Gamete-Type Dependent Crossover Interference Levels in a Defined Region of *Caenorhabditis elegans* Chromosome V

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ABSTRACT In certain organisms, numbers of crossover events for any single chromosome are limited ("crossover interference") so that double crossover events are obtained at much lower frequencies than would be expected from the simple product of independent single-crossover events. We present a number of observations during which we examined interference over a large region of *Caenorhabditis elegans* chromosome V. Examining this region for multiple crossover events in heteroallelic configurations with limited dimorphism, we observed high levels of crossover interference in oocytes with only partial interference in spermatocytes.

KEYWORDS

recombination
crossover interference
meiosis
Caenorhabditis elegans

Despite their occurrence in several systems, interference phenomena in which one crossover event on a chromosome reduces the probability of a second crossover have remained something of a mystery (Berchowitz and Copenhaver 2010). *Caenorhabditis elegans* provides an outstanding model for meiotic regulation and a system of choice to study detailed genetic properties of meiosis (Zetka 2009). Although interference certainly occurs in *C. elegans* (Hodgkin *et al.* 1979), literature reports tend to differ regarding levels of interference depending on age, temperature, gender, linkage group, and experimental design. Several individual observations are described below:

a. A complete or near-complete lack of double crossovers is seen on the X chromosome in oocytes (Hodgkin *et al.* 1979; Hayashi *et al.* 2010).
b. Hodgkin *et al.* (1979) show double crossovers during spermatogenesis in a region of LGIV, albeit with ~55% interference (Hodgkin *et al.* 1979).
c. Zetka and Rose (1995) find double crossover in males in a region of LGI at a frequency that suggested little or no interference during spermatogenesis (Zetka and Rose 1995).

d. Meneely *et al.* (2002) fail to detect double crossovers during spermatogenesis in a region of LGIII (Meneely *et al.* 2002).
e. Analyses of double crossover frequencies detected in the case of fused chromosomes performed by Hillers and Villenueve (2003) suggest that the *C. elegans* crossover control mechanism recognizes a DNA unit defined by a region capable of continuous homologous synapsis (Hillers and Villeneuve 2003).
f. Nabeshima *et al.* (2004) report almost complete crossover interference in oocytes on chromosomes I and X (Nabeshima *et al.* 2004).
g. Henzel *et al.* (2011) found crossover distribution along chromosome IV portion of mnT12 (fusion of IV and X chromosomes) to be significantly different between males and hermaphrodites. Their findings in males indicate that crossovers in adjacent chromosome intervals are discouraged, with the strength of crossover interference diminished by distance (Henzel *et al.* 2011).
h. Hammarlund *et al.* (2005) report essentially complete interference for both oogenesis and spermatogenesis in hermaphrodite animals, on chromosomes V and X in spite of a large homology gap created by a heterozygous large insertion. The homology gap apparently alters crossover distribution but not the interference (Hammarlund *et al.* 2005).
i. A number of studies provide data consistent with differential regulation of crossover interference in the two gamete lines (oocytes and sperm) (Howell *et al.* 1987; K. S. McKim and M. C. Zetka unpublished results cited in Zetka and Rose 1995; Lim *et al.* 2008).

In addition to differences in the region assayed for interference, we noted that the diverse nature of experimental designs somewhat limits direct comparisons between studies. Some studies used strains in

which extensive polymorphic differences between chromosomes could conceivably affect pairing dynamics, whereas others used strains with known differences limited to small numbers of experimentally induced mutant alleles. Other growth and assay conditions also differed between studies in this area (including growth temperature, which is known to affect recombination frequencies in *C. elegans*) (Lim *et al.* 2008). We describe crossover interference levels in oogen-

esis and spermatogenesis observed using three widely spaced markers on *C. elegans* LGV (Figure 1A).

MATERIALS AND METHODS

Mapping markers LGV: *unc-60(e723)*, *dpy-11(e224)*, *rol-9(sc148)* Used for production of feminized animals LGII: *tra-2(q122gf)* Other mutations present (in post-recombination tester strains)
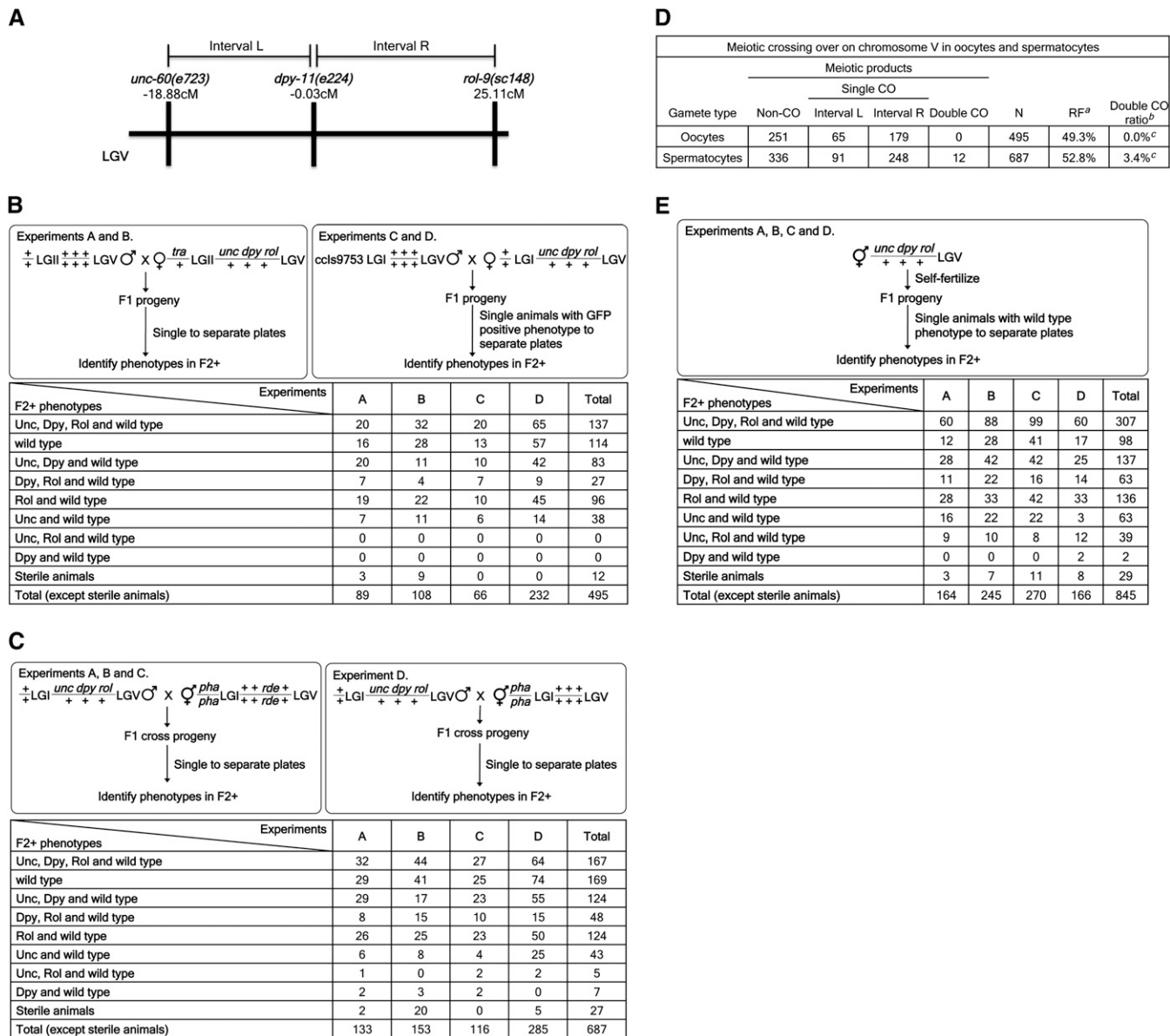

Figure 1 (A) Map positions of the genetic markers used in crossover assays. Schematic diagram indicates the markers used in recombination analysis and their corresponding genetic map positions. The markers used and the intervals assessed (L, R) for the three-marker analysis are indicated above the map. Map positions given in the figure are from Wormbase (Harris *et al.* 2010). (B) Crossover frequencies in oogenesis. Table entries are counts of F1 genotypes from each cross, as detected by examination of F1 progeny. Experiments A, B, and D were conducted at 23°, and experiment C was conducted at 16°. *tra* = *tra-2(q122gf)*; *unc* = *unc-60(e723)*; *dpy* = *dpy-11(e224)*; *rol* = *rol-9(sc148)*; ccIs9753, integration on chromosome I of *myo2::gfp*, *pes-10::gfp* and *gut::gfp*. (C) Crossover frequencies in spermatogenesis. Table entries are counts of F1 genotypes from each cross, as detected by examination of F1 progeny. Experiments A, B, and D were conducted at 23°, and experiment C was conducted at 25°. *rde* = *rde-1(ne300)*; *unc* = *unc-60(e723)*; *dpy* = *dpy-11(e224)*; *rol* = *rol-9(sc148)*; *pha* = *pha-1(e2123ts)*. (D) Meiotic crossing-over on chromosome V in oocytes and spermatocytes. [a][(Total number of crossover events)/(number of meiotic products analyzed)] × 100. [b][(Number of double crossovers)/(number of single crossovers + number of double crossovers)] × 100. [c]Oocytes and spermatocytes differed significantly with respect to the relative incidence of double crossovers *vs.* single crossovers among meiotic products with crossover. Fisher exact test for independence p-value = 0.002. (E) Crossover frequencies in self-fertilization. Table entries are counts of F1 genotypes resulting from the self-fertilization, as detected by examination of F1 progeny. All experiments were conducted at 23°. *unc* = *unc-60(e723)*; *dpy* = *dpy-11(e224)*; *rol* = *rol-9(sc148)*.

D — Meiotic crossing over on chromosome V in oocytes and spermatocytes

Gamete type	Non-CO	Single CO Interval L	Single CO Interval R	Double CO	N	RF[a]	Double CO ratio[b]
Oocytes	251	65	179	0	495	49.3%	0.0%[c]
Spermatocytes	336	91	248	12	687	52.8%	3.4%[c]

B — Experiments A, B, C and D

F2+ phenotypes	A	B	C	D	Total
Unc, Dpy, Rol and wild type	20	32	20	65	137
wild type	16	28	13	57	114
Unc, Dpy and wild type	20	11	10	42	83
Dpy, Rol and wild type	7	4	7	9	27
Rol and wild type	19	22	10	45	96
Unc and wild type	7	11	6	14	38
Unc, Rol and wild type	0	0	0	0	0
Dpy and wild type	0	0	0	0	0
Sterile animals	3	9	0	0	12
Total (except sterile animals)	89	108	66	232	495

E — Experiments A, B, C and D

F2+ phenotypes	A	B	C	D	Total
Unc, Dpy, Rol and wild type	60	88	99	60	307
wild type	12	28	41	17	98
Unc, Dpy and wild type	28	42	42	25	137
Dpy, Rol and wild type	11	22	16	14	63
Rol and wild type	28	33	42	33	136
Unc and wild type	16	22	22	3	63
Unc, Rol and wild type	9	10	8	12	39
Dpy and wild type	0	0	0	2	2
Sterile animals	3	7	11	8	29
Total (except sterile animals)	164	245	270	166	845

C — Experiments A, B and C / Experiment D

F2+ phenotypes	A	B	C	D	Total
Unc, Dpy, Rol and wild type	32	44	27	64	167
wild type	29	41	25	74	169
Unc, Dpy and wild type	29	17	23	55	124
Dpy, Rol and wild type	8	15	10	15	48
Rol and wild type	26	25	23	50	124
Unc and wild type	6	8	4	25	43
Unc, Rol and wild type	1	0	2	2	5
Dpy and wild type	2	3	2	0	7
Sterile animals	2	20	0	5	27
Total (except sterile animals)	133	153	116	285	687

LGI: ccIs9753(myo2::gfp, pes-10::gfp, gut::gfp) LGIII: pha-1(e2123ts) LGV: rde-1(ne300).

Standard methods were used for growing and handling animals. Homozygous triple-mutants having unc-60(e723), dpy-11(e224), and rol-9(sc148) mutations on LGV were generated (Brenner 1974) and used to measure the crossover frequency in the experiments described. Crossover interference values are presented as [1−(observed double crossover events)/(expected double crossover events)]×100.

We note the formal possibility that a fraction of apparent double crossovers may actually reflect gene conversion events. An upper bound on gene conversion for C. elegans can be deduced from two-point mapping data (Moerman and Baillie 1979; McKim et al. 1988), with data relevant to the dpy-11 lesion e224 available from Rosenbluth et al. (1988). In all cases, gene conversions can be inferred to represent a sufficiently rare category of events to be negligible in our interference analysis.

Oogenesis double-crossover frequency

To measure recombination frequency in oogenesis, we performed two parallel experiments with a similar experimental outline. First, a cross was performed between males with a wild-type chromosome V and unc-60(e723) dpy-11(e224) rol-9(sc148) V heterozygous triple-mutant hermaphrodites. Progeny were singled onto separate plates. Genotypes of the animals resulting from this cross were determined through subsequent self-fertilization and examination of subsequent generations for appearance of Unc, Dpy, and Rol phenotypes. Selection of cross progeny in the first generation cross is critical for interpretation of the gametic source of recombination.

In a first set of experiments, the heterozygous triple-mutant unc-60 (e723) dpy-11(e224) rol-9(sc148)/+++ were crossed with males having integrated GFP markers on chromosome I, allowing specific selection of the cross progeny. Cross-progeny animals were singled onto separate plates and their progeny were used to quantify different crossover events. Note in this case that introduction of the transgene postdates the inferred recombination events.

The second set of experiments initiated from a cross of homozygous triple-mutants to a tra-2(q122gf) males resulted in a population of heterozygous XX females carrying the feminizing mutation tra-2 (q122gf). Because these hermaphrodites are not able to self-fertilize because of the tra-2(q122gf) mutation, a subsequent cross with wild-type (N2) males resulted in only cross-progeny animals. Cross-progeny animals were singled onto separate plates and their progeny were used to identify parental genotypes and thus to quantify recombination frequencies. Singled animals carrying the tra-2(q122gf) mutation on chromosome II were discarded because of their inability to self-fertilize.

Male spermatogenesis double-crossover frequency

To quantify double-crossover frequency in male spermatogenesis, we crossed unc-60(e723) dpy-11(e224) rol-9(sc148)/+++ heterozygous triple-mutant males to pha-1(e2123ts) hermaphrodites at the nonpermissive temperature. Homozygous pha-1(e2123ts) embryos failed to survive growth at temperatures more than 23°, ensuring survival of only cross progeny. Cross-progeny animals were singled onto separate plates, with occurrence of phenotypes in subsequent generations used to determine genotypes and to detect crossover events.

Self-fertilization double-crossover frequency

To quantify double crossovers in self-fertilization, we allowed unc-60 (e723) dpy-11(e224) rol-9(sc148)/+++ heterozygous triple-mutant animals to self-fertilize and then singled to separate plates their pheno-

typically wild-type progeny. Although not all double-crossover events can be detected (because of the potential for recombinant chromosomes originating from both gametes), one of the genotypes (dpy-11(e224)/+) can only be produced through a double-crossover–type event.

Confidence Intervals

The confidence intervals (C.I.s) were calculated using the Clopper and Pearson exact method for binomial proportion (Clopper and Pearson 1934).

RESULTS

Measurements of interference entail a comparison of the product of individual two-point recombination frequencies between markers with the corresponding double-crossover frequency. We used standard genetic crosses to measure the frequency of single and multiple crossover events. Because C. elegans is a hermaphroditic species, we assayed frequencies in hermaphrodite oogenesis, male spermatogenesis, and in an aggregate measure that combines hermaphrodite spermatogenesis and oogenesis (Figure 1, B, C, and E, respectively).

Figure 1B presents the set of results observed in oogenesis. The observed oocyte two-factor recombination frequencies in our experiments were 13.1% [in agreement with results reported by Hammarlund et al. (2005)] and 36.2%, respectively, for the unc-60(e723)-to-dpy-11 (e224) (interval L) and dpy-11(e224)-to-rol-9(sc148) (interval R) intervals (95% C.I.s, 10.3%–16.42% and 31.9%–40.6%, respectively) (Clopper and Pearson 1934). In the absence of crossover interference, one would expect 4.75% double recombinants in our experimental setup. Given 495 examined animals, 24 double recombinants would have been expected. We detected no double recombinants in oocyte meiosis, indicating high crossover interference (95% C.I., 87.5%–100% crossover interference).

Figure 1C presents results obtained in male spermatogenesis. The observed male spermatocyte two-factor recombination frequencies in our experiments were 15.0% and 37.8%, respectively, for the intervals L and R (95% C.I.s, 12.4%–17.9% and 34.2%–41.6%, respectively). The 12 cases of double crossovers in a population of 687 animals suggested partial, but not complete, crossover interference in the range of 69.2% (95% C.I., 48.7%–84.6% interference).

This analysis provides evidence for gender-specific interference effects. Despite comparable levels of total recombination (49.3% and 52.8%, respectively, for the assayed portion of chromosome V), we found an extreme difference in proportion of double-crossover events with double-crossover products not detected in oocyte meiosis while accounting for 3.4% of total crossover products from male spermatocyte meiosis. The difference between oocytes and male spermatocytes is statistically significant, with p-value of 0.002 (Figure 1D).

In these experiments, the interrogated oocytes and sperm differ in both cell biology and chromosome content (oocytes were from 2X:2A animals, sperm were from 1X:2A animals). An additional self-fertilization experiment was performed to assess double crossovers in XX sperm. As diagrammed in Figure 1E, we could unambiguously distinguish a class of double-crossover events from self-fertilization. Assuming complete crossover interference in oogenesis and 69.2% interference in spermatogenesis, the number of expected double recombinants that could be detected in our approach in the examined population of 845 animals would be three. In our self-fertilizing experiment, we have detected two double-recombinant animals. Because the previous outcross experiments had ruled out substantial double-crossover events during XX hermaphrodite oogenesis, we inferred that the self-fertilization–observed double crossovers were likely to have occurred during hermaphrodite (XX) spermatogenesis.

CONCLUSION

The use of distant genetic markers on LGV allowed us to monitor double crossovers and interference intensity in oogenesis and spermatogenesis. We found interference for LGV during oogenesis to be very high (with the observation of zero detected double recombinants among 495 sampled meioses compared to an expectation of 24 for no interference), suggesting complete or at least near-complete interference. Crossover interference during male spermatogenesis was not complete, with 12 double-recombinant animals detected among 687 sampled (expected number for no interference, 39; interference, 69.2%). The cytological mechanism underlying the dichotomy of high interference in oogenesis and lower interference in spermatogenesis remains a question for future work.

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