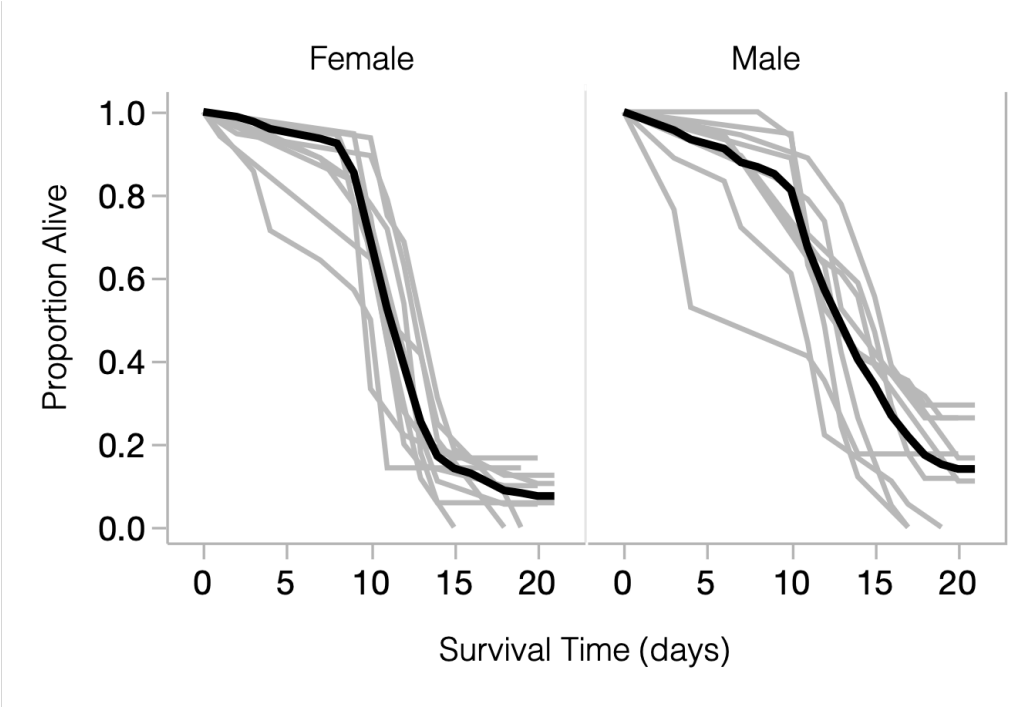

Fig S1. Survival of males and females from the 10 genetic backgrounds following system infection with DCV by pricking. Genetic backgrounds (grey lines) demonstrate the presence of variation in susceptibility to DCV infection through comparisons with the mean (black line).

Genetic Background	Sex	Median Lifespan	Lifespan SE
59	Male	14	1.62
	Female	14	1.22
75	Male	13.5	1.28
	Female	12	1.77
138	Male	14	1.63
	Female	13	0.99
373	Male	13	0.94
	Female	11	1.0
379	Male	16	0.95
	Female	13	0.8
380	Male	12	0.48
	Female	10	1.52
502	Male	15	1.2
	Female	11	1.25
738	Male	15	1.35
	Female	11.5	1.1
765	Male	11.5	1.11
	Female	11	2.1
818	Male	11	2.06
	Female	12.5	0.64

Table S3. Summary statistics of the survival data presented in Figure S1. The susceptibility rank is derived from a treatment groups median lifespan.

Social aggregation experiment

Social aggregation was measured in 55mm Petri dishes with 2% agar poured in until 3mm from the lid in order to limit flight. Flies were pricked with DCV or TRIS as described in the main methods and transferred to Petri dishes in groups of twelve after 72 hours of infection, under light CO_2 anaesthesia. Due to reducing anaesthesia as much as possible to curtail behavioural defects associated with over-exposure to CO_2 [1], and experimenter error, some flies escaped Petri dishes before they were closed. A total of 448 dishes contained twelve flies, while 113 and 19 contained eleven and ten, respectively. Flies within a Petri dish were the same genetic background, sex and infection treatment. Once transferred, flies were left in Petri dishes to acclimate for 30 minutes. This acclimation period was identified in a prior experiment where it was observed that after 30 minutes, fly movement in arenas was minimal, as shown previously [2,3]. A single image was recorded of each Petri dish using a 13 Megapixel camera, followed by a second image (10-20 minutes later). Using these images we calculated the NND using *ImageJ* software [4], by marking flies in the centre of their thorax with the multi-point tool. We calibrated the distance between flies in photos using the 55mm width of the Petri dish and calculated the nearest neighbour distance between each pair of flies in millimetres using the 'NND' package in *ImageJ*. These values were used to calculate the median NND for each petri dish [2,5]. To account for differences in body lengths between lines and sexes, we also calculated the NND using body lengths by dividing millimetre distances by the mean body length of a randomly selected group of 30-40 individuals from each genetic background and sex combination (Figure S1). We also tested for differences in body lengths between males and females from these 10 genetic backgrounds. This model tested an interaction between sex (male/female) and DGRP line (10 genetic backgrounds), all modelled as fixed effects. Incorporating this size difference into measures of social aggregation, by measuring body lengths between individuals did not alter the results qualitatively (Figure S2).

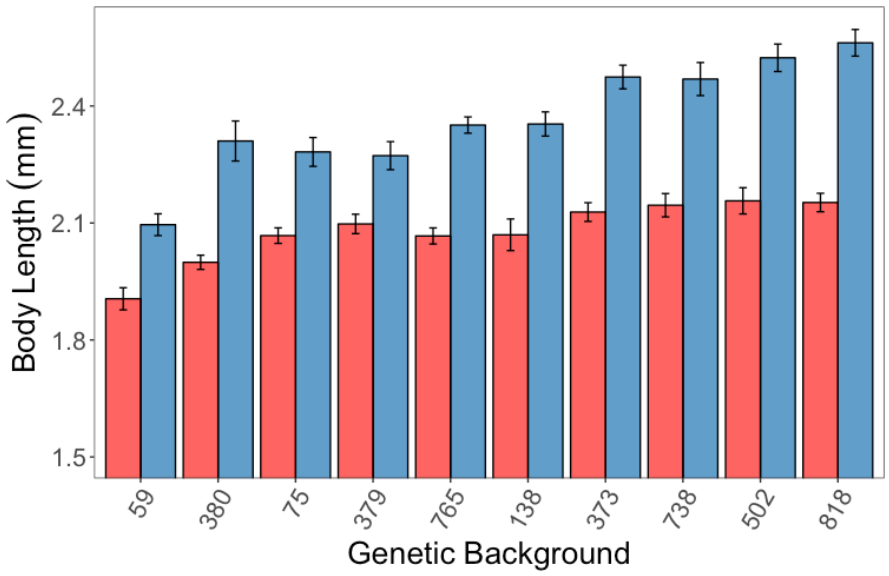

Figure S2. Mean±SE body length of flies calculated from 30 flies per line for males (red) and females (blue).

Response	Predictor	Df	F	p
Body Length	Genetic Background	9	28.5	<0.0001
	Sex	1	440.8	<0.0001
	Genetic Background * Sex	9	3.44	<0.001

Table S4. Model outputs for statistical tests performed on body lengths of treatment groups comprised of each combination of sex and genetic background.

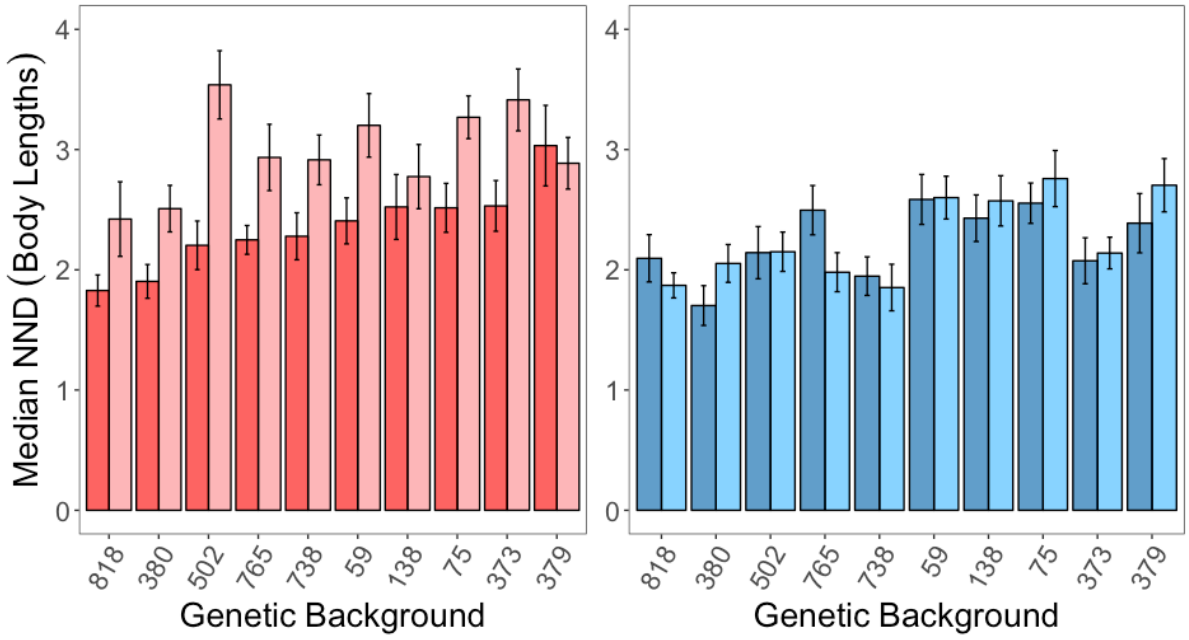

Figure S3. Mean±SE median nearest neighbour distance (NND) in body lengths of adult flies placed in Petri dishes for at least 30 minutes until settled. (a) Uninfected female-only arenas shown in blue, and infected female-only arenas in pale blue. (b) Uninfected male-only arenas are shown in red, and infected male-only arenas in pink. The x-axis of both panels is ordered from the lowest to highest mean median NND of female flies of a single genetic background.

Response	Predictor	Df	F	p
Median NND (body length)	Genetic Background	9	6.55	<0.0001
	Sex	1	38.74	<0.0001
	Infection	1	24.3	<0.0001
	Genetic Background * Sex	9	1.56	0.12
	Genetic Background * Infection	9	0.99	0.45
	Sex * Infection	1	20.94	<0.0001
	Genetic Background * Sex * Infection	9	1.58	0.12

Table S5. Model outputs for statistical tests performed on social aggregation when measured using body lengths, testing the causes of variation in sociality in males and females of 10 *D. melanogaster* genetic backgrounds.

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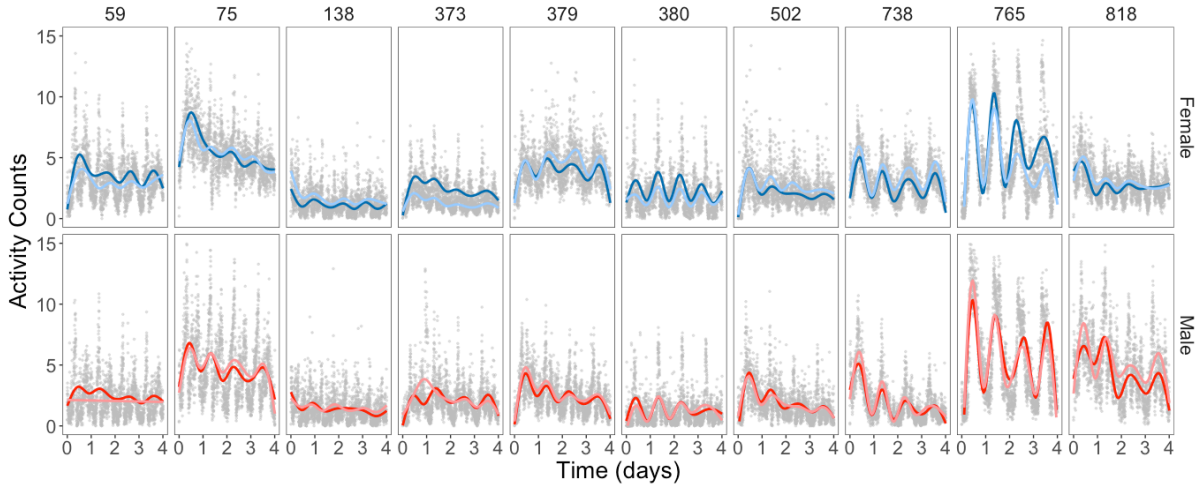

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114 **Figure S4.** Activity counts of adult flies for the first 4 days locomotor activity was measured in
115 the DAM. The mean activity counts of DAM tubes containing single flies of the same sex and
116 DCV infection status are represented by generalised additive model lines where uninfected
117 females are shown in blue, infected females in pale blue, uninfected males are shown in red,
118 and infected males in pink.

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References

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