**Figure S2** Sexual orientation of Or67d<sup>Gal4</sup> mutants. CI values were measured in single-choice courtship assays with mature males of indicated genotypes and (A) intact or decapitated Ore-R virgins (♀, filled columns) and males (♂, hatched columns) in daylight, (C) intact Ore-R virgins (♀, filled columns) and dewinged males (♂, hatched columns) in the dark, and (D) decapitated Ore-R virgins (♀, filled columns) and males (♂, hatched columns) in the dark. The number of couples observed is shown below each column. Error bars represent double s.e.m. (B) Copulation efficiencies of wild-type couples in single-choice courtship assays in daylight. The percentage of copulating Canton-S (blue graph) and Ore-R couples (red graph) out of 10 couples each is plotted as a function of time they spent in a cylindrical chamber of 9 mm height x 16 mm diameter.

In addition to single-choice courtship assays with intact flies, we conducted experiments with virgins and males that had been decapitated (A,D). In daylight, Or67d<sup>Gal4</sup> compared to Ore-R males did not increase their CI towards decapitated males (p=0.11), as observed with intact object flies (A). By contrast, in the dark (D) the CI towards decapitated males of Or67d<sup>Gal4</sup> compared to wild-type males was increased significantly (p=0.001) and was the same as that towards decapitated females (p=0.77). However, this phenotype of Or67d<sup>Gal4</sup> males could not be rescued by expressing Or67d under the control of Gal4 (D). Since the CI towards decapitated males of heterozygous UAS-Or67d/+; Or67d<sup>Gal4</sup>/+ males was increased as well, we conclude that the Or67d<sup>Gal4</sup> insertion or the genetic background of this fly stock generates this dominant courtship phenotype.

It should be emphasized that the Or67d<sup>Gal4</sup> stocks, kindly provided by Barry Dickson, were verified for the replacement of the open reading frame of Or67d by that of Gal4 [28] by isolation of their DNA, followed by PCR and DNA sequencing of the insertion site.