

RESEARCH NOTE

Sex-specific asymmetry in eye development in interspecific hybrids in the *Drosophila bipectinata* species complex

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There are a number of species pairs, especially in the genus *Drosophila*, which despite having acquired distinct species status, can still be hybridized. Such species can be very useful for studying hybrid incompatibilities which ultimately can lead to speciation genes (the genes that have diverged to the greatest extent between the species). *D. bipectinata* species complex, comprising four closely related species, *D. bipectinata*, *D. parabipectinata*, *D. malerkotliana* and *D. pseudoananassae*, is one example where the members can be cross-fertilized, but only in the laboratory. However, consistent with Haldane's rule among the hybrids, the females are fertile (homogametic sex) and males are sterile (heterogametic sex) (Bock 1971a). We performed studies on fluctuating asymmetry (FA) in the interspecific hybrids of this complex with the thought of comparing the level of the same with the respective parental species. During the course of our investigation, we came across certain abnormalities in the hybrids having *D. pseudoananassae* as one of the parental species. One of them was the occurrence of asymmetrical eyes in hybrid males. This note gives a description of the asymmetrical eyes and the possible explanation(s) for such an abnormality.

Taxonomically, *D. bipectinata* species complex belongs to the subgroup *ananassae* of the widely diverged *melanogaster* species group. The females of the members are morphologically indistinguishable but the males can be distinguished on the basis of pigmentation of abdominal tip and sex comb arrangements (Bock and Wheeler 1972). A number of population genetics, behaviour, taxonomic and evolutionary studies have been carried out in the complex (Bock 1971a,b; Banerjee and Singh 1997; Kopp and Barmina 2005; Kopp *et al.* 2006; Mishra and Singh 2006, 2007; Singh and Sisodia 2008; Nanda and Singh 2012; Banerjee and Singh 2012; Santosh and Krishna 2013; Singh and Singh 2013). It is evident

from these studies that this complex provides a good model for studies dealing with evolution and speciation. In earlier studies attempting to derive a phylogenetic relationship among the members of this complex, it has been proven beyond doubt that while *D. bipectinata*, *D. parabipectinata* and *D. malerkotliana* are closely related to each other, *D. pseudoananassae* is distantly related. Presently, we are pursuing evolutionary studies in this complex with particular reference to sexual isolation, FA and inversion polymorphism. We have found incomplete sexual isolation and based on the asymmetric mode of mating preference, we have derived a phylogenetic relationship among the members of this complex (Banerjee and Singh 2012). Further, the pattern of mating preference of F₁ hybrid females with the parental males have been studied and found to be different in the hybrid females of each pair (Banerjee and Singh 2014). It has also been concluded that the level of FA remains nearly the same in the four species of the complex. Therefore, irrespective of their evolutionary divergence, the four species do not seem to differ much in the system controlling developmental stability (Banerjee and Singh 2015).

With a view of comparing the level of FA of parental species with their hybrids, we made 12 crosses including reciprocals (four by four factorial design). One strain of each of the four species was involved and crosses were made by keeping seven days aged virgin females and males in food vials (20 pairs per vial) for five days before being transferred to another set of vials. For scoring of FA, 50 sons and daughters were included from all the crosses except the ones involving *D. pseudoananassae*. In some crosses, like those involving *D. bipectinata* and *D. parabipectinata*, sufficient number of progeny could be obtained in the first set of vials, but in most of the other crosses, we could complete 50 only by taking progeny from both the sets. In some crosses involving *D. pseudoananassae* as one of the parents, very few progeny emerged and that too only from the second set of vials.

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In the crosses, *D. pseudoananassae* females \times *D. bipectinata* males and *D. pseudoananassae* females \times *D. parabiptinata* males, a number of hybrid sons were found having severely compromised eye symmetry (all the sons emerging were examined for eye asymmetry). While, on one side, the eyes were properly developed but on the other side it had various abnormalities. In the reciprocal crosses, however, (*D. bipectinata* females \times *D. pseudoananassae* males and *D. parabiptinata* females \times *D. pseudoananassae* males) all sons were normal. The abnormalities had several nuances (figure 1). We could also spot a fly which did not have an eye on one side. The asymmetry did not exhibit any side specificity and it was either right or left side which manifested the abnormalities. Such sons also had a poor viability and could not survive for more than two days. The number of sons having abnormal eyes is presented in table 1. The number of males analysed refers to the total male progeny size of the concerned crosses (table 1) and the data show that the frequency varies in two crosses. Also, in the later cross, i.e. *D. pseudoananassae* females \times *D. parabiptinata* males, there ratio being 100 daughters for a single son. Therefore,

Haldane's rule is not only reflected faithfully in the sterility of the hybrid sons (of all the crosses), but also in inviability and morphological abnormalities (eye asymmetry) of sons from certain crosses involving *D. pseudoananassae* as one of the parents. The sex-ratio distortion may be due to lethality at the embryonic/larval/pupal level(s) of development in the males which does not allow sufficient males to emerge and among the ones that are able to eclose too, there are a few in which the eyes are affected and viability is poor. Daughters, on the other hand did not exhibit any visible morphological abnormality (apparent from outside) and no effect could be seen on their viability. For the asymmetric eyes, it is clear that recessive epistatic X-autosomal interactions are involved as none of the daughters were found to have asymmetrical eyes and sons from only one of the reciprocal crosses were affected. The X chromosome of the maternal species, *D. pseudoananassae* (inherited by the sons in both the crosses), is perhaps incompatible with the autosomal genes of the paternal species *D. bipectinata* and *D. parabiptinata*, which is causing the left-right signalling during eye formation to go awry. There must be some X-linked gene(s)



Figure 1. Photographs of eyes of hybrid males showing variation in shape. (a) Normal eyes; (b–h) abnormal eyes.

Table 1. Number of hybrid males showing abnormal eyes.

Females	Crosses		Males analysed	Males with abnormal eyes
		Males		
<i>D. pseudoananassae</i> × <i>D. bipectinata</i>			38	02
<i>D. pseudoananassae</i> × <i>D. parabipectinata</i>			45	20

which along with certain autosomal genes are involved in the left–right signalling cascade during eye formation. The X-linked genes in *D. pseudoananassae* must have diverged to such an extent that in the hybrid background, they cannot work in a coordinated manner along with the autosomal genes from the other species, leading to asymmetrical eye development (recessive epistatic incompatibilities). Indeed, X–Y incompatibilities between *D. pseudoananassae* and the other three species have also been implicated in hybrid male sterility in earlier studies in our laboratory (Mishra and Singh 2006). Not just in *bipectinata* complex, but the faster evolution of the X chromosome was remarked as being the general pattern in the genus *Drosophila* and the genes having the most profound effect on viability and sterility were discovered as being X-linked (Dobzhansky 1936; Coyne and Orr 1989). However, Wu and Davis (1993) pointed out that the involvement of X chromosome becomes more prominent due to its hemizygoty in the males. The factors involved, mostly being recessive are bared in the hemizygous X, while the recessive autosomal incompatible genes may go undetected, having a compatible copy in the F₁ hybrids. The incompatible autosomal factors may be revealed in the F₂ (or backcross) hybrids. However, at this point, we are unable to further dissect the X chromosome (to arrive at the precise incompatible loci), due to lack of X-linked markers. We are also not in a position to detect autosomal recessive epistatic incompatibilities because there is a certain degree of sterility in the hybrid daughters which would impede backcrossing. It would also be interesting to study the expression pattern of genes in the eye imaginal discs both in the parents and the hybrids, which we are unable to accomplish immediately and hope to sort out sometime in the future. Therefore, as of now, we can only say that genes of the X chromosome of *D. pseudoananassae*, be it those involved in sterility or normal eye development have undergone profound divergence from the other three species of the complex.

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