THE UNIVERSITY of EDINBURGH

Edinburgh Research Explorer

Viral infection causes sex-specific changes in fruit fly social aggregation behaviour

Citation for published version:
Siva-Jothy, J & Vale, P 2019, 'Viral infection causes sex-specific changes in fruit fly social aggregation behaviour', Biology letters.

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Biology letters

Publisher Rights Statement:
This is the accepted version of the following article: [full citation], which has been published in final form at [Link to final article].

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 26. Sep. 2019

Viral infection causes sex-specific changes in fruit fly social aggregation behaviour

Jonathon A. Siva-Jothy[1], Pedro F. Vale[1,2*]

[1]Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, EH9 3FL Edinburgh, United Kingdom

[2] Centre for Immunity, Infection and Evolution, University of Edinburgh, EH9 3FL Edinburgh, United Kingdom

Abstract

Host behavioural changes following infection are common and could be important determinants of host behavioural competence to transmit pathogens. Identifying potential sources of variation in sickness behaviours is therefore central to our understanding of disease transmission. Here, we test how group social aggregation and individual locomotor activity vary between different genotypes of male and female fruit flies (*Drosophila melanogaster*) following septic infection with *Drosophila* C Virus (DCV). We find genetic-based variation in both locomotor activity and social aggregation, but we did not detect an effect of DCV infection on fly activity or sleep patterns within the initial days following infection. However, DCV infection caused sex-specific effects on social aggregation, as male flies in most genetic backgrounds increased the distance to their nearest neighbour when infected. We discuss possible causes for these differences in the context of individual variation in immunity and their potential consequences for disease transmission.

Key words: Social aggregation, locomotor activity, Drosophila Activity Monitor, Drosophila, Drosophila C Virus, sexual dimorphism

Introduction

Infection-induced changes to host physiology, and immunity in particular, following infection are well known, but it is equally striking that many animals experience similar behavioural changes following infection [1,2]. Common behavioural responses to infection include eating and moving less, as well as foregoing social and sexual interactions [1,3–5]. Whether these behavioural changes in response to infection are evolved host responses, parasite manipulations, or a coincidental by-product of infection[6,7], they have potentially important consequences for disease transmission [8]. This is particularly clear for behaviours such as individual locomotor activity or group social aggregation, which will directly determine how frequently susceptible and infected individuals interact. Assessing how host behaviours that influence contact rates might change following infection is therefore central to understanding the spread of infectious disease.

The extent to which host behaviours are modified during infection is likely to depend on genetic and environmental factors. Even in the absence of infection, individuals of some genetic backgrounds are more likely to aggregate than others [9,10], while males and females in a broad range of host species often exhibit distinct behavioural profiles [11,12]. How these different sources of variation influence infection-induced behavioural changes

42 is relatively understudied [8]. Measuring how males and females of different genetic
43 backgrounds modify their behaviour during infection may highlight groups of individuals
44 with higher contact rates and offer insight into the potential causes of heterogeneity in
45 pathogen spread.
46
47 Testing if locomotor and aggregation behaviours change following infection, and if these
48 changes differ between genetic backgrounds, is not straightforward for most host species.
49 It requires knowledge of how individuals within a population differ in their genetic
50 backgrounds and the ability to expose many individuals of the same background to
51 infection in controlled conditions, while comparing their behavioural responses to infection
52 with individuals of the same background that do not experience infection. For many animal
53 species, and certainly in human populations, this type of intervention is either extremely
54 challenging or not feasible. One alternative is to leverage the tools offered by model
55 systems. *Drosophila melanogaster*, for example, has been widely used as a model system
56 for behavioural genetics [13,14], and used specifically to study social aggregation and
57 locomotor activity [9,15,16]. Further, *D. melanogaster* is a powerful model of immunity in
58 response to a range of bacterial and viral pathogens [17]. Previous work has shown that
59 *D. melanogaster* exhibits a range of behavioural changes following *Drosophila* C Virus

60 (DCV) infection, including pathogen avoidance during oviposition [18], foraging [19] and
61 changes in locomotor [20–22]. Here, we test whether DCV infection changes social
62 aggregation and locomotor activity in *D. melanogaster*, and if these effects vary with
63 genetic background and sex.

Materials & Methods

Flies and Rearing Conditions

We used males and females from 10 lines sourced from the Drosophila Genetic Resource Panel (DGRP) [23], which are among the most and least susceptible genetic backgrounds to systemic Drosophila C Virus infection [24]. Genetic variation in DCV susceptibility was confirmed in a separate experiment where survival was measured following DCV infection in males and females from these lines (Figure S1; Table S3). Flies were reared in plastic vials on a standard diet of Lewis medium at 18±1˚C with a 12 hour light:dark cycle with stocks tipped into new vials every 14 days. One month before the experiment, flies were transferred to incubators and maintained at 25±1˚C with a 12 hour light:dark cycle at low density (~10 flies per vial) for two generations.

Virus Culture and Infection

The Drosophila C Virus (DCV) isolate was originally isolated in Charolles, France [25] and the stock used in this experiment was grown in Schneider Drosophila Line 2 (DL2) as previously described [20], diluted one hundred-fold (10^8 infectious units per ml) in TRIS-HCl solution (pH=7.3), aliquoted and frozen at -70˚C until required. Given the extensive dilution of DL2 cells in TRIS buffer, 100% TRIS buffer was used as a control for the infection

82 solution. It is important to note that while our laboratory stocks are routinely screened for
83 viruses and contaminants, unknown contaminants may be harboured by the DL2 cells.
84 However, given the many orders of magnitude in the titers of possible contaminants
85 compared to the titre of DCV, it is unlikely that these would cause effects confounded with
86 the effect of DCV. To infect with DCV, 3-5-day old flies were pricked in the thorax in the
87 mesopleura with a 0.15mm diameter pin, bent at 90º ~0.5mm from the tip, dipped in DCV
88 (or TRIS-HCl for controls). Using this infection protocol establishes a systemic infection that
89 results in increased viral titres within the first 3 days of infection [22,26–28].
90
91 **Measuring Drosophila social aggregation**
92 Social aggregation was measured in a separate experiment, by calculating the nearest
93 neighbour distance (NND) between individuals within a 12-fly group of the same sex and
94 genetic background that were contained within a Petri dish for 30 minutes [10,16,29]. The
95 experiment was conducted over five experimental blocks, each carried out over a single
96 day, where each genetic background, sex and infection treatment was measured. Flies in
97 infected treatment groups, were pricked with DCV 72 hours before their NND was
98 measured. The NND was calculated by image analysis of pictures recorded of each group
99 using the 'NND' package in ImageJ [30]. In total, we measured social aggregation in 580

groups of flies, with n=14-16 replicate groups of 12 flies for each genetic background, sex and infection status combination. To consider the effect of body size on social aggregation, we also measured the body length of a subset of individuals from each treatment group (Figure S1). NND measures were converted from millimetres to body lengths by dividing values by the average body length of individuals from treatment groups (Figure S2). A more detailed description of the experimental setup and analysis can be found in electronic supplementary material.

Measuring Drosophila activity

The activity of single flies was measured during 4 continuous days using a Drosophila Activity Monitor (DAM2 System, TRIKinetics), in an incubator maintained at 25°C in a 12:12 light:dark cycle [15]. Over the course of the experiment, we measured the activity of 872 flies, with n=18-28 flies for each combination of sex and genetic background (Table S1). Raw activity data was processed using the DAM System File Scan Software [15], and the resulting data was manipulated using Microsoft Excel. We analysed fly activity data using three metrics: total locomotor activity, proportion of time spent asleep and the average activity when awake, as described previously [20]. A more detailed description of the experimental setup and analysis can be found in electronic supplementary material.

118

119 **Statistical Analysis**

120 We tested if differences in locomotor activity and social aggregation could be attributed
121 to fly genetic background or sex using Generalized Linear Models (GLMs). Models used a
122 full factorial 3-way interaction between infection status (control/infected), sex
123 (male/female) and DGRP line (10 lines), all modelled as fixed effects. Analysis of social
124 aggregation used a model listing only the median nearest neighbour distance of each dish
125 as its response variable. To assess locomotor activity, we analysed 3 response variables in
126 separate GLMs (total activity, proportion of time asleep, awake activity), adjusting the
127 significance threshold to 0.01667 using Bonferroni correction to account for multiple-
128 testing. All statistical analyses and graphics were carried out and produced in R 3.3.0 [31]
129 using the *ggplot2* [32] and *lme4* [33] packages.

Results

Social aggregation

We found a significant effect of genetic background on the median nearest neighbour distances (NND) (Figure 1; Table 1). We found no evidence of sexual dimorphism in social aggregation across multiple genetic backgrounds, with no significant interaction between sex and genetic background. However, we observed that while female aggregation was not affected by infection, infected males aggregated further apart from each other compared to uninfected males (Figure 1; Table 1). This increase in the NND following infection was generally observed in males, regardless their genetic background (Figure 1; Table 1). We also detected an expected sexual dimorphism in body size, as female *D. melanogaster* are typically larger than males (Figure S1, Table S3). Incorporating this size difference into measures of social aggregation, by measuring body lengths between individuals did not alter the results qualitatively (Figure S2, Table S4).

Locomotor activity

All three parameters of total locomotor activity, the proportion of time spent asleep and the average activity when awake, were affected by a combination of sex and genetic background (Figures 2 and S3; Table 2). However, there was no detectable difference in

how much infected and healthy flies moved or slept, and hence no evidence that infection impacted on any parameter of fly locomotor activity (Figures 2 and S3; Table 2).

Discussion

Identifying changes in host behaviour following infection is important to understand heterogeneity in disease transmission. Overall, our results indicate a significant sex difference in the effect of infection on social aggregation but no effect of infection on locomotor activity in either sex.

We observed that how closely flies aggregate with one another differs with their genetic background. The genetic variation we observed is similar to other studies that have measured nearest neighbour distance [10], as well as other aspects of *Drosophila* social behaviour, such as group size preference [9] and group composition [34]. Group composition is affected by the natural *foraging* gene polymorphism, where larvae are either sitters, which aggregate toward conspecifics at food sources or rovers, who are more prone to independent food searching behaviour. Larger groups of larvae on food patches are more likely to be comprised of sitters, as rovers leave food patches after overcrowding

166 [34]. Genetic components of social behaviour have also been identified in a number of
167 mammal species, including humans [35]. In a number of vole species, variation in oxytocin
168 [36] and arginine vasopressin [37] receptor density is associated with between-species
169 variation in pair-bonding and monogamy.
170
171 While aggregation between healthy males and females did not differ, once infected, males
172 moved further apart from one another, while female aggregation did not change. One
173 possible explanation for why males aggregate further apart following infection is a sex
174 difference in immunity and the costs of social aggregation [38]. Sexually dimorphic
175 immunity may be particularly relevant as male *D. melanogaster* exhibit a stereotyped suite
176 of aggressive behaviours [39–41]. While fighting can gain males access to valuable
177 resources, it often incurs substantial costs [42,43]. DCV infection could exacerbate the cost
178 of male aggregation, as resources would also need to be spent on fighting infection, which
179 could lead to males aggregating less. Despite females also fighting one another, this
180 aggression is generally less costly [44,45]. Females may therefore still be able to aggregate
181 relatively closely while fighting DCV infection.
182

183 Irrespective of the metric used, we found no measurable effect of DCV infection on
184 locomotor activity. Other work has shown decreases in *Drosophila* daily movement
185 following injection with DCV, where a marked reduction is seen after 4 days of infection
186 [21]. Reduced daily locomotor activity has also been observed in *Drosophila* after 3 days
187 of infection with the DNA virus Kalithea virus [46]. Injecting, rather than pricking, flies with
188 viral suspension, allows more precise control of infectious dosage, which could also
189 increase infection severity [47]. Another potential explanation is that we infected flies via
190 thoracic pricking, as opposed to abdominal injection which has been shown to reduce
191 resistance to infection in *Drosophila* [48]. The injury produced by thoracic pricking may
192 obscure subtler changes to activity produced by DCV infection. Orally infecting flies shows
193 a range of sex-specific behavioural symptoms, with sub-lethal doses reducing daily
194 locomotor activity in males after 3-6 days of infection [22]. Conversely, following oral
195 infection with larger doses of DCV, females, but not males, have been shown to sleep
196 more [20]. These studies suggest we may not have seen an effect of DCV infection on
197 activity, because infections were not severe enough to elicit behavioural symptoms.
198 Measuring the activity of flies later in infection might address these explanations, as this
199 will enable flies to heal from thoracic injury and accrue a greater viral burden.
200

201 We measured social aggregation in groups of individuals composed of the same genetic
202 background, sex and infection status in order to dissect their influence on social
203 aggregation. However, in more heterogenous wild populations these characteristics can
204 produce population structure that could affect contact between individuals. Individuals
205 with shared genotypes can be more likely to interact due to predispositions to traits such
206 as group size preference [34,49] and aggression [50]. Similarly, sexual interactions between
207 males and females, as well as fighting and other forms of sexual competition, further alter
208 contact networks within populations [51,52]. When present together, healthy hosts might
209 also be able to avoid infected conspecifics by detecting the pathogen or cues of its
210 pathology [53]. Future work aiming to characterise the influence of these sources of
211 variation on heterogeneity in contact rate should consider how they change with, and are
212 changed by, population structure.
213
214 The contrasting ways social aggregation and locomotor activity change following infection
215 highlight the complexity of sources determining between-individual variation in disease
216 transmission. This is complicated further by sex differences across and within these genetic
217 backgrounds. The change induced by DCV infection on social aggregation but not
218 locomotor activity also demonstrates the importance of considering multiple host

219 behaviours. Central to understanding the effect of this genetic and sex-specific variation
220 in social aggregation and locomotor activity on heterogeneity in disease transmission is
221 characterising their effect on contact rates. Additionally, future work should consider how
222 these traits interact with other key determinants of transmission, such as infectiousness
223 and infection duration, as these three components ultimately define disease transmission
224 in conjunction with one another.
225
226 **Acknowledgements**
227 We are grateful to Katy Monteith for general lab assistance, Angela Reid and Lucinda
228 Rowe for preparation of all fly media and the Regan, Obbard, Walling and Vale lab
229 members for constructive discussion of these ideas.
230
231 **Funding**
232 J.A.S.J. was supported by a NERC E3 DTP PhD studentship. P.F.V. was supported by a
233 Branco Weiss fellowship (https://brancoweissfellowship.org/) and a Chancellor's Fellowship
234 (School of Biological Sciences, University of Edinburgh).
235
236 Data Accessibility:

237 All data and R code can be accessed on Data Dryad:

238 https://datadryad.org/review?doi=doi:10.5061/dryad.9232648

239 DOI: https://doi.org/10.5061/dryad.9232648
240

241

242 Authors' contributions:

243 JSJ and PFV conceived and designed the study; JSJ carried out the experimental work,

244 acquired and analysed the and drafted the manuscript; JSJ and PFV wrote the

245 manuscript. JSJ approved the final version to be published. JSJ and PFV agree to be

246 accountable for all aspects of the work in ensuring that questions related to the accuracy

247 or integrity of any part of the work are appropriately investigated and resolved.

248

249

250

251

Figure 1 – Mean±SE median nearest neighbour distance (NND) in millimetres (mm). (a) Uninfected female-only arenas shown in blue, and infected female-only bars 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.

Figure 2 – Mean±SE (A) total locomotor activity, (B) proportion of time flies spent sleeping and (C) mean activity while flies were awake, during the first 96 hours following DCV infection. Across all panels, sex and infection status are represented by colour with uninfected females shown in blue, infected females in pale blue, uninfected males in red, and infected males in pink. The order of genetic backgrounds on the x-axis of each of panel follows the ascending order of female flies.

Tables

Response	Predictor	Df	F	p
Median NND	Genetic Background	9	5.0249	<0.0001***
	Sex	1	2.7870	0.13
	Infection	1	21.1301	<0.0001***
	Genetic Background × Sex	9	1.4112	0.19
	Genetic Background × Infection	9	0.9654	0.49
	Sex × Infection	1	19.6600	<0.0001***
	Genetic Background × Sex × Infection	9	1.6729	0.12

Table 1 - Model outputs for statistical tests performed on social aggregation, testing the causes of variation in sociality in males and females of 10 *D. melanogaster* genetic backgrounds. Significant predictors are marked with asterisks (p<0.05=*, p<0.01=** and p<0.001=***).

Response	Predictor	Df	F	p
Total Activity	Genetic Background	9	14.83	<0.0001***
	Sex	1	1.537	0.21
	Infection	1	0.117	0.73
	Genetic Background × Sex	9	3.0485	0.0013*
	Genetic Background × Infection	9	1.4125	0.18
	Sex × Infection	1	3.9707	0.047
	Genetic Background × Sex × Infection	9	1.9471	0.043
Proportion of Time Spent Asleep	Genetic Background	9	25.1759	<0.0001***
	Sex	1	77.9823	<0.0001***
	Infection	1	0.6939	0.41
	Genetic Background × Sex	9	3.444	<0.001**
	Genetic Background × Infection	9	0.8021	0.61
	Sex × Infection	1	0.7513	0.39
	Genetic Background × Sex × Infection	9	1.4612	0.16
Awake Activity	Genetic Background	9	8.1673	<0.0001***
	Sex	1	0.6641	0.54
	Infection	1	0.0008	0.86
	Genetic Background × Sex	9	5.2153	0.0013*
	Genetic Background × Infection	9	0.8716	0.58
	Sex × Infection	1	0.8430	0.44
	Genetic Background × Sex × Infection	9	1.2998	0.61

272

273 **Table 2** – Model outputs for statistical tests performed on host activity data, testing the
274 causes of variation in locomotor activity, sleep patterns and average awake activity in males
275 and females of 10 *D. melanogaster* genetic backgrounds. Significance thresholds are
276 corrected for multiple testing using Bonferroni correction, with significant predictors are
277 marked with asterisks ($p<0.01667$=*, $p<0.001$=** and $p<0.0001$=***).

References

1. Hart BL. 1988 Biological basis of the behavior of sick animals. *Neuroscience & Biobehavioral Reviews* **12**, 123–137. (doi:10.1016/S0149-7634(88)80004-6)

2. Poulin R. 1995 'Adaptive' changes in the behaviour of parasitized animals: a critical review. *Int. J. Parasitol.* **25**, 1371–1383.

3. Kazlauskas N, Klappenbach M, Depino AM, Locatelli FF. 2016 Sickness Behavior in Honey Bees. *Front Physiol* **7**. (doi:10.3389/fphys.2016.00261)

4. Hart BL. 2011 Behavioural defences in animals against pathogens and parasites: parallels with the pillars of medicine in humans. *Phil. Trans. R. Soc. B* **366**, 3406–3417. (doi:10.1098/rstb.2011.0092)

5. Lopes PC. 2014 When is it socially acceptable to feel sick? *Proceedings of the Royal Society of London B: Biological Sciences* **281**, 20140218. (doi:10.1098/rspb.2014.0218)

6. de Roode JC, Lefèvre T. 2012 Behavioral Immunity in Insects. *Insects* **3**, 789–820. (doi:10.3390/insects3030789)

7. Vale PF, Siva-Jothy J, Morrill A, Forbes M. 2018 The influence of parasites on insect behavior. In *Insect Behavior: from mechanisms to ecological and evolutionary consequences*, pp. 274–291. OUP Oxford.

8. Barron DG, Gervasi SS, Pruitt JN, Martin LB. 2015 Behavioral competence: How host behaviors can interact to influence parasite transmission risk. *Current Opinion in Behavioral Sciences* **6**, 35–40. (doi:10.1016/j.cobeha.2015.08.002)

9. Saltz JB. 2011 NATURAL GENETIC VARIATION IN SOCIAL ENVIRONMENT CHOICE: CONTEXT-DEPENDENT GENE-ENVIRONMENT CORRELATION IN DROSOPHILA MELANOGASTER. *Evolution* **65**, 2325–2334. (doi:10.1111/j.1558-5646.2011.01295.x)

10. Anderson BB, Scott A, Dukas R. 2016 Social behavior and activity are decoupled in larval and adult fruit flies. *Behavioral Ecology* **27**, 820–828. (doi:10.1093/beheco/arv225)

11. Manoli DS, Foss M, Villella A, Taylor BJ, Hall JC, Baker BS. 2005 Male-specific *fruitless* specifies the neural substrates of *Drosophila* courtship behaviour. *Nature* **436**, 395–400. (doi:10.1038/nature03859)

12. Walum H *et al.* 2008 Genetic variation in the vasopressin receptor 1a gene (AVPR1A) associates with pair-bonding behavior in humans. *PNAS* **105**, 14153–14156. (doi:10.1073/pnas.0803081105)

13. Dubnau J. 2014 *Behavioral Genetics of the Fly (Drosophila Melanogaster)*. Cambridge University Press.

14. Sokolowski MB. 2001 Drosophila: genetics meets behaviour. *Nature reviews. Genetics* **2**, 879–90. (doi:10.1038/35098592)

15. Pfeiffenberger C, Lear BC, Keegan KP, Allada R. 2010 Processing Sleep Data Created with the Drosophila Activity Monitoring (DAM) System. *Cold Spring Harb Protoc* **2010**, pdb.prot5520. (doi:10.1101/pdb.prot5520)

16. Simon AF, Chou M-T, Salazar ED, Nicholson T, Saini N, Metchev S, Krantz DE. 2012 A simple assay to study social behavior in Drosophila: measurement of social space within a group. *Genes, brain, and behavior* **11**, 243–52. (doi:10.1111/j.1601-183X.2011.00740.x)

17. Troha K, Buchon N. 2019 Methods for the study of innate immunity in Drosophila melanogaster. *Wiley Interdiscip Rev Dev Biol* , e344. (doi:10.1002/wdev.344)

18. Siva-Jothy JA, Monteith KM, Vale PF. 2018 Navigating infection risk during oviposition and cannibalistic foraging in a holometabolous insect. *Behav Ecol* (doi:10.1093/beheco/ary106)

19. Vale PF, Jardine MD. 2016 Viral exposure reduces the motivation to forage in female Drosophila melanogaster. *Fly* , 1–7. (doi:10.1080/19336934.2016.1207029)

20. Vale PF, Jardine MD. 2015 Sex-specific behavioural symptoms of viral gut infection and Wolbachia in Drosophila melanogaster. *Journal of Insect Physiology* **82**, 28–32. (doi:10.1016/J.JINSPHYS.2015.08.005)

21. Arnold PA, Johnson KN, White CR. 2013 Physiological and metabolic consequences of viral infection in Drosophila melanogaster. *The Journal of Experimental Biology* **216**, 3350–3357. (doi:10.1242/jeb.088138)

22. Gupta V, Stewart CO, Rund SSC, Monteith K, Vale PF. 2017 Costs and benefits of sublethal Drosophila C virus infection. *Journal of Evolutionary Biology* **30**, 1325–1335. (doi:10.1111/jeb.13096)

23. Mackay TFC *et al.* 2012 The Drosophila melanogaster Genetic Reference Panel. *Nature* **482**, 173–8. (doi:10.1038/nature10811)

24. Magwire MM, Fabian DK, Schweyen H, Cao C, Longdon B, Bayer F, Jiggins FM. 2012 Genome-Wide Association Studies Reveal a Simple Genetic Basis of Resistance to Naturally Coevolving Viruses in Drosophila melanogaster. *PLoS Genetics* **8**. (doi:10.1371/journal.pgen.1003057)

25. Jousset F-X, Bergoin M, Revet B. 1977 Characterization of the Drosophila C Virus. *Journal of General Virology* **34**, 269–283. (doi:10.1099/0022-1317-34-2-269)

26. Gupta V, Vale PF. 2017 Nonlinear disease tolerance curves reveal distinct components of host responses to viral infection. *R Soc Open Sci* **4**, 170342. (doi:10.1098/rsos.170342)

27. Dostert C, Jouanguy E, Irving P, Troxler L, Galiana-Arnoux D, Hetru C, Hoffmann JA, Imler J-L. 2005 The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of drosophila. *Nat Immunol* **6**, 946–953. (doi:10.1038/ni1237)

28. Rij RP van, Merkling SH. 2015 Analysis of resistance and tolerance to virus infection in *Drosophila*. *Nature Protocols* **10**, 1084. (doi:10.1038/nprot.2015.071)

353 29. Clark PJ, Evans FC. 1954 Distance to Nearest Neighbor as a Measure of Spatial
354 Relationships in Populations. *Ecology* **35**, 445–453. (doi:10.2307/1931034)

355 30. Schneider CA, Rasband WS, Eliceiri KW. 2012 NIH Image to ImageJ: 25 years of Image
356 Analysis. *Nat Methods* **9**, 671–675.

357 31. R Core Team. 2017 R: A language and environment for statistical computing. *R*
358 *Foundation for Statistical Computing, Vienna, Austria*

359 32. Wickham H. 2016 *ggplot2: Elegant Graphics for Data Analysis.* Springer-Verlag New
360 York.

361 33. Bates D, Mächler M, Bolker B, Walker S. 2015 Fitting Linear Mixed-Effects Models
362 Using **lme4**. *Journal of Statistical Software* **67**. (doi:10.18637/jss.v067.i01)

363 34. Philippe A-S, Jeanson R, Pasquaretta C, Rebaudo F, Sueur C, Mery F. 2016 Genetic
364 variation in aggregation behaviour and interacting phenotypes in Drosophila.
365 *Proceedings. Biological sciences* **283**, 20152967–20152967.
366 (doi:10.1098/rspb.2015.2967)

367 35. Anacker AMJ, Beery AK. 2013 Life in groups: the roles of oxytocin in mammalian
368 sociality. *Front Behav Neurosci* **7**. (doi:10.3389/fnbeh.2013.00185)

369 36. Ophir AG, Gessel A, Zheng D-J, Phelps SM. 2012 Oxytocin receptor density is
370 associated with male mating tactics and social monogamy. *Hormones and Behavior* **61**,
371 445–453. (doi:10.1016/j.yhbeh.2012.01.007)

372 37. Winslow JT, Hastings N, Carter CS, Harbaugh CR, Insel TR. 1993 A role for central
373 vasopressin in pair bonding in monogamous prairie voles. *Nature* **365**, 545.
374 (doi:10.1038/365545a0)

375 38. Kelly CD, Stoehr AM, Nunn C, Smyth KN, Prokop ZM. 2018 Sexual dimorphism in
376 immunity across animals: a meta-analysis. *Ecol. Lett.* **21**, 1885–1894.
377 (doi:10.1111/ele.13164)

378 39. Dierick H a, Greenspan RJ. 2006 Molecular analysis of flies selected for aggressive
379 behavior. *Nature genetics* **38**, 1023–31. (doi:10.1038/ng1864)

380 40. Dierick HA. 2007 A method for quantifying aggression in male Drosophila melanogaster.
381 *Nature protocols* **2**, 2712–8. (doi:10.1038/nprot.2007.404)

382 41. Baxter CM, Barnett R, Dukas R. 2015 Aggression, mate guarding and fitness in male
383 fruit flies. *Animal Behaviour* **109**, 235–241. (doi:10.1016/j.anbehav.2015.08.023)

384 42. Chen S, Lee AY, Bowens NM, Huber R, Kravitz EA. 2002 Fighting fruit flies : A model
385 system for the study of aggression. *Proceedings of the National Academy of Sciences of*
386 *the United States of America* **99**, 5664–5668.

387 43. Lim RS, Eyj??lfsd??ttir E, Shin E, Perona P, Anderson DJ. 2014 How food controls
388 aggression in Drosophila. *PLoS ONE* **9**. (doi:10.1371/journal.pone.0105626)

389 44. Ueda A, Kidokoro Y. 2002 Aggressive behaviours of female Drosophila melanogaster
390 are influenced by their social experience and food resources. *Physiological Entomology*
391 **27**, 21–28. (doi:10.1046/j.1365-3032.2002.00262.x)

392 45. Nilsen SP, Chan Y-B, Huber R, Kravitz EA. 2004 Gender-selective patterns of
393 aggressive behavior in Drosophila melanogaster. *PNAS* **101**, 12342–12347.
394 (doi:10.1073/pnas.0404693101)

395 46. Palmer WH, Medd NC, Beard PM, Obbard DJ. 2018 Isolation of a natural DNA virus of
396 Drosophila melanogaster, and characterisation of host resistance and immune responses.
397 *PLOS Pathogens* **14**, e1007050. (doi:10.1371/journal.ppat.1007050)

398 47. Neyen C, Bretscher AJ, Binggeli O, Lemaitre B. 2014 Methods to study Drosophila
399 immunity. *Methods (San Diego, Calif.)* **68**, 116–28. (doi:10.1016/j.ymeth.2014.02.023)

400 48. Chambers MC, Jacobson E, Khalil S, Lazzaro BP. 2014 Thorax Injury Lowers Resistance
401 to Infection in Drosophila melanogaster. *Infect Immun* **82**, 4380–4389.
402 (doi:10.1128/IAI.02415-14)

403 49. Brown CR, Brown MB. 2000 Heritable basis for choice of group size in a colonial bird.
404 *PNAS* **97**, 14825–14830. (doi:10.1073/pnas.97.26.14825)

405 50. Lea AJ, Blumstein DT, Wey TW, Martin JGA. 2010 Heritable victimization and the
406 benefits of agonistic relationships. *Proc Natl Acad Sci U S A* **107**, 21587–21592.
407 (doi:10.1073/pnas.1009882107)

408 51. Perkins SE, Ferrari MF, Hudson PJ. 2008 The effects of social structure and sex-biased
409 transmission on macroparasite infection. *Parasitology* **135**, 1561–1569.
410 (doi:10.1017/S0031182008000449)

411 52. Silk MJ, Weber NL, Steward LC, Hodgson DJ, Boots M, Croft DP, Delahay RJ,
412 McDonald RA. 2018 Contact networks structured by sex underpin sex-specific
413 epidemiology of infection. *Ecology Letters* **21**, 309–318. (doi:10.1111/ele.12898)

414 53. Curtis VA. 2014 Infection-avoidance behaviour in humans and other animals. *Trends in*
415 *Immunology* **35**, 457–464. (doi:10.1016/j.it.2014.08.006)

416