A new spontaneous chromosomal inversion in a classical laboratory strain of *Drosophila subobscura*.

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*Drosophila subobscura* stands out for its rich chromosomal polymorphism in natural populations. Krimbas (1992) reviewed up to 66 spontaneous chromosomal inversions that combined into 79 arrangements. Some of these inversions are common in the whole range of the species distribution, but others are only present either at low frequencies across the species distribution area or in a restricted geographical area. In addition, a set of inversions should have been discovered shortly after its appearance in nature. This is the case of inversions E17, E18, E21 and O26 found after the New World colonization by *D. subobscura* (Balanyà et al., 2003), and also that of some inversions recorded only once in populations widely and repeatedly studied, such as E19, E20, and U12 in Zürich (Gosteli and Hauschteck-Jungen, 1989) and O25 in Barcelona (Orengo and Prevosti, 1992).

Figure 1. Polytene E chromosomes of an heterokaryotype for chromosomal arrangements E,t and E24 of *Drosophila subobscura*. Arrows indicate the cytological location of both inversion E24 breakpoints. C: Centromere; T: Telomere; dot: the small chromosome.

Here we report a new spontaneous inversion (Figure 1) that arose in the *ch cu* laboratory strain. This strain that was obtained in the Krimbas’ laboratory over 40 years ago (Zouros and Krimbas, 1973) is homokaryotypic for its five long chromosomes (As, Jst, Ust, Est, and O23,4). The *ch cu* strain has been maintained and used in our department for over 35 years to determine the chromosomal polymorphism in natural populations samples by crossing wild males to *ch cu* virgin females and subsequently observing polytene chromosomes from F1 larvae (e.g., Prevosti et al., 1982; Orengo 1994). In addition, we have commonly used polytene chromosome preparations from this strain to map DNA probes by *in situ* hybridization (e.g., Segarra and Aguadé, 1992; Orengo et al., 2015).
Despite the many thousands of \textit{ch cu} chromosomes that we have observed either in heterokaryotypic F$_1$ larvae or directly in \textit{ch cu} strain larvae—over the long period elapsed, we had never detected any discordant arrangement in \textit{ch cu} chromosomes.

During one of our \textit{in situ} hybridization experiments, we realized that the pair of E chromosomes from one \textit{ch cu} larva was heterokaryotypic for an inversion (Figure 1). We could readily discard an accidental contamination of the \textit{ch cu} strain from other \textit{D. subobscura} strains of the Barcelona area maintained in our laboratory, since the rest of chromosomes were homokaryotypic for the \textit{ch cu} strain arrangements Ast, Jst, Ust, and O3+4, which are at rather low frequency in the Barcelona area. Moreover, upon closer inspection of the inversion span, we could confirm that this was a new inversion, since its cytological breakpoints correspond to sections 63C/64A and 70C/70D of the Kunze-Mühl and Müller (1958) map, which are not shared by any other spontaneous known inversion. We named this inversion E$_{24}$. The spontaneous origin of a new inversion in a laboratory strain that is normally used to determine the karyotype of wild-caught individuals might raise concerns relative to the identification of inversions newly originated in natural populations. Indeed, if the rate of origin of inversions in laboratory strains were high—which does not seem to be the case for the \textit{ch cu} strain—, some of the inversions newly described as having originated in natural populations might have actually originated in the laboratory strain used to karyotype wild-caught individuals.


\textbf{Abnormal ovipositor in a \textit{Drosophila melanogaster} female.}

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While collecting virgin females from a wild stock, we found a female with an abnormal ovipositor (Figures 1, abnormal; Figure 2, normal). The stock was obtained from wild \textit{D. melanogaster} flies collected at the Font Groga site, near Barcelona, in autumn 2012 (Canals \textit{et al.}, 2013). Unfortunately, it was not possible to cross this female, and we did not have any information on her parents because she appeared in a mass culture.

Figure 1. Abnormal ovipositor (ventral and lateral views).