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Abstract

BRCA1 and BRCA2 function in homologous meiotic recombination, and mutations in these genes cause human breast and ovarian cancer. Nevertheless, disruptive mutations in these genes are present at an unexpectedly high frequency in human populations. A recent study suggested that BRCA1/2 mutations are maintained because they increase the early-life fertility of their carriers (Smith et al., 2011). However, further study of this unexpected fitness advantage in humans is impeded by family planning strategies. Just like its human counterpart, Drosophila BRCA2 (dmbrca2) interacts with RAD51 and functions in DNA repair by homologous recombination (Klovstad et al., 2008) and thus may experience similar selective pressures. We produced flies heterozygous for a BRCA2 knockout and compared fecundity to flies not bearing the mutation in the first two days postcopulation to test for a fitness advantage similar to that observed in humans. In contrast to humans, Drosophila heterozygous for a disruption of BRCA2 do not show increased...
numbers of eggs compared to their wild-type counterparts, indicating that disruption of this gene does not confer a similar fecundity advantage across species.

Introduction

Human germline mutation carriers of BRCA1 and BRCA2 mutations have up to an 80% lifetime risk of developing breast cancer (Thorlacius et al., 1998). The protein products of tumor-suppressor genes BRCA1/2 are ubiquitously expressed and function in transcription, DNA repair and recombination, and checkpoint control of cell cycle (Venkitaraman, 2002). Women with BRCA1/2 mutations may develop cancer before menopause, resulting in moderate negative selection against these alleles (Smith et al., 2011; da Silva, 2012). In spite of this selective pressure, these mutations remain curiously prevalent in human populations.

A recent study suggested that BRCA1/2 mutations are maintained in human populations because they increase the fertility of their carriers (Smith et al., 2011). BRCA1/2 mutations correlated with increased fertility in human populations. However, family planning makes further examination of this unexpected fitness advantage in humans challenging (Smith et al., 2011). Understanding whether BRCA1/2 mutations have the same effect in model species would facilitate the use of these model species to understand better the genetics that control human cancer susceptibility and possibly treatment.

*Drosophila melanogaster* is an ideal model for the study of reproductive advantages conferred by BRCA2 mutations. The *Drosophila* transcript corresponding to CG30169, hereafter known as *dmbrca2*, is a homolog for human BRCA2 (Lo et al., 2003; Klovstad et al., 2008). *Drosophila dmbrca2* is known to function in DNA repair by homologous recombination (Klovstad et al., 2008) and interact with Rad51 just like its human counterpart (Brough et al., 2008). Also similar to humans, *D. melanogaster* exhibit naturally occurring nonsynonymous polymorphisms in *dmbrca2* that appear to have been maintained by balancing selection (Langley et al., 2012). However, *Drosophila* are easy to rear and can be subjected to controlled mating to study the impact of BRCA2 mutations. Identifying a reproductive advantage associated with BRCA2 mutations would not only support the result found in human populations, but would serve as the foundation for the use of *D. melanogaster* as a model species for the study of the evolutionary forces acting to maintain variation at this disease-relevant gene in humans.

In the present study we tested whether disruption of *dmbrca2* increased the fecundity of heterozygous females.

Methods

Three strains of *Drosophila melanogaster* were used in this study. We used two wild type strains, Zimbabwe 29 and Zimbabwe 30 (kindly provided by C. Aquadro and C.-I Wu), and Bloomington Drosophila Stock Center stock number 13272. Stock number 13272 has a P-element inserted immediately upstream of the coding region of *dmbrca2* that prevents expression of the protein product (y[1] w[67c23]; P{y+[mDint2] w [BR.E.BR]=SUPor-P}CG30169[KG03961]). Barnwell et al. (2008) showed this P-element insertion disrupts expression of the transcript. For simplicity, the stocks used will be referred to as “Z29”, “Z30”, and “P-dmbrca2”.

Several crosses were employed to measure the fertility of *dmbrca2* heterozygotes without subjecting the lines to a potential loss in fertility due to inbreeding depression. First, P-*dmbrca2* was crossed to Z30. This cross produced two classes of progeny, half with the P-element disruption
upstream of \textit{dmbrca2}, and half with the balancer. The female progeny of this cross that did not bear the balancer were individually pair mated to a Z29 male. The second cross ensured that the resultant progeny would not be homozygous for segments derived from any single strain.

To assay the fecundity of \textit{dmbrca2} heterozygotes, female progeny of the Z29 male cross were individually paired with two Z30 males for one day. The females were then transferred to a grape agar petri dish. We scored the number of eggs and larvae produced by each female one and two days after the female flies were transferred to the petri dish. To confirm fertilization, a subset of females that did not produce larvae were dissected, and presence of sperm was assayed by microscopy. We performed PCR to determine whether these mothers were heterozygous for the P-element disruption of \textit{dmbrca2} (F: 5'-TGGGATAGCCTGTTCGGATGAC-3'; R: 5'-GCAACGCTTACCAACAC-TGCA-3'; P-element: 5'-GCTATCGACGGGACCACCTTATG-3'). This three-primer combination yielded a 130 bp band for chromosomes bearing the P-element insertion and a 152 bp band for chromosomes without a P-element. PCR products were visualized on 3% TBE agarose gels. We scored 177 mothers for fecundity and genotype. The fecundity difference between mutant and wild type mothers was tested with a nonparametric Mann-Whitney U-test.

**Results and Discussion**

We estimated an overall fertilization rate of 91% based on the combined percentage of females that had eggs hatch to larvae or bore sperm upon dissection (N = 111). Despite this high fertilization rate, there was extensive day-to-day variation in the number of eggs laid by both \textit{dmbrca2} heterozygotes and wild type females (Figure 1). A Kruskal-Wallis test found that this variation in egg laying was predominantly explained by the day that the flies were allowed to lay eggs (N = 176, p < 0.0001).

![Figure 1](image.png)

Figure 1. Both \textit{dmbrca2} heterozygotes and wild type females showed large variation in the number of eggs they laid.

Both with and without controlling for the daily variance, females heterozygous for the P-element disruption of \textit{dmbrca2} did not lay significantly more eggs than wild type females (see Figure
The lack of difference in fecundity between \textit{dmbrca2} heterozygotes and wild type extended to the number of larvae found on the second day (Mann-Whitney U-test, N = 155, p = 0.28). In fact, wild type females had slightly more larvae on average than the \textit{dmbrca2} heterozygotes.

Overall, we fail to see a fecundity advantage associated with heterozygosity of a \textit{dmbrca2} disruptive mutation. In contrast, flies homozygous for this disruption laid few eggs and exhibited reduced meiotic recombination (2008). Hence, the high nonsynonymous polymorphism in \textit{dmbrca2} across \textit{D. melanogaster} natural populations (Langley et al., 2012) is not readily explained by overdominant effects on fecundity, at least in the first two days after mating and for this particular mutation. We cannot exclude the possibility that fitness differences do exist but were not apparent because of specific conditions in which the flies were raised and/ or fecundity differences that may have occurred later after mating.

Furthermore, our result contrasts the previous finding that human BRCA2 disease-causing mutation heterozygotes have significantly more children (Smith et al., 2011). This contrast may represent interspecies differences in the function of BRCA2, or it may be a result of the nature of the specific mutation used to disrupt \textit{dmbrca2} in our \textit{Drosophila} study. Like humans, \textit{D. melanogaster} show naturally occurring nonsynonymous polymorphisms in \textit{dmbrca2} across different populations that appear have been maintained by balancing selection possibly due to antagonistic pleiotropy (Langley et al., 2012). Indeed, a human BRCA2 variant has been associated both with increased breast cancer risk and increased fetal viability (Healey et al., 2000). This contrast lends itself to the hypothesis that it is not the disruption of BRCA2 that might cause a fecundity advantage in heterozygotes, but instead the type of disruption, its interaction with other species-specific factors, or both.

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**Electrophoretic variants of xanthine dehydrogenase enzyme in natural populations of \textit{Drosophila ananassae}.*

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Allozymes are allelic variants of enzymes which are encoded by structural genes. Polymorphism at enzyme loci arise when mutation occurs followed by the action of evolutionary