

RESEARCH NOTE

The *Drosophila bipectinata* species complex: degree of sterility and dystrophied ovaries in interspecific hybrid females

PARUL BANERJEE and BASHISTH N. SINGH*

Genetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005, India[Banerjee P. and Singh B. N. 2016 The *Drosophila bipectinata* species complex: degree of sterility and dystrophied ovaries in interspecific hybrid females. *J. Genet.* **95**, 167–170]

Introduction

The failings of the heterogametic sex, quite customary in interspecific hybridizations, have been called Haldane's rule. Haldane (1922) said that, in general, when one sex of hybrids between species is sterile or inviable, it is more frequently the heterogametic one. As suggested by numerous workers, the reason behind this phenomenon is attributed to the X chromosome, which occurs in hemizygous condition in the heterogametic sex. It is always incompatible interactions between the X and either the autosomes or cytoplasmic factors that are involved in the failings of the heterogametic sex (Dobzhansky 1936; Coyne and Orr 1989a, b; Presgraves 2003; Barbash *et al.* 2004; Mishra and Singh 2005; Chang and Noor 2007).

The interspecific hybrids between members of the *Drosophila bipectinata* species complex, comprising four closely related species namely *D. bipectinata*, *D. parabipectinata*, *D. malerkotliana* and *D. pseudoananassae*, are no exception to this well-recognized tenet (the hybrid sons are sterile) and its connection with the X chromosome (Bock 1978; Mishra and Singh 2006, 2007). It has also been found that the hybrid sons are not just sterile but from certain crosses they show other abnormalities too, like poor viability and asymmetrical eyes (Singh and Banerjee 2015).

Interestingly, while conducting studies on fluctuating asymmetry (FA) in this complex, we came across a number of interspecific hybrid daughters with dystrophied ovaries from crosses involving *D. pseudoananassae* as one of the parental species (Banerjee and Singh 2015). This was especially more pronounced in crosses *D. bipectinata* × *D. pseudoananassae* and *D. malerkotliana* × *D. pseudoananassae*, with several daughters showing dystrophied ovaries, holding fibrous bundles in place of ovarioles (figure 1). From the very beginning, no questions have been raised as to the fertility of

the hybrid daughters in this complex. Yet, females with dystrophied ovaries cannot be fertile. This prompted us to study fertility of the F₁ hybrid daughters in the complex and check the degree of sterility in the hybrid daughters.

Materials and methods

In the present study, one strain of each of the four species of *D. bipectinata* species complex was used. They are: *D. bipectinata*, PN 99; *D. parabipectinata*, Mys; *D. malerkotliana*, RC 91; *D. pseudoananassae*, KB284. All the stocks are being maintained in the laboratory on simple yeast–agar medium at ~24°C and 12 h cycle of light and darkness. They have spent a number of generations in the laboratory.

Twelve crosses were set up using the four by 4 × 4 factorial design by putting 20 pairs of seven-days-aged virgin females and males in food vials. The parents were transferred to a fresh set of vials after five days. They were discarded from the second set after another five days. When progeny emerged, females were collected. From crosses where it was possible, 50 females were collected and from the ones in which very few progeny emerged (involving *D. pseudoananassae* as one of the parents), we collected as many females as could be collected. Females were aged for seven days before being used for setting up crosses.

For fertility assay, females were kept individually in food vials with four males (two each from the respective parental species, to increase the probability of matings). They were transferred to a fresh set of vials after five days, and allowed to remain there without being discarded. After about 10 days, the first set of vials were examined for larval activity. Vials in which larval activity was found were noted as having a fertile female. The vials in which no larval activity was observed were kept for a few more days and regularly checked for larval activity. The corresponding second set of vials were also examined for larval activity. Vials in which no larval

*For correspondence. E-mail: bashisthsingh2004@rediffmail.com; bnsingh@bhu.ac.in.

Keywords. interspecific hybridization; dystrophied ovaries; female sterility; *Drosophila bipectinata* species complex.

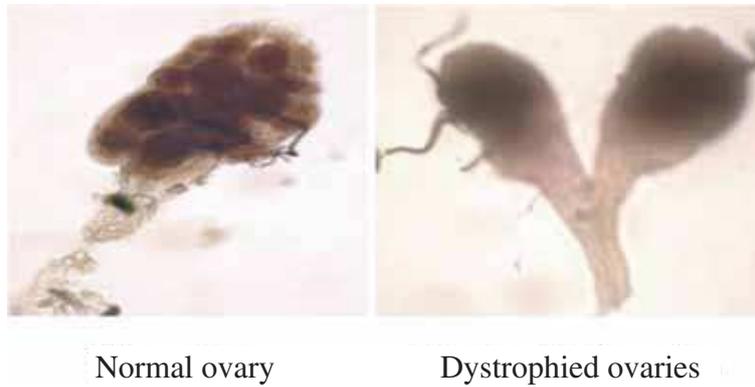


Figure 1. Normal and dystrophied ovaries of hybrid females.

activity was noted in both the sets were scored as having sterile females. Hence, the number of fertile and sterile females from each cross was scored. Likewise, fertility of females of pure species was also scored.

Chi-square values were calculated from R×C contingency table to check whether there is a significant difference in the number of fertile and sterile females among the hybrid daughters and the respective parental females and also to check whether the numbers of fertile and sterile females differ in reciprocal crosses. To test whether fertile and sterile females are distributed randomly in all the six crosses

(values across reciprocal crosses were added), R×C contingency chi-square values were also calculated.

Results and discussion

The numbers of fertile and sterile daughters from crosses *D. bipectinata* × *D. parabipectinata* and *D. bipectinata* × *D. malerkotliana* were not found to differ significantly from the respective numbers in parental females ($P > 0.05$, table 1). Daughters from all the other crosses were found to have

Table 1. R×C contingency chi-square test to check the difference in fertility between hybrid females from reciprocal crosses and between hybrid females and parental males in the *D. bipectinata* species complex.

Type of female	<i>n</i>	No. of fertile females	No. of sterile females	Chi-square
Daughters of bi and pa	50	48	02	} 0.385 } 1.11
Daughters of pa and bi	52	51	01	
bi	50	47	03	
pa	50	48	02	
Daughters of bi and ma	50	45	05	} 1.33 } 3.88
Daughters of ma and bi	50	41	09	
bi	50	47	03	
pa	50	45	05	
Daughters of pa and ma	47	16	31	} 3.46 } 96.7**
Daughters of ma and pa	51	09	42	
ma	50	45	05	
pa	50	48	02	
Daughters of ps and bi	50	01	49	} 43.1** } 102**
Daughters of bi and ps	32	22	10	
bi	50	47	03	
ps	50	39	11	
Daughters of ps and pa	54	04	50	} 1.02 } 110**
Daughters of pa and ps	13	00	13	
pa	50	48	02	
ps	50	39	11	
Daughters of ps and ma	50	13	37	} 4.88* } 71.3**
Daughters of ma and ps	15	00	15	
ma	50	45	05	
ps	50	39	11	

n, Number of females tested; * $P < 0.05$ and ** $P < 0.001$; bi, *D. bipectinata*; pa, *D. parabipectinata*; ma, *D. malerkotliana*; ps, *D. pseudoananassae*; The species represented first in a cross is the female parent.

Table 2. R×C contingency chi-square test to check the difference in fertility among the hybrid females from six interspecific crosses of the *D. bipectinata* species complex.

Type of female	<i>n</i>	No. of fertile females	No. of sterile females	Chi-square
Daughters of bi and pa	102	99	03	257*
Daughters of bi and ma	100	86	14	
Daughters of pa and ma	98	25	73	
Daughters of bi and ps	82	23	59	
Daughters of pa and ps	67	04	63	
Daughters of ma and ps	65	13	52	

n, Number of females tested; * $P < 0.001$; bi, *D. bipectinata*; pa, *D. parabipectinata*; ma, *D. malerkotliana*; ps, *D. pseudoananassae*.

drastically reduced fertility compared to the parental females ($P < 0.001$, table 1). Further, the numbers of fertile and sterile females also differed significantly among daughters from the six crosses ($P < 0.001$, table 2). The number of daughters with dystrophied ovaries was not found to be directly proportional to the number of sterile daughters so, even daughters with normal-looking ovaries could be sterile: for example, in *D. parabipectinata* × *D. malerkotliana* crosses, the females had normal ovaries, but most were sterile.

In other studies undertaken to investigate divergence among the four species, taking into account sexual isolation, and divergence at certain nuclear and mitochondrial loci, even some Y chromosomal loci, it has been proven that, while *D. bipectinata*, *D. parabipectinata* and *D. malerkotliana* are closely related to each other, *D. pseudoananassae* is distantly related to them (Bock 1978; Kopp and Barmina 2005; Lewis *et al.* 2005; Kopp *et al.* 2006; Banerjee and Singh 2012). Since *D. pseudoananassae* diverged earliest, it must have accumulated greater differences, and hence sterility has gone beyond affecting only the hybrid sons to affect the hybrid daughters too.

Coyne and Orr (1989b) said that in *Drosophila* hybridizations in which females are also sterile involve more distantly related species than those in which only males are sterile. Therefore, divergence between certain species pairs in the *D. bipectinata* complex has reached such a level that even the hybrid females have become sterile.

Sterility of the homogametic sex in interspecific hybridizations is far less known in *Drosophila* and hence has been far less studied than in the heterogametic sex. A few studies that have tried to unearth the genetic basis of hybrid female sterility are Lancefield (1929) in two races of *D. obscura*; Orr (1987) in the species pair *D. pseudoobscura*–*D. persimilis*; Davis *et al.* (1994) in *D. simulans*–*D. mauritiana*–*D. sechellia*; Heikkinen and Lumme (1991) in *D. virilis*–*D. lumme*. Sterility of hybrid females in F₁ itself is rare, and understanding the precise genetic mechanism requires complex genetic backcrossing for cointrogression of certain chromosomal blocks from one species into the genetic background of another species. Owing to pronounced operation of Haldane's rule, the effect of hybridization on female

fertility may often get overlooked. Male sterility and reduced female fertility have nevertheless been associated in interspecific hybridizations (Johnson and Wu 1993), indicating that the pathways involved in fertility may be overlapping in the two sexes. Like in case of hybrid male sterility, most of the studies done so far on hybrid female sterility have implicated the X chromosome. In this complex, X chromosome may be a player in epistatic interactions in the species pair *D. bipectinata*–*D. pseudoananassae*. In the other pairs, we are not in a position to point out involvement of the X chromosome. Unfortunately, owing to lack of suitable markers in *D. pseudoananassae*, we cannot go beyond F₁ to study accurately the genetic basis of female sterility in this complex. However, this is the first report of female sterility in this complex and we are certainly in a position to expressly state that among the interspecific hybrids in the *D. bipectinata* species complex the males are sterile and the females (from some crosses) are only partially fertile.

Acknowledgements

Financial assistance in the form of Meritorious Fellowship to PB and UGC-BSR Faculty Fellowship Award to BNS, from the University Grants Commission, New Delhi, is gratefully acknowledged. The authors also thank the handling editor, Dr N. G. Prasad, and the anonymous reviewer for their helpful comments on the original draft of the manuscript.

References

- Banerjee P. and Singh B. N. 2012 Interspecific sexual isolation and phylogeny among different members of the *Drosophila bipectinata* species complex. *Genetica* **140**, 75–81.
- Banerjee P. and Singh B. N. 2015 Interspecific hybridization does not affect the level of fluctuating asymmetry (FA) in the *Drosophila bipectinata* species complex. *Genetica* **143**, 459–471.
- Barbash B. A., Awadalla P. and Parone A. M. 2004 Functional divergence caused by ancient positive selection of a *Drosophila* hybrid incompatibility locus. *PLoS Biol.* **2**, 839–848.
- Bock I. R. 1978 The *bipectinata* complex: a study in interspecific hybridization in the genus *Drosophila* (Insecta: Diptera). *Aust. J. Biol. Sci.* **31**, 197–208.

- Chang A. E. and Noor M. A. F. 2007 The genetics of hybrid male sterility between the allopatric species pair *Drosophila persimilis* and *D. pseudoobscura bogotana*: dominant sterility alleles in collinear autosomal regions. *Genetics* **176**, 343–349.
- Coyne J. A. and Orr H. A. 1989a “Patterns of speciation in *Drosophila*” revisited. *Evolution* **51**, 295–303.
- Coyne J. A. and Orr H. A. 1989b Two rules of speciation. In: *Speciation and its consequences* (ed. D. Otte and J. A. Endler), pp. 180–207. Sinauer associates, Sunderland, USA.
- Davis A. W., Noonberg E. G. and Wu C. I. 1994 Evidence for complex genic interactions between con specific chromosomes underlying hybrid female sterility in the *Drosophila simulans* clade. *Genetics* **137**, 191–199.
- Dobzhansky T. 1936 Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura*, hybrids. *Genetics* **21**, 113–135.
- Haldane J. B. S. 1922 Sex-ratio and unidirectional sterility in hybrid animals. *J. Genet.* **12**, 101–109.
- Heikkinen E. and Lumme J. 1991 Sterility of male and female hybrids of *Drosophila virilis* and *D. lumme*. *Heredity* **67**, 1–11.
- Johnson N. A. and Wu C.-I. 1993 Evolution of postmating reproductive isolation: measuring the pleiotropic fitness effects associated with hybrid male sterility factors. *Am. Nat.* **142**, 213–223.
- Kopp A. and Barmina O. 2005 Evolutionary history of the *Drosophila bipectinata* species complex. *Genet. Res. Camb.* **85**, 23–46.
- Kopp A., Frank A. K. and Barmina O. 2006 Interspecific divergence, intrachromosomal recombination and phylogenetic utility of Y-chromosomal genes in *Drosophila*. *Mol. Phyl. Evol.* **38**, 731–741.
- Lancefield D. E. 1929 A genetic study of crosses of two races or physiological species of *Drosophila obscura*. *Z. Indukt. Abstam. Vererbung.* **52**, 287–317.
- Lewis R. L., Beckenbach A. T. and Mooers A. O. 2005 The phylogeny of the subgroups within the *melanogaster* species group: Likelihood tests on *COI* and *COII* sequences and a Bayesian estimate of phylogeny. *Mol. Phyl. Evol.* **37**, 15–24.
- Mishra P. K. and Singh B. N. 2005 Why hybrid males are sterile in *Drosophila*? *Curr. Sci.* **89**, 1813–1819.
- Mishra P. K. and Singh B. N. 2006 Genetic interactions underlying hybrid male sterility in the *Drosophila bipectinata* species complex. *Genes Genet. Syst.* **81**, 193–200.
- Mishra P. K. and Singh B. N. 2007 Assessing the putative roles of X-autosome and X–Y interactions in hybrid male sterility of the *Drosophila bipectinata* species complex. *Genome* **50**, 653–659.
- Orr H. A. 1987 Genetics of male and female sterility in *Drosophila pseudoobscura* and *D. persimilis*. *Genetics* **116**, 555–563.
- Presgraves D. C. 2003 A fine scale genetic analysis of hybrid incompatibilities in *Drosophila*. *Genetics* **163**, 955–972.
- Singh B. N and Banerjee P. 2015 Sex-specific asymmetry in eye development in the interspecific hybrids in the *Drosophila bipectinata* species complex. *J. Genet.* **94**, 493–495.

Received 7 May 2015, in final revised form 12 June 2015; accepted 6 July 2015

Unedited version published online: 8 July 2015

Final version published online: 12 January 2016