RESEARCH ARTICLE

Sub-strains of *Drosophila* Canton-S differ markedly in their locomotor behavior [v1; ref status: indexed, http://f1000r.es/3is]

Julien Colomb[1], Björn Brembs[2]

[1]Institute of Biology – Neurobiology, Freie Universität, Berlin, Germany
[2]Institute of Zoology – Neurogenetics, Universität Regensburg, Regensburg, Germany

v1 **First published:** 30 Jul 2014, **3**:176 (doi: 10.12688/f1000research.4263.1)
Latest published: 30 Jul 2014, **3**:176 (doi: 10.12688/f1000research.4263.1)

Abstract

We collected five sub-strains of the standard laboratory wild-type *Drosophila melanogaster* Canton Special (CS) and analyzed their walking behavior in Buridan's paradigm using the CeTrAn software. According to twelve different aspects of their behavior, the sub-strains fit into three groups. The group separation appeared not to be correlated with the origin of the stocks. We conclude that founder effects but not laboratory selection likely influenced the gene pool of the sub-strains. The flies' stripe fixation was the parameter that varied most. Our results suggest that differences in the genome of laboratory stocks can render comparisons between nominally identical wild-type stocks meaningless. A single source for control strains may settle this problem.

Open Peer Review

Referee Status: ✓ ✓ ✓

	Invited Referees 1	2	3
version 1 published 30 Jul 2014	✓ report	✓ report	✓ report

1 **Gregg Roman**, University of Houston USA

2 **Josh Dubnau**, Cold Spring Harbor Laboratory USA

3 **Hiromu Tanimoto**, Tohoku University Japan, **Vladimiros Thoma**, Tohoku University Japan

Discuss this article

Comments (0)

Corresponding author: Björn Brembs (bjoern@brembs.net)

How to cite this article: Colomb J and Brembs B. **Sub-strains of *Drosophila* Canton-S differ markedly in their locomotor behavior [v1; ref status: indexed, http://f1000r.es/3is]** *F1000Research* 2014, **3**:176 (doi: 10.12688/f1000research.4263.1)

Copyright: © 2014 Colomb J and Brembs B. This is an open access article distributed under the terms of the Creative Commons Attribution Licence, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

Grant information: The author(s) declared that no grants were involved in supporting this work.

Competing interests: No competing interests were disclosed.

First published: 30 Jul 2014, **3**:176 (doi: 10.12688/f1000research.4263.1)
First indexed: 15 Aug 2014, **3**:176 (doi: 10.12688/f1000research.4263.1)

Introduction

In our quest for understanding gene function, we commonly manipulate gene expression and compare the phenotypes of the manipulated versus control organisms. For technical reasons and to facilitate comparison as well as reproducibility between different experiments, a limited number of control strains have been established in most model organisms. For instance, the C57BL, 129 and FVB strains are commonly used in mouse studies; N2 is the common control strain used in *Caenorhabditis elegans*; and Canton-Special (CS) is one of the most-used wild-type strains in *Drosophila melanogaster* genetics studies. The CS stock was established by C. B. Bridges[1] and chosen because of its low mutation rate. S. Benzer introduced CS to what was to become neurogenetics in his landmark study in 1967[2], because of its strong fast-phototaxis response. The strain has been used as a control in neurogenetics studies ever since.

With time and reproductive isolation, populations of laboratory control strains can diverge, in spite of ideal breeding conditions and seemingly little selective pressure. Several studies comparing the behavior of sub-strains of mice showed that their behavior differs[3]. For instance, Paylor and colleagues measured that one sub-strain of C57 mice showed a higher startle amplitude after tactile stimulation than another[4]. Similarly, the behavior of different N2 *C. elegans* sub-strains was found to vary to a considerable extent[5].

In this study, we tested five different CS *Drosophila melanogaster* sub-strains in Buridan's paradigm[6–8], where flies walk between two stripes on a platform surrounded by a water moat. We could separate the CS sub-strains into three groups according to their behavior during the experiment. In addition, we found that the between-strain variability in the stripe fixation score is particularly high. We discuss possible solutions to prevent sub-strain related problems.

Materials and methods

Fly care

Flies were kept in vials (68 ml, Art.-Nr. 217101, Greiner Bio-One GmbH, Maybachstr.2, 72636 Frickenhausen) in a controlled density[9] on standard cornmeal/molasses medium[10] at 25°C in a 12 h:12 h dark/light cycle for one generation before being tested. Flies were collected 0–1 day after hatching and put in new food vials for one day. Approximately ten female flies (N=11-12 in each group) were then CO_2-anaesthetized and their wings were cut with surgical scissors at two thirds of their length, before being taken back to their vial to recover overnight. They were then captured individually using a fly aspirator and put in the experimental setup to be tested.

Fly strains

Five sub-strains of CS wild-type *Drosophila melanogaster* were collected in the lab from 2008 to 2011. Troy Zars took his CS_TZ stock to Columbia, MO, USA in 2002 when leaving Martin Heisenberg's lab in Würzburg, Germany. It arrived in our laboratory in Berlin in 2008. The CS_TP stock was separated from Tim Tully's strain in Waltham, MA, USA in 1992 and moved to Paris, France. The CS_JC stock was derived from the CS_TP stock in 2007 when one of the authors (JC) was in Thomas Préat's lab. Both strains (TP and JC) arrived in Berlin in 2009. The CS_BS stock was separated by Bruno van Swinderen from Ralf Greenspan's stock in San Diego, CA, USA in 1999 and brought to Brisbane, Australia. From there it arrived in Berlin in 2008. Finally, Henrike Scholz received her stock from Ulrike Heberlein in San Francisco, CA, USA. The CS_HS strain arrived in Berlin in 2007. Flies of all strains were kept at 18°C until being tested in 2012 and 2013.

Buridan's paradigm

Experimental details are described in detail elsewhere[7]. Briefly, two black stripes producing 11° wide landmarks were positioned 293 mm from the center of a platform with a diameter of 117 mm, surrounded by water and illuminated with bright white light from behind. The centroid position of the fly was recorded via custom software (BuriTrack, http://buridan.sourceforge.net). If flies jumped from the platform, they were taken back to the platform with a brush and the tracker was reinitialized. Each data file represents five minutes of uninterrupted walking. We measured two replicates of the same five sub-strains in consecutive years, 2012 and 2013.

For more than three decades, experiments in Buridan's paradigm demonstrated that wild-type flies typically walk back and forth between the landmarks. We performed 5 minute long walking experiments with five different wild type Canton S (CS) sub-strains: CS_TP, CS_TZ, CS_JC, CS_BS and CS_HS. The locomotion parameters we calculated can be divided into three broad categories: temporal (activity/pause structure), spatial (stripe fixation, thigmotaxis, trajectory straightness) and mixed (speed, number of walks between stripes, distance travelled) measures (Table 1)[7].

Experimental differences between the replicates

The experiments in 2012 were done according to the previously published setup[7], while the 2013 experiments were performed in four new setups. In the new setups, illumination is slightly brighter (10–11 klx in the new setup, 7.5–8.5 klx in the old setup). We did not detect any difference in the temperature on the platforms (27°C for all machines). The platform was cleaned between flies in the 2012 replicate, while the platform was rotated between two tests in the 2013 replicate, and cleaned only after a series of five flies had been tested.

Analysis

The data was analyzed using CeTrAn v.4 (https://github.com/jcolomb/CeTrAn/releases/tag/v.4). Data with a mean distance travelled smaller than 50 mm/min was excluded to avoid outliers (2 data points were excluded in the second replicate, one for CS_JC and one for CS_HS).

Twelve different parameters were calculated (Table 1) and a Principle Components Analysis (PCA) was performed to visualize the results and identify potential groupings of the sub-strains. The effects of genotype and replicate were analyzed with an ANOVA (in R) using the second principal component, since the first and the third components were not normally distributed (assessed with a Shapiro test). Transition plots and the stripe deviation plot have not been tested statistically.

Raw trajectory data (including outliers) of the results of the CeTrAn analysis and the PCA result table are available on figshare: http://dx.doi.org/10.6084/m9.figshare.1014264.

Table 1. Brief description of the twelve parameters calculated from the trajectory of the flies used in this paper. A more detailed description is available at http://dx.doi.org/10.6084/m9.figshare.844624.

Parameter	Description
Median speed	Median of the speed of the animal while walking
Mean distance travelled per min	Distance travelled during the experiment divided by the duration of the experiment
Turning angle	Median of the angle difference between two movements
Meander	Median of the turning angle divided by instantaneous speed
Thigmotaxis while moving	Proportion of time spent on the edge of the platform versus the center of the platform (equal surfaces) while moving
Thigmotaxis while sitting	Proportion of time spent on the edge of the platform versus the center of the platform (equal surfaces) while being immobile
Stripe deviation	Median deviation angle between walking direction and direction toward the stripes
Number of walks	Number of times a fly walks between the two stripes during the experiment
Number of pauses	Number of times a fly stopped walking for more than 1s during the experiment
Activity bouts duration	Median duration of activity phases
Pause length	Median duration of pauses
Total time active	Sum of the length of activity phases during the experiment

Results

In Buridan's paradigm, wild-type flies typically walk back and forth between two inaccessible landmarks and their walking behavior is then analyzed. We performed 5 minutes long walking experiments with five different Canton S (CS) sub-strains: CS_TP, CS_TZ, CS_JC, CS_BS and CS_HS. We tested them in two replicates in two consecutive years using different hardware and under slightly varying experimental details (see Materials and Methods). The locomotion parameters that we calculated can be divided into three broad categories: temporal (activity/pause structure), spatial (stripe fixation, thigmotaxis, trajectory straightness) and mixed (speed, number of walks between stripes, distance travelled) measures (Table 1[7]). Flies' walking behavior was also visualized in transition plots, where the frequency of passage at each platform position is indicated by a heatmap. A distinction between sub-strains, which is consistent between the two replicates, can be seen in the visualization of this purely spatial parameter (Figure 1).

Using CeTrAn 4.0, we took twelve measurements of the flies' walking behavior and analyzed them using PCA. For simplicity of representation, we plotted the mean and standard error of the three first principal components, while pooling the replicates (Figure 2). Since the first and third principle components were not normally distributed (Shapiro test), we performed an ANOVA for the second component with the fly sub-strains and the replicates as factors. This analysis demonstrated significant effects of the sub-strain (F value = 37.315 < 2e-16), the replicate (4.155 0.04374), and the interaction sub-strain × replicate (F value = 3.891 0.00527). A post hoc test of the sub-strain effect, together with a non-parametric test for the third component of the PCA, showed three groups: CS_TZ and CS_TP together in one group, CS_BS and CS_HS together and CS_JC alone (CS_TZ and CS_JC could be separated only on PC3).

Strikingly, the stripe fixation behavior covered the full range from strong fixation (10° average deviation from the stripe) to almost no fixation at all (30°: a random walk generates a 44° score,[7]) (Figure 3). We did not perform any statistical tests on this data, as they are already included in the PCA.

In order to estimate the variability range of the CS behavior on a larger scale, we have set up a trajectory database to receive data from CS flies in different laboratories, using similar machines and protocols. Figure 4 currently visualizes the result of a PCA analysis over our data and one additional data set, contributed by the Botella lab in Regensburg. However, in future versions of this article, data collected from other laboratories will be continuously fed into this database, with Figure 4 providing real-time visualizations of this incoming data. Four additional labs have already agreed to run these experiments using similar machines and protocols; researchers that are interested in contributing to this project can contact the corresponding author, who will provide them with further details about how to participate.

Discussion

By analyzing the trajectories of five nominally identical CS sub-strains of *Drosophila melanogaster* in Buridan's paradigm, we were able to distinguish three different groups of sub-strains. All strains were treated similarly in the same laboratory conditions for

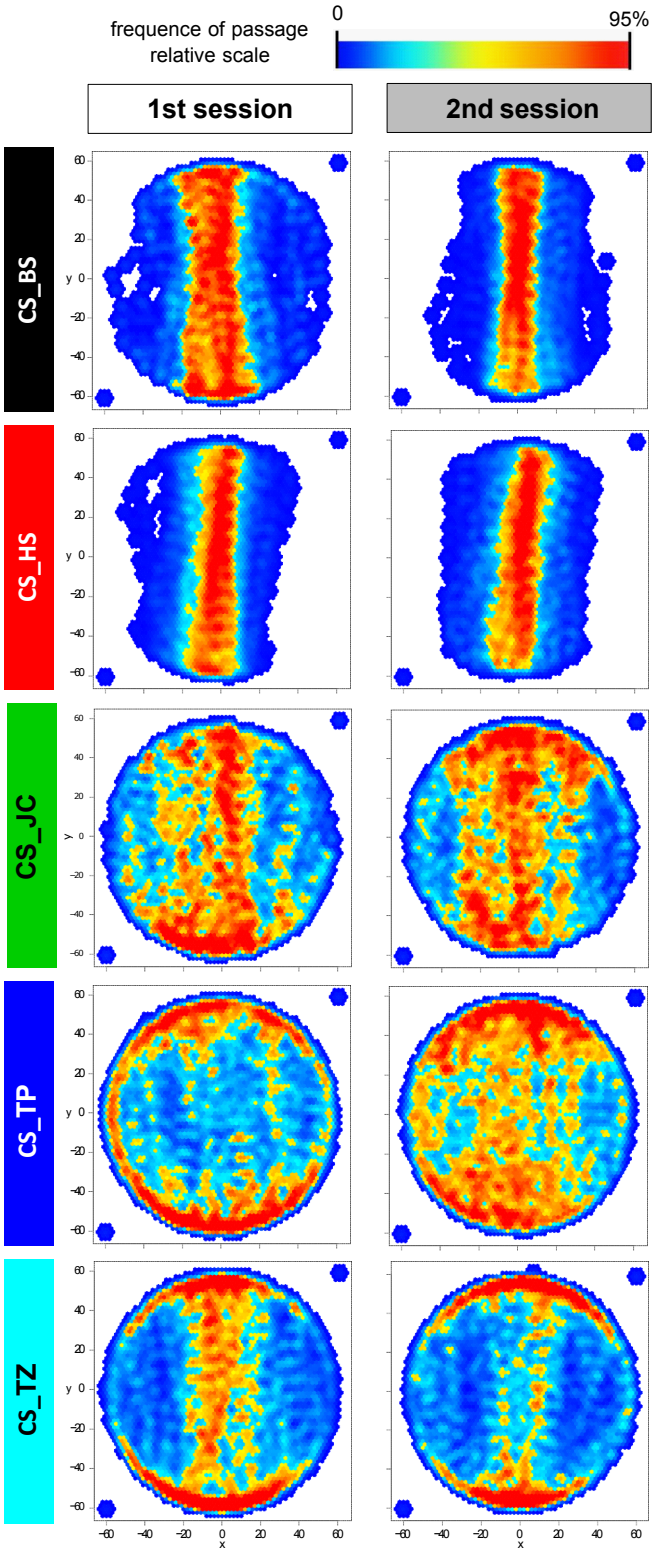

Figure 1. In transition plots, the behavior of each sub-strain looks different from the other strains and similar between the two experimental sessions. Transition plots represent the position of the fly on the platform, excluding the time when the fly was immobile. The scale is proportional, with red points meaning that the number of times the fly was in that position is at least 95% of the maximal score obtained for any position. A Gaussian smooth was applied to the resulting heat map. The two points outside the platform were added manually to assure orthogonal axes of the representation. Sample size is 11-12 for each plot.

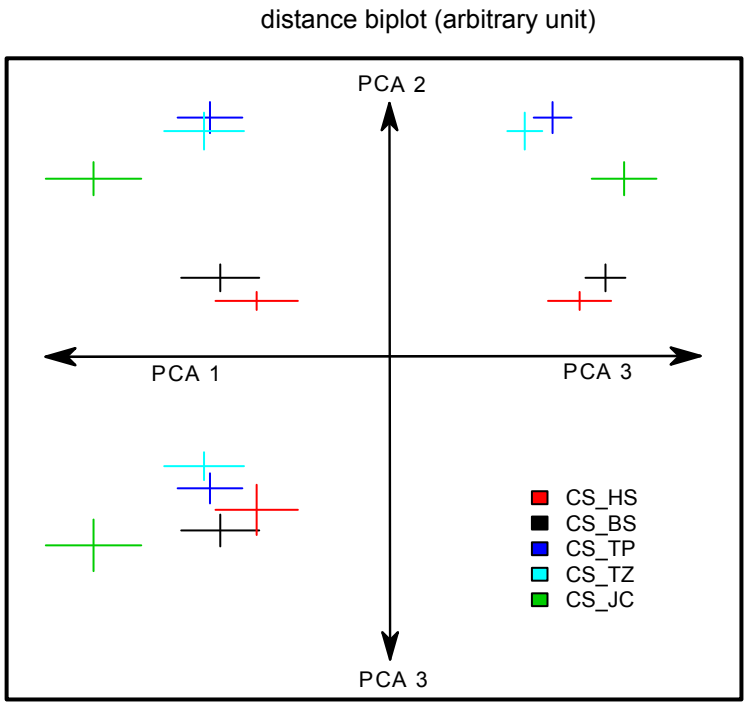

Figure 2. The CS sub-strains can be separated into three groups according to their overall behavior in Buridan's paradigm. A PCA was performed over the 12 measured variables capturing the flies' locomotion. The three first principal components are plotted against each other: from the center of the axes; PC1 to the left, PC2 up and PC3 down and to the right. Since units are arbitrary, they were not indicated. For each genotype, we represent the mean and standard error of the mean for the different PCs as a colored cross (data from the two replicates were pooled). The three groups are best visualized separately on the PC2-PC3 plot (upper-right). Sample size for each group is 23-24.

many generations (4 to 6 years) before being tested. There was no difference in rearing or experimental conditions between the different groups of flies. Therefore, it is a straightforward assumption that the differences in behavior we report here are genetic in origin. The sub-strain differences were comparable in the two replicates conducted one year apart, indicating that the genetic differences between the sub-strains were stable over this time span, although the CS_BS sub-strain appeared to have been modified during that time.

From the twelve parameters of walking that we tested, stripe deviation showed the most striking variability. Stripe fixation likely depends on multiple parameters, such as the fly's light/dark preference, their anxiety state, visual acuity, leg motor coordination or effects of wing clipping. It was used as a determining behavioral feature of Buridan's paradigm[11]. Our results call for special care with the genetic background of the tested strains when analyzing this behavioral feature.

The numerically small but statistically significant difference between the two replicates (see raw data for individual variables) may be attributed to the differences in test setups and conditions. Since the behavior of the flies did not tend to converge (at least not over the one year time-frame we covered), the different strains apparently did not evolve particular traits to cope with our particular laboratory conditions. It is therefore plausible that such micro-evolution played little role in differentiating the sub-strains in the first place; the major cause for the difference between sub-strains might therefore be founder effects produced when a new fly stock is established, or population bottlenecks in the history of each strain. This hypothesis is also supported by the fact that common descent fails to explain the grouping we found in the PCA. In particular, two strains originating from the Paris lab (CS_TP and CS_JC) showed strikingly different locomotor behavior. This suggests that founder effects or bottlenecks were leading to dramatic alterations of behavior in Buridan's paradigm. These results raise the question of which other phenotypes might be affected in the numerous CS sub-strains present in laboratories throughout the world.

Interestingly, the use of a control line may lead to inaccurate interpretation of the data. For example, *crammer* mutant flies were reported to either show[12] or not show[13] an appetitive short term memory deficit with identical memory retention scores, because the scores of the control "CS" flies were different in the two studies. Our results further emphasize the need for a more systematic scheme addressing control populations. Existing genetic background differences

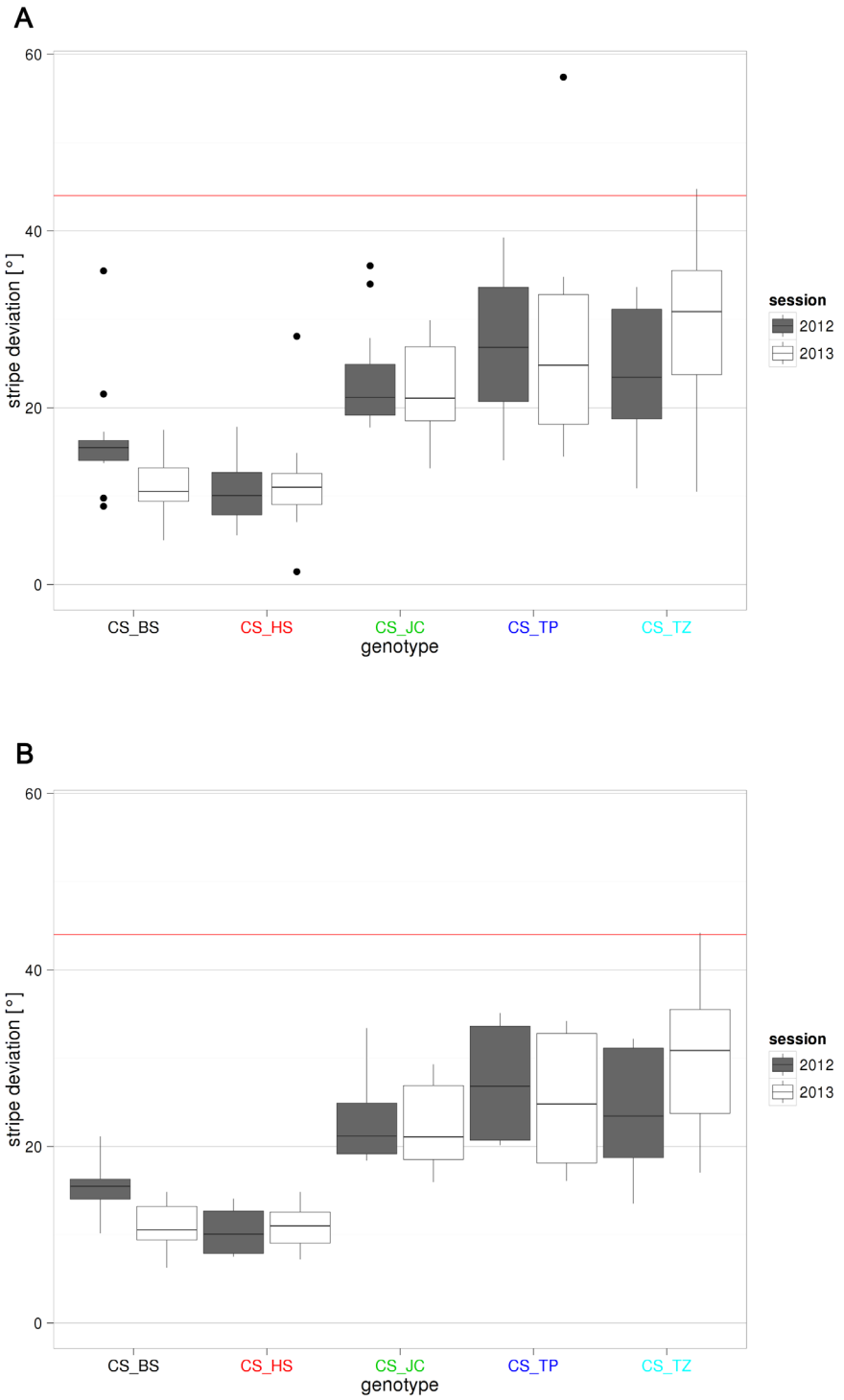

Figure 3. The different sub-strains show a large spectrum of values for the stripe deviation parameter. For every movement of the fly, the angle between its direction and the direction toward the stripes was calculated. The median of these angles was calculated for each fly, representing a quantification of stripe fixation by the fly. The value of each sub-strain in each session is depicted in boxplots: for each group, we represent the median, 25–75% quantiles and the total spread of the values (excluding outliers) as line, box and whiskers, respectively. The version of this figure on the *F1000Research* website is interactive, readers can define the type of whiskers displayed as either the 10^{th}–90^{th} percentiles (**A**) or Tukey whiskers (1.5 x IQR from 1^{st}/3^{rd} quartile; **B**). The text color code used for the genotypes is analogous to that used in Figure 2. The red horizontal line corresponds to the median value for random walks: 44°. Sample size is 11–12 for each boxplot. No statistical analysis was performed.

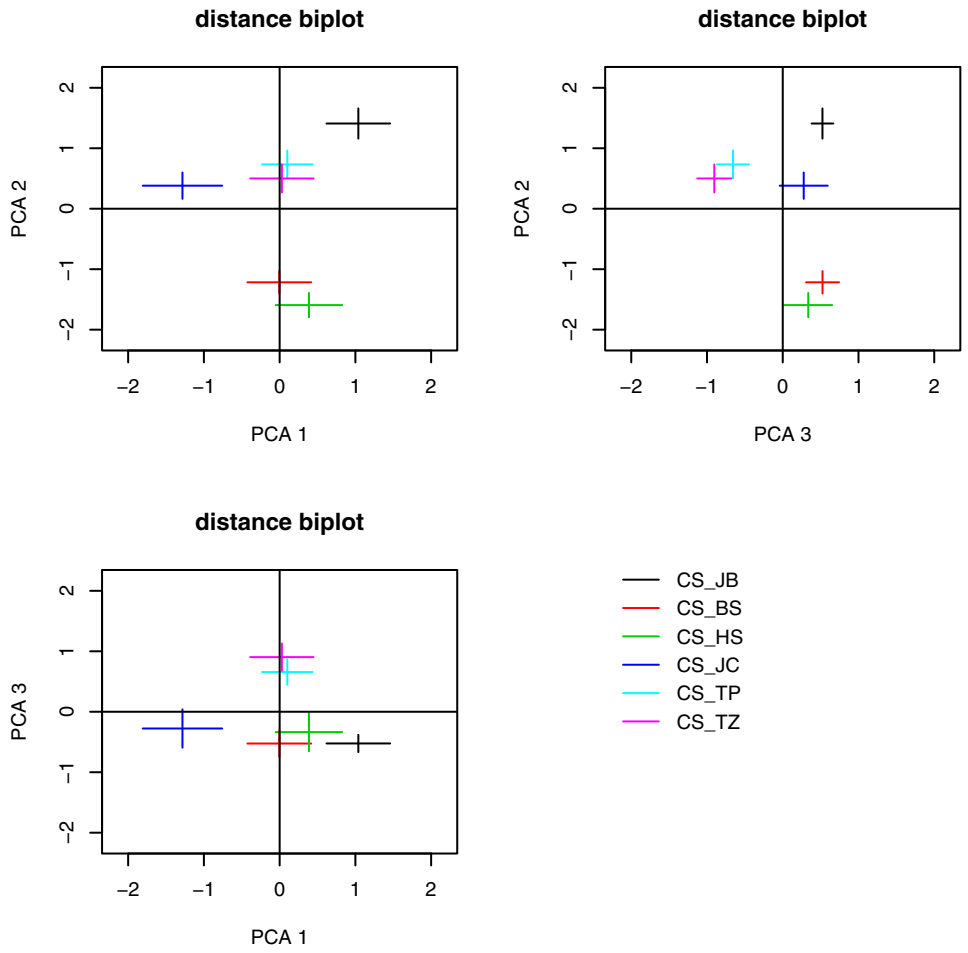

Figure 4. Principal component analysis of one additional Canton S strain submitted by José Botella, tested in Regensburg (CS_JB) with the five previously tested Canton S strains for comparison. This CS_JB strain was ordered from the Bloomington stock center (stock #1) approx. seven years ago. CS_JB falls within the range of variability seen so far, but does not appear to clearly group with any of the previously measured strains. In future versions, this figure will be updated with Canton S data from other laboratories, tested according to the same parameters as described in the Materials and Methods section. Please email BB for instructions on how to submit. The final version of this figure will have final instructions for data submission.

may indeed explain discrepancies between results obtained in different laboratories, and that the use of the "CS" as a control strain is not enough to achieve comparability or reproducibility. A homogenization of the genetic backgrounds of 'standard' control strains would indeed be required.

Fortunately, our experiments suggest that the primary cause for differences in wild-type strains come from founder effects and not laboratory selection. One possible, but logistically challenging solution might be to have a common source for lines used for out-crossing events (including control lines), kept in massive, randomly interbreeding populations, for each lab to purchase at regular intervals. Large stock centers such as the Bloomington stock center would in principle be the candidate locations to implement such a solution. However, the phenotypes of mutations can vary depending on the genetic background within which the mutation is embedded[14–16]. The choice of one or multiple reference wild-type strain(s) is therefore not without implications for the future of the field and should be carefully investigated.

Data availability

figshare: Buridan raw data: Sub-strains of Drosophila Canton-S differ markedly in their locomotor behaviour, doi: 10.6084/m9.figshare.1014264[17]

Author contributions

JC and BB conceived the study, discussed the data and co-wrote the manuscript. JC performed the experiments and analyzed the data.

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

Acknowledgments

We thank Troy Zars, Thomas Préat, Henrike Scholz and Bruno van Swinderen for generously sharing their Canton S and many other strains with us over the years.

References

1. Stern C, Schaeffer EW: **On Primary Attributes of Alleles in *Drosophila melanogaster*.** *Proc Natl Acad Sci U S A.* 1943; **29**(11): 351–61.
PubMed Abstract | Free Full Text
2. Benzer S: **Behavioral mutants of *Drosophila* isolated by countercurrent distribution.** *Proc Natl Acad Sci U S A.* 1967; **58**(3): 1112–9.
PubMed Abstract | Publisher Full Text | Free Full Text
3. Crawley JN, Belknap JK, Collins A, *et al.*: **Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies.** *Psychopharmacology (Berl).* 1997; **132**(2): 107–24.
PubMed Abstract | Publisher Full Text
4. Paylor R, Crawley JN: **Inbred strain differences in prepulse inhibition of the mouse startle response.** *Psychopharmacology (Berl).* 1997; **132**(2): 169–80.
PubMed Abstract | Publisher Full Text
5. Gems D, Riddle DL: **Defining wild-type life span in *Caenorhabditis elegans*.** *J Gerontol A Biol Sci Med Sci.* 2000; **55**(5): B215–9.
PubMed Abstract | Publisher Full Text
6. Buelthoff H, Gotz KG, Herre M: **Recurrent inversion of visual orientation in the walking fly *Drosophila melanogaster*.** Springer Berlin/Heidelberg. *J Comp Physiol A.* 1982; **148**(4): 471–81.
Publisher Full Text
7. Colomb J, Reiter L, Blaszkiewicz J, *et al.*: **Open source tracking and analysis of adult *Drosophila* locomotion in Buridan's paradigm with and without visual targets.** *PLoS One.* 2012; **7**(8): e42247.
PubMed Abstract | Publisher Full Text | Free Full Text
8. Götz KG: **Visual guidance in *Drosophila*.** *Basic Life Sci.* 1980; **16**: 391–407.
PubMed Abstract | Publisher Full Text
9. Brembs B: **Operant learning of *Drosophila* at the torque meter.** *J vis Exp.* 2008; 16. pii: 731.
PubMed Abstract | Publisher Full Text | Free Full Text
10. Guo A, Liu L, Xia SZ, *et al.*: **Conditioned visual flight orientation in Drosophila: dependence on age, practice and diet.** *Learn Mem.* 1996; 3(1): 49–59.
PubMed Abstract | Publisher Full Text
11. Phillips AM, Smart R, Strauss R, *et al.*: **The *Drosophila* black enigma: the molecular and behavioural characterization of the black1 mutant allele.** *Gene.* 2005; **351**: 131–42.
PubMed Abstract | Publisher Full Text
12. Colomb J, Kaiser L, Chabaud MA, *et al.*: **Parametric and genetic analysis of *Drosophila* appetitive long-term memory and sugar motivation.** *Genes Brain Behav.* 2009; **8**(4): 407–15.
PubMed Abstract | Publisher Full Text
13. Krashes MJ, Waddell S: **Rapid consolidation to a radish and protein synthesis-dependent long-term memory after single-session appetitive olfactory conditioning in *Drosophila*.** *J Neurosci.* 2008; **28**(12): 3103–13.
PubMed Abstract | Publisher Full Text | Free Full Text
14. Ivanco TL, Greenough WT: **Altered mossy fiber distributions in adult Fmr1 (FVB) knockout mice.** *Hippocampus.* 2002; **12**(1): 47–54.
PubMed Abstract | Publisher Full Text
15. Mineur YS, Sluyter F, de Wit S, *et al.*: **Behavioral and neuroanatomical characterization of the Fmr1 knockout mouse.** *Hippocampus.* 2002; **12**(1): 39–46.
PubMed Abstract | Publisher Full Text
16. Lipp HP, Wolfer DP: **Genetic background problems in the analysis of cognitive and neuronal changes in genetically modified mice.** *Clin Neurosci Res.* 2003; **3**(4–5): 223–31.
Publisher Full Text
17. Colomb J, Brembs B: **Buridan raw data: Sub-strains of Drosophila Canton-S differ markedly in their locomotor behavior.** *figshare.* 2014.
Data Source

Open Peer Review

Current Referee Status: ✓ ✓ ✓

Version 1

Referee Report 02 September 2014

doi:10.5256/f1000research.4564.r5633

Hiromu Tanimoto, **Vladimiros Thoma**
Graduate School of Life Sciences, Tohoku University, Sendai, Japan

Colomb and Brembs reported an important piece of information that highlights large differences in the walking trajectory of six substrains of a wild-type *Drosophila* strain (Canton S) in the so-called Bridan paradigm. Drawing conclusions from behavioral comparisons of strains could therefore be moot, if the tested strains do not share the same genetic background (at least for the Buridan paradigm).

This report is presented in a clear and succinct way. In addition, the authors invite submission of data by other labs, and the addition of these new data will be plotted in Fig. 4. This is an interesting endeavor which nicely uses the function of this journal. One has yet to keep in mind that fly behavior can be dramatically affected by 'unwritten' lab conditions (e.g. fly food, rearing conditions), and therefore the new data from other labs might not be comparable to the current dataset. One suggestion for the contributing labs to circumvent this caveat is to use one (or more) of the strains analyzed in this study and to check the reproducibility.

In addition, I have a few minor comments that may be addressed:

1. The authors argue that the basis of the behavioral variability is differences in the genetic background, but other reasons (e.g. epigenetic differences) can conceivably contribute to the variability as well.

2. The authors state that there was a significant effect of the replicate on Principal Component 2 (page 3, left column, third line from bottom), but later state that "(…) sub-strain differences were comparable in the two replicates conducted one year apart" (page 5, left column, line 5 from top). Either of the statements should be amended.

3. "A" in "PCA" stands for analysis. Use "PCA" instead of "PCA analysis". My understanding of PCA is "Principal Component Analysis" rather than "Principle Component Analysis" the authors use in the paper. Use PC1, PC2, PC3 in the axes of Fig.2 and Fig. 4.

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Referee Report 15 August 2014

doi:10.5256/f1000research.4564.r5635

Josh Dubnau
Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA

This is an interesting manuscript that I hope will be read widely. In this field, we give a lot of lip service to controlling genetic background because we all know it is important. But often, "wild type" lines such as CS are assumed to be equivalent and this turns out to be quite problematic conceptually. Now Colomb and Brembs have put some empirical evidence forward that confirms our fears about this practice. CS is a commonly used outbred strain, but my CS and your CS are different due to founder effects, drift, and selection. In this particular study, the founder effect has a massive impact on Buridan's paradigm, a relatively simple locomotor behavior. And this would likely be true for any quantitative trait.

I have one comment on the conclusion; the effects seem to be mostly due to founder effects rather than selection (drift should be considered as well). This seems like a likely explanation in this current comparison, but this might change when we deal with mutants and transgenes maintained on an outbred strain. Such mutants or transgenes may cause accumulation of modifiers, which I would consider to be selection based.

Regardless of the underlying mechanism, I like the fact that the authors of this study propose a solution, in which a stock center might maintain a large population of an outbred strain such as CS, and then we all would outcross our mutants and transgenes to that line. This could work, and offers the advantage that results from different groups would be comparable. Without such a mechanism, it is important, at a minimum, that all labs at least back cross all mutants/transgenes to their own CS or equivalent w.t. line. On the other hand, I would argue that genetic screens and collections of large populations of Gal4 and other such lines should be generated on an INBRED background which eliminates or reduces founder effects. This provides a more useful reagent for the community to use because it eliminates the onerous need to outcross every single line from a large collection.

Overall, this is an important issue, and this study does a nice job of shedding light with actual data.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Referee Report 08 August 2014

doi:10.5256/f1000research.4564.r5630

Gregg Roman
Department of Biology and Biochemistry, University of Houston, Houston, TX, USA

This manuscript does an excellent job demonstrating significant strain differences in Burdian's paradigm. Since each Drosophila lab has their own wild type (usually Canton-S) isolate, this issue of strain differences is actually a very important one for between lab reproducibility. This work is a good reminder for all geneticists to pay attention to the population effects in the background controls, and presumably the mutant lines we are comparing.

I was very pleased to see the within-isolate behavior was consistent in replicate experiments one year apart. The authors further argue that the between-isolate differences in behavior arise from a Founder's effect, at least in the differences in locomotor behavior between the Paris lines CS_TP and CS_JC. I believe this is a very reasonable and testable hypothesis. It predicts that genetic variability for these traits exist within the populations. It should now be possible to perform selection experiments from the original CS_TP population to replicate the founding event and estimate the heritability of these traits.

Two other things that I liked about this manuscript are the ability to adjust parameters in figure 3, and our ability to download the raw data. After reading the manuscript, I was a little disappointed that the performance of the five strains in each 12 behavioral variables weren't broken down individually in a table or figure. I thought this may help us readers understand what the principle components were representing. The authors have made this data readily accessible in a downloadable spreadsheet.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.