



# Peer Community In Evolutionary Biology

## Peer Community in Evolutionary Biology Editorial correspondance

Recommender : Ana Rivero

Reviewers : Ana Rivero and Silvie Huijben

<http://dx.doi.org/10.24072/pci.evolbiol.100030>

Dawson E, Bailly T, Dos Santos J, Moreno C, Devilliers M, Maroni B, Sueur C, Casali A, Ujvari B, Thomas F, Montagne J, Mery F. 2017. **An interaction between cancer progression and social environment in *Drosophila***. *BiorXiv*, 143560, ver. 3 of 19th September 2017. doi: [10.1101/143560](https://doi.org/10.1101/143560)

### Round #1

#### Decision round #1

Recommendation contingent on addressing the statistical issues raised by both referees

by Ana Rivero, 2017-07-19 12:01

Manuscript: <https://doi.org/10.1101/143560>

#### Decision & reviews

Dear Authors, This is a very interesting article that addresses an important issue on the correlation between cancer and social environment, using a relevant experimental system. However, both referees have raised issues with the statistical analyses: they lack clarity and at times they seem to be at odds with what it's written in the text. Would be happy to recommend provided the statistical analyses are clarified and all the individual points raised by the referees (statistical, or otherwise) addressed.

Best Ana

#### Reviewed by Ana Rivero round #1, 2017-07-10 23:56

Review Dawson et al The relationship between cancer progression and social environment in *Drosophila*

This is an original manuscript looking at the effects of sociality on non-infection disease progression and vice-versa. Please see below for a detail list of comments and suggestions which I hope will increase the clarity of the manuscript (because the lack of line numbers I found it easier to list them in order of appearance, rather than importance). The short format makes for a slightly frustrating read, as I found myself wanting to know much more about these tumours, and about the potential mechanistic and adaptive explanations behind these rather sophisticated behaviours (cancerous *Drosophila* flies that prefer to mix with other cancerous flies rather than healthy ones - wow). Other than that, and as you will see, my main comments relate to the statistical analyses, which I think could be clarified pretty much throughout.

#### Introduction

Page 5 (first para.), (1) Previous examples of the correlation between cancer progression and social isolation have been described in intrinsically social mammals such as humans and mice. I have a hard time thinking about *Drosophila* as a stereotypically social animal, perhaps a few lines about adult *Drosophila* social behaviour would help to better understand why this is a good model for addressing this issue. (2) To understand the adaptive nature of *Drosophila* faced with cancerous vs non cancerous flies I also feel the need to know more about these "intestinal-like cancers" (which is a peculiar way to call them – do you mean "intestinal cancer-like tumours?"). Do they occur naturally or are they a laboratory construct? What are their fitness effects on the flies (if any?). It seems surprising that these tumours have no effect on fly performance or longevity (page 24)–

so what do they do? And why would you expect *Drosophila* to have evolved adaptive strategies to deal with them?

#### Results

Page 5 (Biological model) (3) What is MARCM and what does it mean that “The flies contained [...] MARCM clones”?

Page 6 (first para.) (4) Substitute “bred in tubes” for “kept in tubes” (there was no breeding involved) (5) The last couple of sentences “After 21 days...” and “More surprisingly...” require a statistical test (in the form of a post-hoc contrast – you can do this by e.g. lumping together the alone and heterogeneous treatment and checking whether there’s a significant change in deviance in the model)

Page 6 (Social interactions para.) (6) A general comment I have throughout the paper is that there is very little information about the statistical analyses. It would help if the Methods had a Statistical Analysis section detailing how the analyses were made (what program? How were the models built? Were they simplified? that kind of thing). Here, for example, you have 3 different F and p values associated to the analyses in figures 2B. Are these 3 different statistical analyses (I hope not) or post-hoc contrasts after fitting a full mixed model (to account for the tube effect) with group composition and fly state (and their interaction) as fixed explanatory variables? This seems to be the appropriate way to do this analysis. (7) Same goes for analyses of data in 2C. (8) I’m also wondering how was the data handled, particularly in the heterogeneous group, where you have a higher replication for the control flies (n=7 per tube) than for the cancerous flies (n=1). Unless I’m mistaken, you do not seem to mention how many true replications (tubes) of each there are.

Page 7 (Social environment choice) (9) (second para.) I see how what you are saying in this paragraph fits well with what I see in Figure 3, however I got confused by the stats. For example, it looks like the target fly effect is only significant 7 days post induction, so I find it surprising that you have a significant target fly main effect but a not significant target fly\*age interaction. As I read on, I’m not even sure whether what is being tested is the difference between control and cancerous targets, or between observed vs expected (random) choice. Where does this second chi-square value for age come from? A bit more info about how was this analysis done would help understand.

(10) Figure 4 (there is no Figure 5) could be clearer: I would change X axis label to “Stimulus flies (days post-induction)” and add “Target flies” to the cancerous and control labels. I would also call these latter ones “empty” rather than control. (11) The exact same comments regarding the stats as for the dual experiment apply here.

#### Discussion

Page 8 (12) (second para.) I do not understand why, if social isolation is the sole issue, cancerous flies do better in the presence of other cancerous flies rather than in the presence of healthy flies. I find this result very interesting but also very perplexing. Any thoughts? (13) You also suggest (page 9) that the attraction of cancerous flies to other cancerous flies may be an adaptive strategy aimed at reducing cancer progression. To be convinced by these adaptive explanations, I would need to know more about the conditions under which these tumours originated (see comments on Intro).

#### Methods

Page 12 (last para) (14) The people that’ve named these *Drosophila* lines seem to have some serious psychological issues (!). Because of all the colons and semicolons it took me a while to understand this. You may want to cater for people like me by modifying the initial sentence like thus: “We used two different *Drosophila* lines: a cancerous line (yw, HS-flp....) and a control line (yw, HS-flp....) which were balanced over co-segregating...”. Please also explain what does this last part of the sentence mean (“balanced over...”). (15) “In all experiments flies were...” this seems to be a repeat of what has already been said (or else the subtlety was lost on me).

Page 13 (first para) (16) This would indeed have been the perfect control for your experiment, as otherwise the temperature treatment is confounded with the Apc-Ras mutation. To what extent could your results be simply explained by the heat shock instead of the tumour? A quick check of the literature shows that hsp’s can have multiple pleiotropic effects in the organism, including on fly behavior. This is not discussed in the manuscript, but I think it should. Would an alternative have been to have a heat-shocked control line in the behavioural experiments?

Page 13 (social breeding) (17) It may be a good idea to call this “social environment” as per the Results section (18) How many food tubes were there for each of the 3 treatments? (19) Was the “tube” effect taken into account in the model? i.e. is this a mixed model with treatment as a fixed factor and tube as a random one?

Page 14 (Social environment choice) (20) I had to read the experimental set up a few times to understand it. To avoid confusion between the different types of cages, it may be a good idea to refer to the outer cage as a “plastic box”.

## Supplementary Information

Page 24 (21) “and locomotor” ...activity? (22) How many groups of cancerous / control females were used for each of the 3 post-induction treatments?

(23) Please tell us what was used as the random variable (tube?) in the mixed model. (24) You seem to have three fixed parameters (instead of 2): fly state, time and age. Not sure what you mean by “age” though (is this the post-induction treatment? i.e. 7, 14 and 21 days? – if so it may be a good idea to keep terminology constant) (25) “no difference between cancerous or control flies could be observed at any age ( $p > 0.5$  for each age tested)” – how were these multiple tests done? Are these post-hoc contrasts? This sentence is misleading (as it sounds like you have done separate statistical tests for each age, which would not be a good idea). Stating that you do not have a significant interaction between fly state and age (if this indeed the case), would obviate the need to do either multiple tests or contrasts.

## Reviewed by Silvie Huijben round #1, 2017-07-13 13:02

This is an interesting study using the drosophila model system to study the effects of social interactions on cancer progression; a study which would be extremely difficult if not impossible to conduct in the human population. The experiments are well-thought-out, however, I do have doubts regarding some statistical analyses.

Major comments:

Statistical analysis:

- I am confused with the statistical analysis in the choice experiments. The authors show the results of a logistic regression in the text, where they ask the questions whether age, cancerous state/stimulus, or the interaction between them affects choice. They are thus comparing different groups in their choice, but they do not directly test for one group whether they are more attracted to a certain cage over the other (just differences between groups). This is more or less tested in individual tests for deviation of random choice (0.5) with asterisks in figures 3-4, however, the method of this testing is not described but seems to be performed for each individual point separately and p-values should thus be adjusted for multiple testing. Was this done? I suggest the authors to have the principle analysis be done on whether cancerous flies are attracted to a certain social group, a secondary analysis would be whether there are differences between cancerous vs control and age of the fly.

- P7, paragr3: “This was especially pronounced when flies were young i.e. at the very beginning of the tumor development”. However, the interaction between age and target fly is not significant so this is not a significant effect

- P7, paragr3: “However, at later ...  $P < 10^{-3}$ .” I believe this p-value must be based on the individual datapoint analysis that is not described. This becomes confusing because you first report a non-significant interaction (see point above), but here you don't talk about a difference between groups, but a difference from random choice. Please rewrite the results so these distinctions become clearer.

- P7, paragr4: “Cancerous flies showed ...  $P = 0.44$ ”, same point as above, you report the non-significant result but mention a significant effect. Report statistical analysis.

- P6, paragr1: “More surprisingly, we ... together (Fig. 1).”: report statistical results backing up this result.

In the concluding paragraph of the discussion there is a referral to the contribution of this study to the evolutionary ecology of cancer, it would be great if the authors could expand a few sentences on this. What are the evolutionary benefits? Could such behavior be adaptive for cancer or is it an unintended consequence of a non-specific infection avoiding behavior? The tumor cells in this study do not impact fitness, could this bias any conclusions drawn from this study?

Minor comments:

P4, paragr1: remove comma behind “it is therefore,”

P4, paragr1: social overcrowding has been found to induce psychiatric and metabolic disorders. How about communicable diseases? That would be important to include since this study specifically highlights non-communicable diseases as opposed to communicable diseases.

P4, paragr2: remove comma after “non-transmissible ones),”

P5, paragr3: rephrase first sentence: “biological model: ... progenitor cells”

P6, paragr2, social interactions (fig 2): Contact duration and number of contracts seem very correlated, is there an explanation for this? Is this correlated in individual flies as well? I trail length and number of contacts was

similar that would make sense (the more they move, the more likely they interact), but if contact duration is longer, it seems there would be less time for meeting other flies. Thus, this suggests a double effect? Would it be possible to see this extra strong effect by analyzing amount of time spent alone?

P7,paragr1: The small size of the arena did not allow the authors to disentangle the direction of social contact. Could the size of the arena thus also have affected the conclusions in this study?

P7,paragr4: Fig 5 = Fig 4

P8,paragr4 "Even if not... with being sick": if this is a general response, shouldn't cancerous flies also avoid other cancerous flies (which they don't)? If it is a general response, they may want to avoid flies with contagious infection despite themselves having cancerous cells. Please discuss.

P13, paragr3: Do the authors have data that wing-clipping (left or right) does not affect behavior?

Figure 1: adding asterisks showing statistical differences between groups would be helpful

Figure 3: remove "28" from x-axis

Figure 4: A header would be helpful. Interaction plotting such as figure 3 for consistency would also help the reader.

## Author's Reply round #1:

We are grateful for the productive and interesting comments made by both reviewers. Please find our responses and corrections below and in the manuscript.

Sincerely,

Fred Mery

This is an original manuscript looking at the effects of sociality on non-infection disease progression and vice-versa. Please see below for a detail list of comments and suggestions which I hope will increase the clarity of the manuscript (because the lack of line numbers I found it easier to list them in order of appearance, rather than importance). The short format makes for a slightly frustrating read, as I found myself wanting to know much more about these tumours, and about the potential mechanistic and adaptive explanations behind these rather sophisticated behaviours (cancerous *Drosophila* flies that prefer to mix with other cancerous flies rather than healthy ones - wow). Other than that, and as you will see, my main comments relate to the statistical analyses, which I think could be clarified pretty much throughout.

We apologize for the missing line numbers...they have now been added. More details are now provided concerning the nature of the tumours. We also provide more details concerning the stats as suggested by both reviewers

Introduction

Page 5 (first para.), (1) Previous examples of the correlation between cancer progression and social isolation have been described in intrinsically social mammals such as humans and mice. I have a hard time thinking about *Drosophila* as a stereotypically social animal, perhaps a few lines about adult *Drosophila* social behaviour would help to better understand why this is a good model for addressing this issue. (2) To understand the adaptive nature of *Drosophila* faced with cancerous vs non cancerous flies I also feel the need to know more about these "intestinal-like cancers" (which is a peculiar way to call them – do you mean "intestinal cancer-like tumours?").

Do they occur naturally or are they a laboratory construct? What are their fitness effects on the flies (if any?). It seems surprising that these tumours have no effect on fly performance or longevity (page 24)– so what do they do? And why would you expect *Drosophila* to have evolved adaptive strategies to deal with them?

1) While *Drosophila* is more traditionally considered a model organism from a genetic perspective, there is now a substantial number of studies and evidence that demonstrates the importance and prevalence of social interactions in *Drosophila* (Battesti et al, Sarin & Dukas, 2009, Mery et al 2009). We have now included a sentence in the introduction which elaborates on this point.

2) While we use mutant flies with induced tumours in our study, it has been shown that cancer occurs in natural populations of *Drosophila* making them a good model to investigate adaptive strategies against cancer. While these tumours are shown not to affect locomotor behaviour or survival of flies, cancer may affect other fitness traits. A recently published study showed that the presence of cancer affects the reproductive strategies of female flies. This has now been highlighted in the manuscript (intro and discussion).

Results Page 5 (Biological model) (3) What is MARCM and what does it mean that "The flies contained [...] MARCM clones"?

This has been corrected. We now provide further details about these clones

Page 6 (first para.) (4) Substitute “bred in tubes” for “kept in tubes” (there was no breeding involved) (5) The last couple of sentences “After 21 days...” and “More surprisingly...” require a statistical test (in the form of a post-hoc contrast – you can do this by e.g. lumping together the alone and heterogeneous treatment and checking whether there’s a significant change in deviance in the model)

Bred has been changed to kept now throughout the manuscript.

Figure 1 initially contained results of a Tukey’s post hoc classification (mentioned in the figure legend). They have now been added to the figure.

Page 6 (Social interactions para.) (6) A general comment I have throughout the paper is that there is very little information about the statistical analyses. It would help if the Methods had a Statistical Analysis section detailing how the analyses were made (what program? How were the models built? Were they simplified? that kind of thing). Here, for example, you have 3 different F and p values associated to the analyses in figures 2B. Are these 3 different statistical analyses (I hope not) or post-hoc contrasts after fitting a full mixed model (to account for the tube effect) with group composition and fly state (and their interaction) as fixed explanatory variables? This seems to be the appropriate way to do this analysis. (7) Same goes for analyses of data in 2C. (8) I’m also wondering how was the data handled, particularly in the heterogeneous group, where you have a higher replication for the control flies (n=7 per tube) than for the cancerous flies (n=1). Unless I’m mistaken, you do not seem to mention how many true replications (tubes) of each there are.

We have now expanded and explained in more detail how we performed the statistical analyses. In particular in the previous version we forgot to mention that, to avoid the problem of unequal sample size, before analysis, that we averaged the values measured to obtain, for each group, one value for cancerous state and/or control state

Page 7 (Social environment choice) (9) (second para.) I see how what you are saying in this paragraph fits well with what I see in Figure 3, however I got confused by the stats. For example, it looks like the target fly effect is only significant 7 days post induction, so I find it surprising that you have a significant target fly main effect but a not significant target fly\*age interaction.

As I read on, I’m not even sure whether what is being tested is the difference between control and cancerous targets, or between observed vs expected (random) choice. Where does this second chi-square value for age come from? A bit more info about how was this analysis done would help understand.

We now provide more information on the way the analyses were done. We apologize but found an error in the statistical analysis of the dual choice (age is in fact strongly significant, no change in the results of the other factors). We observed a general decrease in preference for the cancerous stimulus group, but we could detect such decrease only in cancerous target flies. This may explain the lack of significant interaction target fly\*age.

(10) Figure 4 (there is no Figure 5) could be clearer: I would change X axis label to “Stimulusflies (days post-induction)” and add “Target flies” to the cancerous and control labels. I would also call these latter ones “empty” rather than control. (11) The exact same comments regarding the stats as for the dual experiment apply here.

Figure 5 has been changed to figure 4. Figure 3 & 4 have now been changed.

Discussion

Page 8 (12) (second para.) I do not understand why, if social isolation is the sole issue, cancerous flies do better in the presence of other cancerous flies rather than in the presence of healthy flies. I find this result very interesting but also very perplexing. Any thoughts? (13) You also suggest (page 9) that the attraction of cancerous flies to other cancerous flies may be an adaptive strategy aimed at reducing cancer progression. To be convinced by these adaptive explanations, I would need to know more about the conditions under which these tumours originated (see comments on Intro).

The point we tried to make is that cancerous flies get more social contact with other cancerous individuals than with healthy ones. Healthy (non-cancerous) flies avoid cancerous flies which may lead to a perceived social isolation in cancerous flies, whereas cancerous flies significantly choose to be close to each other leading to more social contact.

We have added explanation about tumour induction in the introduction and in the M&Ms.

Methods

Page 12 (last para) (14) The people that’ve named these Drosophila lines seem to have some serious psychological issues (!). Because of all the colons and semicolons it took me a while to understand this. You may want to cater for people like me by modifying the initial sentence like thus: “We used two different Drosophila lines: a cancerous line (yw, HS-flp....) and a control line (yw, HS-flp....) which were balanced over co-segregating....”. Please also explain what does this last part of the sentence mean (“balanced over...”). (15) “In all experiments flies were...” this seems to be a repeat of what has already been said (or else the subtlety was lost on me).

We apologize for the genetic jargon, and we fully understand the difficulty for a non-Drosophila researcher to follow. The point is that we have to respect the genetic code so that Drosophila geneticists know the exact genetics of the lines used. There are no cancerous and noncancerous line. Cancerous and control flies are offspring from crosses of stable lines. Because of homozygous lethality the parental lines are stabilized using the so-called balancer chromosomes; due to length limitation, we cannot describe all these genetic tools. However to make it clearer for none- Drosophila people we have added references and explanation of the clonal strategy.

Page 13 (first para) (16) This would indeed have been the perfect control for your experiment, as otherwise the temperature treatment is confounded with the Apc-Ras mutation. To what extent could your results be simply explained by the heat shock instead of the tumour? A quick check of the literature shows that hsp's can have multiple pleiotropic effects in the organism, including on fly behavior. This is not discussed in the manuscript, but I think it should. Would an alternative have been to have a heat-shocked control line in the behavioural experiments?

We agree that heat shock can have multiple pleiotropic effects. However, in our case it is a consequence of leaky expression of the HS promoter that may direct very low levels of flipase, potentially inducing clones at low but unpredictable frequency. Although, we have observed a low rate of none heat shocked flies exhibiting tumors of rather smaller size, it was obvious for us that these flies might strongly interfere and bias the results, when analysing the effect of social interactions. This is why in all experiments control and cancerous flies were heat shock at 3 days post emergence. We tried to make this clear.

Page 13 (social breeding) (17) It may be a good idea to call this "social environment" as per the Results section  
This has been changed

(18) How many food tubes were there for each of the 3 treatments?

This has been added

(19) Was the "tube" effect taken into account in the model? i.e. is this a mixed model with treatment as a fixed factor and tube as a random one?

Because in the heterogeneous environment only one cancerous fly was present, we grouped fly guts randomly from different tubes (this is now mentioned in the manuscript). Thus tube was not a random factor and only treatment was included as a fixed factor.

Page 14 (Social environment choice) (20) I had to read the experimental set up a few times to understand it. To avoid confusion between the different types of cages, it may be a good idea to refer to the outer cage as a "plastic box".

This has been corrected

Supplementary Information

Page 24 (21) "and locomotor" ...activity?

This has been corrected

(22) How many groups of cancerous / control females were used for each of the 3 post-induction treatments?

This is now mentioned in the figure legend

(23) Please tell us what was used as the random variable (tube?) in the mixed model.

This is now clarified, tube is our level of replication

(24) You seem to have three fixed parameters (instead of 2): fly state, time and age. Not sure what you mean by "age" though (is this the post-induction treatment? i.e. 7, 14 and 21 days? – if so it may be a good idea to keep terminology constant)

Age is days post induction. This is now clarified

(25) "no difference between cancerous or control flies could be observed at any age ( $p > 0.5$  for each age tested)" – how were these multiple tests done? Are these post-hoc contrasts? This sentence is misleading (as it sounds like you have done separate statistical tests for each age, which would not be a good idea). Stating that you do not have a significant interaction between fly state and age (if this indeed the case), would obviate the need to do either multiple tests or contrasts.

We agree that presenting the full model (which include age and state) is a better option. it is now presented this way.

Reviewer 2:

This is an interesting study using the drosophila model system to study the effects of social interactions on cancer progression; a study which would be extremely difficult if not impossible to conduct in the human population. The experiments are well-thought-out, however, I do have doubts regarding some statistical analyses.

Major comments:

Statistical analysis:

We now provide more analyses (especially concerning treatment comparisons) and present a more detailed explanation of how we performed our statistical analyses.

- I am confused with the statistical analysis in the choice experiments. The authors show the results of a logistic regression in the text, where they ask the questions whether age, cancerous state/stimulus, or the interaction between them affects choice. They are thus comparing different groups in their choice, but they do not directly test for one group whether they are more attracted to a certain cage over the other (just differences between groups). This is more or less tested in individual tests for deviation of random choice (0.5) with asterisks in figures 3-4, however, the method of this testing is not described but seems to be performed for each individual point separately and p-values should thus be adjusted for multiple testing. Was this done? I suggest the authors to have the principle analysis be done on whether cancerous flies are attracted to a certain social group, a secondary analysis would be whether there are differences between cancerous vs control and age of the fly.

Stars on figure 3 and 4 represent significant deviation from random choice calculated for each line and age. We disagree with the necessity of doing the adjustment for multiple testing as we are not comparing the treatments among them in this analysis (compared to figure 1 and 2). We believe that this representation allows the reader to see at which age and state there is a significant effect and can conclude that there is for example aversion of the cancerous flies by the control ones when tumours are well developed. We clarified each analysis done and detail the statistics.

- P7, paragr3: "This was especially pronounced when flies were young i.e. at the very beginning of the tumor development". However, the interaction between age and target fly is not significant so this is not a significant effect

We made this clear that here we were not comparing cancerous vs control

- P7, paragr3: "However, at later ...  $P < 10^{-3}$ ." I believe this p-value must be based on the individual datapoint analysis that is not described. This becomes confusing because you first report a non-significant interaction (see point above), but here you don't talk about a difference between groups, but a difference from random choice. Please rewrite the results so these distinctions become clearer.

We hope this is now clarified

- P7, paragr4: "Cancerous flies showed ...  $P = 0.44$ ", same point as above, you report the nonsignificant result but mention a significant effect. Report statistical analysis.

We apologize, the significance of attraction towards the social stimulus could only be understood by mentioning the p value of the intercept. It has been added.

- P6, paragr1: "More surprisingly, we ... together (Fig. 1).": report statistical results backing up this result.

This is now clearly shown in figure 1 with the post-hoc analysis

In the concluding paragraph of the discussion there is a referral to the contribution of this study to the evolutionary ecology of cancer, it would be great if the authors could expand a few sentences on this. What are the evolutionary benefits? Could such behavior be adaptive for cancer or is it an unintended consequence of a non-specific infection avoiding behavior? The tumor cells in this study do not impact fitness, could this bias any conclusions drawn from this study?

We modified the discussion accordingly to this comment

Minor comments:

P4, paragr1: remove comma behind "it is therefore," P4, paragr1: social overcrowding has been found to induce psychiatric and metabolic disorders. How about communicable diseases? That would be important to include since this study specifically highlights non-communicable diseases as opposed to communicable diseases.

Correction done. We had initially the feeling that entering into a 'transmissible' vs 'non transmissible' discussion would be out of the scope of the manuscript and preferred not mentioning it too much

P4, paragr2: remove comma after "non-transmissible ones),"

This has been corrected

P5, paragr3: rephrase first sentence: "biological model: ... progenitor cells"

This has been corrected

P6, paragr2, social interactions (fig 2): Contact duration and number of contracts seem very correlated, is there an explanation for this? Is this correlated in individual flies as well? I trail length and number of contacts was similar that would make sense (the more they move, the more likely they interact), but if contact duration is longer, it seems there would be less time for meeting other flies. Thus, this suggests a double effect? Would it be possible to see this extra strong effect by analyzing amount of time spent alone?

This correlation between number of contact and contact duration suggest that flies in an homogeneous group of cancerous flies are more aggregated than flies of an heterogeneous group or a group of control individuals.

We clarified this in the manuscript

P7,paragr1: The small size of the arena did not allow the authors to disentangle the direction of social contact. Could the size of the arena thus also have affected the conclusions in this study?

We agree that the size of the arena might be a constrained to disentangle the direction of social contact. This is mainly a technical and logistic constrain. We do not really see however how it could have bias the conclusion of the study (for breeding the flies were kept in even smaller tube). We found clear variation in contact duration and number depending on group composition.

P7,paragr4: Fig 5 = Fig 4

This has been corrected

P8,paragr4 “Even if not... with being sick”: if this is a general response, shouldn’t cancerous flies also avoid other cancerous flies (which they don’t)? If it is a general response, they may want to avoid flies with contagious infection despite themselves having cancerous cells. Please discuss.

Even if we have not observed clear ‘avoidance’ of the cancerous flies by other cancerous ones we still see a decrease in preference. We believe that there could be a balance between avoidance of the potentially contagious individual and attraction of individual of the same type. This balance may vary with cancer progression.

P13, paragr3: Do the authors have data that wing-clipping (left or right) does not affect behavior?

In previous studies we showed that wing clipping does not affect the interactive behaviour of the flies (Battesti et al 2015). Note that in the present study all flies are wing clipped (left or right) which should also eliminate any potential variation in wing clipping effect

Figure 1: adding asterisks showing statistical differences between groups would be helpful

In the previous version we forgot to present the results of a post-hoc classification. This has now been corrected

Figure 3: remove “28” from x-axis

This has been corrected

Figure 4: A header would be helpful. Interaction plotting such as figure 3 for consistency would also help the reader.

This has been corrected

## Round #2

### Decision and reviews of round #2

Further clarifications needed

by Ana Rivero, 2017-09-01 13:13

Manuscript: <https://doi.org/10.1101/143560>

Decision & reviews

Dear authors,

Thank you for the replies to our comments and suggestions. There are still some issues that the reviewers feel are important to improve the quality of the paper and that were not fully addressed in your earlier replies.

If you choose to answer to these points, and to facilitate our work, I would ask you to please indicate in your replies where in the manuscript the changes have been made (provide line numbers)

#### REVIEWER 1

Results

Page 7 (Social environment choice)

(9) (second para.) I see how what you are saying in this paragraph fits well with what I see in Figure 3, however I got confused by the stats. For example, it looks like the target fly effect is only significant 7 days post induction, so I find it surprising that you have a significant target fly main effect but a not significant target fly\*age interaction. As I read on, I’m not even sure whether what is being tested is the difference between control and cancerous targets, or between observed vs expected (random) choice. Where does this second chi-square value for age come from? A bit more info about how was this analysis done would help understand.

AUTHOR’S REPLY

We now provide more information on the way the analyses were done. We apologize but found an error in the



statistical analysis of the dual choice (age is in fact strongly significant, no change in the results of the other factors). We observed a general decrease in preference for the cancerous stimulus group, but we could detect such decrease only in cancerous target flies. This may explain the lack of significant interaction target fly\*age.

**RECOMMENDER REPLY #1 The analyses of the choice experiments still lack clarity. These issues have also been raised by REVIEWER 2, please see below.**

**(19) Was the “tube” effect taken into account in the model? i.e. is this a mixed model with treatment as a fixed factor and tube as a random one?**

AUTHOR’S REPLY

Because in the heterogeneous environment only one cancerous fly was present, we grouped fly guts randomly from different tubes (this is now mentioned in the manuscript). Thus tube was not a random factor and only treatment was included as a fixed factor.

**RECOMMENDER’S REPLY #2**

**Does this mean that for the “alone” and the “heterogeneous” treatments the % of cancerous flies is given for an individual cancerous fly and that for the “homogeneous” treatment it is the mean for the 8 cancerous flies in the tube? But then what does it mean that you “grouped fly guts randomly from different tubes”? (and where in the manuscript is this point clarified? Please provide line numbers).**

REVIEWER 2

Major comments

- I am confused with the statistical analysis in the choice experiments. The authors show the results of a logistic regression in the text, where they ask the questions whether age, cancerous state/stimulus, or the interaction between them affects choice. They are thus comparing different groups in their choice, but they do not directly test for one group whether they are more attracted to a certain cage over the other (just differences between groups). This is more or less tested in individual tests for deviation of random choice (0.5) with asterisks in figures 3-4, however, the method of this testing is not described but seems to be performed for each individual point separately and p-values should thus be adjusted for multiple testing. Was this done? I suggest the authors to have the principle analysis be done on whether cancerous flies are attracted to a certain social group, a secondary analysis would be whether there are differences between cancerous vs control and age of the fly.

AUTHOR’S REPLY

Stars on figure 3 and 4 represent significant deviation from random choice calculated for each line and age. We disagree with the necessity of doing the adjustment for multiple testing as we are not comparing the treatments among them in this analysis (compared to figure 1 and 2).

We believe that this representation allows the reader to see at which age and state there is a significant effect and can conclude that there is for example aversion of the cancerous flies by the control ones when tumours are well developed. We clarified each analysis done and detail the statistics.

**RECOMMENDER REPLY #3**

**3.1. We agree that this is a useful way of looking at the data, but the question asked by reviewer to was how was the significant deviation from random choice tested. Please state what statistical test was used.**

**3.2. Contrary to what the authors state, the adjustment for multiple testing is required irrespective of whether you’re comparing treatments with each other or, as is the case here, comparing observed vs expected (random) 6 different times (one for each day x fly state combination). You could easily do a (back of the envelope) Bonferroni correction by dividing your critical alpha (0.05) by the number of tests (0.05/6 = 0.008). Thus, you would then only consider tests with  $p < 0.008$  (or to round it up  $p < 0.01$ ) to be significant.**

- P7, paragr3: “This was especially pronounced when flies were young i.e. at the very beginning of the tumor development”. However, the interaction between age and target fly is not significant so this is not a significant effect.

AUTHOR’S REPLY We made this clear that here we were not comparing cancerous vs control

**RECOMMENDER REPLY #4**

**Not that clear, actually. The analyses in Lines 178-179 seem to be comparing control vs cancerous (this is the “target fly” effect, right?). As stated by the reviewer, since the interaction age x target fly is not significant, stating that “this was especially pronounced when flies were young” is, statistically speaking, incorrect (even if there seems to be a trend in that direction).**

- P7, paragr3: “However, at later ...  $P < 10^{-3}$ .” I believe this p-value must be based on the individual datapoint analysis that is not described. This becomes confusing because you first report a non-significant interaction (see point above), but here you don’t talk about a difference between groups, but a

difference from random choice. Please rewrite the results so these distinctions become clearer.

AUTHOR'S REPLY

We hope this is now clarified

**RECOMMENDER REPLY #5**

**Not really. Please clarify what analyses have been done (i.e. what is it exactly that you're testing?) in lines 180-181 and lines 183-184 and how they differ from the analysis reported in lines 178-179. I'm guessing 178-179 is cancerous vs target, while the other analyses test for a departure from randomness for cancerous (180 181) and control (183-184) flies. If so, this is far from clear from the text. Please see also #6 regarding clarifying the significance of the intercept.**

- P7, paragr4: "Cancerous flies showed ...  $P=0.44$ ", same point as above, you report the nonsignificant result but mention a significant effect. Report statistical analysis.

AUTHOR'S REPLY

We apologize, the significance of attraction towards the social stimulus could only be understood by mentioning the p value of the intercept. It has been added.

**RECOMMENDER REPLY #6**

**6.1 Thanks for the clarification. As an intercept can be interpreted in many different ways depending on the type of model, the fact that in a logistic regression a significant intercept indicates a departure from randomness (which may or may not be explained by the explanatory variables) needs to be clearly stated here (Line 454-455 is lost in the depths of the m&m and does not suffice). If possible, please provide a reference.**

**6.2 I take it the individual departure from each of the treatment combinations (indicated by the stars in Figure 4) was done separately? If so please state how (as per #3.1 and #3.2).**

**6.3 Why are there no stats comparing Cancerous vs Control target flies?**

- In the concluding paragraph of the discussion there is a referral to the contribution of this study to the evolutionary ecology of cancer, it would be great if the authors could expand a few sentences on this. What are the evolutionary benefits? Could such behavior be adaptive for cancer or is it an unintended consequence of a non-specific infection avoiding behavior? The tumor cells in this study do not impact fitness, could this bias any conclusions drawn from this study?

AUTHOR'S REPLY We modified the discussion accordingly to this comment

**RECOMMENDER REPLY #7**

**I have failed to see any substantial change to the discussion. Could you please be more precise as to how and where (line numbers) these changes have been made?**

Minor comments:

- P8, paragr4 "Even if not... with being sick": if this is a general response, shouldn't cancerous flies also avoid other cancerous flies (which they don't)? If it is a general response, they may want to avoid flies with contagious infection despite themselves having cancerous cells. Please discuss.

AUTHOR'S REPLY

Even if we have not observed clear 'avoidance' of the cancerous flies by other cancerous ones we still see a decrease in preference. We believe that there could be a balance between avoidance of the potentially contagious individual and attraction of individual of the same type. This balance may vary with cancer progression.

**RECOMMENDER REPLY #8**

**Please clarify whether this clarification has been included in the manuscript and if so where.**

Author's Reply round #2:

Review Dawson et al. The relationship between cancer progression and social environment in *Drosophila*  
We are grateful for the productive and interesting comments made by the reviewers. We believe that we have now addressed all the points and we apologize for the lack of clarity. Please find our responses and corrections below and in the manuscript.

Sincerely,

On behalf of all co-authors, Frederic Mery

Minor general correction: For simplicity and clarity we have now replaced "target" with "focal".

Statistical clarification: The reviewers were mainly concerned about the lack of clarity in the statistical analyses (especially in the choice experiment) and the possible wrong interpretation of some results. We have now clarified these points, as detailed below:

- How was the significant deviation from random choice tested? Please state what statistical test was used.

Deviation from random choice was tested using a binomial test (comparing the number of events a focal fly was observed on a specific cage (stimulus cancerous flies for the dual experiment, social cage for the aversion/attraction experiment) and the total number of landings on a cage). This is mentioned in the figure legends and L466-468.

-Does this mean that for the "alone" and the "heterogeneous" treatments the % of cancerous flies is given for an individual cancerous fly and that for the "homogeneous" treatment it is the mean for the 8 cancerous flies in the tube? But then what does it mean that you "grouped fly guts randomly from different tubes"? (and where in the manuscript is this point clarified? Please provide line numbers).

In order to do the measure of GFP-positive cells, for each replicate we took a total of 5 guts, but each gut originated from a fly from a separate tube of the same treatment. So for example, 1 replicate of the homogenous treatment consisted of 5 guts of cancerous flies, each of these 5 flies randomly taken from 5 different homogeneous tubes. This procedure was carried out for ALL treatments i.e. the 'heterogeneous' and 'alone' groups. This is written in lines 413-416.

-Contrary to what the authors state, the adjustment for multiple testing is required irrespective of whether you're comparing treatments with each other or, as is the case here, comparing observed vs expected (random) 6 different times (one for each day x fly state combination). You could easily do a (back of the envelope) Bonferroni correction by dividing your critical alpha (0.05) by the number of tests ( $0.05/6 = 0.008$ ). Thus, you would then only consider tests with  $p < 0.008$  (or to round it up  $p < 0.01$ ) to be significant.

We disagree with the need to do a Bonferroni correction. Using a general linear model and a binary logistic regression we first show that there is a trend for decreased preference towards cancerous flies.

We then did a binomial test for each measure independently and show a deviation from random choice for multiple points. This follows classic statistical analysis and we provide robust P values. The following papers state in more detail why corrections for multiple comparisons are not required when there are multiple significant data points and clear trends in the data.

- M. D. Moran 2003 "Arguments for Rejecting the Sequential Bonferroni in Ecological Studies" *Oikos* Vol. 100, No. 2
- R.J. Cabin and R.J. Mitchell 2000 "To Bonferroni or Not to Bonferroni: When and How Are the Questions" *Bulletin of the Ecological Society of America* Vol. 81, No. 3
- S. Nakagawa 2004 "A farewell to Bonferroni: the problems of low statistical power and publication bias" *Behavioral Ecology*, Vol. 15, Issue 6

- "This was especially pronounced when flies were young i.e. at the very beginning of the tumor development". However, the interaction between age and target fly is not significant so this is not a significant effect "However, at later ...  $P < 10^{-3}$ ." I believe this p-value must be based on the individual datapoint analysis that is not described. This becomes confusing because you first report a non-significant interaction (see point above), but here you don't talk about a difference between groups, but a difference from random choice. Please rewrite the results so these distinctions become clearer.

We agree that this was unclear and potentially misleading. We have now removed the interpretation of the data and present only the general analysis which show a decreased preference for cancerous flies with age. We also provide more explanation on the way stats were done L183-194.

-As an intercept can be interpreted in many different ways depending on the type of model, the fact that in a logistic regression a significant intercept indicates a departure from randomness (which may or may not be explained by the explanatory variables) needs to be clearly stated here (Line 454-455 is lost in the depths of the m&m and does not suffice). If possible, please provide a reference.

To make things clear we have modified figure 4 and believe that the effect is much more visually shown (i.e. stats are now presented in a way which fits with the graph. This was not the case before).

I take it the individual departure from each of the treatment combinations (indicated by the stars in Figure 4) was done separately? If so please state how (as per #3.1 and #3.2)

6.3 Why are there no stats comparing Cancerous vs Control target flies?

The stars on figure 3 and 4 are the results of a binomial test done for each state and age (12 or 16 replicates per measure). This is stated in the figure legend and the material and methods. Again, no multiple comparison corrections were done as these tests are also combined with a generalized linear model showing clearly trends in the data (see references and point above).

Concerning the comparison Cancerous vs control, the experiment includes 2 focal types x 2 stimulus type x 2 ages which would make any interpretation of the data (3 way interactions) complex. This is why we split the analyses in two as shown now on figure 4.

In the concluding paragraph of the discussion there is a referral to the contribution of this study to the evolutionary ecology of cancer, it would be great if the authors could expand a few sentences on this.

Could such behavior be adaptive for cancer or is it an unintended consequence of a non-specific infection avoiding behavior? The tumor cells in this study do not impact fitness, could this bias any conclusions drawn from this study?

What are the evolutionary benefits?

L210-215 now states that potentially there is an adaptive advantage for the cancer but that the avoidance of cancerous flies could also be the manifestation of adaptive behaviour on the part of healthy individuals (i.e. infection avoiding behaviour).

The last paragraph goes into detail about the potential adaptive benefits of cancerous individuals seeking out an optimal social environment. Lines 228-231 address the point of fitness which the reviewer raised.

Lines 231-233 state that the behaviour of the cancerous flies could be seen as adaptive.

P8, paragr4 "Even if not... with being sick": if this is a general response, shouldn't cancerous flies also avoid other cancerous flies (which they don't)? If it is a general response, they may want to avoid flies with contagious infection despite themselves having cancerous cells. Please discuss.

This is now discussed L216-220.

Sincerely

Frederic Mery