PROCEEDINGS OF THE ROYAL SOCIETY B

Proc. R. Soc. B (2007) **274**, 417–424
doi:10.1098/rspb.2006.3739
Published online 14 November 2006

Selection against males in *Caenorhabditis elegans* under two mutational treatments

Diogo Manoel[1], Sara Carvalho[1], Patrick C. Phillips[2] and Henrique Teotónio[1,*]

[1]*Centro de Biologia do Desenvolvimento, Instituto Gulbenkian de Ciência, Apartado 14, 2781-901 Oeiras, Portugal*
[2]*Centre for Ecology and Evolutionary Biology, 5289 University of Oregon, Eugene, OR 97403-5289, USA*

Within populations with mixed mating systems, selfing is expected to be favoured over outcrossing unless a countervailing process such as severe inbreeding depression is present. In this study, we consider the relationship between the expression of deleterious alleles and the maintenance of outcrossing in the nematode species, *Caenorhabditis elegans*. This species is characterized by an androdioecious breeding system composed of males at low frequency and self-fertilizing hermaphrodites that can only outcross via males. Here, we find that experimentally increasing the mutational load in four different isogenic wild isolates using 10 generations of Ethylmethane sulphonate (EMS) and UV irradiation mutagenesis significantly diminishes the cost of males. Males are maintained at higher frequencies in mutagenized versus non-mutagenized populations. Nevertheless, males still tend to be driven to low frequencies within isolates that are known to be prone to lose males. Further, we determine the viability effects of a single round of mutagen exposure and find that, for EMS, outcrossing overcomes the almost completely recessive and nearly lethal effects generated. We briefly interpret our results in light of current evolutionary theory of outcrossing rates.

Keywords: *Caenorhabditis elegans*; self-fertilization; outcrossing; inbreeding depression; mutation effects

1. INTRODUCTION

Current evolutionary theory relies on two classes of selective factors for the evolution of outcrossing rates: reproductive assurance in its most general sense and the expression of deleterious alleles (Jarne & Charlesworth 1993; Charlesworth & Charlesworth 1998; Pannel 2002). Considering only the expression of deleterious mutations, when the level of inbreeding depression (defined as the difference in fitness among selfing and outcrossing lineages) generated by partially recessive alleles is strong (greater than 0.5), selfing is disadvantageous relative to outcrossing despite its possible transmission advantage (e.g. Fisher 1941; Lande & Schemske 1985; cf. Stewart & Phillips 2002). However, if inbreeding depression is not strong, deleterious recessive mutations can be purged from a population via selfing since more homozygotes will be produced than with outcrossing (Lande & Schemske 1985; Charlesworth *et al.* 1993; Byers & Waller 1999; Crnokrak & Barrett 2002). Moreover, the distributions of both inbreeding depression and heterozygous and homozygous selective coefficients within populations will determine the specific conditions under which outcrossing rates evolve (Holsinger 1988; Lande 1994; Schultz & Willis 1995; Charlesworth & Charlesworth 1998). In general, then, it is expected that inbreeding depression will constrain the evolution of outcrossing rates.

In this study, we use the nematode, *Caenorhabditis elegans*, as an experimental model to test the hypothesis that increasing levels of inbreeding depression should favour increasing levels of outcrossing via the retention of males. *Caenorhabditis elegans* is ideal for this question both because of its ease of cultivation and because it shows an androdioecious breeding system in which populations are composed of hermaphrodites and males (Brenner 1974). Hemizygous sex determination results from X chromosome number, hermaphrodites having two and males only one. Males are produced either from male–hermaphrodite breeding or from the fertilization of aneuploid gametes, in which the meiotic non-disjunction of the X chromosome has occurred, with normal gametes. The presence of males above the very low non-disjunction threshold is therefore a measure of outcrossing within *C. elegans*. Previously, it has been shown that males are selected against in laboratory environments (Stewart & Phillips 2002; Cutter 2005; Teotónio *et al.* 2006). Here, we demonstrate that the expression of deleterious partially recessive alleles diminishes the strength of selection against males in four different genetic backgrounds, thereby demonstrating the importance of deleterious mutations in the evolution of outcrossing rates.

2. MATERIAL AND METHODS

(a) *Selection against males under two mutational environments*

Stewart & Phillips (2002) have shown that selection against males occurred in the reference N2 strain, observing that populations with approximately 50% of males rapidly lose them in the span of less than 10 generations in the laboratory (see also Cutter 2005; Teotónio *et al.* 2006). Here, we used a similar experimental design for four different wild strains: CB4856 and N2 obtained from the *Caenorhabditis* Genetics Center, JU440 obtained from Marie-Anne Félix and PX174 obtained from B. White and P. C. Phillips (sampled in Oregon in 2002). To ensure isogenicity, wild strains were inbred by single individual selfing for 10 generations and stocks cryogenically frozen for posterior experimental use

* Author for correspondence (teotonio@igc.gulbenkian.pt).

Received 28 August 2006
Accepted 19 September 2006

(Stiernagle 1999). These strains were chosen based on our previous genetic characterization of outcrossing characters (Teotónio *et al.* 2006) and gene diversity data (Koch *et al.* 2000; Haber *et al.* 2005; Cutter 2006) to represent extreme phenotypes and the extant genetic variation in this species.

Our standard laboratory environment is different from the one described in Stewart & Phillips (2002). Briefly, it consists of the maintenance of approximately 1000 individuals in a 9 cm diameter Petri dish with NGM-light agar (US Biological) with a lawn of HT115 *Escherichia coli* as the source of food. In each generation, gravid adults are killed by a hypochlorite/sodium hydroxide solution, so that only eggs survive (Stiernagle 1999). These are then maintained in an M9 buffer solution for 16–18 h until all individuals hatch and developmentally arrest at the first larval stage (L1). To propagate the next generation, L1 individuals are placed onto fresh Petri dishes at the appropriate density. Completion of the life cycle takes 4 days at 20°C and 80% relative humidity.

For each isogenic strain, eight replicate lines were obtained by placing several hermaphrodites with an excess of males to ensure outcrossing and a high proportion of males at generation zero of the experiments (more than 30%), for a total of 32 separate lines. Half of the replicate lines were exposed to an external mutagen treatment during day 3 of the life cycle when most individuals are at the late L4/early adulthood phase and when gametogenesis has started. Ethylmethane sulphonate (EMS) at 50 mM for 2 h and 254 nm UV radiation at 10 J m^{-2} were applied in alternate generations to minimize direct adaptation to the mutagen. Preliminary experiments identified a decrease in egg to adult viability of *ca* 10%. The remaining replicate lines were maintained as above but without mutagen exposure, and thus serve as controls. Following 10 generations of treatment, generation 11 was scored for male proportions by counting approximately 1000 individuals per line.

An estimate of mutational input per diploid genome (U) can be given for EMS (cf. Davies *et al.* 1999). For our experimental populations, and using the same rationale as Davies *et al.* (1999), calibrated for 2 h of EMS exposure, there are *ca* 3.8×10^{-6} transitions per GC base pair (EMS is known to mostly generate G/C to A/T transitions), giving $U=61$ transitions per diploid genome per generation. U estimated from phenotypic assays in mutation accumulation experiments (e.g. Vassilieva *et al.* Evolution 2000), is lower than 1, which means that most mutations are unaccounted for, and that most mutations should have small selective coefficients (see table 3 of Davies *et al.* 1999).

Since measurements made at generation 11 could reflect the expression of maternal mutagen environmental effects, both mutagen and non-mutagen treatments were measured again for male proportions after three generations of maintenance in a common environment. Specifically, eggs laid by generation 11 lines were allowed to grow until they depleted their food over the next two weeks. After this period, all lines were transferred to fresh Petri dishes and maintained under standard conditions until generation 13 adult individuals could be counted. Egg to adult viability was also assayed at generation 13 to assess the accumulation of deleterious mutations during the first 10 generations. Here, 100 eggs were established on a fresh plate and allowed to develop and grow. Viability was scored as the number of live adults. Four replicate plates were used per replicate line and per wild strain.

To determine whether the mutagen treatment increases the rates of non-disjunction, and therefore the number of males, and/or increases the rate of beneficial mutations associated with male function, a similar set of selection and mutagenesis experiments were performed following the initial set. In these experiments, three separate hermaphrodites were taken from frozen isogenic stocks and used to establish three different replicate lines for the N2 and CB4856 strains, for a total of 12 lines. Therefore, males were initially at a high frequency in the first set of experiments, whereas in the second set of experiments, males could only appear as a consequence of meiotic non-disjunction of the X chromosome during hermaphrodite gametogenesis. Male frequency was scored for each replicate by counting approximately 10 000 individuals after 10 generations of mutagen treatment.

(b) *Inbreeding depression generated by a single round of mutagen exposure*

Inbreeding depression is known to be negligible within natural isolates of *C. elegans* (Johnson & Hutchinson 1993; E. Dolgin *et al.* 2006, personal communication). In order to address the effects of mutation accumulation in the experimental populations, inbreeding effects were measured as egg to adult viability after a single round of exposure to either EMS (50 mM for 2 h) or 254 nm UV radiation (10 J m^{-2}). Male-enriched populations were obtained as before from CB4856, PX174, N2 and JU440. EMS or UV light was applied to each of these populations and F1 offspring either allowed to self-fertilize or forced to outcross with sibling males. Viability was estimated in the F2 offspring. Contemporaneously, the parental lines without mutagen exposure and an F1 generation whose parents had been exposed were assayed to account for any intergenerational directional environmental effects. There were thus seven different groups of individuals per wild strain assayed: unexposed parentals, EMS or UV F1 individuals, EMS or UV selfed F2 individuals and EMS or UV outcrossed F2 individuals. Viability was assayed as above. Replicates were divided over 2 consecutive days.

(c) *Statistical analysis*

The unit of observation for the 10 generation mutagen exposure treatment was each of the four replicate populations within each treatment (a total of 32 data points at each generation). All data were obtained as proportions and thus several transformations were tested for conformity with linear model assumptions. Normality of residuals was tested with Kolmogorov–Smirnov test and homocedasticity with Bartlett's test. The log ($X\times1000$) transformation gave the best-fit models for all data on male proportions, while viability was best modelled when left untransformed. Data in figures are shown in the original proportions for clarity. A single two-way ANOVA was modelled to generation 11 and generation 13 separately, with strain as a four-level fixed factor (CB4856, JU440, PX174 and N2) and treatment as a two-level (mutagen and non-mutagen exposure) fixed factor. Interaction between strain and treatment was also assessed. Posterior contrasts testing mutagen effects within each strain were done with Tukey tests, but only when the interaction effects between the two factors were significant.

The experimental design used in the inbreeding experiments allows for the partitioning of phenotypic variance into mutational and environmental effects. Inbreeding depression for viability is estimated as $\delta=[1-(\text{viability of F2 selfed}/\text{viability of F2 outcrossed})]$. Data for the F2 generations were standardized by subtracting the average value of both the parental and the F1 generations for each mutagen. Each assay

Table 1. (top) Regression coefficients in a genetic model of heterozygous and homozygous mutational effects on viability. (bottom) Results for each strain, after a single round of EMS. (Estimates of dominance (h) and homozygous (s) selection coefficients are shown. Regression coefficients as different from zero are $^{*}p<0.05$ and $^{**}p<0.001$ (two-tailed Student's t-tests).)

strain	intercept	heterozygosity	homozygosity	$F_{2,23}$	R^2	h	s
parentals	1	0	0				
F1	1	1	0				
F2 selfed	1	0.5	0.25				
F2 outcrossed	1	1	0				
CB4856	0.863^{**}	-0.099^{*}	-0.902^{**}	24.17	59.5%	0.095	1.039
JU440	0.837^{**}	−0.053	-0.947^{**}	24.83	70.3%	0.048	1.110
N2	0.945^{**}	-0.084^{*}	-0.825^{**}	20.04	65.6%	0.096	0.880
PX174	0.895^{**}	-0.083^{*}	-0.848^{**}	16.90	61.7%	0.087	0.953
mean strains	0.885	−0.080	−0.881			0.081	0.996
s.d.	0.047	0.019	0.056			0.023	0.100

plate was taken as the unit of observation. To this F2 data, and separately for each mutagen, a two-way ANOVA was done with strain as a four-level fixed factor (CB4856, JU440, PX174 and N2) and breeding treatment as a two-level fixed factor (self and outcross). Interaction between factors was also assessed for significance. Day of set-up was modelled as a covariate.

Multiple regression models were also employed to estimate heterozygous and homozygous mutation effects, according to the model of table 1, for each strain separately and taking data from all generations. Based on these estimates, the selective coefficient under homozygocity (s) and the dominance coefficient (h) were estimated using the standard diploid model, in which heterozygous lineages will have a lower viability than the parental lineages by the quantity hs, while homozygous lineages will have lower viability than the parentals by a quantity s (Crow & Kimura 1970). This model assumes equality of effects among mutations and no epistasis if more than one mutation is present per genome.

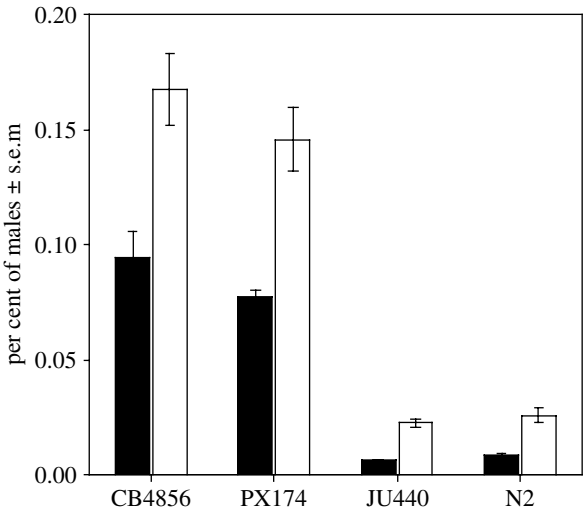

Figure 1. Male proportions in four isogenic strains subject to mutagen exposure (white bars) or control (solid bars) after 10 generations of laboratory maintenance. Data are shown as mean values of the four replicates with standard error of the mean as the error bars. There are significant treatment, strain and interaction effects. Differences within each strain between the two treatments are all significant after multiple comparison correction.

3. RESULTS

(a) *Selection against males*

As in the previous studies (Stewart & Phillips 2002; Cutter 2005), we find that males are selectively costly, since their proportion fell to less than 10% from initial proportions of more than 30%. However, males were kept at higher proportions in mutational treatments when compared with controls (figure 1; mutagen treatment: $F_{1,24}=197.04$, $p<0.001$). Similarly, there were differences among the four different strains ($F_{3,24}=393.24$, $p<0.001$), with N2 and JU440 males being driven to much lower frequencies than CB4856 and PX174 (see Teotónio *et al.* 2006). The interaction between strain and treatment was significant as well ($F_{3,24}=7.08$, $p=0.001$). Posterior contrasts by Tukey tests revealed differences within all strains between treated and untreated replicates (all $p<0.01$).

The observed differences in male proportions were not due to directional maternal (environmental) effects caused by the mutagens, since male proportion differences measured at generation 13 continue to be significantly explained by mutagen treatment (figure 2; $F_{1,24}=6.43$, $p=0.018$). Strain effects are also still significant ($F_{3,24}=135.83$, $p<0.001$), but the interaction no longer is ($F_{3,24}=0.99$, $p=0.415$, figure 2). Differences in male proportions are smaller than in generation 11 since purging of deleterious mutations must have occurred during the two generations of common environment.

Further, viability measurements at generation 13 demonstrate that populations which experienced mutagen treatment were less viable than the controls (figure 2; $F_{1,24}=8.33$, $p=0.008$), probably as a result of the accumulation of deleterious mutations. Differences among strains were also significant ($F_{3,24}=8.51$, $p=0.001$), but not the interaction term ($F_{3,24}=0.37$, $p=0.774$).

Finally, the observed differences in the number of males in the mutagen treatments are not due to an increase in the rates non-disjunction of the X chromosome and/or an increase in the rates of beneficial mutations associated with male phenotypes. Experiments starting with replicates of the CB4856 and N2 strains from single hermaphrodites did not show a significant increase in the number of males after 10 generations of mutagen exposure (figure 3; treatment effect: $F_{1,8}=0.88$, $p=0.376$; strain effect: $F_{1,8}=3329.19$, $p<0.001$; interaction: $F_{1,8}=2.18$, $p=0.178$).

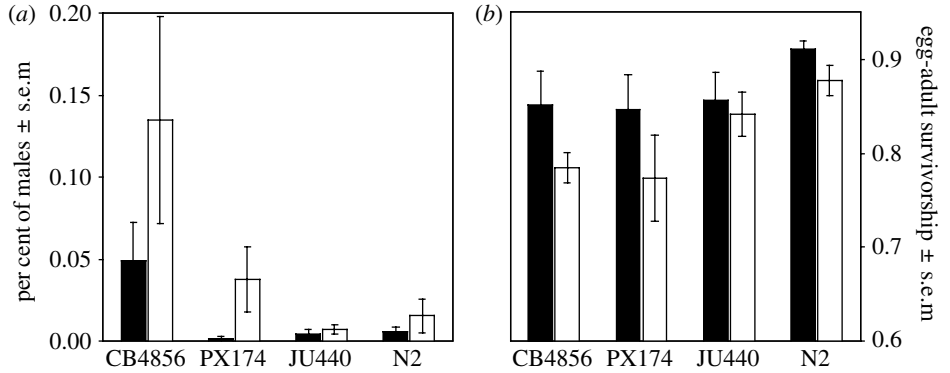

Figure 2. (*a*) Male proportions and (*b*) viability are shown for generation 13, three generations after stopping the mutagen treatment. Black bars indicate mean values of four replicates for control treatment and white bars for mutagen treatment, with associated standard error of the mean. For both characters, there are significant mutagen treatment and strain effects.

(b) *Inbreeding depression*

A single round of EMS exposure generated mutations with strong deleterious effects, such that the average inbreeding depression for viability across strains was $\delta = 0.22 \pm 0.01$ s.e.m. (figure 4). Progeny resulting from outcrossing have significantly higher viability than those from self-fertilization ($F_{1,39} = 50.01$, $p < 0.001$). There were no differences among the four different strains ($F_{3,39} = 0.63$, $p = 0.599$) or in the interaction among strains and breeding treatment ($F_{3,39} = 0.85$, $p = 0.477$). Replication across days was also not significant ($F_{1,39} = 3.42$, $p = 0.072$).

To estimate the dominance (h) and recessive (s) selective coefficients, a multiple regression model was employed to each strain independently (table 1). It is clear that mutations created by EMS are nearly lethal when homozygous, and that they are also partially recessive, with heterozygous lineages being approximately 8% less viable than parentals. Results for a single round of UV light exposure are more complex (figure 4). Day of assay set-up was a significant covariate ($F_{1,39} = 6.55$, $p = 0.014$), as well as strain ($F_{3,39} = 3.63$, $p = 0.021$) and breeding treatment ($F_{1,39} = 17.2$, $p < 0.001$). Here, however, the outcrossed individuals were less viable than selfed individuals, which is indicative of underdominant effects among different mutations. The interaction between strain and treatment was not significant. The ANOVA model has however a poor fit ($R^2 = 6.43\%$). The multiple regression models also have a very poor fit (R^2 for all strains below 10%, not shown), so estimates of h and s were not calculated.

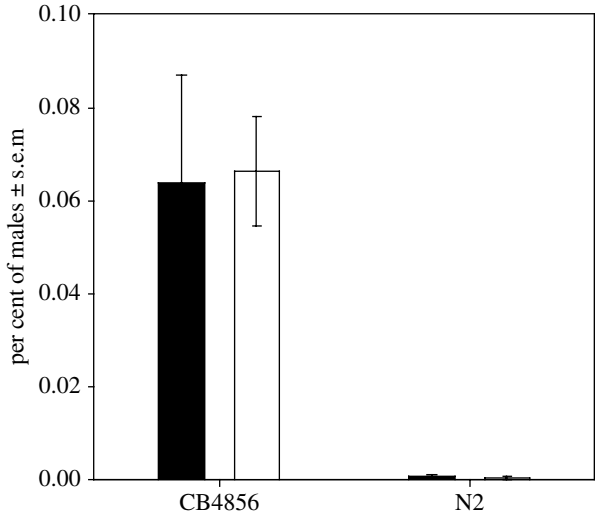

Figure 3. Male proportions after 10 generations of mutagen exposure with production of males in the initial generation being solely due to the meiotic non-disjunction of X chromosome in hermaphrodite gametogenesis. Black bars indicate mean values of three replicates for control treatment and white bars for mutagen treatment, with associated standard error of the mean. There are no detectable differences among treatments.

4. DISCUSSION

The role of males in *C. elegans* populations has been something of a conundrum. Are they evolutionary relics (Chasnov & Chow 2002) or does outcrossing via males have an important impact on variation within and between populations (Stewart & Phillips 2002; Cutter 2005)? It has previously been demonstrated that outcrossing in *C. elegans* is selected against in laboratory environments (Stewart & Phillips 2002), which agrees well with the very low proportions of males and outcrossing observed in natural isolates (Barrière & Félix 2005; Teotónio *et al.* 2006), as well as with the negligible inbreeding depression found for several life-history characters in *C. elegans* (Johnson & Hutchinson 1993; E. Dolgin *et al.* 2006, personal communication). The experiments presented here study the effects of increased mutational load, as defined by a decrease in population fitness due to the expression of induced mutations, on outcrossing rates. We show that the selective cost of outcrossing and the production of males can diminish under conditions of increased mutational loads. Cutter (2005) has found a similar effect, under different laboratory conditions, when increasing mutational loads through genetic disruption of a DNA repair pathway. We extend his study to more than one natural isolate, while controlling for male reproductive success and genotype by environment effects, as well as estimating the selective properties of the induced mutations.

We find that after 10 generations of EMS/UV mutagen exposure, experimental populations have higher male frequencies than controls, and therefore higher rates of outcrossing. These differences are not due to inadvertent environmental effects generated by the mutagen, since mutagen-treated and control populations maintain male proportion differences after two full generations of maintenance in a common environment. These differences are nevertheless lower at generation 13 than generation 11, undoubtedly reflecting the purging of a significant number

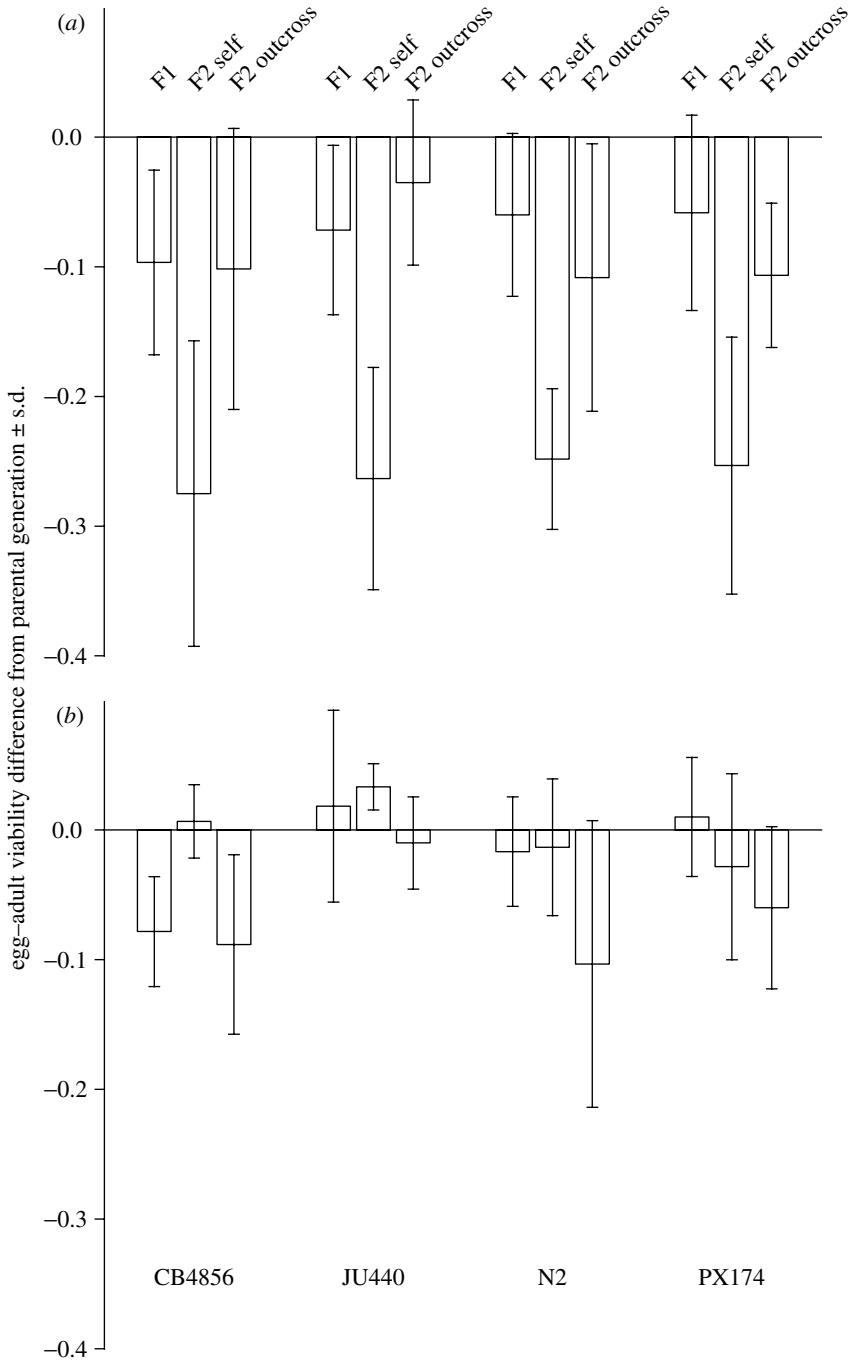

Figure 4. Inbreeding and outcrossing effects after a single round of mutagen exposure to (*a*) EMS and (*b*) UV light, for four isogenic strains, and shown as the difference from the parental viability with standard deviations as error bars.

of the accumulated mutations under mixed selfing and outcrossing following cessation of additional mutational input (see below). Further, deleterious mutations have accumulated in the treated populations, since their egg to adult viability is low relative to control populations. We also do not detect any evidence that rates of either X chromosome non-disjunction during gametogenesis, the mechanism by which males can be generated from unmated hermaphrodites, or mutations that could increase male reproductive success, increase in mutagen-treated populations relative to controls. While the accumulation of deleterious mutations causes the selective cost of outcrossing to diminish, it is not clear that it allows the maintenance of mixed outcrossing rates, since experimental populations have yet to reach equilibrium and male frequencies are still fairly low.

Available phenotypic models predict that males will be maintained whenever the effectiveness of male mating (discounted by selection against males) can overcome the selfing advantage of hermaphrodites (discounted by the effects of inbreeding depression), as in the relationship $\alpha(1-\sigma) > 2\beta(1-\delta)$, where α is male reproductive success; σ is the viability difference among males and hermaphrodites; β is the proportion of oocytes that are self-fertilized; and δ is inbreeding depression (Stewart & Phillips 2002; Cutter *et al.* 2003). Since our experimental populations are not at equilibrium, we cannot fully address this relationship, but we can test for the existence of an association between male reproductive success and inbreeding depression. First, we find that higher male reproductive successes are associated with a decrease in egg to adult viability (the overall correlation between log male

proportion and viability with all mutagen-treated and control populations, at generation 13 of the experiment, is $r_{\text{Pearson}} = -0.517$, $n = 32$, $p = 0.002$). This observation can only be interpreted as increased mutation accumulation in populations with higher rates of outcrossing. An alternative interpretation is that the lower viabilities observed in those populations with higher male reproductive success reflect lower viability of males relative to hermaphrodites, since males are hemizygous for the X chromosome. At generation 13, however, male numbers are so low that even large differential viability among genders does not change the results (not shown). Second, the significant interaction term at generation 11 between strain and mutagen treatment also suggests that mutation accumulation is higher in the two strains that have higher male proportions, CB4856 and PX174, relative to the two that have lower male proportions, N2 and JU440, since the differences observed between mutagen-treated and control populations are larger. Taken together, it appears that variation in male reproductive success and outcrossing rates influences the magnitude of mutational loads and presumably inbreeding depression as well (see also Charlesworth *et al.* 1993; Schultz & Willis 1995; for genetic models with varying outcrossing rates).

The selective effects of mutations generated by a single round of either EMS or UV exposure were also estimated. For EMS, we find nearly lethal mutations ($s = 0.996$), these being close to fully recessive ($h = 0.08$), across the four strains. For UV, the ANOVA models fitted were significant but poorly predictive. There is a suggestion of underdominance, which can be explained if UV generates small rearrangements, such as deletions, duplication and translocations (cf. Anderson 1995; Johnsen & Baillie 1997), which in turn impair the proper segregation of chromosomes during the meiosis of heterozygotes (cf. Villeuneuve 1994; Villeuneuve & Hillers 2001). If real, however, underdominance has hampered our power to observe higher male frequency in the mutagen treatments, since outcrossing will be selected against to an even larger extent than in controls. For this reason, and because UV models were poorly fitted, we only interpret the five generations of EMS mutational input for the remaining of discussion.

The critical element in the theories for the maintenance of outcrossing is the level of inbreeding depression in the population (Lloyd 1979; Lande & Schemske 1985; Charlesworth *et al.* 1990). We have shown that the EMS treatment is capable of inducing a large amount of inbreeding depression within a single generation ($\delta = 0.22$), whereas the UV treatment would appear to generate little inbreeding depression or perhaps outbreeding depression, instead. While the per-generation rate of inbreeding depression is less than the $\delta > 0.5$ needed for the deterministic maintenance of outcrossing in most models (review in Charlesworth & Charlesworth 1998), this value represents the standing level of inbreeding depression, not the rate of input as measured here. Further, this result is for the general case in which selfers and outcrossers have equal mating availability. For the asymmetrical mating system of *C. elegans* (outcrossing only via male reproduction), variation in male mating success can have a large influence on the equilibrium frequency of males (see above; Stewart & Phillips 2002). This is equivalent to extreme 'pollen discounting', which facilitates the persistence of intermediate levels of outcrossing (Nagylaki 1976; Holsinger 1991; Harder & Wilson 1998; Porcher & Lande 2005). Finally, the distribution of mutational effects will also have a large influence on the standing level of inbreeding depression, as mutations with large effects, such as those observed here (and which have been routinely observed in natural mutation accumulation studies in *C. elegans*; e.g. Vassilieva *et al.* 2000; Ajie *et al.* 2005), are more readily purged from partially selfing populations than mutations of smaller effect (Heller & Maynard Smith 1979; Lande & Schemske 1985; Holsinger 1988; Hedrick 1994; Lynch *et al.* 1995; Wang *et al.* 1999).

Although the average effect of mutations generated by EMS detected under laboratory conditions can be quite large, the distribution of effect sizes appears to be very skewed, with the majority of mutations (perhaps 90% or more) having small effects ($s < 0.1$; cf. Davies *et al.* 1999; Keightley *et al.* 2000). Inbreeding depression is driven primarily by mutation rate rather than effect size, with mutations of intermediate effect having the largest impact on finite populations (Lynch *et al.* 1995). The increased mutation rate used here is therefore likely to have generated substantial inbreeding depression within the experimental populations. Further, dominance coefficients (h) can also decrease the mean fitness of selfing lineages to an extent that outcrossing will be favoured. For example, for alleles with $h < 0.1$, inbreeding depression can be well above 50% (e.g. Latta & Ritland 1994; Peters *et al.* 2003). With overdominance ($h < 0$) on the other hand, outcrossing alleles can be favoured even if inbreeding depression is low (Holsinger 1988; Charlesworth & Charlesworth 1990). In the best empirical study of the heterozygous effects of mutants generated by EMS to date, Peters *et al.* (2003) have shown that on average $h = 0.1$, which is very close to our own estimate of $h = 0.08$. Further, variation of h around this mean was found to be significant with several alleles showing overdominant effects. Hence, in our experimental populations, mutants with $h < 0.08$ should have been generated, contributing to an increase in inbreeding depression in the experimental populations. With these strongly recessive mutations, males are maintained at higher frequencies in the high mutation treatments because the outcrossing they induce effectively complements the mutations' deleterious effects, thereby increasing the relative fitness of outcrossed (and male producing) versus selfed progeny.

Overall, then, increasing the rate of deleterious mutations can lead to an increase in the frequency of males and a concomitant increase in the level of outcrossing within these nematode populations. However, increasing the rate of mutation is not sufficient to preserve males in all backgrounds. Male mating ability must be sufficiently high so that the rate of male production can overcome the rate of purging of mutations of large effect via selfing. It is therefore not surprising that increasing the rate of deleterious mutation is more effective at maintaining males in genetic backgrounds in which the rate of loss of males is relatively slow under control conditions, as predicted by theory (figure 2; Stewart & Phillips 2002; Teotónio *et al.* 2006). Such mutation by background interactions are likely to prove critical for our understanding of the variable levels of outcrossing observed in natural populations (primarily plants; Goodwille *et al.* 2005). The experimental circumstances explored here are decidedly non-equilibrium in nature; therefore, more

theory needs to be developed before the precise balance factors necessary for long-term maintenance of males in the face of continual mutational input and purging via selfing. However, we have demonstrated that level of mutational input and strain-specific characteristics such as male mating are important in determining whether or not males will persist within these partially selfing populations.

We thank R. Koroloff and A. Homem for their technical help. We also thank J. Anderson, A. Cutter, M.-A. Félix, I. Gordo, L. Morran, J. Thompson, and especially some careful reviewers for comments regarding the project and the manuscript. This work was supported by the National Science Foundation grant DEB-0236180 (to P.C.P.), and Fundação para a Ciência e a Tecnologia (FCT/FEDER/POCTI/BIA-BDE/61127/2004) and Fundação Calouste Gulbenkian (to H.T.).

REFERENCES

Ajie, B., Estes, S., Lynch, M. & Phillips, P. C. 2005 Behavioral degradation under mutation accumulation. *Genetics* **170**, 655–660. (doi:10.1534/genetics.104.040014)

Anderson, P. 1995 Mutagenesis. *Methods Cell Biol.* **48**, 31–58.

Barrière, A. & Félix, M. 2005 High local genetic diversity and low outcrossing rate in *Caenorhabditis elegans* natural populations. *Curr. Biol.* **15**, 1176–1184. (doi:10.1016/j.cub.2005.06.022)

Brenner, S. 1974 The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71–94.

Byers, D. L. & Waller, D. M. 1999 Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annu. Rev. Ecol. Syst.* **30**, 479–513. (doi:10.1146/annurev.ecolsys.30.1.479)

Chasnov, J. R. & Chow, L. K. 2002 Why are there tales in the hermaphroditic species *Caenorhabditis elegans*? *Genetics* **160**, 983–994.

Charlesworth, B. & Charlesworth, D. 1998 Some evolutionary consequences of deleterious mutations. *Genetica* **102/103**, 3–19. (doi:10.1023/A:1017066304739)

Charlesworth, D. & Charlesworth, B. 1990 Inbreeding depression with heterozygote advantage and its effect on selection for modifiers changing outcrossing rate. *Evolution* **44**, 870–888.

Charlesworth, B., Charlesworth, D. & Morgan, M. T. 1990 Genetic loads and estimates of mutation rates in highly inbred plant populations. *Nature* **347**, 380–382. (doi:10.1038/347380a0)

Charlesworth, D., Morgan, M. T. & Charlesworth, B. 1993 Mutation accumulation in finite outbreeding and inbreeding populations. *Genet. Res. Camb.* **61**, 39–56.

Crnokrak, P. & Barrett, S. C. 2002 Perspective: purging the genetic load: a review of the experimental evidence. *Evolution* **56**, 2347–2358. (doi:10.1554/0014-3820(2002)056[2347:PPTGLA]2.0.CO;2)

Crow, J. F. & Kimura, M. 1970 *An introduction to population genetics theory*. New York, NY: Harper & Row.

Cutter, A. D. 2005 Mutation and the experimental evolution of outcrossing in *Caenorhabditis elegans*. *J. Evol. Biol.* **18**, 27–34. (doi:10.1111/j.1420-9101.2004.00804.x)

Cutter, A. D. 2006 Nucleotide polymorphism and linkage disequilibrium in wild populations of the partial selfer *Caenorhabditis elegans*. *Genetics* **172**, 171–184. (doi:10.1534/genetics.105.048207)

Cutter, A. D., Avilés, L. & Ward, S. 2003 The proximal determinants of sex ratio in *C. elegans* populations. *Genet. Res. Camb.* **91**, 91–102.

Davies, E. K., Peters, A. D. & Keightley, P. D. 1999 High frequency of cryptic deleterious mutations in *Caenorhabditis elegans*. *Science* **285**, 1748–1751. (doi:10.1126/science.285.5434.1748)

Fisher, R. A. 1941 Average excess and average effect of a gene substitution. *Ann. Eug.* **11**, 53–63.

Goodwillie, C., Kalisz, S. & Eckert, C. G. 2005 The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annu. Rev. Ecol. Syst.* **36**, 47–79. (doi:10.1146/annurev.ecolsys.36.091704.175539)

Haber, M., Scüngel, M., Putz, A., Müller, S., Hasert, B. & Schulenburg, H. 2005 Evolutionary history of *Caenorhabditis elegans* inferred from microsatellites: evidence for spatial and temporal genetic differentiation and the occurrence of outbreeding. *Mol. Biol. Evol.* **22**, 160–173. (doi:10.1093/molbev/msh264)

Harder, L. D. & Wilson, W. G. 1998 A clarification of pollen discounting and its joint effects with inbreeding depression on mating system evolution. *Am. Nat.* **152**, 684–695. (doi:10.1086/286199)

Hedrick, P. W. 1994 Purging inbreeding depression and the probability of extinction: full-sib mating. *Heredity* **73**, 363–372.

Heller, R. & Maynard Smith, J. 1979 Does Muller's ratchet work with selfing? *Genet. Res. Camb.* **32**, 289–293.

Holsinger, K. E. 1988 Inbreeding depression doesn't matter: the genetics basis of mating system evolution. *Evolution* **42**, 1235–1244. (doi:10.2307/2409007)

Holsinger, K. E. 1991 Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems. *Am. Nat.* **138**, 606–622. (doi:10.1086/285237)

Jarne, P. & Charlesworth, D. 1993 The evolution of the selfing rate in functionally hermaphrodite plants and animals. *Annu. Rev. Ecol. Syst.* **24**, 441–466. (doi:10.1146/annurev.es.24.110193.002301)

Johnsen, R. C. & Baillie, D. L. 1997 Mutation. In *C. elegans* II (ed. D. L. Riddle, T. Blumenthal, B. J. Meyer & J. R. Priess), pp. 79–95. New York, NY: Cold Spring Harbor Laboratory Press.

Johnson, T. E. & Hutchinson, E. W. 1993 Absence of strong heterosis for life span and other life history traits in *Caenorhabditis elegans*. *Genetics* **194**, 465–474.

Keightley, P. D., Davies, E. K., Peters, A. D. & Shaw, R. G. 2000 Properties of ethylmethane sulfonate-induced mutations affecting life-history traits in *Caenorhabditis elegans* and inferences about bivariate distributions of mutation effects. *Genetics* **156**, 143–154.

Koch, R., VanLuenen, H. G. A. M., Van der Horst, M., Thijssen, K. L. & Platerk, R. H. A. 2000 Single nucleotide polymorphisms in wild isolates of *Caenorhabditis elegans*. *Genome Res.* **10**, 1690–1696. (doi:10.1101/gr.GR-1471R)

Lande, R. 1994 Risk of population extinction from fixation of new deleterious mutations. *Evolution* **48**, 1460–1469. (doi:10.2307/2410240)

Lande, R. & Schemske, D. W. 1985 The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* **39**, 24–40. (doi:10.2307/2408514)

Latta, R. & Ritland, K. 1994 Conditions favoring stable mixed mating systems with jointly evolving inbreeding depression. *J. Theor. Biol.* **170**, 15–23. (doi:10.1006/jtbi.1994.1165)

Lloyd, D. G. 1979 Some reproductive factors affecting the selection of self-fertilization in plants. *Am. Nat.* **113**, 67–79. (doi:10.1086/283365)

Lynch, M., Conery, J. & Burger, R. 1995 Mutation accumulation and the extinction of small populations. *Am. Nat.* **146**, 489–518. (doi:10.1086/285812)

Nagylaki, T. 1976 A model for the evolution of self-fertilization and vegetative reproduction. *J. Theor. Biol.* **58**, 55–58. (doi:10.1016/0022-5193(76)90138-7)

Pannel, J. R. 2002 The evolution and maintenance of androdioecy. *Annu. Rev. Ecol. Syst.* **33**, 397–425. (doi:10.1146/annurev.ecolsys.33.010802.150419)

Peters, A. D., Halligan, D. L., Whitlock, M. C. & Keightley, P. D. 2003 Dominance and overdominance of mildly deleterious induced mutations for fitness traits in *Caenorhabditis elegans*. *Genetics* **165**, 589–599.

Porcher, E. & Lande, R. 2005 The evolution of self-fertilization and inbreeding depression under pollen discounting and pollen limitation. *J. Evol. Biol.* **18**, 497–508. (doi:10.1111/j.1420-9101.2005.00905.x)

Schultz, S. T. & Willis, J. H. 1995 Individual variation in inbreeding depression: the roles of inbreeding history and mutation. *Genetics* **141**, 1209–1223.

Stewart, A. D. & Phillips, P. C. 2002 Selection and maintenance of androdioecy in *Caenorhabditis elegans*. *Genetics* **160**, 975–982.

Stiernagle, T. 1999 Maintenance of *C. elegans*. In *C. elegans* a practical approach (ed. I. Hope), pp. 51–67. Oxford, UK: Oxford University Press.

Teotónio, H., Manoel, D. & Phillips, P. C. 2006 Genetic variation for outcrossing among *Caenorhabditis elegans* isolates. *Evolution* **60**, 1300–1305. (doi:10.1554/06-085.1)

Vassilieva, L. L., Hook, A. M. & Lynch, M. 2000 The fitness effects of spontaneous mutations in *Caenorhabditis elegans*. *Evolution* **54**, 1234–1246. (doi:10.1554/0014-3820(2000)054[1234:TFEOSM]2.0.CO;2)

Villeneuve, A. M. 1994 A *cis*-acting locus that promotes crossing over between X chromosomes in *Caenorhabditis elegans*. *Genetics* **136**, 887–902.

Villeneuve, A. M. & Hillers, K. J. 2001 Whence meiosis? *Cell* **106**, 647–650. (doi:10.1016/S0092-8674(01)00500-1)

Wang, J., Hill, W. G., Charlesworth, D. & Charlesworth, B. 1999 Dynamics of inbreeding depression due to deleterious mutations in small populations: mutation parameters and inbreeding rate. *Genet. Res.* **74**, 165–178. (doi:10.1017/S0016672399003900)