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Skewed sex ratios after partial or complete deletion of the *Obp50a-d* gene cluster in *Drosophila melanogaster*

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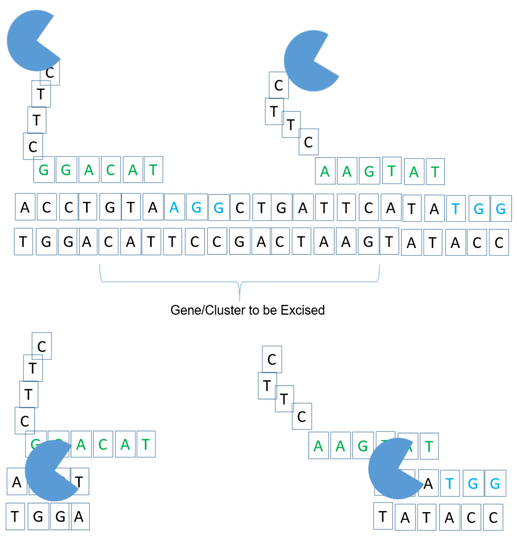
Abstract

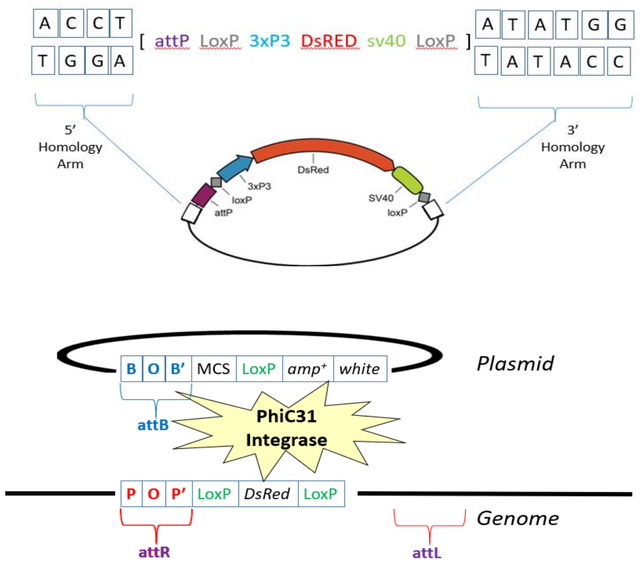
Odorant binding proteins (OBPs) in *Drosophila melanogaster* are generally thought to be associated with olfactory responses, but identification of OBPs in non-chemosensory tissues (*e.g.* accessory glands) suggests that some members of this family may have evolved to acquire different functions. The functions of many OBPs remain, however, poorly defined. We used CRISPR technology to delete a group of four members of the *Obp50* cluster, *Obp50a-d*, that are in close physical proximity without intervening genes, while introducing a PhiC31 integration site. We encountered difficulty in obtaining females from the CRISPR knock-out line when flies were reared at 25ºC but slowing their growth at 18ºC rescued females. We quantified sex ratio at 25ºC in the CRISPR knockout line versus the co-isogenic Canton-S control. We then reinserted all combinations of intact members of this group in their endogenous location through PhiC31 integration. Deletion of the entire *Obp50a-d* cluster resulted in a female-male sex ratio of 0.33 compared to a ratio of 0.46 after reinsertion of an intact *Obp50a-d* cluster. Reinsertion of constructs with intact *Obp50b*, *Obp50c* and/or *Obp50d* genes failed to restore a normal sex ratio. However, reinsertion of constructs that contained an intact *Obp50a* gene resulted in an average sex ratio of 0.43, close to the control. Thus, the *Obp50* cluster has undergone functional diversification during evolution and *Obp50a* has acquired a function that is essential for enabling balanced development of males and females. The mechanism of sex ratio bias from the *Obp50a-d* cluster remains to be determined.

Background: Line generation



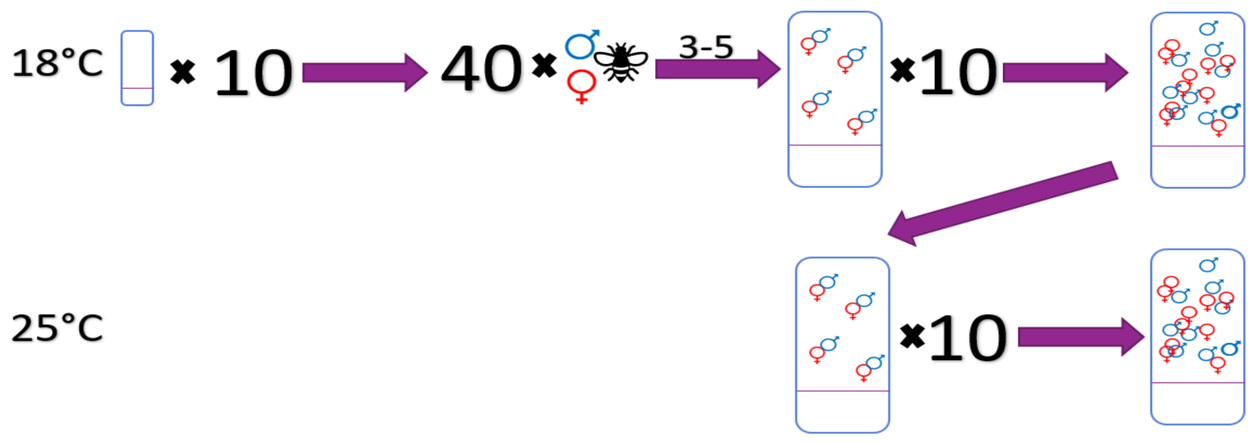
In order to remove the *Obp50a-d* gene cluster, we made use of CRISPR/Cas9 technology. After identification of PAM sites, we introduced guide RNAs, which bind upstream of the PAM sites, allowing the Cas9 endonuclease to excise the *Obp50a-d* region.

Background: Line generation



We co-injected a donor plasmid with our other CRISPR components. This donor plasmid possessed two homology arms, which allowed the donor plasmid to bind to the region of interest, along several other elements that were key to the reinsertion of our gene cluster. The key elements being the DsRed fluorescent marker and an *attP* site, a viral docking site that facilitates re-insertion of individual genes. The final phase was the reinsertion of a single functional gene within the cluster. This was accomplished using the PhiC31 injection method.

Methods



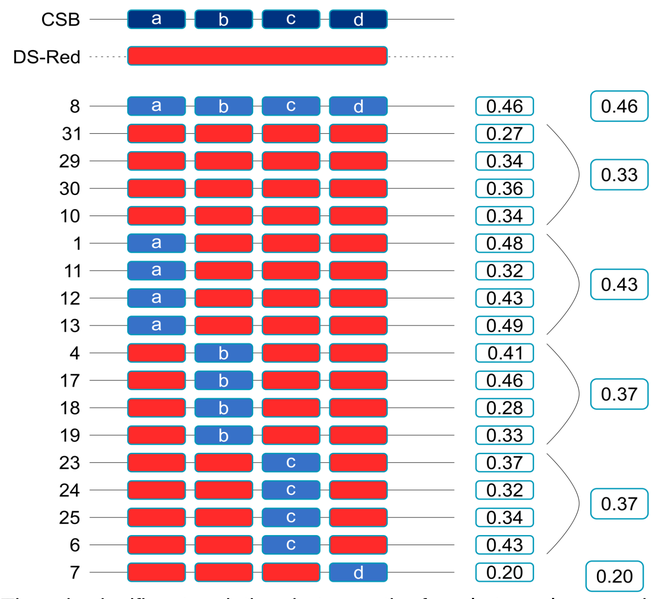
At 18°C the fly stocks for each line are expanded to 10 vials each by changing food every 6 days. Following this expansion males and females are collected separately as they emerge. The males and females are then aged 3-5 days in collection vials. 10 vials of 4X4 CADs are set up for each line, after 6 days the vials are cleared. The male and female flies are counted for each vial on each day as they emerge. The counted flies are then used to set up the 25° C portion of the assay and the entire process is repeated.

Results: Initial observations, statistical analysis

Line	SE	Diff	LCI	UCI	P value
10	0.041	-0.044	-0.21	0.12	1.0
29	0.036	-0.039	-0.21	0.13	1.0
30	0.047	0.18	0.020	0.35	0.016*
31	0.036	-0.10	-0.27	0.060	0.53
1	0.033	0.10	-0.060	0.27	0.53
11	0.038	-0.067	-0.23	0.096	0.96
12	0.037	0.0054	-0.16	0.17	1.0
13	0.038	0.080	-0.084	0.24	0.87
4	0.032	0.040	-0.12	0.20	1.0
17	0.042	0.045	-0.12	0.21	1.0
18	0.043	-0.083	-0.25	0.080	0.83
19	0.042	-0.013	-0.18	0.15	1.0
6	0.044	0.069	-0.10	0.24	0.98
23	0.034	0.0043	-0.16	0.17	1.0
24	0.037	-0.063	-0.23	0.10	0.98
25	0.044	-0.064	-0.23	0.099	0.98
7	0.052	-0.21	-0.40	-0.021	0.018*

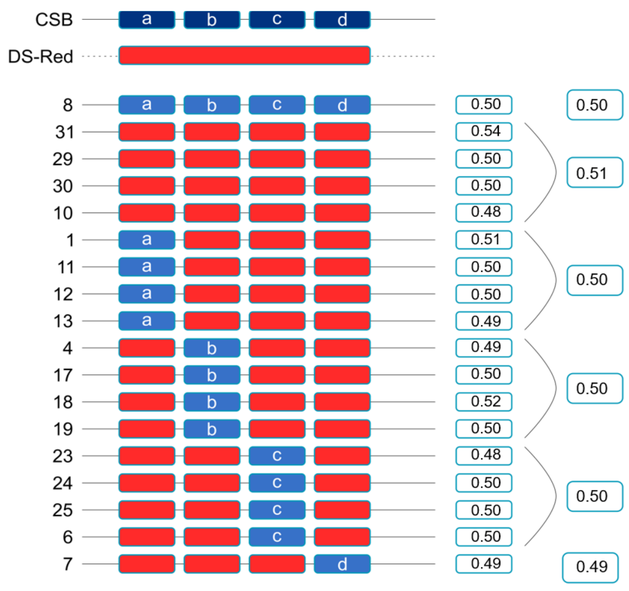
Note: P< 0 '***' 0.001. '**' 0.01. '*' 0.05. '.' 0.1. ' ' 1. The grouped lines are genetically identical but were created independently. All lines are being compared to the positive control reinsertion line which is line 8, using a Dunnett's test.

Initial observations at 25°C



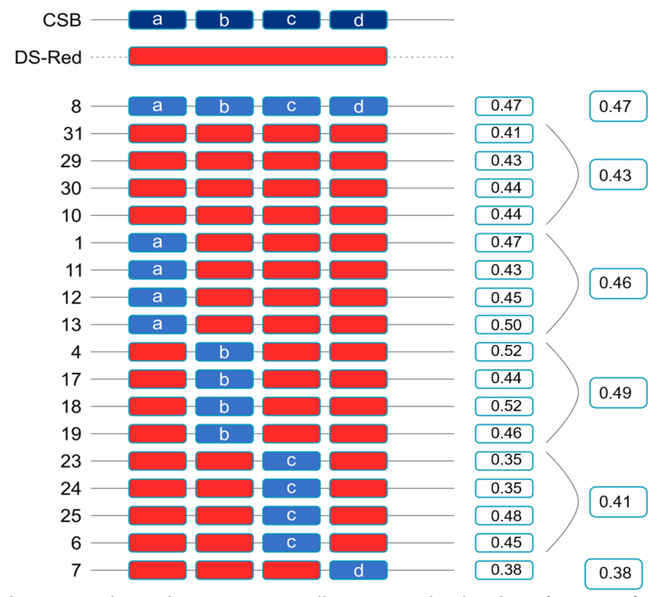
There is significant variation, between the female to male sex ratio of the reinsertion lines and the positive control. These are the result that led to further investigation.

18°C sex ratio analysis



There is no significant variation between the reinsertion lines. This is to be expected as the females were saved 18° C.

25°C sex ratio analysis



The 25°C data shows greater divergence in the female to male sex ratios between the reinsertion lines and the positive control. In particular line 7, 23 and 24.

Conclusion

Deletion of the *Obp50*a-d cluster with CRISPR results in variation of female to male sex ratios at 18°C and 25°C. Across data sets only constructs containing an intact *Obp50*a led to the consistent restoration of a normal female to male sex ratio. This indicates that the paralogs of the *Obp50* cluster have undergone functional diversification during evolution.

ACKNOWLEDGMENT

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