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Sex-specific responses to sexual familiarity, and the role of olfaction in *Drosophila*: a new analysis confirms original results

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One of the key findings from our paper on sex-specific responses to sexual familiarity in *Drosophila melanogaster* [1] was an olfaction-mediated male courtship preference for sexually novel females over sexually familiar females. Over a series of seven experiments, we assayed male courtship preferences for novel or familiar females and analysed the data using generalized linear models (GLMs) with a binomial error distribution. It has been brought to our attention that the statistical models we used for analysing male courtship behaviour did not take into account temporal correlations in courtship events within males. Consequently, the variance in courtship events was higher than predicted by the model, and the excess dispersion could potentially result in errors in conclusions. This highlights the general potential for high-frequency sampling of behaviours to give rise to high temporal correlations of event counts within a dataset, and the importance of correcting dispersion factors when analysing this type of data.

We first verified that the male courtship data were overdispersed. We then re-ran GLMs with a binomial error distribution—in which each male was represented twice in the dataset—and adjusted the dispersion parameter to account for both duplication and overdispersion [2]. The results of the new analyses, which are therefore corrected for overdispersion and duplication, are presented in table 1. The significance of familiarity effects was reduced in the new analyses compared with the original paper (table 1), but the results qualitatively confirm those originally reported [1]. Overall, the new analyses support our original biological interpretation of the experiments: males preferentially court novel females, and require olfaction to do so, in *D. melanogaster*.

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References

1. Tan CKW, Løvlie H, Greenway E, Goodwin SF, Pizzari T, Wigby S. 2013 Sex-specific responses to sexual familiarity, and the role of olfaction in *Drosophila*. *Proc. R. Soc. B* **280**, 20131691. (doi:10.1098/rspb.2013.1691)
2. McCullagh P, Nelder JA. 1989 Binary data. In *Generalized linear models* (eds DR Cox, DV Hinkley, D Rubin, BW Silverman), pp. 124–128. New York, NY: Chapman & Hall.

Table 1. Results from the original paper [1] and from the re-analyses.

experiment and figure in original paper	male type	female marking method	explanatory variables	overdispersion factor	<i>p</i> -value originally reported	re-analysis estimate	re-analysis s.e.	re-analysis z-value	re-analysis <i>p</i> -value
direct novelty figure 1a	wild-type	eye colour	familiarity (novel) eye colour (white)	2.45	<0.001 <0.001	0.6481 2.7899	0.3643 0.3643	1.78 7.66	0.075 <0.001
direct novelty figure 1a	wild-type	paint	familiarity (novel) paint status (painted)	2.5	<0.001 <0.001	0.7854 0.4587	0.2315 0.2315	3.39 1.98	<0.001 0.048
direct novelty figure 1a	wild-type	females not marked (decapitated)	familiarity (novel)	10.34	<0.001	1.5075	0.4903	3.07	0.002
phenotypic novelty figure 1c	wild-type	paint colour	familiarity (novel) paint colour (yellow)	2.37	<0.001 0.073	0.9972 -0.5631	0.4257 0.4257	2.34 -1.32	0.019 0.186
phenotypic novelty figure 1c	<i>Orco</i> mutant	paint colour	familiarity (novel) paint colour (yellow)	1.69	0.873 0.294	-0.0060 -0.3191	0.3289 0.3289	-0.02 -0.97	0.986 0.332
phenotypic novelty figure 1c	wild-type	females not marked (decapitated)	familiarity (novel)	5.90	<0.001	1.0736	0.2185	4.91	<0.001
phenotypic novelty figure 1c	<i>Orco</i> mutant	females not marked (decapitated)	familiarity (novel)	2.92	<0.001	0.6170	0.2120	2.91	0.004