

Postcopulatory Sexual Selection Reduces Genetic Diversity in Experimental Populations of *Caenorhabditis elegans*

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Abstract

Postcopulatory sexual selection affects the evolution of numerous features ranging from mating behavior to seminal fluid toxicity to the size of gametes. In an earlier study of the effect of sperm competition risk on sperm size evolution, experimental populations of the nematode *Caenorhabditis elegans* were maintained either by outcrossing (sperm competition present) or by selfing (no sperm competition), and after 60 generations, significantly larger sperm had evolved in the outcrossing populations. To determine the effects of this selection on population genetic variation, we assessed genetic diversity in a large number of loci using random amplification of polymorphic DNA-PCR. Nearly 80% of the alleles present in parental strain populations persisted in the 6 experimental populations after the 60 generations and, despite a 2.2-fold difference in expected heterozygosity, the resulting levels of genetic variation were equivalent between the outcrossing and selfing experimental populations. By inference, we conclude that genetic hitchhiking due to sexual selection in the experimental populations dramatically reduced genetic diversity. We use the levels of variation in the selfing populations as a control for the effects of drift, and estimate the strength of sexual selection to be strong in obligatorily outcrossing populations. Although sequential hermaphrodites like *C. elegans* probably experience little sexual selection in nature, these data suggest that sexual selection can profoundly affect diversity in outcrossing taxa.

Sexual selection continues after copulation in the forms of sperm competition and female sperm manipulation (Birkhead and Møller 1998). Male adaptations to postcopulatory sexual selection include copulatory plugs (Thornhill and Alcock 1983), penile brushes, and scrapers for removing preexisting sperm (Waage 1979), toxic seminal secretions (Chapman et al. 1995; Rice 1996; Wigby and Chapman 2005) increased ejaculate size during high-risk matings (Baker and Bellis 1993; Gage and Barnard 1996), and production of sperm with greater competitiveness (LaMunyon and Ward 1998, 1999). Recent selection experiments have shown that manipulating the intensity of sperm competition results in evolution of sperm morphology in nematodes (LaMunyon and Ward 2002) and of ejaculate investment and composition in *Drosophila* (Pitnick et al. 2001), establishing a causal link between these adaptations and postcopulatory sexual selection.

As with other forms of positive selection, postcopulatory sexual selection on a given gene will also affect neighboring-linked loci by dragging linked neutral polymorphisms to fixation, resulting in reduced genetic variation surrounding

the target of selection (Maynard Smith and Haigh 1974; Braverman et al. 1995). Such genetic hitchhiking in response to selection on a polygenic trait will cause a reduction in population genetic diversity, mimicking the effect of a reduction in population size (Foley 1992). In the absence of coincident demographic effects, the magnitude of the resulting decline in polymorphism reflects the strength of selection.

Here, we investigate the effect of sexual selection on genetic variation using *Caenorhabditis elegans* as a model. In a previous study of experimental evolution in *C. elegans*, 2 treatments of sexual selection intensity were considered by genetically manipulating the mode of reproduction (LaMunyon and Ward 2002). Populations were composed of 1) self-fertilizing hermaphrodite worms, resulting in the absence of sexual selection or 2) obligately outcrossing males and functional females, allowing sexual selection to occur (LaMunyon and Ward 2002). At the end of 60 generations, sperm volume had increased by 20% in the outcrossing populations but not in the selfing populations, indicating the presence of postcopulatory sexual selection in the form of sperm competition. Male

body size also increased slightly in the outcrossing populations, suggesting that some precopulatory sexual selection also took place (LaMunyon and Ward 1999, 2002). In the present study, we assess the amount of neutral population genetic variation in these 2 experimental treatments with random amplification of polymorphic DNA (RAPD)-PCR (Williams et al. 1990) and use the resulting estimates of polymorphism to measure the effect of this selection on genetic diversity and to infer the strength of sexual selection in this experiment. These results illustrate the potential for intense sexual selection to dramatically reduce genetic variation within populations.

Materials and Methods

Experimental Populations

Populations of *C. elegans* are normally androdioecious, being composed of both self-fertile hermaphrodites and rare males. In the selection experiment of LaMunyon and Ward (2002), the mode of reproduction was altered to examine the effect of permitting sperm competition on the evolution of sperm morphology. Reproduction was manipulated using the *spe-8(bc53)* mutation that disrupts spermiogenesis in hermaphrodites, but leaves male sperm unaffected (L'Hernault et al. 1988; Shakes and Ward 1989; Muhrad and Ward 2002). Therefore, the *spe-8(bc53)* mutation renders *C. elegans* populations male/female and results in increased risk of sperm competition as well as other pre- and postcopulatory forms of sexual selection that might not occur in selfing populations.

The experimental populations of worms were constructed from 5 inbred strains (CB4855, DR1345, DR1350, AB1, and N2) that were originally collected from different geographic locations worldwide (Hodgkin and Doniach 1997). These strains have different RAPD-PCR profiles from each individual and, because of intense inbreeding in the laboratory, are taken to be homozygous at all of their loci within a given strain. Therefore, by initiating experimental populations with similar numbers of individuals from each founder strain (or derivative thereof), the initial genetic diversity was equalized among the populations.

There were 2 types of experimental treatments constructed: cross-fertilizing populations and self-fertilizing populations. We constructed 3 replicate cross-fertilizing populations (Cross1, Cross2, and Cross3), each being homozygous for *spe-8(bc53)* and each founded with 60 hermaphrodites and 120 males, taken equally from the 5 inbred strains (see LaMunyon and Ward 2002 for details). Three replicate self-fertilizing experimental populations (Self1, Self2, and Self3) were each founded with 60 hermaphrodites that were heterozygous for *spe-8(bc53)*. As with the crossing populations, the selfing populations were initiated with equal numbers of worms taken from the wild isolates, yielding equivalent average genetic diversity among the hermaphrodites in the initial experimental populations.

The populations were maintained at 20 °C in petri dishes on agar seeded with *Escherichia coli* strain OP50 (Brenner 1974) for 60 generations. Each new generation was founded by transferring to new plates 60 subadult (L4) hermaphrodites or females, and for the outcrossing populations, an additional 100 L4 males. Consequently, we calculate the

effective size (N_e) of populations in selfing and outcrossing treatments as 30 and 150, respectively (Hedrick 2000). Note that this estimate of N_e in the selfing lines is upwardly biased because *spe-8(bc53)* homozygotes created in the course of the experiment would be sterile, thus reducing the number of transferred individuals capable of self-fertilization; the minimum N_e for the selfing lines is 22.5. In addition, because it was critical that opportunity for sexual selection existed in each generation, the interval between transfers was 4 days that exceeds the generation time by 1 day at 20 °C. The transferred progeny were thus produced 1 full day after their parents were paired and were likely to have been fertilized after significant mating activity and competition among the ejaculates of different males (LaMunyon and Ward 2002). At this worm density, mating activity is intense: periodic observations revealed that $34 \pm 2\%$ (mean \pm standard error of the mean) of hermaphrodites have one or more males in attendance actively mating or attempting to mate. Furthermore, hermaphrodites exposed to males for 1 day contain on average 434 ± 62 ($n = 13$) sperm, whereas unmated hermaphrodites have only 180 ± 10 sperm ($n = 15$). Thus, in the crossing populations, hermaphrodites received nearly 250 sperm over the course of 1 day of mating activity, and the number of sperm transferred in a given copulation is small, averaging less than 50 (Hodgkin and Doniach 1997). Therefore, mate competition and sperm competition were likely intense in the outcrossing populations that led to the evolution of larger, more competitive sperm (LaMunyon and Ward 2002).

The RAPD-PCR

We used RAPD-PCR (Williams et al. 1990) to randomly assess population genetic variation. All PCR reactions were conducted in 25 μ l volumes with the following components: 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM MgCl₂, 2 mM each deoxynucleotide triphosphate, 0.8 μ M primer, 0.02 U/ μ l Taq polymerase. The PCR primers were 10mer oligonucleotides available in sets OPA and OPB from Operon Technologies®. Cycling conditions were as follows: an initial denaturation at 94 °C for 60 s, then 45 cycles of 94 °C for 30 s, 38 °C for 30 s, and 72 °C for 60 s. PCR products were separated on 2% agarose gels in a TBE buffer system and visualized by ethidium bromide staining.

Applying the above protocol to single worm PCR (Williams 1995) of the experimental populations (Self1, Self2, Self3, Cross1, Cross2, Cross3), we assessed genetic variation within and between the populations and breeding systems. Single L4 hermaphrodites (or females) were transferred to the wells of 96-well PCR plates in 2.5 μ l of worm lysis buffer using the protocol of Plasterk (1995). The PCR plates were incubated at -80 °C for 30 min, 65 °C for 1 h, and 95 °C for 15 min, after which the PCR cocktail was added and cycling commenced as described above (Plasterk 1995). Eight worms from each experimental population were used per PCR primer, totaling 48 worms per primer. The primers included OPA-3, OPA-17, OPA-18, OPA-19, OPB-7, OPB-15, OPB-17, and OPB-18. Nine RAPD-PCR bands that were present in both *E. coli* OP50 DNA

Table 1. Estimates of diversity from Shannon's diversity index (H'_j)

Primer	Number of products	Number polymorphic	H'_j								H'_{pop}	H'_{tot}	H'_{pop}/H'_{tot}	$1-H'_{pop}/H'_{tot}$
			Self1	Self2	Self3	Self mean	Cross1	Cross2	Cross3	Cross mean				
OPA-19	14	13	3.87	3.67	3.95	3.83	4.07	3.85	4.64	4.18	4.01	4.88	0.82	0.18
OPA-17	13	11	2.32	2.60	2.50	2.48	2.90	2.65	1.44	2.33	2.40	3.37	0.71	0.29
OPB-15	9	9	1.54	1.57	1.17	1.43	2.03	3.06	2.76	2.62	2.02	2.89	0.70	0.30
OPB-17	9	7	1.29	0.54	1.21	1.01	0.17	0.92	0.88	0.66	1.24	1.56	0.80	0.20
OPA-3	6	5	1.52	1.04	1.26	1.28	1.18	1.54	1.25	1.33	1.30	1.64	0.80	0.20
OPA-18	6	5	1.70	1.68	0.68	1.35	1.66	1.00	0.46	1.04	1.20	1.48	0.81	0.19
OPB-7	4	4	0.67	0.50	0.17	0.44	0.34	0.59	0.81	0.58	0.51	0.72	0.71	0.29
OPB-18	2	2	0.62	0.74	0.17	0.51	0.17	0.92	0.88	0.66	0.58	0.80	0.73	0.27
Mean	7.8	7.0	1.69	1.54	1.39	1.54	1.71	1.97	1.65	1.78	1.66	2.17	0.76	0.24

extractions and *C. elegans* preparations were excluded from analysis.

We also compared population-wide polymorphism in the 6 experimental crossing and selfing strains with polymorphism in the strains originally used to construct them: AB1, CB4855, DR1350, DR1345, and BA786. DNA was bulk extracted from populations of L1 hatchling worms, after bleach and NaOH isolation of fertilized eggs from large cultures (Sulston and Hodgkin 1988; Plasterk 1995), after which the lysate was used in PCR reactions. The primers included OPA-2, OPA-3, OPA-4, OPA-12, OPA-13, OPA-15, OPA-18, OPA-19, OPB-15, OPB-17, and OPB-18.

Data Analysis

RAPD-PCR patterns are multilocus fingerprints in which each band represents a locus with 2 states (presence/absence), and genetic polymorphisms are indicated by differences in RAPD-PCR profiles between individuals. We calculated Shannon's diversity index (H'_j) according to Bussell (1999) Equation 1 to quantify variation among populations and between the selfing and outcrossing sets of populations. The average diversity over all populations (H'_{pop}) was calculated for each locus using Bussell (1999) Equation 2. H'_{pop} enables comparison of the levels of diversity among the populations for all primers together or for each primer individually. Overall diversity across populations (H'_{tot}) was computed using Equation 3 in Bussell (1999). For each locus or for all loci together, the component of diversity within populations is H'_{pop}/H'_{tot} and the component of diversity between populations is $1 - H'_{pop}/H'_{tot}$ (Chalmers et al. 1992; Bussell 1999).

Although Shannon's index is a measure of diversity, it is not a direct estimator of genetic variation. Therefore, we applied a method that employs estimated allele frequencies to approximate the genetic diversity within populations (Lynch and Milligan 1994). The frequencies of null alleles (\hat{q} , absence of a PCR product) were calculated for each locus in each crossing population (Lynch and Milligan 1994, Equation 2a), based on the proportion of N individuals in the sampled population that lacks the PCR product (\hat{x}). Only loci that had null allele frequencies greater than zero were used in calculations. Estimating \hat{q} is based on an assumption of random mating (Lynch and Milligan 1994) that is not appropriate for

the selfing populations. Consequently, we applied a correction for complete inbreeding in the selfing populations such that $\hat{q} = \hat{x}$ (Lynch and Milligan 1994).

The Lynch and Milligan (1994) method to calculate gene diversity within populations provides an estimate of heterozygosity in the populations and accounts for the fact that RAPD-PCR generates dominant markers. We computed gene diversity for our selfing and crossing populations (Lynch and Milligan 1994, Equation 4a), where $\hat{H}_j(i)$ is the probability that 2 genes drawn randomly from population j differ at the i th locus. The average gene diversity over all loci in our selfing and crossing populations (\hat{H}_j) was computed from Equation 5 in Lynch and Milligan (1994).

Markers with high frequency in a population may cause biased estimates of \hat{q} and, thus, of gene diversity. We therefore estimated gene diversity in a second set of calculations restricted to loci with markers whose frequency is less than $1 - (3/N)$ to obtain unbiased estimates (Lynch and Milligan 1994). Finally, standard errors (SEs) of the estimates of gene diversity were calculated using the variance estimate from Equation 1 of Lynch and Milligan (1994).

Results and Discussion

RAPD-PCR of single worms from the 6 experimental populations identified 63 scorable loci, 56 of which were polymorphic (Table 1). On average, each primer detected 7.8 loci (range: 2–14), 7.0 of which were polymorphic (range: 2–13). The experimental populations both gained and lost marker bands compared with the 5 parental strains from which the experimental lines were constructed. In population-wide screens, we identified 42 scorable bands (henceforth loci) in the parental strains. However, 10 unique loci were found within the experimental lines, indicating that recombination among the parental strains during construction of the experimental populations created new RAPD loci (Figure 1). The experimental populations also lost completely 5 loci during the course of 60 generations, retaining an average of 26 products per experimental population (Figure 1).

Shannon's diversity index (H') revealed that 76% of RAPD diversity occurred within the 6 experimental populations (Table 1), indicating that populations did not differ

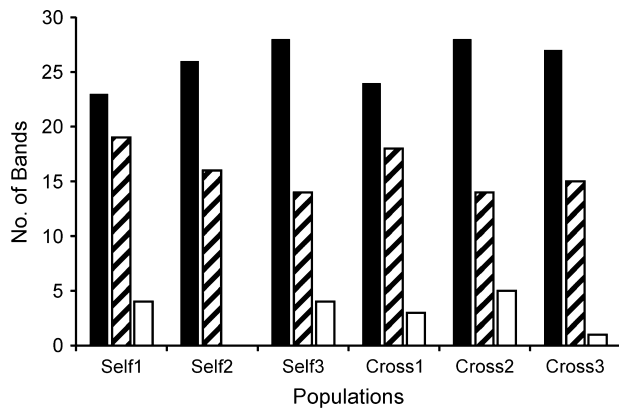


Figure 1. The number of loci (PCR products) identified in population-wide RAPD-PCR of the experimental populations when compared with the original strains used to construct the experimental populations. Closed bars represent products that were conserved from the parental strains, hatched bars represent the number of loci lost from the parental strains, and open bars represent novel loci not found in the original strains.

dramatically in overall levels of polymorphism. Furthermore, the average H' values for the selfing and crossing populations were very similar (Table 1), with the crossing populations retaining only nominally more diversity (Mann–Whitney $U = 8$; $P = 0.127$). Examining the populations with the Lynch and Milligan (1994) method revealed that gene diversity, \hat{H}_j , in the crossing populations was indistinguishable from that in the selfing populations (Table 2), corroborating the Shannon's diversity estimates (Mann–Whitney $U = 6$; $P = 0.513$). Removing loci with high marker frequency resulted in estimates of gene diversity for the selfing populations that were slightly greater than for the crossing populations (Table 2). The uniformly small, yet overlapping, SEs of the gene diversity estimates indicate that the lack of difference between crossing and selfing populations is a robust result (Table 2). The numbers of conserved and new bands also did not differ between crossing and selfing populations (Figure 1).

RAPD-PCR allowed us to sample genetic diversity across a large number of random loci in the *C. elegans* genome. Given the 56 polymorphic loci, this corresponds to approximately 9 loci on each of the 6 chromosomes with each locus separated by an average of ~ 5.4 cM or ~ 1.8 MB. Although these strengths of RAPD-PCR have led to its widespread use in studies of genetic diversity (Shapiro et al. 1997; Bussell 1999; Prathepha and Baimai 1999; Arnholdt-Schmitt 2000; da Silva et al. 2000; Zhou et al. 2000; Engelen et al. 2001; Moya et al. 2001), this method can be subject to certain weaknesses depending on the application. One issue is that products of similar size do not necessarily derive from a common locus, leading to an underestimate of diversity; however, this should occur to an equivalent extent in both of our experimental treatments and therefore should not bias the comparison of selfing and crossing populations. Although the repeatability of RAPD-PCR between laboratories can be a concern for certain kinds of studies, this study avoids this

Table 2. Estimates of gene diversity (\hat{H}_j) in the experimental populations

Population	Null frequency > 0		0 < Null frequency $\leq 1 - 3/N$	
	\hat{H}_j (SE)	Number of loci	\hat{H}_j (SE)	Number of loci
Self1	0.286 (0.004)	51	0.246 (0.007)	35
Self2	0.274 (0.004)	45	0.259 (0.006)	34
Self3	0.273 (0.004)	42	0.239 (0.008)	28
Mean self	0.278 (0.003)	46.0	0.248 (0.004)	32.3
Cross1	0.328 (0.005)	46	0.234 (0.006)	32
Cross2	0.300 (0.004)	49	0.192 (0.005)	34
Cross3	0.256 (0.005)	45	0.166 (0.005)	33
Mean cross	0.295 (0.015)	46.7	0.198 (0.014)	33.0

The estimates were calculated using all loci with null allele frequencies greater than zero. A second set of estimates were calculated using loci with null allele frequencies greater than zero but less than $1 - 3/N$ (Lynch and Milligan 1994).

issue by having used standardized conditions in a single laboratory. Finally, to address the fact that RAPD-PCR markers are dominant and therefore lack information on heterozygotes, we employed statistical methods developed to account for the dominance of markers (Lynch and Milligan 1994).

What processes could result in statistically similar levels of polymorphism between selfing and crossing populations, given that the crossing lines were founded each generation with a population size ($N_e = 150$) at least 5-fold larger than the selfing lines ($N_e \leq 30$)? A potentially lower effective size in the selfing lines (induced by production of *spe-8(bc53)* sterile homozygotes) would only exacerbate this discrepancy. Following Kimura (1964) and Maynard Smith and Haigh (1974), this difference in N_e should lead to at least 2.2 times greater heterozygosity in the crossing populations after 60 generations due to genetic drift alone. Stochastic variation in male reproductive success could reduce N_e in the crossing populations, but for N_e in the crossing population to equal that of selfing populations, more than 90% of males would have had to fail to reproduce in a manner unrelated to their relative fitness.

Although stochastic factors may have had some role in constraining polymorphism in the crossing lines, we propose instead that sexual selection was the principal constraining factor. It has been shown that intense postcopulatory sexual selection by sperm competition led to the evolution of larger sperm in the crossing lines (LaMunyon and Ward 2002). Sperm competition together with other potential forms of post- and precopulatory sexual selection likely generated a disproportionate loss of variation in the crossing populations. For this scenario to explain the observed reduction in genetic variation, many loci must have been subject to selection. This seems plausible considering the number of genes involved in spermatogenesis alone: DNA microarray studies show that $\sim 7.5\%$ of the transcriptome is expressed preferentially during spermatogenesis (>1300 genes), of which 105 genes show male-specific expression (Reinke et al. 2000, 2004). These genes are unlikely to currently experience strong selection

pressure from sperm competition in nature, given available estimates of low outcrossing rates in the wild (Barrière and Félix 2005; Sivasundar and Hey 2005; Cutter 2006). Nonetheless, *C. elegans* sperm genes do show elevated rates of protein evolution, whereas genes expressed principally in the male soma do not, perhaps reflecting historical patterns of differential selection on sperm genes, if selfing evolved relatively recently (Cutter and Ward 2005). Indeed, the increase in sperm volume in the experimental crossing populations coupled with observations of larger sperm sizes among nematode species with greater sperm competition risk makes a compelling case that sperm size is a principal target of postcopulatory sexual selection in nematodes (LaMunyon and Ward 1999, 2002).

Several studies have shown that selection through sperm competition can maintain genetic diversity at selected loci due to nontransitive interactions between males and females (Clark et al. 1995, 2000; Clark 2002; Birkhead et al. 2004). This can occur if, for example, the sperm displacement ability conferred by a given male genotype depends on the female genotype and the genotypes of the male competitors (Clark et al. 2000; Clark 2002), thus allowing multiple alleles to be maintained at selected loci. Although our study was not designed to test for such an effect, the pattern observed here is more consistent with a simpler genetic architecture that also may be compatible with the general lack of strong epistasis for fitness-related traits in *C. elegans* (Peters and Keightley 2000). It remains to be determined whether male–female species of *Caenorhabditis*, which likely experience ongoing sexual selection, might show a pattern more similar to the *C. elegans* or the *Drosophila melanogaster* models.

The strength of sexual selection that occurred in the crossing populations can be roughly estimated using the observed levels of diversity and N_e in the populations. Applying Maynard Smith and Haigh's (1974) Equation 27, we calculate that an effective selection coefficient, s , of magnitude ~ 0.25 (~ 0.34 when using diversity estimates with common markers removed, Table 2) in the crossing populations is consistent with the observed levels of genetic variation and N_e (assuming initial frequency of all alleles = 0.2 and average recombination rate 2.98 cM/MB [Barnes et al. 1995]). If these rough calculations reasonably describe the strength of selection operating on features associated with sperm competitive ability or other sexually selected traits, then selection in the crossing populations was exceptionally strong.

Postcopulatory sexual selection driven by female multiple mating is nearly ubiquitous (Birkhead and Møller 1998) and has resulted in the evolution of a number of traits, for example, toxic ejaculates (Rice 2000) and giant sperm (Miller and Pitnick 2002). Although opportunities for sexual selection by sperm competition are likely limited in natural populations of *C. elegans*—given the low incidence of outcrossing (Barrière and Félix 2005; Sivasundar and Hey 2005; Cutter 2006) and selection for optimization of self-fertilization (Hodgkin and Barnes 1991; Cutter 2004)—these experimental results illustrate the power of sexual selection to reduce genetic variation within populations. These experiments also suggest that sexual selection may prove particularly intense during and

immediately after evolutionary transitions from hermaphroditism to separate sexes.

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